

IONIC REGULATION OF THE  
BALTIC AND FRESH-WATER RACES OF THE ISOPOD  
*MESIDOTEA (SADURIA) ENTOMON* (L.)

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INTRODUCTION

A number of the mechanisms involved in the adaptation of fresh-water animals to their environment have now been established. By comparison with marine and brackish-water species, fresh-water forms tend to have a lower surface permeability, increased capacity to transport inorganic ions into the body from the medium, a higher affinity of the transport mechanism for ions and, in some cases, the capacity to produce urine less concentrated than the blood. Further, many fresh-water forms have a markedly lower blood concentration than related forms living in dilute brackish water.

*Mesidotea* provides an opportunity to study the course of the evolution of some of these features. This animal entered the Scandinavian region from the Arctic Seas following the regression of the ice (Ekman, 1940, 1953; Segerstråle, 1956, 1957). In the Scandinavian region it is found in brackish water throughout the Baltic Sea and in fresh water in Lake Ladoga in Russia and in eight Swedish lakes. It does not occur in Scandinavian rivers and, since in fresh water it is not normally found in water shallower than a few metres, it is most improbable that it can have been introduced from one fresh-water habitat to another by birds or other biotic agency. The lake populations therefore probably represent relict forms derived from the animals present in the area when the lakes were originally separated from the Baltic. Seven of the Swedish lakes where *Mesidotea* occurs, Mälaren, Vättern, Vänern, Mjörn and three other small lakes near the west coast, lie on the line of the original connexion between the North Sea and the Baltic (Charlesworth, 1957, for review). The eighth lake is on the island of Ornö in the Baltic (information supplied by B-O. Jansson). A gradual isostatic rise of the Swedish mainland has resulted in the successive isolation of the lakes from the Baltic Sea.

It has been claimed that the Baltic race cannot be acclimatized to fresh water (Bogucki, 1932; Lockwood & Croghan, 1957). The fresh-water races must therefore differ physiologically from the Baltic race. Adaptation of the lake races has not, however, proceeded as far as in typical fresh-water species, since it has been shown (Lockwood & Croghan, 1957) that they retain two features typical of brackish-water animals; a high blood concentration (*c.* 250 mM/l. chloride) and the ability to tolerate saline media up to and including full-strength sea water.

In the present investigation the earlier work of Lockwood & Croghan (1957) has been extended by comparing brackish-water animals from the Baltic and fresh-water animals from Mälaren in respect of permeability and ion-transport properties.

## THEORETICAL CONSIDERATIONS

*Haemolymph volume*

It is possible to calculate the proportion of the total body water which is in the haemolymph and in the cells from the concentration of potassium and chloride in the haemolymph and in the total body water, if it is assumed that these ions are in equilibrium (Donnan Equilibrium) between haemolymph and cells. That is:

$$K_C/K_B = Cl_B/Cl_C, \quad (1)$$

where subscript *C* refers to the concentration in the cell and subscript *B* refers to the concentration in the haemolymph. This assumption is correct in the case of *Carcinus* (Shaw, 1955 *a, b*) except when the haemolymph concentration is considerably lowered but is not correct for all crustaceans (Robertson, 1961).

Now for each ion (*X*)

$$X_T = X_B V_B + X_C V_C, \quad (2)$$

where  $X_T$  is the concentration of the ion in the total body water and  $V_B$  and  $V_C$  are the relative volumes of the haemolymph and cells respectively, or more strictly the proportion of the body water that is in the haemolymph and cells respectively. By combining equations (1) and (2) the relative volume of the haemolymph can be defined

$$V_B = \frac{K_T Cl_T - K_B Cl_B}{K_B Cl_T + K_T Cl_B - 2K_B Cl_B}. \quad (3)$$

Using values of  $V_B$  and  $V_C$  derived from equation (3) the concentrations of the ions in the cells can also be calculated.

*Permeability considerations*

It will be shown that net sodium loss into a dilute medium is across the outer surface of the animal (presumably across the gill surface) and that losses in the urine are insignificant. It seems clear that this loss of sodium represents diffusion of NaCl, as in both the haemolymph and medium NaCl is the predominate solute and there is no considerable change in the Na:Cl ratio when haemolymph concentration changes. Thus a diffusion equation can be written

$$r_{O(\text{net NaCl diffusion})} = \frac{P_{\text{NaCl}} A}{W} (C_{\text{NaCl}}^B - C_{\text{NaCl}}^M), \quad (4)$$

where  $r_{O(\text{net NaCl diffusion})}$  is the efflux of NaCl (moles.sec.<sup>-1</sup> g.<sup>-1</sup>),  $P_{\text{NaCl}}$  the permeability coefficient of the surface for NaCl (cm.sec.<sup>-1</sup>),  $A$  the area of permeable surface (cm.<sup>2</sup>),  $W$  the weight of the animal (g.) and  $C_{\text{NaCl}}^B$  and  $C_{\text{NaCl}}^M$  the concentration of NaCl in the haemolymph and medium respectively (moles.cm.<sup>-3</sup>). This equation is unaffected by any electrical potential difference between haemolymph and medium. However, as the permeable area is unknown, eqn. (4) can be written

$$r_{O(\text{net NaCl diffusion})} = K_{\text{NaCl}} (C_{\text{NaCl}}^B - C_{\text{NaCl}}^M), \quad (5)$$

where  $K_{\text{NaCl}}$  is another coefficient (cm.<sup>3</sup> sec.<sup>-1</sup> g.<sup>-1</sup>). This is the coefficient that is used later to describe the permeability of the animal. To convert  $K_{\text{NaCl}}$  to  $P_{\text{NaCl}}$ , the

true permeability coefficient,  $K_{\text{NaCl}}$  must be divided by  $A/W$ . In one Askö animal the silver-staining area of the gills was determined roughly. Assuming that this represents the permeable part of the gills then  $A/W \sim 0.8 \text{ cm.}^2 \text{ gm.}^{-1}$ . Thus numerically  $K_{\text{NaCl}}$  should approximate to  $P_{\text{NaCl}}$ .

If the animal is placed in de-ionized water eqn. (5) simplifies to

$$r_{O(\text{diffusion})} = K_{\text{NaCl}} C_{\text{NaCl}}^B \quad (6)$$

A determination of  $r_{O(\text{diffusion})}$  can be made from the change of concentration in known volume of initially de-ionized water before the increase of NaCl concentration in the medium is sufficient to allow significant active uptake of NaCl to occur.

A determination of  $r_{O(\text{diffusion})}$  can also be made from the rate of loss of  $^{22}\text{Na}$  from the animal. Again this should be determined in de-ionized water, in this case to avoid complications due to possible exchange diffusion. Then

$$-dC_A^*/dt = K_{\text{NaCl}} C_B^* \quad (7)$$

where  $C_A^*$  is activity in the animal (counts per 100 sec.  $\text{g.}^{-1}$ ) and  $C_B^*$  the activity in the haemolymph (counts per 100 sec.  $\text{ml.}^{-1}$ ).

But if almost all the sodium in the animal is in the haemolymph, then

$$C_B^* V = C_A^* \quad (8)$$

where  $V$  is the proportion of haemolymph in the body ( $\text{ml.} \cdot \text{g.}^{-1}$ ). Using this relationship eqn. (7) can be integrated

$$C_A^*/C_{A(u=0)}^* = \exp(-K_{\text{NaCl}} t/V), \quad (9)$$

$$k_D = K_{\text{NaCl}}/V, \quad (10)$$

where  $k_D$ , the rate constant, is easily obtainable from the fall of activity in time  $t$ . Then from eqns. (6) and (10)

$$r_{O(\text{diffusion})} = k_D C_A \quad (11)$$

where  $C_A$  is the concentration of sodium in the animal.

#### *Influx of sodium*

The rate of uptake of  $^{22}\text{Na}$  can be expressed by the equation

$$\frac{dC_A^*}{dt} = r_I \frac{C_M^*}{C_M} - r_O \frac{C_A^*}{C_A} \quad (12)$$

where  $C_A^*$  is the activity inside the animal (counts per 100 sec.  $\text{g.}^{-1}$ ),  $C_M^*$  is the activity of the medium (counts per 100 sec.  $\text{ml.}^{-1}$ ),  $C_A$  the sodium concentration in the animal ( $\mu\text{M.} \cdot \text{g.}^{-1}$ ),  $C_M$  the concentration of sodium in the medium ( $\mu\text{M.} \cdot \text{ml.}^{-1}$ ),  $t$  time in hours (hr.) and  $r_I$  and  $r_O$  the influx and efflux of sodium respectively ( $\mu\text{M.} \cdot \text{hr.}^{-1} \cdot \text{g.}^{-1}$ ).

Equation (12) can be integrated if it is assumed  $C_M^*$ ,  $C_M$ ,  $C_A$  and  $r_O$  are constant.  $C_M^*$  and  $C_M$  can be kept effectively constant by using a large volume of medium. Although  $C_A$  will vary in non-steady-state conditions, it is unlikely to vary sufficiently during a series of loading experiments to invalidate the integration. If  $C_M$  and  $C_A$  are constant it would also be expected that  $r_O$  is constant. Then

$$r_I = \frac{r_O C_M}{C_A} \frac{[C_{A(u=0)}^* - C_A^* \exp(tr_O/C_A)]}{[C_M^* - C_M^* \exp(tr_O/C_A)]} \quad (13)$$

where  $C_A^*$  is the activity in the animal at the start of the loading period and  $C_A^*$  is the activity in the animal after time  $t$ . There is negligible further error in using a simplified integration of eqn. (12):

$$r_I = \frac{C_M}{C_M^*} \left( \frac{\Delta C_A^*}{t} + r_O \frac{C_A^*}{C_A} \right). \quad (14)$$

To determine  $r_I$  it is necessary to know the efflux  $r_O$ . The efflux is composed of two components:

$$r_O = r_{O(\text{diffusion})} + r_{O(\text{exchange diffusion})}. \quad (15)$$

$r_{O(\text{diffusion})}$  can be determined using eqn. (11).

$r_{O(\text{exchange diffusion})}$  can be determined from the increase in rate of loss of  $^{22}\text{Na}$  when the animal is transferred from de-ionized water to inactive 40 mM/l. NaCl solution.

$$-\frac{dC_A^*}{dt} = r_O \frac{C_A^*}{C_A} \quad (16)$$

integrating 
$$C_A^*/C_{A(t=0)}^* = \exp(-r_O t/C_A), \quad (17)$$

then 
$$r_O = kC_A, \quad (18)$$

where  $k$  is the rate constant.

But this  $r_O$  is composed of exchange diffusion and diffusion components. Assuming that exchange diffusion is proportional to external concentrations (Croghan, 1958),

$$r_{O(\text{exchange diffusion})} = K_E C_M. \quad (19)$$

The coefficient  $K_E$  can be defined

$$K_E = \frac{1}{40} (r_O - r_{O(\text{diffusion})}). \quad (20)$$

Thus the influx  $r_I$  should be determinable. But

$$r_I = r_{I(\text{active})} + r_{I(\text{diffusion})} + r_{I(\text{exchange diffusion})}. \quad (21)$$

$r_{I(\text{active})}$  can be calculated therefore if the other two components of the influx are known:

$$r_{I(\text{diffusion})} = r_{O(\text{diffusion})} (C_M/C_B). \quad (22)$$

And by definition 
$$r_{I(\text{exchange diffusion})} = r_{O(\text{exchange diffusion})}. \quad (23)$$

There is, however, a problem in this calculation of influxes. Although the potential difference between haemolymph and medium should not affect the net rate of diffusion of NaCl from the animal, this is not true about the unidirectional fluxes  $r_{O(\text{diffusion})}$  and  $r_{I(\text{diffusion})}$  in media apart from de-ionized water. House (1963) uses an equation derived from the Goldman theory that defines the ratio of  $r_{O(\text{diffusion})}$  in two media as a function of potential difference. Unfortunately there is no data on the potential differences in media between 40 mM/l. NaCl and de-ionized water. Thus it must be admitted that there may be errors in defining  $r_{O(\text{diffusion})}$  and  $r_{I(\text{diffusion})}$  using eqns. (11) and (22). However, unless something extraordinary happens to the potential difference in this range of media the sum of  $r_{O(\text{diffusion})} + r_{O(\text{exchange diffusion})}$  should not be greatly affected and hence  $r_O$  and  $r_I$  should be reasonably accurate.

## MATERIAL AND METHODS

Baltic *Mesidotea* were obtained close to the island of Askö (lat. 58° 49' N.) by trawling or by means of traps baited with dead fish. One of the localities where Askö animals were caught was investigated using a National Institute of Oceanography Salinometer: depth 18.5 m., bottom salinity 6.6‰, bottom temp. 4.9° C. (July 1966). Fresh-water animals were obtained by trawling in the Lilla Ullevifjärden, a northern branch of L. Mälaren.

The majority of the animals used in the experiments were in the size-range 0.5–1 g. The animals were kept in their natural media or adapted to media made by diluting Askö sea water, Kristineberg sea water (Swedish west coast) or Bay of Biscay sea water with distilled water. Acclimatization and permeability experiments were carried out at 5° C. at the Biological Laboratory on Askö (Askölaboratoriet). Later some animals were transferred to Southampton, where isotope experiments were carried out at 10° C. Animals were fed occasionally on fragments of *Mytilus* or *Macoma*. They were not fed during or immediately preceding an experiment. Apart from some determinations of haemolymph composition, experiments were not carried out on egg-bearing females, as it was felt that the presence of a large egg-filled brood pouch might affect the results. Haemolymph samples were obtained as described by Lockwood & Croghan (1957). As far as possible samples were pipetted for analysis before clotting occurred.

The composition of the whole animal was also studied. The water content of an animal was determined after carefully removing adherent medium with filter paper and drying the animal at 100° C. overnight. The animal was then ground up with 10 ml. distilled water, centrifuged and the supernatant used for analysis. As a sizeable haemolymph sample was taken for analysis before drying, the analyses of the supernatants were corrected to compensate for the amounts of material removed in the haemolymph sample, the volume of which was determined by weighing the animal before and after removing the haemolymph sample.

Sodium and potassium concentrations were determined using a Beckman Model B flame-spectrophotometer. Chloride was determined using the first method of Ramsay, Brown & Croghan (1955) using a Radiometer PHM 3 h valve voltmeter.

Analyses were carried out as far as possible in duplicate on haemolymph samples from single animals. It was usually possible to obtain the sodium, potassium and chloride concentrations in the haemolymph of a single animal when required. Analyses were also carried out in duplicate on supernatants from single animals and on samples of the media.

Conductivity determinations were carried out on samples of media and were used to calculate salinity and sodium concentration. Measurements of the potential between the haemolymph and medium were attempted using Hg-calomel-sat. KCl electrodes drawn out to fine tips and a Radiometer PHM 3 h valve voltmeter.

Isotope studies were carried out using <sup>22</sup>Na. The activity in the animal or medium was determined in a well-type scintillation counter and either an IDL or Panax Scaler. The efficiency of the counting system was the same for whole animals and for samples of media.

## RESULTS

*Concentration of sodium in the haemolymph after adaptation to various media*

The results are summarized in Fig. 1. Some of these data refer to animals in which haemolymph sodium concentration was determined after they had lost some sodium into de-ionized water. The initial haemolymph sodium concentration was calculated

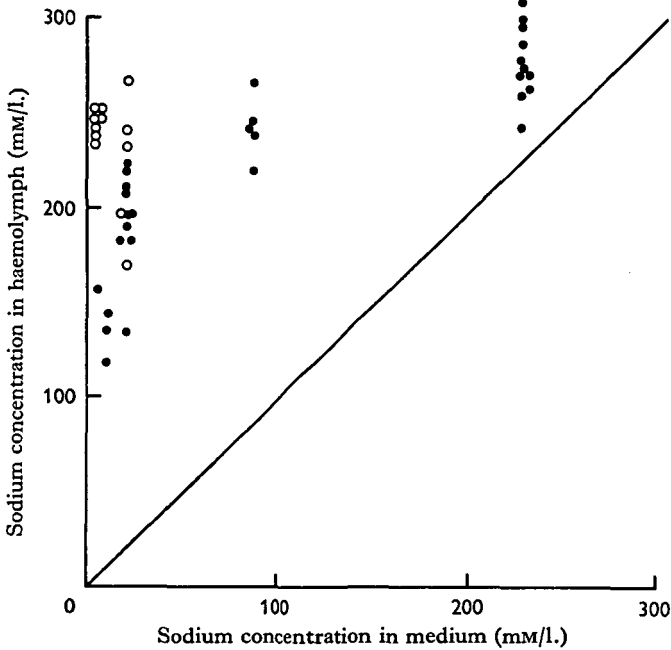


Fig. 1. Relation between the concentration of sodium in the haemolymph and in the medium. Each point represents a sample from a single animal. ●, Askö animals; ○, Mälaren animals.

from the final haemolymph sodium concentration and from the total amount of sodium that had left the animal, using the relation

$$\Delta C_A = V_W \Delta C_B, \quad (24)$$

where  $\Delta C_A$  is the amount of sodium leaving the animal ( $\mu\text{M.g.}^{-1}$ ),  $V_W$  is the water content of the animal ( $\text{ml.g.}^{-1}$ ) and  $\Delta C_B$  is the change of haemolymph sodium concentration ( $\mu\text{M.g.}^{-1} = \text{mm.l.}^{-1}$ ). The use of  $V_W$  rather than some estimate of haemolymph volume will be justified subsequently (Croghan, in preparation).

The acclimatization of Baltic *Mesidotea* to dilute media was carried out over a somewhat longer period than was attempted previously (Lockwood & Croghan, 1957). Animals were transferred from Askö sea water (salinity 6.5‰) and acclimatized at 5° C. in a series of stages in diluted Askö sea water. After 7 days the animals were in 10% Askö sea water. There was some mortality in this medium. After 9 days in this medium six survivors were transferred to 5% Askö sea water (salinity 0.43‰, 5.5 mm/l. Na). After 5 days only two small animals were active. A haemolymph sample was obtained from one of these animals and was found to contain 157 mm/l. Na. This

value is plotted on Fig. 1 and is well below the normal levels for Baltic and fresh-water *Mesidotea*.

### *Ionic ratios in the haemolymph*

In a number of cases chloride and potassium as well as sodium were determined in haemolymph samples. The concentration of chloride and potassium are plotted as a function of haemolymph sodium concentration in Fig. 2. The concentrations of sodium and chloride in the haemolymph are approximately equal although there is a tendency for the Cl:Na ratio to rise above unity when the concentration of the haemolymph is high.

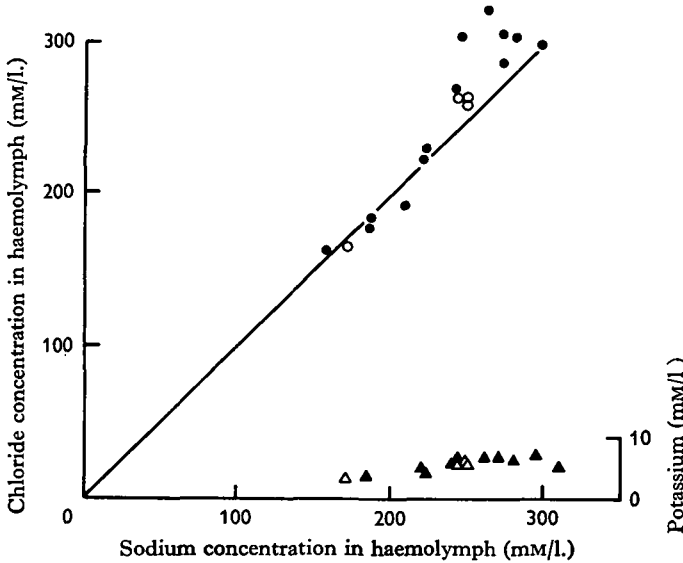


Fig. 2. Chemical composition of the haemolymph. Chloride: ●, Askö animals; ○, Mälaren animals. Potassium: ▲, Askö animals; △, Mälaren animals.

### *Haemolymph volume and cell ionic concentrations*

Data from determinations of the concentrations of sodium, potassium and chloride in haemolymph and total body water are summarized in Table 1. The sodium and chloride concentrations in the total body water are considerably higher in the Mälaren animals than in the Askö animals. As the haemolymph concentrations are comparable and as these ions are usually found only in low concentrations in cells, this suggests that the Mälaren animals have a considerably higher haemolymph volume than the Askö animals. The higher sodium content of Mälaren animals is also apparent from the isotope data in Table 4. Using data of Table 1 the relative volume of the haemolymph ( $V_B$ ) and the concentrations of these ions in the cells were calculated using eqns. (3) and (2). The data are plotted as a function haemolymph sodium concentration in Fig. 3. Some averaged data on the water content of the body and haemolymph volume are given in Table 2.

The Mälaren animals appear to have a considerably larger haemolymph volume than Askö animals.

Comparing Askö and Mälaren animals on the basis of the same dry weight, the

water content of the Mälaren animals is 79% greater than Askö animals and the haemolymph volume of the Mälaren animals is 136% greater than the Askö animals. Of the increased water content 87% is in the haemolymph.

*Potential difference between haemolymph and medium*

An attempt was made to measure the electrical potential difference between haemolymph and medium in Askö animals in Askö sea water. The microelectrode was

Table 1. *Concentration of ions in haemolymph and total body water*

Medium	Medium concentration (mm/l. Na)	Haemolymph concentration (mm/l.)			Concentration in total body water (mm/kg.)			Proportion water in body ( $V_w$ ) (ml./g.)	Proportion of water in haemolymph ( $V_B$ ) (ml./ml.)
		Na	K	Cl	Na	K	Cl		
Askö animals									
20% Askö sea water	20	183	3.8	185	92	60.5	83	0.68	0.43
		220	5.3	225	116	65	108	0.70	0.45
Askö sea water	86	241	6.5	275	133	47	133	0.77	0.44
50% Kristineberg sea water	227	261	6.9	324	148	64.5	185	0.74	0.55
		244	7.5	305	146	75	182	0.69	0.58
		271	7.3	307	168	69.5	185	0.72	0.58
Mälaren animals									
2½% Askö sea water	2.6	248	6.3	260	168	52.6	180	0.78	0.68
		248	5.7	266	203	45.5	179	0.82	0.66
20% Askö sea water	20	242	5.6	266	207	41.5	198	0.84	0.73
		170	3.7	167	130	39.7	126	0.86	0.75

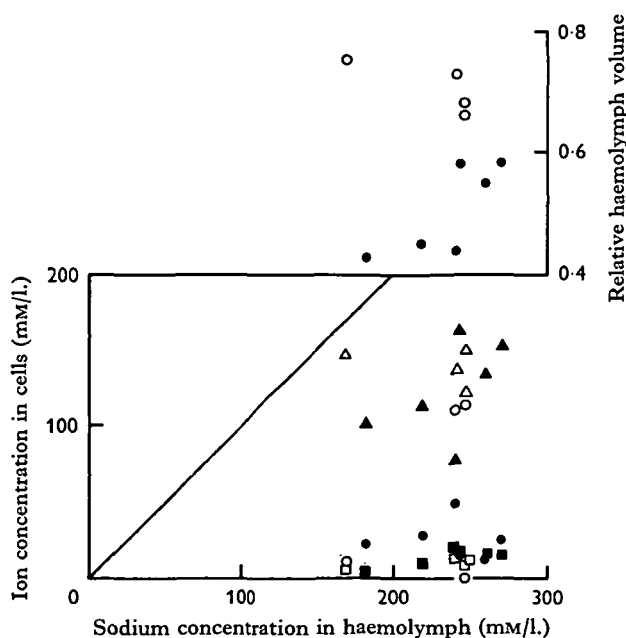


Fig. 3. Relative haemolymph volume and ion concentrations in cells. Relative haemolymph volume: ●, Askö animals; ○, Mälaren animals. Concentrations, Askö animals: ●, Na; ▲, K; ■, Cl; Concentrations, Mälaren animals: ○, Na; △, K; □, Cl.



inserted either through an arthrodial membrane between two tergites or through a small hole previously made in a tergite. Although care was taken to keep the region round the point of insertion dry to avoid short-circuiting, the potentials obtained were irregular and only a few mV. positive or negative. These experiments were regarded as inconclusive.

Table 2. *Distribution of water in the animal*

	Proportion of water in body ( $V_w$ ) (ml./g.)	Proportion of water in haemolymph ( $V_B$ ) (ml./ml.)	Proportion of haemolymph in body (ml./g.)
Askö animals	0.72	0.5	0.36
Mälaren animals	0.82	0.7	0.57

*Rate of sodium loss into de-ionized water*

Animals were taken from the medium to which they had been adapted, rinsed in de-ionized water and placed individually in 20 ml. of de-ionized water in small polythene vessels. A 1 ml. sample was immediately removed and subsequent similar samples were removed at intervals of 1-3 hr., usually for a period of 6 hr. Sodium concentration was determined using the flame photometer. The initial loss rate of sodium was determined before the sodium concentration of the medium had risen to a level at which back flux into the animal could be significant. The data are summarized in Fig. 4.

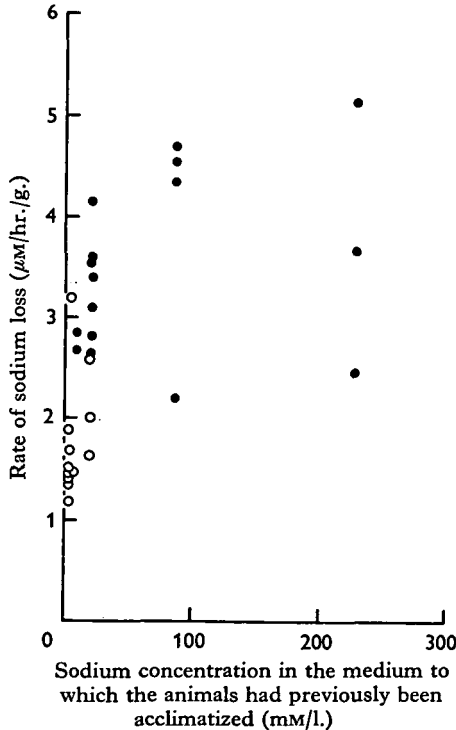


Fig. 4. Loss of sodium into de-ionized water, 5° C. ●, Askö animals; ○, Mälaren animals.

At the end of the experiment the haemolymph sodium concentration was determined.

In order to decide whether a significant part of the sodium loss was in the urine, the rate of loss from Askö animals taken from Askö sea water was compared in successive 1 hr. periods in de-ionized water and in an isosmotic solution of sucrose in de-ionized water. The isosmotic sucrose solution would be expected greatly to reduce or to abolish urine production. The results are summarized in Table 3. There is no significant difference in the rates of loss into the two media. Thus sodium loss in the urine is regarded as negligible, and net loss will be regarded as entirely by diffusion across the outer surface of the animal.

Table 3. *Loss of sodium from Askö animals into de-ionized water and isosmotic sucrose solution*

(Loss determined over one hour period at 15° C. and calculated as  $\mu\text{M} \cdot \text{hr}^{-1} \text{g}^{-1}$ .)

	De-ionized water	Isosmotic sucrose	De-ionized water
Animal 1	5.3	5.7	5.1
	Isosmotic sucrose	De-ionized water	Isosmotic sucrose
Animal 2	5.5	6.4	6.6

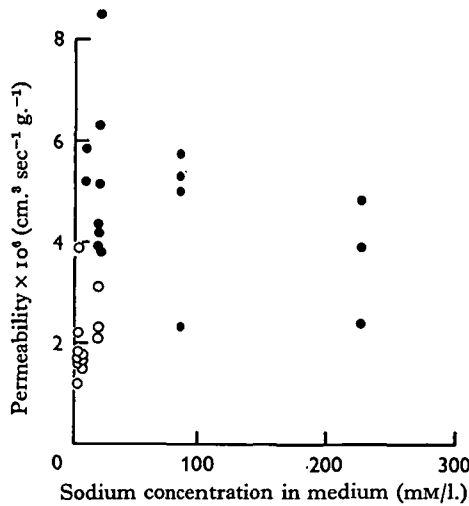


Fig. 5. Permeability ( $K_{\text{NaCl}}$ ) as function of concentration of medium to which animals had previously been adapted. 5° C. ●, Askö animals; ○, Mälaren animals.

The coefficient  $K_{\text{NaCl}}$  was calculated from the data of Fig. 4 using eqn. (6) and the initial haemolymph sodium concentration was calculated using eqn. (24). The permeability data are summarized in Fig. 5 as a function of the medium concentration to which the animal had previously been adapted and as a function of the initial haemolymph concentration in Fig. 6. It seems clear that the permeability of the Mälaren animals is about half that of the Askö animals. Substituting these values of  $K_{\text{NaCl}}$  into eqn. (5) it is possible to calculate the rate of loss of NaCl by diffusion from the animal in the medium to which the animal had previously been adapted. This data is

summarized in Fig. 7. This indicates the rate at which the active uptake mechanism must operate in various media to maintain a steady state.

Some measurements have also been made of the decrease of  $^{22}\text{Na}$  activity in animals washed in slowly flowing de-ionized water. Experiments were carried out at  $10^\circ\text{C}$ . The mean half-time for Askö animals (previously adapted to 20% Bay of Biscay sea water) was  $10.7 \pm 1.0$  (s.e.) hr. and for Mälaren animals (previously adapted to 1% Bay of Biscay sea water) was  $20.7 \pm 2.1$  (s.e.) hr. It is impossible to convert these data to

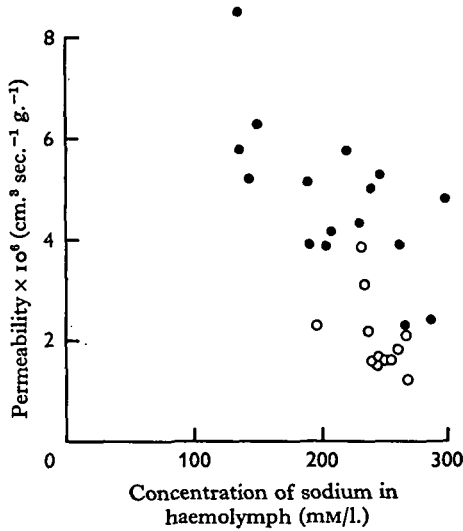


Fig. 6. Permeability ( $K_{\text{NaCl}}$ ) as function of initial haemolymph concentration.  $5^\circ\text{C}$ .  
●, Askö animals; ○, Mälaren animals.

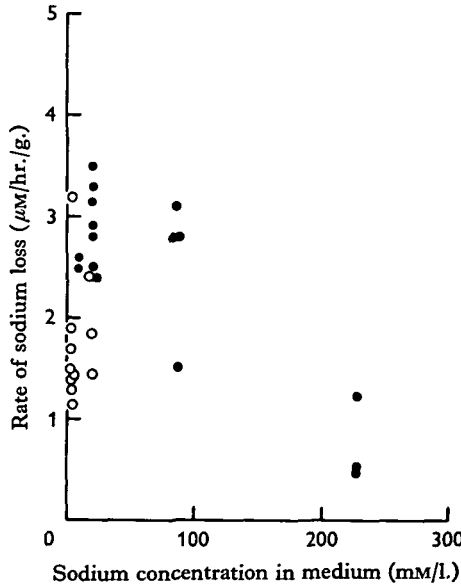


Fig. 7. Net diffusion loss of NaCl in medium to which the animals had been adapted.  $5^\circ\text{C}$ . ●, Askö animals; ○, Mälaren animals.

sodium flux, as no determination of the amount of sodium in the bodies of these particular individuals was made. This information was available in the case of a few animals, the data for which are summarized in Table 4. In a few cases the rate of loss of  $^{22}\text{Na}$  was compared in de-ionized water and in isosmotic sucrose solution. The rates of loss are identical. Some examples are given in Fig. 8.

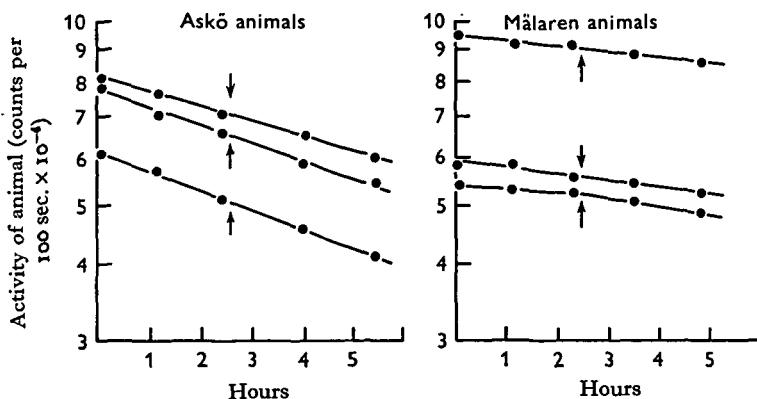


Fig. 8. Loss of  $^{22}\text{Na}$  into isosmotic sucrose and into de-ionized water. Animals transferred from isosmotic sucrose to de-ionized water at arrow.  $10^\circ\text{C}$ .

#### Rate of sodium uptake

Askö animals previously acclimatized to 20% Bay of Biscay sea water and Mälaren animals previously acclimatized to 1% Bay of Biscay sea water were placed in de-ionized water for  $1\frac{1}{4}$ – $1\frac{1}{2}$  hr. to lower haemolymph concentration and to activate maximal transport capacity (Shaw, 1959). In the case of Askö animals an animal was then transferred for 0.5 hr. to 100 ml. of 0.625 mM/l. solution labelled with  $^{22}\text{Na}$ . The animal was then rinsed and counted and transferred to labelled 1.25 mM/l. NaCl solution for 0.5 hr. and again rinsed and counted. This loading and counting procedure was repeated in 2.5, 5.0, 10, 20 and 40 mM/l. NaCl solutions. All solutions were made up to the same specific activity (counts per 100 sec. mM.  $\text{Na}^{-1}$ ). In the case of Mälaren animals the procedure was similar except that, because of a faster net uptake rate at low medium concentrations, they were placed in de-ionized water for  $1\frac{1}{4}$  hr. after loading in 5, 10, 20 and 30 mM/l. NaCl in order to ensure that the haemolymph concentration was kept below the normal level.

In all cases the animal was then returned to labelled 40 mM/l. NaCl solution for a period of about 40 hr., which is sufficient to allow their specific activity to approximate to that of the medium. Then the sodium concentration in the animal ( $C_A$ ) can be determined:

$$C_A = C_M(C_A^*/C_M^*). \quad (25)$$

The animal was then transferred to unlabelled 40 mM/l. NaCl solution and from the loss of activity in a known time the rate of sodium loss determined using eqn. (18). The animal was finally transferred to de-ionized water and from the loss of activity in a known time the rate of sodium loss was determined using eqn. (11).

From these uptake and loss data the fluxes were calculated: total sodium influx using eqn. (14), diffusion influx using eqn. (22), exchange diffusion influx using eqn. (23),

the active component of the influx using eqn. (21) and the net diffusion efflux as the difference between diffusion efflux and diffusion influx.

Unfortunately the full procedure was only carried out on a small number of animals. Examples of influx curves are given in Figs. 9 and 10. The results of the influx experiments are summarized in Table 4. The balance point is the medium concentration where the active uptake curve crosses the net diffusion efflux line and indicates the concentration of the medium where the animal would be in a steady state under the conditions of the experiments. It will be noted from the data in Table 4

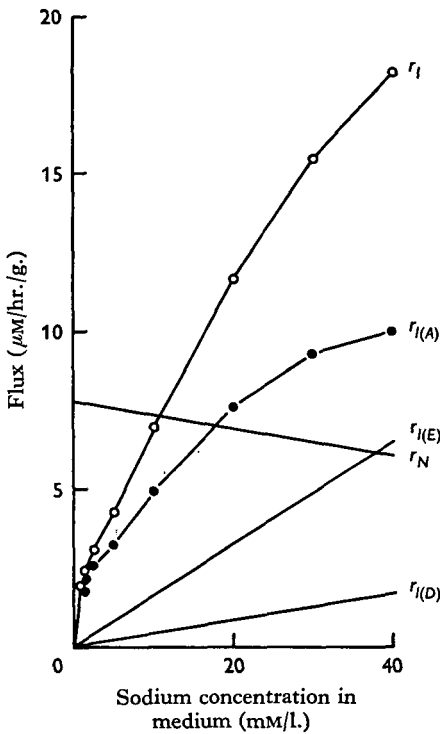


Fig. 9

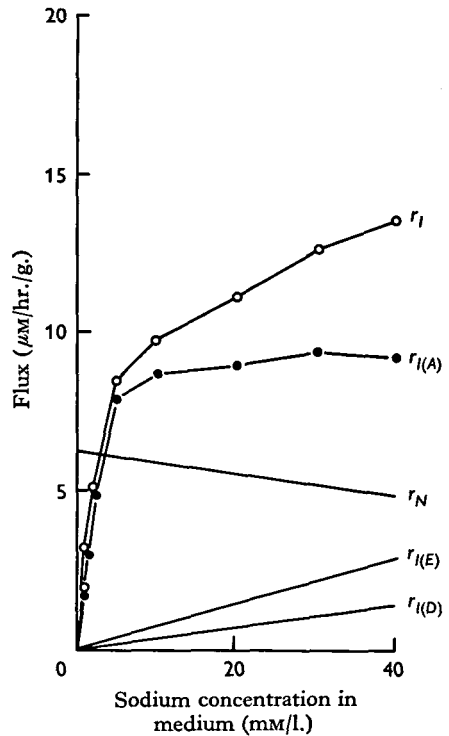


Fig. 10

Fig. 9. Relation between sodium fluxes and external sodium concentration in an Askö animal. 10° C.  $r_I$ , Total influx;  $r_{I(A)}$ , active influx;  $r_{I(E)}$ , exchange diffusion influx;  $r_{I(D)}$ , diffusion influx;  $r_N$ , net diffusion efflux.

Fig. 10. Relation between sodium fluxes and external sodium concentration in a Mälaren animal. 10° C.  $r_I$ , Total influx;  $r_{I(A)}$ , active influx;  $r_{I(E)}$ , exchange diffusion influx;  $r_{I(D)}$ , diffusion influx;  $r_N$ , net diffusion efflux.

Table 4. *Uptake data*

	Sodium concentration in animal (µM/g.)	$K_M$ (mM/l. Na)	Maximal rate of active uptake (µM/hr./g.)	Balance point (mM/l. Na)
Askö animal A	62.5	14	7.0	28
Askö animal B	66	10	10	18
Mälaren animal A	103	2.5	9.4	3.5
Mälaren animal B	80	4	6.3	4.5

that the concentration of the medium required to half-saturate the transporting mechanism ( $K_M$ ) is markedly lower in the Mälaren animals than in those from the Baltic. This finding was confirmed by additional experiments, using a less rigorous technique, on a further five Mälaren and four Askö animals.

#### DISCUSSION

The evolution of *Mesidotea* as an inhabitant of fresh water in Scandinavia is of particular interest since the fresh-water habitats are all post-glacial. Further, the lakes in which it occurs are known to have been isolated from the Baltic at different periods (for summary see Charlesworth 1957). It seems clear that this species must have become adapted to fresh water several times. The adaptations shown by the different isolated races may well be different. The present paper is limited to discussing the physiological differences between animals from the Lilla Ullevifjärden, a northern branch of L. Mälaren (0.67 mM/l. Na) that has been isolated from the Baltic relatively recently, and from the Baltic close to the island of Askö (salinity 6.5‰, 86 mM/l. Na).

Earlier work had suggested that the Baltic race could not be adapted to fresh water (Bogucki, 1932; Lockwood & Croghan, 1957). Following progressive adaptation over a longer time period a number of animals survived for a considerable period in 10% Askö sea water (salinity 0.82‰, 10.5 mM/l. Na). However, in 5% Askö sea water (salinity 0.43‰, 5.5 mM/l. Na) mortality was high. Haemolymph sodium analyses on animals from 10% Askö sea water and on one of the survivors in 5% Askö sea water showed that the haemolymph sodium concentration had been considerably lowered compared to the normal values for Baltic and fresh-water animals. The 5% Askö sea-water medium is within the Venice classification level for fresh water (salinity 0.5‰, Venice Symposium, 1959), but is nevertheless considerably more concentrated than the fresh water in the Lilla Ullevifjärden. It appears that 5% Askö sea water is right on the edge of the normal range of individual adaptation of the Baltic race. However, the process of isolation of the fresh-water habitats would have occurred much more slowly and there would be time for genetic selection also to occur.

The data on the relation between the sodium concentration in the haemolymph and in the medium are similar to those obtained for chloride previously (Lockwood & Croghan, 1957) and indicate that the adaptations of the fresh-water animals enable them to maintain a high haemolymph concentration in fresh water (2½% Askö sea water) similar to that of Baltic animals in considerably more concentrated media.

Analyses of the total composition of the animals have been used to derive the haemolymph volume and intracellular ion concentrations. The Mälaren animals appear to have a much larger relative haemolymph volume than the Askö animals. The reason for this difference is not clear. Possibly the fresh-water animals in an environment where the difference between the haemolymph concentration and medium concentration is so great may occasionally suffer a severe loss of NaCl from the body, such as for example might follow injury. Then a high haemolymph volume might minimize the osmotic effects on the cells of the rapid loss of a given amount of NaCl from the body, and also provide more volume into which cells could swell osmotically before cutting off the circulation. The calculated intracellular ion concentrations are as might be expected. The potassium concentration is high and the sodium and chloride

concentrations low. There would be little error in assuming that all the sodium and chloride is in the haemolymph. If the cells are isosmotic with the haemolymph, there must be a considerable concentration of other osmotically active material in the cells.

The rates of loss of sodium into de-ionized water indicate that the permeability of the outer surface of the animals (presumably the gills on the pleopods) is considerably less in Mälaren animals than in Askö animals. The extent to which this difference is due to differences in the relative permeable area or to the permeability coefficient of the surface membranes is unknown. However, the difference of permeability means that the Mälaren animals lose NaCl by diffusion appreciably more slowly in dilute media than Askö animals. This must be a significant factor in adaptation to fresh water. Something similar may occur in *Gammarus duebeni* (Shaw & Sutcliffe, 1961; Sutcliffe, 1967b) but the data here are complicated by significant losses of NaCl in the urine, and external permeability was not expressed as a coefficient. There is no evidence of any adaptations of the permeability of *Mesidotea* when the animals are adapted to various medium concentrations. This failure to adapt permeabilities must be a factor in the limitation of the ability of Baltic *Mesidotea* to maintain a high haemolymph concentration in dilute media. When the permeability is compared with haemolymph concentration a relation is apparent. The permeability, at least of the Baltic animals, is greater in those having low haemolymph concentrations. Consider the steady state

$$K_{\text{NaCl}} (C_{\text{NaCl}}^B - C_{\text{NaCl}}^M) = r_{I(\text{active})}. \quad (26)$$

If the permeability is high, the steady-state haemolymph concentration will be lowered.

Experiments in isosmotic sucrose solutions suggested that the loss of NaCl in the urine in *Mesidotea* is small by comparison with that across the body surface. The isopods have maxillary glands, and in the case of *Mesidotea* these are small. In contrast to the situation with crustacean antennary glands, there appears to be no physiological information on the function of maxillary glands, and they are not mentioned in a recent review on the physiology of invertebrate excretory organs (Kirschner, 1967).

Shaw (1959, 1960, 1961a, b), Shaw & Sutcliffe (1961), Sutcliffe (1967a, b) and Sutcliffe & Shaw (1967) have shown that influx of sodium into the body is determined by the concentration of the medium at low medium concentrations. The relation was fitted to the Michaelis-Menten equation for reactions proceeding via an enzyme-substrate complex. However in almost all of this work the total influx was used. This may be considerably greater than the active influx as appears to be the case in *Mesidotea* in all except dilute media. In the case of *Carcinus* where an attempt was made to isolate the active component (Shaw, 1961a), the procedure by which this was done appears to be very doubtful as possible exchange diffusion was disregarded. Also with the exception of the work on *Astacus* (Bryan, 1960; Shaw, 1959, 1960) and *Carcinus* (Shaw, 1961a) uptake was determined using animals that had not been depleted of NaCl. Uptake might thus be considerably affected by changes in the haemolymph sodium concentration. The general conclusions were that the medium concentration at which the rate of sodium uptake is half the maximal rate ( $K_M$ , Michaelis constant, an inverse measure of the affinity of the uptake mechanism for the substrate transported) may vary between different species. The value of  $K_M$  tends to be markedly lower in fresh-water species than in brackish-water species. It is particularly low in well-

established typical fresh-water species. In *Gammarus pulex* and *G. lacustris*  $K_M$  is in the range 0.10–0.15 mm/l. Na (Sutcliffe, 1967*a*; Sutcliffe & Shaw, 1967). A low  $K_M$  indicates an adaptation of the carrier mechanism increasing its affinity for sodium or chloride and enabling a high rate of active uptake to be maintained even in dilute media.

In the case of *Mesidotea* the maximum rate of active transport (presumably related to the total number of carrier sites) does not differ significantly between the two races. These maximal rates of active uptake are comparable with those found by determination of changes in haemolymph concentration (Lockwood & Croghan, 1957). The values of  $K_M$  in the two races do differ, however. The value of  $K_M$  in the Baltic race is quite high and can be compared with the value estimated for *Carcinus* (Shaw, 1961*a*). The balance point is in consequence also high. In the normal Askö medium the carrier will be saturated and any variation in uptake rate will depend on control of the number of sites active. With the high value of  $K_M$  it is not surprising, however, that the Baltic animals cannot maintain a normal haemolymph concentration and survive in very dilute media. The value of  $K_M$  is much lower in the Mälaren animals than in the Baltic animals. The balance point is in consequence also considerably reduced. However, the value of  $K_M$  is by no means as low as that of some other fresh-water animals that have been studied. It is comparable with the brackish-water and some fresh-water races of *Gammarus duebeni* (Shaw & Sutcliffe, 1961; Sutcliffe, 1967*b*) and with the migrant *Eriocheir* (Shaw, 1961). This indicates that in the normal fresh-water medium the transport mechanism will be operating far below saturation and capable of a maximal transport rate only about 18% of the rate possible in a medium sufficiently concentrated to saturate the sites. The balance point also appears to be well above the concentration of their normal fresh-water medium. However, these animals had been kept for a period of some months in 1% Bay of Biscay sea water (4.6 mm/l Na); although this is fresh water by Venice definition it is considerably more concentrated than their normal medium. Some long-term changes in the permeability and/or uptake mechanism may have occurred. The balance point is certainly below the sodium concentration of the 1% Bay of Biscay sea-water medium.

In their normal environment most species balance the loss of NaCl from the body by active uptake without utilizing the full transport capacity at that external concentration. Thus there is a reserve capacity that can be brought into use if the blood concentration falls for any reason. In the case of typical fresh-water species that have been studied such as *Astacus* (Bryan, 1960; Shaw, 1959) and *Asellus* (Lockwood, 1960) the reserve capacity is several times that required to balance the usual loss. In the fresh-water *Mesidotea* it seems clear that there can be little reserve capacity. Also, unless the animals are in their normal medium the replacement of any temporary NaCl loss will be a slower process in the Mälaren animals than in the Askö animals. In this situation the higher haemolymph volume of the Mälaren animals would be an advantage.

In conclusion the adaptations that have enabled *Mesidotea* to survive in fresh-water media and to maintain the high haemolymph concentration of the brackish-water race involve a reduction in the permeability to NaCl of the external surfaces and an increase in the affinity of the active uptake mechanism, enabling it to continue to take up NaCl rapidly even in the more dilute external media. However, the Mälaren animals appear to be only marginally adapted to their normal medium.



Lake Mälaren has only been isolated from the Baltic relatively recently but most of the other lakes in which *Mesidotea* occurs have been isolated from marine influence for very much longer and also have a lower conductivity. It would be of interest to investigate what further modifications to the mechanism adapting *Mesidotea* to fresh water may have evolved in these races.

SUMMARY

1. The isopod *Mesidotea entomon* has colonized the Baltic and certain Swedish lakes since the end of the last Ice Age.

2. The ionic regulation of Baltic animals and fresh-water animals (L. Mälaren) has been compared.

3. It has been possible to adapt Baltic animals to very dilute media, but 5 % Askö sea water (5.5 mM/l. Na) appears to be the limit of adaptation. The haemolymph sodium concentration of Baltic animals from the very dilute media was considerably lowered.

4. The haemolymph sodium concentration in Mälaren animals is high (250 mM/l. Na) and comparable with that in Baltic animals in much more concentrated solution. The haemolymph ionic ratios of the Baltic and freshwater animals are similar. The Cl:Na ratio rises slightly in the more concentrated haemolymph samples.

5. From the concentration of ions in the haemolymph and in the total body water, the relative volume of the haemolymph was calculated. Mälaren animals appear to have a much larger haemolymph volume.

6. The permeability of the animals was determined from the rate of loss of sodium into de-ionized water. The permeability of the Mälaren animals is considerably reduced compared to the Baltic animals. Permeability is not related to the medium to which the animals had been adapted.

7. The sodium influx was determined using  $^{22}\text{Na}$ . The rate of active uptake was calculated from this. The maximal rate of active uptake was similar in Baltic and Mälaren animals. The sodium concentration of the medium at which active uptake was half maximum ( $K_M$ ) was considerably lower in Mälaren animals than in Baltic animals.

8. The evolution of *Mesidotea* as a fresh-water animal is interpreted as a result of a reduction in permeability of the external surfaces to NaCl and an increase in the affinity of the active transport mechanism enabling the animal to maintain the haemolymph NaCl concentration in a steady state in fresh water.

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