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SODIUM REGULATION IN THE LARVAE OF *CHIRONOMUS DORSALIS* (*MEIG.*) AND *CAMPTOCHIRONOMUS TENTANS* (FABR.): THE EFFECT OF SALT DEPLETION AND SOME OBSERVATIONS ON TEMPERATURE CHANGES

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SUMMARY

Sodium regulation was studied in fourth instar larvae of *Chironomus dorsalis* and *Camptochironomus tentans*. Both maintain a body sodium level well above that of the surrounding medium. The haemolymph contains approximately 90% of total body sodium and approximates to a single compartment freely exchanging sodium with the external medium. The anal papillae play a primary role in sodium regulation, the gut being of secondary importance.

Sodium regulation in both species is comparatively insensitive to alterations in acclimatization temperature.

C. dorsalis and *C. tentans* are capable of maintaining sodium balance in media containing 10 μmole Na and 25 μmole Na respectively.

When exposed to several changes of distilled water, *C. tentans* is capable of reducing sodium loss by elaboration of a more dilute urine. This is apparently, supplemented by a reduction in the permeability of the body surface. Activation of sodium uptake in both species is comparatively sluggish, with influx reaching a maximum only after the loss of > 30% body sodium.

INTRODUCTION

Ionic and osmotic regulation in dipteran larvae has received spasmodic attention since Wigglesworth's (1933*a*, *b*; 1938) work on culicine larvae.

The best studied animal has been the larva of *Aëdes aegypti*, and the nature of the sodium regulatory mechanism of this species is now known in some detail (Treherne, 1954; Stobbart, 1959–1974). Along with other ions, sodium is secreted from the external medium into the haemolymph against a steep concentration gradient, and is kept within narrow concentration limits. The Malpighian tubules contribute to this fine control (Ramsay, 1950, 1951, 1953), although the overriding importance of the anal papillae has long been established (Wigglesworth, 1933*a*, *b*; 1938). The work of Koch (1938) indicates that salt regulation in the larva of *Chironomus plumosus* is somewhat similar to that in *Aëdes* and the culicine larvae.

Although some work exists which relates osmostic regulation in chironomid larvae to their habitat (Sutcliffe, 1959; Strenzke & Neumann, 1960; Neumann, 1961; Lauer,

1969), sodium regulation in these animals has been largely neglected, with the exception of some isolated observations by Koch (1954).

Chironomid larvae, like *A. aegypti* larvae, have a large haemolymph compartment and vermiform shape, and therefore readily lend themselves to a variety of experimental techniques. Their comparatively large size and robust nature, together with their widespread distribution in fresh and brackish waters, make them particularly attractive experimental material. This investigation establishes the basic features of sodium regulation under freshwater conditions in fourth instar larvae of *Chironomus dorsalis* and *Camptochironomus tentans*.

MATERIALS AND METHODS

Experimental animals were collected from the field where residual populations remain throughout the year. *Chironomus dorsalis* larvae were collected from the Sleek Burn at Choppington in Northumberland. Larvae of *Camptochironomus tentans* were mainly collected from Big Waters Pond at Dinnington in Northumberland, although some preliminary experiments were carried out on specimens of this species from Leazes Park Lake, Newcastle upon Tyne. In June 1970, the sodium concentration of water from the Sleek Burn and Big Waters Pond was found to be 7 mM and 8 mM respectively, and that from Leazes Park Lake 1·5 mM. In January 1973, these concentrations had increased to 8 mM, 9·5 mM and 2·2 mM. An attempt was made to culture *C. tentans* (Sadler, 1935), but although several fertile egg masses were laid, there was insufficient recruitment into the fourth instar for experimental needs.

There are no entirely satisfactory works on the identification of chironomid larvae although Bryce (1960) gives some useful guides. Classification of larval types reaches down to minute taxonomic characters, and is at best very tedious, and at worst unreliable. Rearing to adults and identifying these through Edwards's (1929) definitive work was found to be satisfactory. In this way, it was found that each of the collecting sites supported virtually a single breeding species.

Fourth instar larvae were used for all experiments. Pupal recruitment came from a considerable size range, particularly in *C. tentans*, in which animals from 14 mg to > 30 mg pupated. Specimens of *C. tentans* were selected from within this size range. For the smaller species, *C. dorsalis*, the animals used were all over 7 mg. Animals were generally used within 10 days of collection. Unless otherwise stated, the experimental NaCl concentration used was 2 mM. Experiments were carried out at 21 ± 1 °C.

Any short-term storage of animals was at room temperature (unless otherwise stated) in polystyrene photographic trays containing leaves, organic debris from the field, and tapwater with the NaCl concentration brought up to 2 mM. Animals left in this medium for 5 days or more were regarded as steady-state animals.

Terminology

The terms steady-state exchange, influx, outflux (efflux), net uptake and net loss are as used by Stobbart (1959, 1960).

Measurement of sodium flux using ^{22}sodium influx

This could be calculated from steady-state exchange data (cf. Treherne, 1954; Stobbart, 1959). However, for a number of experiments, a short-term loading method

was employed, whereby groups of 6–10 animals were immersed in a labelled solution for a measured period of 1–2 h. Back-flux during this time could reasonably be ignored.

After labelling, animals were washed in distilled water, weighed, and then ashed at 500 °C for a period of 12 h, on marked platinum strips. The ash was dissolved in a drop of N/10 HCl to convert the sodium present to NaCl, and the solution and washings were transferred by a standardized procedure to a planchet for measurement, when dry, of its radioactivity. 4·5 μm of dextrose was used as a spreader.

General tracer techniques

Measurements of radioactivity were made using an I.D.L. low background counter. Fluxes were calculated from a knowledge of the specific activity of the loading medium and of the radioactivity of the samples counted. Whole body sodium fluxes were expressed as mM/kg wet wt./hr.

Tests showed that counts of radioactive solutions varied proportionally with the volumes of solution used in these experiments. Animal material was tested by building up samples from material of known activity. These, too, were satisfactory in this respect. The specific activities of the experimental media were between 0·4 and 1·0 mCi/g Na.

Chemical analyses

Samples for measuring whole body sodium and potassium were prepared from groups of ashed animals as described before.

Sodium and potassium were generally measured with an EEL flame photometer. In the case of very dilute sodium solutions a Unicam SP900 flame spectrophotometer was used.

Haemolymph calcium and magnesium concentrations were measured using a EEL 240 atomic absorption spectrophotometer.

Chloride determinations were made with an Aminco-Cotlove automatic chloride titrator, operated on the range suitable for samples containing 0·2 μm chloride. For whole body chloride measurement, groups of up to five macerated larvae were used in each sample. Maceration was aided by fine grinding sand (containing no soluble Cl^-). Animal debris and sand was present throughout the titration. However, when this debris, leached of chloride, was added to a standard NaCl solution, it did not interfere with the titration.

Haemolymph analysis

Haemolymph was removed by puncturing animals with a glass needle on a waxed slide. Haemolymph was taken up into silicone lined micropipettes. These could only be used about six times each, as a thin haemolymph coating gradually built up on the silicone lining. Calibration with ^{22}Na solutions of known specific activity showed an error of less than 3 % using these pipettes. Animals generally yielded between 5 and 10 μl of haemolymph each.

For measurement of the specific activity of a haemolymph sample, 10 μl of labelled haemolymph were mixed with 2 ml of distilled water. This was split into two parts, one for counting, after spreading and drying, and the other for chemical measurement of sodium after further dilution.

Measurement of freezing-point depression in haemolymph

This was measured by the method of Ramsay & Brown (1955). Haemolymph samples were frozen immediately after extraction, to prevent possible changes in freezing-point.

Depletion of animals

Animals were depleted of salts by subjecting them to two changes of distilled water per day for six days. Some washed, organic debris of negligible sodium content was available for tube building and food. This procedure preceded all net uptake experiments and all sodium influx experiments in this work, except where the steady-state is specified. A distinction was made between sodium balance and sodium depletion in so far as 'balanced' animals were left to find their own sodium level when placed in distilled water. The balancing procedure is that used by Shaw (1959*a*) for *A. pallipes*, except that in the present work densities used were 1 larva/60 ml and 1 larva/40 ml for the larger species (*C. tentans*), and 1 larva/30 ml and 1 larva/20 ml for *C. dorsalis*.

Starvation of animals

It was diffiult to define a 'starving' animal as these species were found to be grazers, readily recycling faeces and showing a tendancy towards cannibalism after about 3 days without food. However, with regular scrubbing of the container, and removal of unhealthy animals, a degree of starvation could be attained after 4–5 days, as judged by an empty digestive tract. In one experiment, a group of animals designated 'gut-blocked' animals, had their mouths blocked with beeswax resin (Krogh & Weis-Fogh, 1951).

Removal of anal papillae

The osmotic shock method of removal of anal papillae from *A. aegypti* larvae (Wigglesworth, 1933*b*), did not work with chronomid larvae. Koch (1938) discussed the merits of $AgNO_3$ for the depapillation of chironomid larvae, but was successful using heat. Cautery was employed here, using a simple heating coil devised by Burtt (1938).

Collection of urine

The term urine is used here to describe the final product of the alimentary tract. Larvae were carefully blotted dry with filter paper whilst being viewed under a binocular microscope. They were then immersed in liquid paraffin which had been extracted with boiling distilled water to remove any salts present. Under paraffin, larvae were seen to exude a discrete droplet of urine. A 1 μl sample was made up from the pooled urine of 3–5 larvae, and suitably diluted for analysis.

RESULTS

Animal size and body sodium concentration

The relationship between body weight and the concentration of total body sodium in *C. tentans* is shown in Fig. 1. The line fitting the data represents the equation: body weight (mg) = $84 - 0{\cdot}97 \times$ (body sodium concentration in m-moles/kg). The correlation coefficient, r ($= -0{\cdot}631$), which is based on 74 degrees of freedom is highly

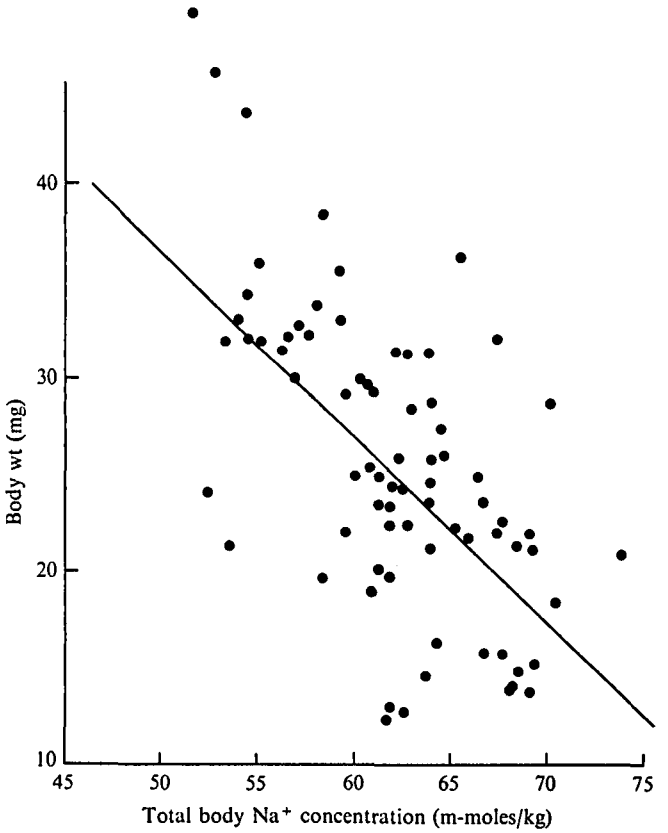

Fig. 1. The relationship between total body sodium concentration and body weight in *C. tentans*. Each point represents a single animal. The line, determined by linear regression analysis, represents the equation; body wet weight (mg) = 84−0·969 (body sodium concentration in m-moles/kg).

significant ($P < 0{\cdot}001$). However, this relationship may be rather more complex than a cursory reference to Fig. 1 suggests. As the animals used in this paper were all less than 30 mg in weight, a separate correlation coefficient was determined for those animals less than 30 mg. In this case $r = 0{\cdot}231$ and was not significant. It could be that the larger larvae represent a distinct group of older animals, perhaps second year larvae, having a lower body sodium concentration. If this were the case, then a simple correlation coefficient would be inappropriate.

Body sodium compartmentation and steady-state exchange

The concentration of the main haemolymph ions of *C. tentans* and *C. dorsalis* (Na only for the latter) are included in Table 1, along with data from other work on dipteran larvae.

An estimation of haemolymph space in *C. tentans* was made by piercing the body wall in several places, including the head capsule, gently squeezing out the haemolymph and blotting it away. This was found for 30 animals to be $60{\cdot}63 \pm 0{\cdot}77$ % (S.E. of the mean) of the body wet weight. This compares with 62·5 % for *A. aegypti* larvae (Ramsay, 1953; Stobbart, 1965). Total body water (wet weight–dry weight)

Table 1. *The major haemolymph ions of* Chironomus *and* Aëdes aegypti

Species	Haemolymph ion concentration (mM) Na	K	Ca	Mg	Cl	O.P as mM-NaCl	Whole body ion concentration (m-moles/kg) Na	K	Cl	Author
Aëdes aegypti	100	3·9	—	—	69	—	70	36	54	Stobbart (1967)
	—	—	—	—	51·3	—	—	—	—	Wigglesworth (1933*b*)
	—	—	—	—	—	129–162	—	—	—	Wigglesworth (1938)
	87	3	—	—	—	—	—	—	—	Ramsay (1953)
Chironomus spp.	104	2·0	5·5	7·5	—	—	—	—	—	Duchateau *et al.* (1953)
C. dorsalis	100	—	—	—	—	—	70	—	—	Present work
C. tentans	93	5·0	3·0	6·3	28·0	105	63	27·5	29·0	Present work

Table 2. *Compartmentation of body sodium and chloride in* C. tentans *using data from Table* 1 *and text*

	Na^+ (mM)	Cl^- (mM)
Extracellular fluid (= haemolymph)	93	28
Intracellular fluid	34	51

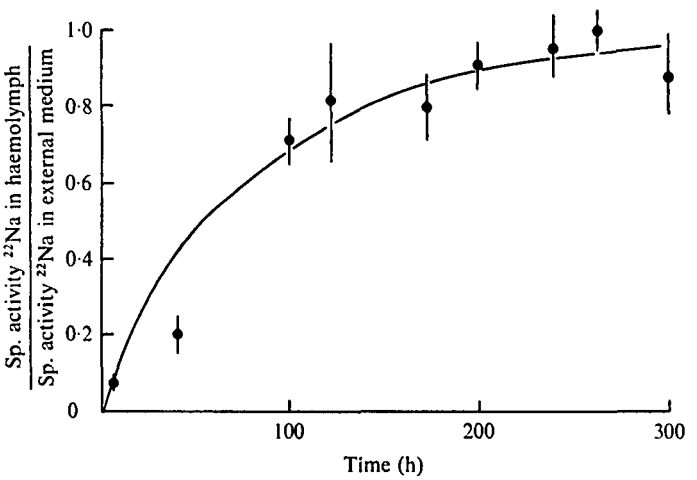

Fig. 2. The steady-state exchange of labelled sodium into the haemolymph of *C. dorsalis* larvae. Each point represents the mean haemolymph sodium determination for four groups of 6–10 larvae. Extent of vertical lines represent ± standard error of the mean.

was found to be 83·50 ± 1·8 % body weight for 4 groups of 6 animals. If it is assumed that the haemolymph represents all but a very small part of the extracellular fluid, the intracellular fluid compartment represents approximately 83·5 − 60·6 = 22·9 % body wet weight.

Assuming the haemolymph sodium concentration in *C. tentans* to be 93 mM and the total body sodium to be 63 m-moles/kg wet weight, it may be seen that 89 % of the body sodium is contained in the haemolymph. A similar situation is found in *C. dorsalis* where the haemolymph sodium compartment comprises approximately 90 % of the total body sodium. Using these data and information from Table 1, estimates may be made of intracellular sodium and chloride concentrations. These are compared

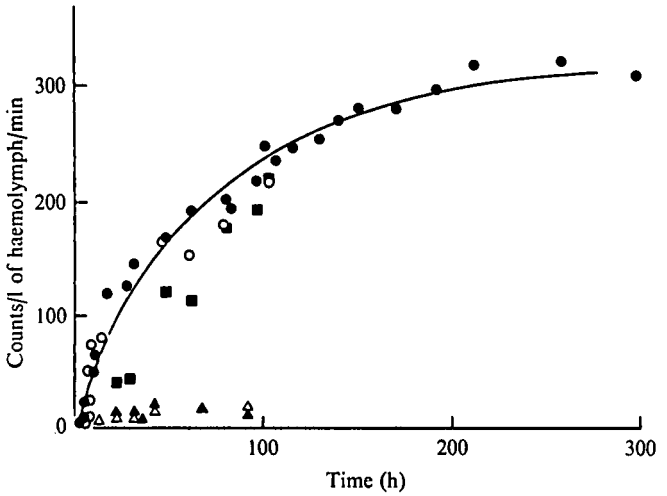

Fig. 3. The steady-state exchange of labelled sodium into the haemolymph of normal, starved and depapillated larvae of *C. dorsalis*. Closed circles represented normal fed animals. The line is fitted by eye to these data. Open circles represent starved normal animals. Squares represent larvae with mouths blocked by beeswax resin. Closed triangles indicate fed, papillae-less animals. Open triangles represent starved papillae-less animals. Each point represents the mean of 10 larvae. Standard errors omitted for clarity. Experimental temperature, 21 °C.

Table 3. *Exchange constants, values for* $T\frac{1}{2}$, *and fluxes derived from data in Fig.* 3

State of larvae	Na^+ in (m-moles/l of haemo-lymph)	*K in (h^{-1})	*K out (h^{-1})	$T\frac{1}{2}$ (h)	Na flux (m-moles/Kg of haemo-lymph/h)
Fed normal	97·5	0·7363	0·0151	45·89	1·51
Starved normal	96·5	0·5472	0·0119	58·23	1·15
Fed papillae-less	79·1	0·0451	0·0011	607·9	1·102
Starved papillae-less	89·2	0·0296	0·0008	888·5	0·069
Gut-blocked (after 3 days' starvation)	95·8	0·33	0·0083	83·47	0·79

* Exchange constants are as used by Stobbart (1959).

with extracellular (haemolymph) sodium and chloride concentrations in Table 2. Such estimates can only be approximate, but it seems likely that intracellular sodium and chloride concentrations are fairly similar.

Fig. 2 shows the increase in specific activity of haemolymph sodium of *C. dorsalis* placed in a 2 mM-NaCl sodium containing labelled sodium. The close approximation of the ratio (sp. activity of haemolymph sodium: sp. activity of sodium in the external medium) to unity at equilibrium indicates that nearly all the haemolymph (or whole body) sodium is freely exchangeable with the external medium. Koch (1938) demonstrated the importance of the anal papillae in salt regulation by chironomid larvae. Fig. 3 confirms that the anal papillae are the principle sites of steady-state sodium exchange in *C. dorsalis*. The effect of starvation upon sodium is also assessed and the data are summarized in Table 3. Exchange constants and values for $T\frac{1}{2}$ and unidirectional fluxes were derived from semi-logarithmic plots of exchange data after Treherne (1954) and Stobbart (1959). The longest lived of the papillae-less animals (Fig. 3)

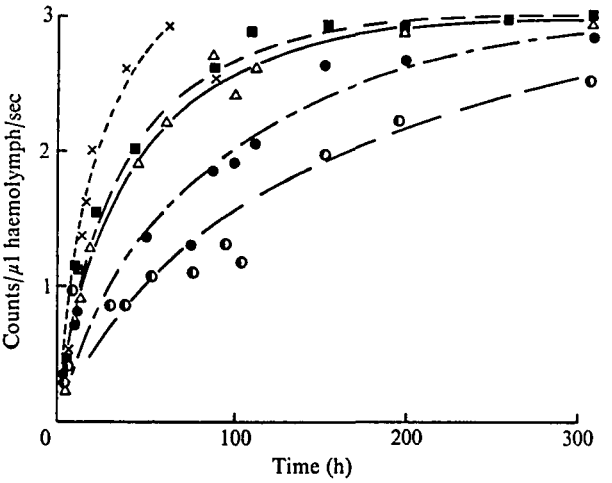

Fig. 4. The effect of temperature on the steady-state exchange of labelled sodium into the haemolymph of *C. dorsalis* larvae. Symbols: - - × - -, 26 °C; — — ■ — —, 24 °C; —△—, 14 °C; — - ● - —, 11 °C; —◐—, 2 °C. Each point represents a single group of 10 animals.

lasted about 100 h. The haemolymph sodium content of these animals was maintained at about 79 mM which, although lower than normal animals, suggested that their low sodium permeability had been largely maintained. The sodium flux in papillae-less animals, however, was reduced by a factor of about 15. This is similar to the situation in *A. aegypti* larvae (Stobbart, 1959) where at least 90 % of the sodium flux occurred through the anal papillae. In contrast to *A. aegypti*, the effect of starvation on sodium flux is much less marked in chironomid larvae. Starvation reduced sodium flux in *Aëdes* larvae by a factor of 6–7 (Stobbart, 1959). In starved *C. dorsalis* however, the reduction in sodium flux was only by a factor of about 0·3. The steady-state exchange of 'gut-blocked' animals (Fig. 3) was not significantly different from that of starved animals over the first 100 h (as determined by regression analysis of semi-logarithmic plots of the exchange curves: $P > 0{\cdot}05$). 'Gut-blocked' animals appeared to deteriorate after about 100 h and their further study was abandoned. Considered with data from depapillated animals, there is sufficient evidence to suggest that the gut plays very little part in the exchange of sodium between the haemolymph and the external medium.

The effect of temperature on the sodium relation of steady-state larvae

The steady-state exchange of sodium in *C. dorsalis* was followed at temperatures varying from 2 to 26 °C. The results are shown in Fig. 4 and summarized in Table 4 and Fig. 5. From Fig. 5 it may be seen that the Q_{10} for sodium influx in *C. dorsalis*, for the temperature range 0–10 °C = 1·31. This represents an activation energy of approximately 5·8 cal M^{-1} as calculated from the Arrhenius equation. Above 20 °C the Q_{10} rises steeply to about 2·2, corresponding to an activation energy of about 12·5 cal M^{-1}.

Owing to the destruction of the breeding population of *C. dorsalis* further observations on the effect of temperature upon sodium regulation were confined to *C. tentans*. Whole body sodium was measured in *C. tentans* acclimatized to a range of

Table 4. *The effect of temperature upon steady-state exchange in* C. dorsalis: *exchange constants, values for* $T\frac{1}{2}$ *and fluxes derived from data in Figs.* 5 *and* 6

Temperature (°C)	Na^+ in (m-moles/l of haemolymph)	K in (h^{-1})	K out (h^{-1})	$T\frac{1}{2}$ (h)	Na flux (m-moles/Kg of haemolymph/h)
2	102·14	0·504	0·010	68·37	1·011
11	101·80	0·670	0·013	52·98	1·345
14	102·75	0·906	0·020	35·24	2·024
21*	97·5	0·737	0·015	45·89	1·510
24	99·6	1·222	0·027	25·39	2·719
26	100·5	1·496	0·033	19·75	3·276

* = Normal animals from Fig. 3.

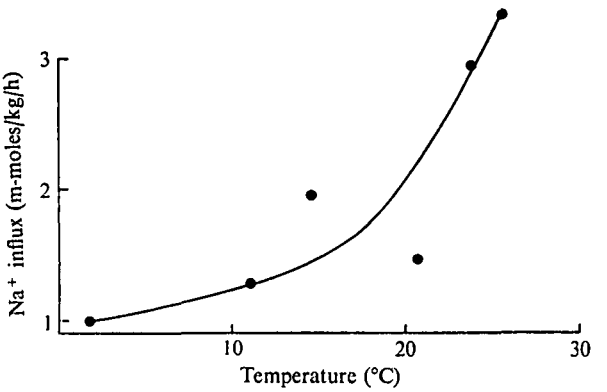

Fig. 5. The relationship between temperature and steady-state influx of ^{22}Na in *C. dorsalis* larvae. Influx at 21 °C is derived from the steady-state exchange curve for normal, fed animals in Fig. 3. Other points are calculated from data presented in Fig. 4.

Table 5. *Whole body sodium concentrations in* C. tentans *larvae acclimatized to different temperatures*

Acclimatization temperature	Body Sodium concentration (m-moles/kg)
5	59·5 ± 1·18 S.E. N = 4 groups of 4–6 larvae
12	58·99 ± 2·00 S.E. N = 4 groups of 4–6 larvae
20	60·96 ± 1·06 S.E. N = 4 groups of 4–6 larvae
28	62·93 ± 1·59 S.E. N = 3 pairs of larvae

temperatures between 2 and 28 °C. All animals were kept for 7 days at the stated temperature, in a 2 mM-NaCl solution. Fewer animals were available for analysis at 28 °C, as many had pupated. No significant difference was found in the total body sodium levels of any of the groups (Table 5). A further experiment measured the short-term change in sodium influx in *C. tentans* following an acute temperature change, with a view to obtaining a temperature coefficient (Motais & Isaia, 1972). Animals acclimatized for a week at 5 °C were split into two batches. In one batch, sodium

Table 6. *Short-term changes in sodium influx from 2 mM-NaCl in* C. tentans *larvae following acute temperature changes*

Batch	Acclimatization temperature = 5 °C	New temperature = 15 °C	Temperature coefficient
A-influx (m-moles/kg/h)	0·608 ± 0·126 S.E.*	1·1198 ± 0·231 S.E.	1·84
	Acclimatization temperature = 10 °C	New temperature = 20 °C	
B-influx (m-moles/kg/h)	0·789 ± 0·110	1·346 ± 0·083	1·71

* N = 4 groups of 6 larvae in all cases.

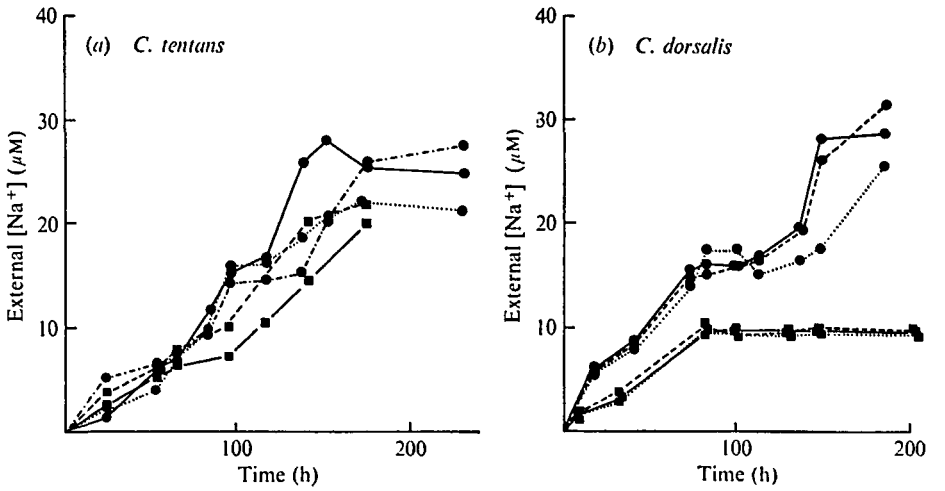

Fig. 6. The time course for the attainment of sodium balance (minimum equilibrium) concentration in the larvae of (*a*) *C. tentans* and (*b*) *C. dorsalis*. For *C. tentans*, each point represents a group of 10 larvae followed at a density of 1 larva/60 ml (●) and 1 larvae/40 ml (■). For *C. dorsalis*, each point represents a group of 10 larvae followed at a density of 1 larva/30 ml (■), and 1 larva/20 ml (●).

influx was measured for an hour immediately after transfer to a solution at 15 °C. A similar experiment was done at 10 °C/20 °C. 2 mM-NaCl solutions were used throughout. The results are recorded in Table 6.

Sodium balance and sodium depletion in distilled water

The ability to balance sodium uptake and loss at low external concentrations is a good indication of an animal's salt regulatory ability in freshwater. The time courses for sodium loss into distilled water from *C. dorsalis* and *C. tentans* are compared in Fig. 6. *C. dorsalis* is able to achieve a satisfactory balance ([Na] out = 10 μM) at a density of 1 larva/30 ml. As this species is generally about half the size of *C. tentans*, it is weight for weight more efficient in this respect than *C. tentans* which, at a density of 1 larva/60 ml, could only achieve a tenuous balance at [Na] out ≃ 25 μM. It may be noted that equilibrium between uptake and loss in chironomid larvae is reached smoothly, and does not involve a large initial inbalance between net loss and net uptake as is found in some other freshwater animals (Stobbart, 1965; Greenaway, 1970). The

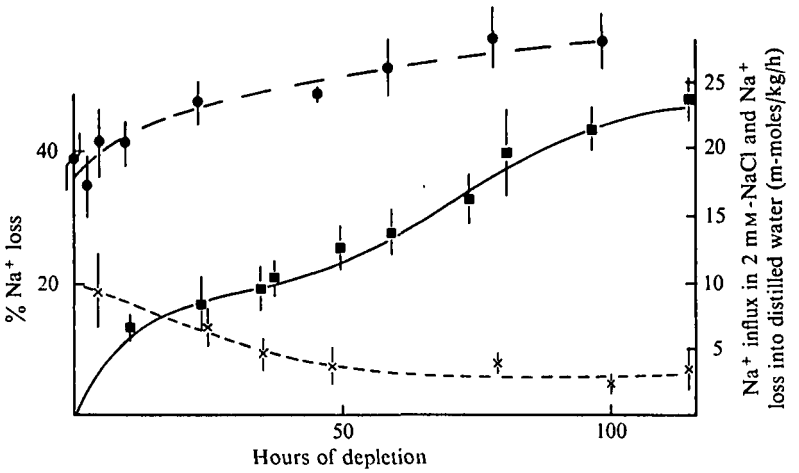

Fig. 7. Sodium loss and sodium influx during the depletion process in *C. dorsalis*. —●—, sodium influx (right-hand ordinate): - - × - -, rate of passive sodium loss into distilled water (right-hand ordinate): —■—, % total body sodium lost (left-hand ordinate). Experimental temperature, 21 °C. Each point represents the mean of 3 or 4 groups of 10 larvae. Vertical lines indicate ± S.E.

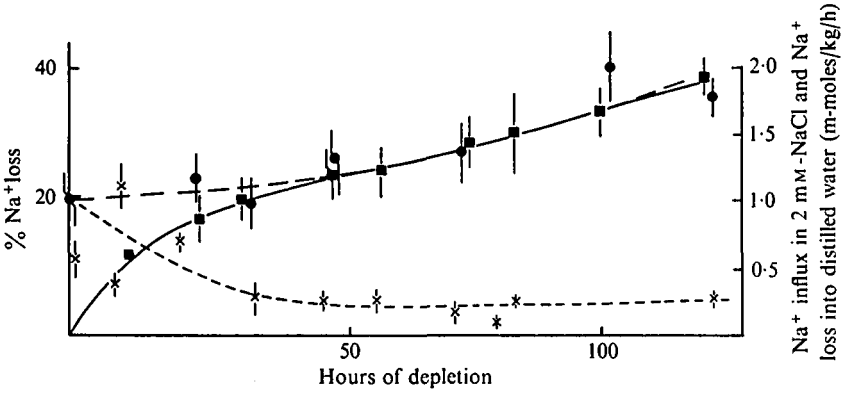

Fig. 8. Sodium loss and sodium influx during the depletion process in *C. tentans*. Experimental temperature, 21 °C. Symbols as in Fig. 7.

balancing process in *C. dorsalis* and *C. tentans* is much slower than in *A. aegypti* (Stobbart, 1965), which achieves a balance at [Na] out = 5 μM after only 20 h, and at a much higher larval density. Moreover, the total drop in body sodium of *A. aegypti* at balance is only 10%, wherease the equivalent loss from *C. dorsalis* at a density of 1 larva/30 ml (Fig. 6*b* (■)) is in excess of 30%.

In many of the following experiments, larvae were required which had their sodium uptake mechanism fully activated. This was achieved by subjecting animals to several changes of distilled water. In order to characterize more fully the nature of this sodium pump activation, measurements of sodium influx and loss were made during the depletion process. Animals were removed for flux measurements from the distilled water, the volume of which was adjusted to maintain a constant larval density. At each change of distilled water, the amount of sodium which had leaked from the

Table 7. *Comparison of excretory fluids in three aquatic larvae*

Species	Acclimatization medium	Intestinal fluid* Na (mM)	Intestinal fluid* K (mM)	Rectal fluid† Na (mM)	Rectal fluid† K (mM)	Author
A. aegypti	Distilled water	24	88	4	25	Ramsay (1951)
S. lutaria	Tap water	1	1	12	4	Shaw (1955)
C. tentans‡	Distilled water	—	—	8·3 ± 2·04 (S.E.)	3·25	Present work
	2 mM-NaCl	—	—	22·7 ± 4·81 (S.E.)	9·6	Present work

* Product of Malpighian tubules.
† Product of rectum (possibly still undergoing modification in some cases).
‡ Mean of 3 groups of 8 animals.

larvae was measured and expressed as a mean passive loss since the previous change. Data for both *C. dorsalis* and *C. tentans* are recorded in Figs. 7 and 8.

It is clear that the activation of the uptake mechanism is a relatively slow process compared with other freshwater animals studied. In *Astacus pallipes* (Shaw, 1959*a*) and *A. aegypti* (Stobbart, 1965), a fall in body sodium of only 6 and 10 % respectively is needed to stimulate fully the sodium transporting system. In the ammocoete larvae of *Lampetra planeri*, a fall in blood sodium of 4–6 % causes an increase in influx by a factor of 2–3 (Morris & Bull, 1970). However, in *C. dorsalis* and *C. tentans* full activation of the sodium pump is only achieved after a fall in body sodium of approximately 40 and 36 % respectively, although in *C. dorsalis* there are signs of increased influx after a 16 % sodium loss. Maximum stimulation of the sodium pump represents, for both species, an increase in influx of between 1·5 and 2 times the steady-state value. The higher influx in *C. dorsalis* may contribute to this species being able to balance at a lower external sodium concentration.

Urine production by C. tentans

It must be borne in mind that the fluid collected represents the final product of the hindgut, and probably bears no resemblance to the fluid secreted by the Malpighian tubules. A coil in the hind gut rendered it impracticable to collect this primary intestinal fluid. However, this has been accomplished by Ramsay (1951) in *A. aegypti*, and data for *A. aegypti* and *C. tentans* have been included in Table 7 for comparison along with *Sialis lutaria*, which is also capable of elaborating a strongly hypotonic urine (Shaw, 1955). The rectal fluids of both *C. tentans* and *S. lutaria* differ from that of *A. aegypti* in having more sodium than potassium. In *C. tentans* the ability to reduce the sodium content of the urine on depletion is indicated. Stobbart (1971*b*) was able to reduce urine flow in the larvae of *A. aegypti* by the application of a ligature between the thorax and abdomen. It was thought that this was due to the removal of a nervous inhibition of the retroperistalses of the mid-gut, which normally cause the circulation of a fraction of the tubule fluid between the tubules and the haemolymph via the mid-gut. A similar phenomenon occurred in *C. tentans* and use was made of this in obtaining an estimate of urine production. This was taken as being equivalent to the gain in weight of ligatured animals/unit time, and was found to represent 23·4 ± 3·9 % body weight/day for 10 individuals. This compares with a maximal

estimate for *A. aegypti* of 33 % body weight/day (Stobbart & Shaw, 1964), and 19·2 % body weight/day for *Corethra* (=*Chaoborus*) *plumicornis* larvae (Schaller, 1949). Using these data, a rough estimate may be made of the part played by the excretory system in sodium loss from steady-state and depleted animals. It is assumed that urine flow in steady-state and depleted animals did not differ appreciably. An immediate problem in attempting to partition sodium loss in *C. tentans* is the variability of the passive loss data, particularly at or close to steady-state (Fig. 8). A steady-state sodium loss of 1·0 m-moles/kg/h is taken as a working estimate, although it is acknowledged that this may be subject to considerable variation. The corresponding loss rate for depleted animals of 0·25 m-moles/kg/h may be taken with greater confidence. From these figures it may be seen that urine sodium represents 23 % of sodium loss in steady-state animals. If the two-thirds reduction in urine sodium, seen on depletion, were the only mechanism operating, a 15 % fall in total sodium loss would be expected. In reality the reduction in sodium loss is about 75 %. After allowing for the lowered total body sodium in depleted animals, a component of loss remains which represents a reduction in permeability of the external epithelium of about 20–25 %. Urine sodium loss apparently accounts for 33 % of the total sodium loss in depleted larvae. This compares with a corresponding value of 26 % for *A. aegypti* calculated using data from Ramsay (1953), Stobbart & Shaw (1964) and Stobbart (1965).

Net uptake of sodium by depleted animals

Fig. 9 shows the time course of net uptake of sodium from 2 mM-NaCl by *C. dorsalis* and *C. tentans*. The curves show considerable difference between the two regarding the initial rate of net uptake, 2·5 m-moles/kg/h for *C. dorsalis* and 1·1 m-moles/kg/h for *C. tentans*. This further emphasizes the superior regulatory ability of *C. dorsalis*.

The relationship between net sodium uptake and simultaneous sodium influx in *C. tentans* is shown in Fig. 10. The same larvae were used for both total body sodium and influx measurements. The curves drawn through the data are fitted in a manner similar to that used by Stobbart (1971*b*). They are based on the assumption that both whole body sodium and sodium influx have an exponential relationship with respect to time. A further assumption is made that the time constants for the curves are identical. Semi-logarithmic plots of the data from the whole body sodium and influx curves (against time) yielded slopes of 0·092 and 0·081 h^{-1} respectively and suggest that, given the variability of the material, this is a reasonable working assumption.

Consider whole body sodium,

$$Na_t = Na_\infty - (Na_\infty - Na_0)\, e^{-kt}, \tag{1}$$

where Na_t = internal sodium concentration at any time t, Na_∞ = internal sodium concentration at $t = \infty$, Na_0 = internal sodium concentration at $t = 0$, k = constant. Then

$$R = R_0 e^{-kt}, \tag{2}$$

where $R = d\,(Na)/dt$ at any time t, and $R_0 = d(Na)/dt$ at $t = 0$. Now if

$$\text{rate of net uptake} = \text{influx} - \text{efflux},$$

i.e.

$$R_0 e^{-kt} = I_\infty + (I_0 - I_\infty)\, e^{-kt} - \text{efflux} \quad \text{(cf. Stobbart, 1971}b\text{)}, \tag{3}$$

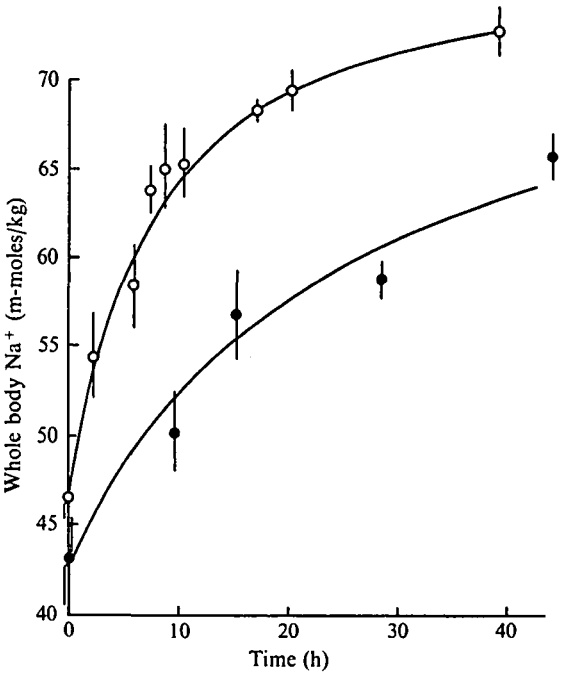

Fig. 9. Net sodium uptake by *C. dorsalis* and *C. tentans* from 2 mM-NaCl. —○—, whole body sodium concentration in *C. dorsalis*; —●—, whole body sodium concentration in *C. tentans*. Experimental temperature, 21 °C. Each point represents the mean of 4 or 5 groups of 6–8 larvae ± S.E.

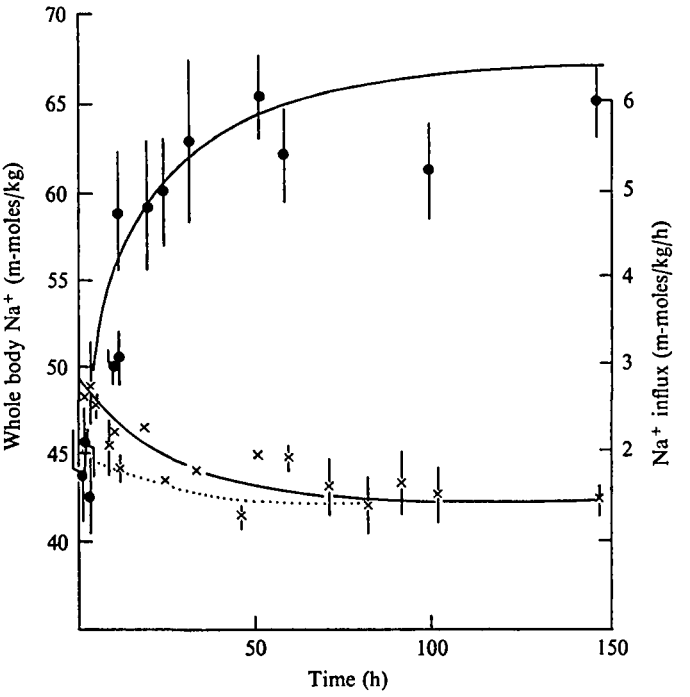

Fig. 10. Net sodium uptake by *C. tentans* from 2 mM-NaCl, with concomitant sodium fluxes. —×—, sodium influx measured directly (right-hand ordinate). —●—, whole body sodium concentration (left-hand ordinate). Each point represents 3 groups of 10 larvae ± S.E. The dotted line indicates the time course of sodium efflux calculated from equation (4).

where I_∞ = influx at $t = \infty$ and I_0 = influx at $t = 0$, then

$$\text{efflux} = I_\infty + (I_0 + I_\infty)\, e^{-kt} - R_0 e^{-kt}$$

$$= I_\infty + (I_0 - I_\infty - R_0)\, e^{-kt}. \quad (4)$$

It may be seen then that the time course of sodium efflux throughout a period of net sodium uptake may be calculated indirectly from influx and total body sodium data (equation 4). A sodium efflux curve derived in this way has been entered as a broken line in Fig. 10. The curve suggests that both efflux and influx are increased upon sodium depletion, although the rise in efflux is apparently very small. However, this may be set against an apparent lowering of integumental permeability as noted earlier and it suggests a degree of coupling of influx and outflux as has been noted in *A. aegypti* (Stobbart, 1959, 1971*b*, 1974). The relationship between net sodium uptake and sodium flux illustrated in Fig. 10 indicates a very efficient negative feedback acting on the sodium pump.

DISCUSSION

The major body ions in *C. tentans* show the familiar discrepancies between the cation and the anion sum in insect haemolymph. The main anion, chloride, (which by reference to Table 1 is partitioned 60 % in haemolymph : 40 % in tissues) reaches only about half the concentration of the major cation, sodium. It therefore makes up rather less of the anion fraction than in *A. aegypti*. Shaw (1955) compiled a more complete list of haemolymph ions for *S. lutaria* and showed that HCO_3^- ions only partly reduced the difference between the cations and the anions. He assumed that the bulk of the deficit was made up of haemolymph amino acids. This is also likely to be the case in *Chironomus* and *Aëdes*. The haemolymph sodium levels in the two species studied here compare reasonably with the findings of other workers. However, the potassium is higher, and the divalent ions a little lower than values presented by Duchateau, Florkin & Leclerq (1953) for *Chironomus*, although these workers do not give the species studied. The activation energies for sodium influx in *C. dorsalis* of 5·8 cal M^{-1} at the lower temperature range and 12·5 cal M^{-1} above 20 °C, compare with values of 6·5 – 11·8 cal M^{-1} for sodium movement across the gill epithelium of *Anguilla anguilla* (Motais & Isaia, 1972). Morris & Bull (1968) found differential temperature effects on sodium influx and efflux in the larvae of *Lampetra planeri*. Efflux was found to be much less temperature dependent than influx which, for the temperature range 0–10 °C, displayed Q_{10} of 2·9, corresponding to an activation energy of about 16 cal M^{-1}. As the temperature effect on any system depends upon the nature of the limiting step, the high Q_{10} in this case indicates that in *L. planeri* at low temperatures, some active process such as the sodium pump itself is limiting. A low Q_{10} such as is seen with *C. dorsalis* at low temperatures, would seem to indicate the mediation of a diffusional (permeability) component. For *C. dorsalis* the temperature effects could be explained in terms of a membrane, limiting at low temperatures, which increases in effective permeability at higher temperatures, thus making more sodium available to the pump. This is, of course, an oversimplification, and disregards such complications as the possible active control over permeability (e.g. by alteration of the structural configuration of the limiting membrane). A comparatively low sensitivity to temperature change is also seen in *A. aegypti* (Treherne, 1954), where between 20 and

Table 8. *Balance concentrations and sodium loss by some freshwater and brackish water animals*

	Habitat (freshwater/ brackish water)	Sodium conc. (mM) of acclimatization medium	Experimental temperature (°C)	Sodium loss into distilled water (m-moles/kg/h)	Balance conc. (mM-Na)	Refere
	F.W.	2·00	10	1·67	—	Sutcliffe & Sh
		0·06	10	0·82	—	
	B.W./F.W.	2·00	10	2·5–6·6	—	Sutcliffe & Sh
		0·15	10	2·2–3·9	—	
		—	9	—	0·1	Sutcliffe (197
	F.W.	2·00	9	1·7	—	Sutcliffe (196
		0·06	9	1·0	—	
		—	9	—	0·05	
	F.W.	Tap water (*ca.* 0·3)	12	0·15	—	Shaw (1959*a*)
		—	12	—	0·04	
	F.W.	—	—	0·8	0·05	Shaw (1959*b*)
	B.W./F.W.	—	—	2·1	0·2–0·5	Shaw (1961)
	F.W.	2·0	28	0·83	—	Stobbart (196
		Dist. water	28	0·21	—	
		—	—	—	0·005	
ntans	B.W./F.W.	2·0	21	1·0	—	Present study
		Dist. water	21	0·25	—	
		—	21	—	0·02–0·03	
	B.W./F.W.	2·0	21	1·0	—	
		Dist. water	21	0 3	—	
		—	21	—	0·01	
	F.W.	0·35	10	0·08	—	Greenaway (1
		0·02	10	0·04	—	
		—	10	—	0·025	
	F.W.	1·2	15	0·13	—	Alvarado & D
		Dist. water	15	0·04	—	
		—	15	—	0·01	
	F.W.	Tap water (*ca.* 0·3)	10	0·36	—	Morris & Bull
		Dist. water	10	0·16	—	
		Tap water (*ca.* 0·3)	1	0·28	—	
		Dist. water	1	0·18	—	
		—	1	—	*ca.* 0·02	

28 °C there is an increase in steady-state flux by a factor of only 1·2. The remarkable stability of the internal concentration in *C. tentans* throughout a large temperature range further illustrates the insensitivity of the sodium mechanism to temperature change. Lockwood (1960) found a similar situation in *Asellus aquaticus* with an external concentration of 2·4 mM, which he assumed was due to an effective feedback mechanism. In the two chironomid species studied here the feedback mechanism is slower, although they are likely to be aided in this respect by their fairly low permeability.

It must be borne in mind that the modes of action of temperature on sodium regulation vary according to the actual temperature range used. Further, the effect may be modified by other factors such as external concentration (Lockwood, 1960; Sutcliffe & Shaw, 1968). With these reservations, Table 8 is constructed to indicate how chironomid larvae compare with other freshwater animals. The only standardization of data concerns the unit of sodium loss, calculated in a number of cases using average or specific animal weights given by the authors. Dashes indicate lack of details of experimental conditions.

Despite the relatively sluggish activation of their sodium uptake mechanism chironomid larvae compare very well with other freshwater animals, showing themselves to be powerful regulators. Moreover, as will be seen in a later paper, the sodium affinity of the pump in *C. tentans* and *C. dorsalis* is only moderate compared with other animals.

C. tentans is a euryhaline species invading brackish water with high sodium concentrations (Palmen & Aho, 1966; Topping, 1971). Considering the collecting sites used here, animals used for the present study can be said to be at the freshwater end of their range. It remains to be seen whether populations from more dilute waters have a more sensitive feedback mechanism, or perhaps tolerate a lower body salt content. The nature of sodium regulation in chironomids in brackish water has yet to be characterized.

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REFERENCES

ALVARADO, R. H. & DIETZ, T. H. (1970). Effect of salt depletion on hydromineral balance in larval *Ambystoma gracile*. II. Kinetics of ion exchange. *Comp. Biochem. Physiol.* **33**, 93–110.

BRYCE, D. (1960). Studies of the larvae of British *Chironomidae* (Diptera), with keys to the *Chrionominae*. *Trans. Soc. Brit. Ent.* **14**, 19–62.

BURTT, E. T. (1938). On the corpora allata of Dipterous insects. *Proc. R. Soc. Lond.* B **126**, 210–23.

DUCHATEAU, G., FLORKIN, M. & LECLERQ, J. (1953). Concentrations des bases fixes et types de composition de la base totale de l'hemolymphe des insects. *Arch. int. Physiol.* **61**, 518–49.

EDWARDS, F. W. (1929). British non-biting midges (Diptera, Chironomidae). *Trans. ent. soc. Lond.* **77**, 279–430.

GREENAWAY, P. (1970). Sodium regulation in the freshwater mollusc, *Limnaea stagnalis* (L.). (Gastropoda: Pulmonata.) *J. exp. Biol.* **53**, 333–49.

KOCH, H. J. (1938). The absorption of chloride ions by the anal papillae of Diptera larvae. *J. exp. Biol.* **15**, 152–60.

KOCH, H. J. (1954). Cholinesterase and active transport of sodium chloride through the isolated gills of the crab *Eriocheir sinensis* (M.Edw). *Recent Developments in Cell Physiology*, 7th Symposium of the Colston Research Society, Bristol, pp. 15–27. New York: Academic Press.

KROGH, A. & WEIS-FOGH, T. (1951). The respiratory exchange of the desert locust (*Schistocerca gregaria*) before, during and after flight. *J. exp. Biol.* **28**, 344–57.

LAUER, G. J. (1969). Osmotic regulation of *Tanypus rubifer*, *Chironomus plumosus* and *Enollagma clausem* in various concentrations of saline lakewater. *Physiol. Zool.* **42**, 381–7.

LOCKWOOD, A. P. M. (1960). Some effects of temperature and concentration of the medium on the ionic regulation of the isopod *Asellus aquaticus* (L). *J. exp. Biol.* **37**, 614–30.

MORRIS, R. & BULL, J. M. (1968). Studies on freshwater osmoregulation in the ammocoete larva of *Lampetra planeri* (Block). II. The effect of de-ionized water and temperature on sodium balance. *J. exp. Biol.* **48**, 597–609.

MORRIS, R. & BULL, J. M. (1970). Studies on freshwater osmoregulation in the ammocoete larva of *Lampetra planeri* (Bloch). III. The effect of external and internal sodium concentration on sodium transport. *J. exp. Biol.* **52**, 257–90.

MOTAIS, R. & ISAIA, J. (1972). Temperature dependance of permeability to water and to sodium of the epithelium of the eel *Anguilla anguilla*. *J. exp. Biol.* **56**, 587–600.

NEUMANN, D. (1961). Osmotische Resistenz und Osmoregulation aquatischer Chironomidenlarven. *Biol. Zbl.* **80**, 693–715.

PALMEN, E. & AHO, L. (1966). Studies on the ecology and phenology of the Chironomidae (Dipt.) of the northern Baltic. 2. *Ann. Zoo. Fenn.* **3**, 217–44.

RAMSAY, J. A. (1950). Osmotic regulation in mosquito larvae. *J. exp. Biol.* **27**, 145–57.

RAMSAY, J. A. (1951). Osmotic regulation in mosquito larvae: the role of the Malpighian tubules. *J. exp. Biol.* **28**, 62–73.

RAMSAY, J. A. (1953). Exchanges of sodium and potassium in mosquito larvae. *J. exp. Biol.* **30**, 78–89.

RAMSAY, J. A. & BROWN, R. J. H. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. Scient. Instrum.* **32**, 372–5.

SADLER, W. O. (1935). Biology of the midge *Chironomus tentans* (Fabr.) and methods for its propagation. *Cornell Univ. Agric. Exp. Stat. Mem.* **173**, 1–24.

SCHALLER, F. (1949). Osmoregulation und Wasserhaushalt der larvae von *Corethra plumicornis* mit besonderer Berucksichtigung der Vorgange am Darmcanal. *Z. vergl. Physiol.* **31**, 684–95.

SHAW, J. (1955). Ionic regulation and water balance in the quatic larva of *Sialis lutaria*. *J. exp. Biol.* **32**, 352–82.

SHAW, J. (1959*a*). The adsorption of sodium ions by the crayfish *Astacus pallipes* Lereboullet. I. The effect of external and internal sodium concentrations. *J. exp. Biol.* **36**, 126–44.

SHAW, J. (1959*b*). Salt and water balance in the East African freshwater crab *Potamon niloticus* (M.Edw). *J. exp. Biol.* **36**, 157–76.

SHAW, J. (1961). Sodium balance in *Eriocheir sinensis*. The adaptation of the Crustacea to freshwater. *J. exp. Biol.* **38**, 557–72.

STOBBART, R. H. (1959). Studies on the exchange and regulation of sodium in the larva of *Aëdes aegypti* (L.). I. The steady-state exchange. *J. exp. Biol.* **36**, 641–53.

STOBBART, R. H. (1960). Studies on the exchange and regulation of sodium in the larva of *Aëdes aegypti* (L.). II. The net transport and fluxes associated with it. *J. exp. Biol.* **37**, 594–608.

STOBBART, R. H. (1955). The effect of some anions and cations upon the fluxes and net uptake of sodium in the larva of *Aëdes aegypti* (L.). *J. exp. Biol.* **42**, 29–43.

STOBBART, R. H. (1967). The effect of some anions and cations upon the fluxes and net uptake of chloride in the larva of *Aëdes aegypti* (L.) and the nature of the uptake mechanisms for sodium and chloride. *J. exp. Biol.* **47**, 35–57.

STOBBART, R. H. (1971*a*). Evidence for Na^+/H^+ and Cl^-/HCO_3^- exchanges during independant sodium and chloride uptake by the larva of the mosquito *Aëdes aegypti*. *J. exp. Biol.* **54**, 19–27.

STOBBART, R. H. (1971*b*). The control of sodium uptake by the larva of the mosquito *Aëdes aegypti* (L.). *J. exp. Biol.* **54**, 29–66.

STOBBART, R. H. (1971*c*). Factors affecting the control of body volume in the larva of the mosquitoes *Aëdes aegypti* (L.) and *Aëdes detritus* Edw. *J. exp. Biol.* **54**, 67–82.

STOBBART, R. H. (1974). Electrical potential differences and ionic transport in the larva of the mosquito *Aëdes aegypti* (L.). *J. exp. Biol.* **60**, 493–533.

STOBBART, R. H. & SHAW, J. (1964). Salt and water balance: Excretion. In *The Physiology of the Insecta* 3 (ed. M. Rockstein), pp. 190–258. New York: Academic Press.

STRENZKE, K. & NEUMANN, D. (1960). Die Variabilitat der abdominalen Korperanhange aquatischer Chironomidenlarven in Abhongigkeit von der lonenzusammensetzung des Mediums. *Biol. Zbl.* **79**, 199–225.

SUTCLIFFE, D. W. (1959). Studies on the biology of some salt-marsh insects. Ph.D. thesis. University of Durham.

SUTCLIFFE, D. W. (1967). Sodium regulation in the fresh-water amphipod, *Gammarus pulex* (L.). *J. exp. Biol.* **46**, 499–518.

SUTCLIFFE, D. W. (1971). Sodium influx and loss in fresh-water and brackish-water populations of the amphipod *Gammarus duebeni* Lilljeborg. *J. exp. Biol.* **54**, 255–68.

SUTCLIFFE, D. W. & SHAW, J. (1967). The sodium balance mechanism in the freshwater amphipod, *Gammarus lacustris* Sars. *J. exp. Biol.* **46**, 519–28.

SUTCLIFFE, D. W. & SHAW, J. (1968). Sodium regulation in the amphipod *Gammarus duebeni* Lilljeborg from freshwater localities in Ireland. *J. exp. Biol.* **48**, 339–58.

TOPPING, M. S. (1971). Ecology of larvae of *Chironomus tentans* in saline lakes in central British Columbia. *Can. Entomol.* **103**, 328–38.

TREHERNE, J. (1954). The exchange of labelled sodium in the larva of *Aëdes aegypti* (L.). *J. exp. Biol.* **31**, 386–401.

WIGGLESWORTH, V. B. (1933*a*). The effect of salts on the anal papillae of mosquito larvae. *J. exp. Biol.* **10**, 1–14.

WIGGLESWORTH, V. B. (1933*b*). The function of the anal gills of mosquito larvae. *J. exp. Biol.* **10**, 16–26.

WIGGLESWORTH, V. B. (1938). The regulation of osmotic pressure and chloride concentration in the haemolymph of mosquito larvae. *J. exp. Biol.* **15**, 235–47.