The detailed distribution of Gammarus duebeni in freshwater habitats is discussed by Hynes (1954) and Sutcliffe (1967a). It occurs in a number of isolated coastal localities situated on the western sea-boards of Britain, including the Isle of Man, and on the Shetlands, the Faroes, Iceland, Norway and Brittany. In most of these localities the amphipod is restricted to only a few of the streams and rivers apparently available for colonization. In Ireland G. duebeni is not confined to coastal localities; it is widely distributed inland in a variety of freshwater habitats (Reid, 1939; Macan & Lund, 1954). Reid was unable to find any morphological differences between specimens from fresh water in Ireland and specimens obtained from brackish water in the south of England. He tried to breed the latter in fresh water, but without success, and so suggested that there may be two distinct physiological races. Support for this was obtained by Beadle & Cragg (1940), who found that G. duebeni from a freshwater locality survived in distilled water longer than did animals from brackish water. However, more extensive experiments of the same kind carried out by Hynes (1954) using animals obtained from the Isle of Man did not confirm this, and other observations by Hynes on the structure of the antennal glands, and the size and number of eggs, also produced no further support for the suggestion that there are two physiological races.

The development of more sensitive methods for determining the uptake and loss of ions has made it possible to reinvestigate the osmoregulatory mechanisms of animals from different habitats. Shaw & Sutcliffe (1961) were able to demonstrate differences in the affinity for sodium ions in the uptake mechanisms of Gammarus pulex from fresh water and G. duebeni from brackish water, and it was suggested that perhaps G. duebeni in freshwater localities has developed an uptake mechanism closer to the type found in G. pulex. G. pulex has a very high affinity for sodium ions at low external concentrations, so that the uptake mechanism situated at the body surface is half-saturated at 0.10-0.15 mM/l. and fully saturated at 1 mM/l. NaCl, whereas in G. duebeni from brackish-water localities the corresponding external concentrations are about ten times higher (Shaw & Sutcliffe, 1961; Sutcliffe, 1967b, c). Consequently when G. duebeni is kept in low external concentrations, below about 1 mM/l. NaCl, the rate of sodium uptake is very low and is barely sufficient.
to replace sodium lost in the urine and by outward diffusion across the body surface. Thus although it is possible to take *G. duebeni* from brackish-water habitats and acclimatize them to fresh water, at least for short periods, it is clear that any mechanism by which the sodium uptake rate was increased at low concentrations would be a considerable advantage for successful colonization of fresh waters, particularly inland waters with a very low salt concentration.

Investigations of sodium regulation in *G. duebeni* from freshwater streams on the Lizard peninsula, Cornwall, on the Kintyre peninsula, Argyll, and on the Isle of Man showed that in animals from these localities the sodium uptake mechanism is essentially the same as the uptake mechanism in animals from brackish-water localities in Britain. Other features of the sodium regulatory mechanism also appear to be similar in animals from the two habitats. But at very low external sodium concentrations (0.1–0.3 mM/l.) the survival of animals from the freshwater localities was distinctly better than the survival of brackish-water animals, and it was suggested that this is due to natural selection in the populations living in freshwater favouring individuals which have a sodium uptake rate higher than the average rate of uptake in the populations living in brackish water (Sutcliffe, 1967c).

In Britain the freshwater streams containing *G. duebeni* have a relatively high salt concentration, usually greater than about 1 mM/l. NaCl. But the fresh waters in many parts of Ireland are relatively low in salts, with sodium concentrations ranging from 0.5 down to about 0.2 mM/l. (Webb, 1947; Sutcliffe, 1967a) and it may be supposed that *G. duebeni* living in these waters is exposed to greater selection pressure on the sodium regulatory mechanism. This could produce adaptive changes in the regulatory mechanism, and initial studies on *G. duebeni* from the River Boyne and the River Liffey in Eire showed that in these animals the sodium-uptake mechanism is indeed distinct from that found in populations of *G. duebeni* in Britain. The investigation was extended to include animals from other parts of Ireland in order to determine whether or not *G. duebeni* living in fresh water in Ireland can be regarded as a single population physiologically distinct from *G. duebeni* living in fresh water in Britain.

**MATERIAL AND METHODS**

*G. duebeni* was collected from a small tributary of the River Boyne near the village of Traminont, Co. Meath, and from a small tributary running into the River Liffey at Clane, Co. Kildare, in June 1961. A further batch of animals was collected from the Boyne tributary in June 1964. The animals were placed in large vacuum flasks and, travelling by air, were transported to the laboratory in Newcastle within 24 hr. of collection in the field.

In March 1965 animals were collected from the shores of Lough Melvin, Co. Leitrim, and from Lough Neagh, near Ballyronan, Co. Londonderry. They were transported by car in buckets containing small amounts of water, and practically all survived a journey to Newcastle lasting 3–4 days.

The concentrations of some of the ions in these waters are given in Table 1.

Experiments with animals from the River Boyne and River Liffey in 1961 were carried out at room temperature, which fluctuated widely between about 14° and 24° C. All later experiments were carried out in a constant-temperature room kept
Sodium regulation in Gammarus duebeni at 10 ± 1°C. The animals were acclimatized to this temperature for about 1 week before commencing experimental work, and between experiments they were fed on leaves of sycamore and elm.

The experimental media were NaCl solutions made with de-ionized water to give a range of sodium concentrations from 0.07 to 6 mM/l., and sea water from Cullercoats diluted with Newcastle tap water to provide a range of concentrations down to 10 mM/l. NaCl (about 2% sea water). These sea-water media are referred to in terms of NaCl solutions having the same freezing points.

The terminology and methods used for determining the uptake and loss of sodium are described in preceding papers (Shaw & Sutcliffe, 1961; Sutcliffe, 1967b, c).

Table 1. The concentrations of sodium, calcium, magnesium and chloride in the localities where Gammarus duebeni was collected in Ireland

<table>
<thead>
<tr>
<th>Locality</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lough Neagh</td>
<td>0.46</td>
<td>1.34</td>
<td>0.66</td>
<td>0.43</td>
</tr>
<tr>
<td>Lough Melvin</td>
<td>0.46</td>
<td>1.08</td>
<td>0.22</td>
<td>0.48</td>
</tr>
<tr>
<td>River Boyne</td>
<td>0.40</td>
<td>2.32</td>
<td>0.64</td>
<td>0.37</td>
</tr>
<tr>
<td>River Liffey</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GAMMARUS DUEBENI FROM THE RIVER BOYNE**

Survival and sodium balance at low external concentrations

When animals from the River Boyne were acclimatized to low external NaCl concentrations at room temperature, mortality was very low and the majority survived acclimatization to 0.15 mM/l. NaCl. At 10°C. survival was even better, and the majority survived in 0.12 mM/l. NaCl for more than 1 week. The ability to maintain sodium balance at very low external concentrations was tested by placing a group of ten animals in about 40 ml. of de-ionized water and allowing the external sodium concentration to increase until sodium uptake was equal to sodium loss, and the external concentration remained constant for at least 24 hr. The water was then replaced by another 40 ml. de-ionized water and the process repeated until the lowest steady-state concentration was found which was maintained for 24 hr. These experiments were carried out at 10°C. One group achieved sodium balance at an external concentration of 0.065 mM/l. sodium, and four other groups at 0.07–0.08 mM/l. sodium. The mean for sixteen groups was 0.10 mM/l. sodium, standard deviation 0.019.

This ability to maintain sodium balance and survive at very low external concentrations is markedly different from that of the brackish-water populations of *G. duebeni* from Meggies Burn and Budle Bay, Northumberland, and from salt-marshes round Morecambe Bay (Sutcliffe, 1967c). It is, in fact, very similar to that of the freshwater species *Gammarus pulex* and *G. lacustris*, except that both of these species are consistently able to maintain sodium balance for long periods at an external concentration of 0.06 mM/l. sodium.
Sodium influx and net uptake

The relation between the sodium influx and the external concentration in animals acclimatized to a variety of external concentrations is shown in Fig. 1. These animals were obtained from the River Boyne in June 1961, average weight 85 mg., and the

Fig. 1. The relation between sodium influx and the external concentration in Gammarus duebeni from the River Boyne acclimatized to various NaCl concentrations at room temperature. A, Groups acclimatized to 2 mM/L (▲), 1 mM/L (●) and 0.5 mM/L (○). B, Groups acclimatized to 0.25 mM/L (●) and 0.15 mM/L (○).

Fig. 2. The relation between sodium influx and the external concentration in Gammarus duebeni from the River Boyne acclimatized to NaCl solutions at 10° C. Groups acclimatized to 0.25 mM/L (●) and 0.12 mM/L (○).
Sodium regulation in *Gammarus duebeni* 343

Experiments were carried out at room temperature. Another collection from the same locality was made in June 1964, average weight 86 mg., and the experiments were carried out at 10°C. The results of these measurements are shown in Fig. 2. It is immediately apparent that the relation between the influx and the external sodium concentration is not the same as that found in *G. duebeni* from Britain, where the influx increased gradually to reach a maximum at an external concentration of about 10 mM/l. NaCl (Shaw & Sutcliffe, 1961; Sutcliffe, 1967c). Instead, the influx increased gradually with increasing external concentrations, but a maximum was reached abruptly at an external concentration of 1–2 mM/l. NaCl, where the sodium-transporting system in the British animals was only half-saturated. With respect to the external concentration at which saturation is reached the Boyne animals resemble

<table>
<thead>
<tr>
<th>Acclimatization concentration (mM/l. NaCl)</th>
<th>Sodium loss (μM/animal/hr.)</th>
<th>No. of groups</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.57</td>
<td>6</td>
<td>0.09</td>
</tr>
<tr>
<td>1.0</td>
<td>0.56</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>0.5</td>
<td>0.45</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>0.25</td>
<td>0.46</td>
<td>9</td>
<td>0.05</td>
</tr>
<tr>
<td>0.15</td>
<td>0.34</td>
<td>7</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 2. The loss rate of sodium into de-ionised water in *Gammarus duebeni* from the River Boyne acclimatized to a range of external concentrations at room temperature

<table>
<thead>
<tr>
<th>External concentration (mM/l. NaCl)</th>
<th>Net uptake (μM/animal/hr.)</th>
<th>Loss rate into de-ionized water (acclimatized to 0.25 mM/l. NaCl) (μM/animal/hr.)</th>
<th>Influx rate (μM/animal/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.11</td>
<td>0.46</td>
<td>0.73</td>
</tr>
<tr>
<td>1.0</td>
<td>0.26</td>
<td>0.46</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Table 3. Sodium uptake from 0.5 and 1 mM/l. NaCl in *Gammarus duebeni* from the River Boyne acclimatized to 0.25 mM/l. NaCl at room temperature

*Gammarus pulex* and *G. lacustris*, although the nature of the relationship between the influx and the external concentration is different. Furthermore, in all cases the sodium transporting system was half-saturated at an external concentration of about 0.4–0.5 mM/l. sodium. This is closer to the half-saturation values found in *G. pulex* and *G. lacustris* (0.1–0.15 mM/l. sodium), and similar to that of the freshwater crayfish, *Astacus pallipes*, where the half-saturation value is 0.2–0.3 mM/l. sodium (Shaw, 1959). Thus in respect of their sodium transporting system the animals from the River Boyne appear to be intermediate between the British populations of *G. duebeni* and the freshwater species *G. pulex* and *G. lacustris*.

In animals acclimatized to 1 and 2 mM/l. NaCl at room temperature the sodium loss rate into de-ionised water was about 0.57 μM/hr. (Table 2), whereas the influx at these external concentrations was 0.9 μM/hr. (Fig. 1 A). This suggests that approximately 33% of the influx was due to the presence of an exchange component, and this is reminiscent of the situation found in the brackish-water animals from Britain (Sutcliffe, 1967c). Furthermore, the net uptake of sodium from 0.5 and 1 mM/l. NaCl
in animals acclimatized to 0.25 mM/L. NaCl was not sufficient to account for differences between influx and loss rates (Table 3), and again it appears that from 20–42% of the influx was due to an exchange phenomenon.

**Regulation of sodium influx and loss rates**

In *G. pulex* and *G. lacustris* it was found that the sodium influx and the sodium-loss rate remained approximately constant in freshwater concentrations greater than 0.2–0.3 mM/L NaCl, but below this the influx was considerably increased and the loss rate was strongly reduced (Sutcliffe, 1967b; Sutcliffe & Shaw, 1967). On the other hand, in *G. duebeni* from brackish-water and freshwater localities in Britain, changes in the influx and loss rates occurred at external concentrations below about 10 mM/L NaCl (Sutcliffe, 1967c). In view of the fact that the saturation of the uptake mechanism in animals from the River Boyne is similar to that in *G. pulex* and *G. lacustris*, it is of interest to see if the fluxes change at low rather than high external concentrations.

Sodium loss rates into de-ionized water in animals acclimatized to a range of external concentrations from 2 to 0.15 mM/L NaCl are given in Table 2. There was no reduction in loss rate when animals were transferred from 2 to 1 mM/L NaCl. In 0.5 mM/L NaCl the loss rate was reduced by about 20%, but there was no further change in animals acclimatized to 0.25 mM/L NaCl. In 0.15 mM/L NaCl the loss rate was then again reduced by 26%. These loss rates were determined with animals which were also used to measure sodium influx rates at room temperature (Fig. 1), and the influx remained constant in animals acclimatized to the range 2–0.5 mM/L NaCl. It was then increased by 30–35% in animals acclimatized to 0.25 mM/L NaCl. In animals acclimatized to 0.25 mM/L NaCl at 10°C, the influx from 0.25 mM/L NaCl was about 0.27 μM/hr. (Fig. 2) and the mean sodium loss into de-ionized water at 10°C from eight groups was 0.28 μM/hr., standard deviation 0.03. When these animals were acclimatized to 0.12 mM/L NaCl the influx was increased by 35–40% and the loss rate was reduced by about 30%. This is distinct from the situation found in *G. duebeni* from brackish water, where the influx rate was not increased at external concentrations below 0.25 mM/L NaCl.

These results show that the influx and loss rates within the range up to 2 mM/L NaCl were changed only at very low external concentrations, and this is yet another point of similarity between these animals on the one hand and *G. pulex* and *G. lacustris* on the other in respect of their sodium transporting systems.

It is also interesting to compare the influx measurements made at room temperature with those made 3 years later at 10°C. The average weight of the animals was the same. At room temperature the maximum influx increased from 0.9 μM/hr. (Fig. 1A) to about 1.25 μM/hr. (Fig. 1B), and the influx was the same in animals acclimatized to 0.25 and 0.15 mM/L NaCl. On the other hand, at 10°C, the maximum influx was only 0.8 μM/hr. in animals acclimatized to 0.25 mM/L NaCl, and this increased to about 1.2 μM/hr. when acclimatized to 0.12 mM/L NaCl (Fig. 2). If a saturation rate of 1.2–1.3 μM/hr. represents the fastest rate possible in animals weighing 85 mg., then at room temperature the maximum rate would already be in operation in 0.25 mM/L NaCl and a further increase would not be possible in animals transferred to lower external concentrations. Very similar observations were made with *G. duebeni*.
Sodium regulation in Gammarus duebeni from brackish water at different temperatures (Sutcliffe, 1967c) where the highest maximum influx also appeared to be about 1'3-1'4 μM/hr. in animals weighing 71 mg. Unfortunately room temperature was not recorded during experiments with animals from the River Boyne, and it may have fluctuated widely between about 14° and 24° C., so it is not possible to estimate the effect of temperature on the influx.

**Blood sodium concentration**

A thorough investigation of the blood concentration over a range of external concentrations has not yet been carried out, but a few measurements of the blood sodium concentration are given in Table 4. Measurements in 1961 were made at the end of a long series of experiments to determine the influx and loss rates, and the lower blood concentrations may be due to the fact that the remaining animals were in poor condition, indicated by an increase in the death-rate. However, it seems clear that the blood sodium concentration is reduced by less than 10% over the range of external concentrations 1-0-25 mM/l. NaCl, and this is distinct from the reduction of about 20% in British animals acclimatized to the same range of external concentrations (Shaw & Sutcliffe, 1961; Sutcliffe, 1967c). It is also interesting to note that the values for blood sodium in these animals from the River Boyne lie between the values found in animals from two brackish-water localities in Britain; that is, the blood sodium concentration is not reduced to a lower level in the Irish freshwater animals.

<table>
<thead>
<tr>
<th>Year</th>
<th>Acclimatization concentration (mM/l. NaCl)</th>
<th>Mean blood sodium concentration (mM/l.)</th>
<th>N</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961</td>
<td>1.0</td>
<td>199</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>214</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>200</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>1964</td>
<td>1.0</td>
<td>233</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>219</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

**Gammarus duebeni from the River Liffey**

**Sodium influx**

The relation between the influx and the external concentration is shown in Fig. 3. The measurements were made at room temperature with animals acclimatized to 1 and 0.25 mM/l. NaCl. The average weight of these animals was about 55 mg. The influx increased rapidly and reached a maximum level at an external concentration of 1.5 mM/l. in animals acclimatized to 1 mM/l. NaCl. The sodium transporting system was half-saturated at an external concentration of about 0.4 mM/l. NaCl, and these results are practically identical with those obtained on animals from the River Boyne.
Sodium loss and net uptake

Sodium loss into de-ionized water was measured in animals acclimatized to 1, 0.25 and 0.15 mM/l. NaCl. The results are given in Table 5. In animals acclimatized to 0.25 following 1 mM/l. NaCl the loss rate was apparently reduced by 22%. This loss rate (0.28 μM/hr.) agrees well with measurements of the influx from 0.25 mM/l. NaCl in animals acclimatized to this concentration (Fig. 3). When acclimatized to 0.15 mM/l. NaCl there was a further reduction in loss rate to 0.20 μM/hr., which balances the influx of 0.20 μM/hr. at this external concentration in animals acclimatized to 0.25 mM/l. NaCl, i.e. the influx rate was probably not increased. Similar results were obtained with animals from the River Boyne, also kept at room temperature.

Since the loss rate in animals acclimatized to 1 mM/l. NaCl is less than the influx, it appears that about 35% of the influx at this concentration is due to an exchange component. However, this exchange component was not observed during net uptake of sodium in animals acclimatized to 0.25 mM/l. NaCl and then transferred to higher external concentrations. Net uptake was measured over a period of 1 hr., and the

Table 5. The loss rate of sodium into de-ionized water in Gammarus duebeni from the River Liffey acclimatized to a range of external concentrations at room temperature

<table>
<thead>
<tr>
<th>Acclimatization concentration (mM/l. NaCl)</th>
<th>Sodium loss (μM/animal/hr.)</th>
<th>No. of groups</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.36</td>
<td>9</td>
<td>0.07</td>
</tr>
<tr>
<td>0.25</td>
<td>0.28</td>
<td>9</td>
<td>0.06</td>
</tr>
<tr>
<td>0.15</td>
<td>0.20</td>
<td>3</td>
<td>—</td>
</tr>
</tbody>
</table>

Fig. 3. The relation between sodium influx and the external concentration in Gammarus duebeni from the River Liffey acclimatized to NaCl solutions at room temperature. Groups acclimatized to 1 mM/l. (●) and 0.25 mM/l. (○).
Sodium regulation in Gammarus duebeni results are shown in Fig. 4. If the initial loss rate into the NaCl solutions was the same as the loss rate into de-ionized water, then this loss and the net uptake account for all of the influx in these animals.

![Graph showing sodium influx and loss](image)

Fig. 4. Net uptake of sodium (vertical bars) from NaCl solutions in Gammarus duebeni from the River Liffey acclimatized to 0.25 mM/L NaCl at room temperature. The horizontal broken line represents the sodium loss rate into de-ionized water. The curve represents sodium influx in animals acclimatized to 0.25 mM/L NaCl.

**GAMMARUS DUEBENI FROM LOUGH NEAGH**

**Sodium influx and loss**

The relation between sodium influx and the external concentration is shown in Fig. 5, using animals acclimatized to 0.5 mM/L NaCl at 10°C. The average weight of these animals was 102 mg. Again the sodium transporting system was saturated at an external concentration of 2 mM/L NaCl, and was half-saturated at about 0.7 mM/L NaCl.

Some measurements of the influx in animals acclimatized to a range of external concentrations at 10°C are given in Table 6, together with determinations of the loss rate into de-ionized water in the same animals. The apparent difference in the influxes from 0.5 mM/L NaCl in animals acclimatized to 0.5 and 0.25 mM/L NaCl is not significant (t = 1.82, P = 0.10). The lower influx (0.21 μM/hr.) in animals acclimatized to 2 mM/L NaCl is probably not significant either, particularly since the loss rate in these animals (0.25 μM/hr.) was not significantly different from the loss rate of 0.22 μM/hr. at 0.5 mM/L NaCl (t = 0.54, P = > 0.5). Hence both the influx and loss rates in animals acclimatized to 0.25 mM/L NaCl are the same as they were previously in 0.5 and 2 mM/L NaCl, although both were probably altered when acclimatized to 0.17 mM/L NaCl. Unfortunately the batch of animals was by now very small, and further experiments were not possible as the animals were
Dying off. However, it appears that both influx and loss rates can remain constant over the range of external concentrations from 2 to 0.25 mM/l. NaCl, again as in *G. pulex* and *G. lacustris*.

**Blood sodium concentration**

The sodium concentrations in the blood of three individuals kept in 2 mM/l. NaCl for nearly 5 weeks at 10° C. were 219, 220 and 227 mM/l. In three animals acclimatized to 0.17 mM/l. NaCl the blood sodium concentrations were 225, 190 and 140 mM/l.

![Graph](image)

**Fig. 5. Sodium influx in *Gammarus duebeni* from Lough Neagh acclimatized to 0.5 mM/l. NaCl at 10° C. Each point represents one measurement on a group of animals.**

<table>
<thead>
<tr>
<th>Acclimatization concentration (mM/l. NaCl)</th>
<th>2.0</th>
<th>0.5</th>
<th>0.25</th>
<th>0.17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influx from 0.5 mM/l. NaCl (μM/animal/hr.)</td>
<td>—</td>
<td>0.21 (3)</td>
<td>0.28 (7) ± 0.03</td>
<td>0.34 (5) ± 0.07</td>
</tr>
<tr>
<td>Influx from 0.25 mM/l. NaCl (μM/animal/hr.)</td>
<td>—</td>
<td>—</td>
<td>0.18 (from Fig. 5)</td>
<td>0.18 (7) ± 0.045</td>
</tr>
<tr>
<td>Loss rate into de-ionized water (μM/animal/hr.)</td>
<td>0.25 (9) ± 0.035</td>
<td>0.22 (12) ± 0.045</td>
<td>0.22 (4)</td>
<td>—</td>
</tr>
</tbody>
</table>

The lowest value was obtained from an animal which appeared to be dying. However, Beadle & Cragg (1940) found that *G. duebeni* obtained from freshwater remained alive in distilled water (changed twice daily) for at least 4 days with a blood chloride concentration of only about 125 mM/l. The blood sodium concentration in animals from Lough Neagh acclimatized to 2 mM/l. NaCl is very similar to that found in animals from the River Boyne.
All of the *G. duebeni* collected from fresh water in Ireland displayed an astonishing ability to tolerate very wide changes in the salt concentration of the external medium, and the following observations apply equally to animals from the River Boyne, the River Liffey and Lough Neagh. Animals repeatedly survived transference straight from 0.5 mM/l. NaCl into undiluted sea water at a temperature of 10° C. Out of sixty-four animals acclimatized to sea water, fifty-four survived direct transference into 0.25 mM/l. NaCl, a far better performance than that usually obtained with animals from brackish-water localities in Britain. After 48 hr. in 0.25 mM/l. NaCl the animals were divided into six groups and each group was placed in about 30 ml. de-ionized water at 10° C. to estimate the sodium-balance concentration. After 24 hr. the mean external concentration was 0.16 mM/l. sodium. The medium was then replaced with a fresh lot of de-ionized water, and 24 hr. later the mean concentration was 0.10 mM/l. sodium. The medium was again changed, and 24 hr. later the mean external sodium concentration was only 0.07 mM/l., standard deviation 0.007. During this experiment only three animals died, and the remainder survived for another 4 days in 0.07 mM/l. NaCl whilst measurements of influx and loss rates were made.

When animals acclimatized to 0.25 mM/l. NaCl at 10° C. were moved to a temperature of 20–22° C. some of them died, and at 25° C. practically all of them showed signs of distress and began to die rather quickly. Animals kept in 0.25 mM/l. NaCl and previously acclimatized to a temperature of about 20° C. for 6 days also behaved in the same way when the temperature was gradually increased up to 25° C. *G. duebeni* has been recorded from hot springs in Iceland at temperatures up to 25° C. (Tuxen, 1944), at 32° C. in coastal pools in Finland, and at 40° C. in hot springs in Greenland (Segerstråle, 1946).

### Sodium influx and loss rates at low external concentrations

The relation between the influx and the external concentration in animals acclimatized to 0.25 mM/l. NaCl at 10° C. is shown in Fig. 6, where the sodium transporting system is half-saturated at an external concentration of 0.5 mM/l. NaCl. Some measurements of influxes from 0.07 mM/l. NaCl in animals acclimatized to a range of external concentrations are given in Table 7, together with loss rates into de-ionized water in the same animals. The average weight of animals from Lough Melvin was 34 mg. When acclimatized to 0.07 mM/l. NaCl the loss rate was equivalent to the influx, and hence internal sodium balance was maintained. Over the range of external concentrations from 2 to 0.25 mM/l. NaCl the loss rate remained constant, although the influx may have been increased in animals acclimatized to 0.25 mM/l. NaCl. When acclimatized to 0.07 mM/l. the loss rate was drastically reduced to about 28% of the loss in 0.25 mM/l. NaCl. Once again this behaviour closely resembles that of *G. pulex* and *G. lacustris*, and it is quite different from that of *G. duebeni* taken from both brackish-water and freshwater localities in Britain.

A few measurements of influx and loss rates were made at a temperature of
21 ± 1°C. In one experiment the influx was measured in animals acclimatized to 0.25 mM/l. NaCl at 10°C. In a second experiment the influx was measured in animals previously acclimatized to a temperature of about 20°C for 6 days. The results are given in Table 8. In both experiments the influxes from 0.5 and 1 mM/l. NaCl were much faster than the comparable rates at a temperature of 10°C. (Fig. 6); in fact the influxes were almost precisely doubled by the 10°C. increase in tempera-

Table 7. Sodium influx and loss rates at 10°C in Gammarus duebeni from Lough Melvin acclimatized to a range of NaCl solutions at 10°C.

<table>
<thead>
<tr>
<th>Acclimatization concentration (mM/l. NaCl)</th>
<th>Influx from 0.07 mM/l. NaCl (μM/animal/hr.)</th>
<th>Loss rate into de-ionized water (μM/animal/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.027 (6) ± 0.006</td>
<td>0.13 (5) ± 0.01</td>
</tr>
<tr>
<td>0.5</td>
<td>0.035 (from Fig. 6)</td>
<td>0.14 (6) ± 0.035</td>
</tr>
<tr>
<td>0.25</td>
<td>0.034 (9) ± 0.003</td>
<td>0.11 (from Fig. 6)</td>
</tr>
<tr>
<td>0.07</td>
<td></td>
<td>0.039 (5) ± 0.005</td>
</tr>
</tbody>
</table>

*Fig. 6. Sodium influx in Gammarus duebeni from Lough Melvin acclimatized to 0.25 mM/l. NaCl at 10°C. Each point represents the mean influx in five to nine groups, except at 0.1 mM/l. NaCl (mean of two groups). Vertical lines indicate extent of standard deviations.*

Table 8. Sodium influx at a temperature of 21°C in Gammarus duebeni from Lough Melvin acclimatized to 0.25 mM/l. NaCl

<table>
<thead>
<tr>
<th>Experimental concentration (mM/l. NaCl)</th>
<th>Sodium influx (μM/animal/hr.) in groups of animals acclimatized at 10°C.</th>
<th>Sodium influx (μM/animal/hr.) in groups of animals acclimatized at 20°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>o.27</td>
<td>o.29</td>
</tr>
<tr>
<td></td>
<td>o.27</td>
<td>o.36</td>
</tr>
<tr>
<td></td>
<td>o.26</td>
<td>o.36</td>
</tr>
<tr>
<td>Mean</td>
<td>o.27</td>
<td>Mean</td>
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<tr>
<td></td>
<td>o.48</td>
<td>o.39</td>
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<td></td>
<td>o.48</td>
<td>o.48</td>
</tr>
<tr>
<td></td>
<td>o.48</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>o.41</td>
</tr>
</tbody>
</table>
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ture. In five groups of animals kept in 0.25 mM/l. NaCl for 6 days at about 20° C. the loss rate into de-ionized water at 21° C. was 0.19 μM/animal/hr., standard deviation 0.03, compared with about 0.11 μM/hr. at 10° C. (from Fig. 6). Hence the loss rate was also doubled by the increase in temperature. A 10° C. rise in temperature also immediately doubled both influx and loss rates in G. duebeni from brackish-water localities in Britain (Sutcliffe, 1967c).

Sodium loss at high external concentrations

In G. duebeni from brackish-water in Britain, acclimatized to a range of external concentrations from 55 to 270 mM/l. NaCl, the sodium loss rate into de-ionized water remained approximately constant. Sodium loss into slightly hyper-osmotic sucrose solutions also remained constant, but at a lower rate, and the difference was attributed to sodium loss in urine with a concentration of about 160-190 mM/l. sodium. In animals acclimatized to concentrations greater than 270 mM/l. NaCl the loss rate into de-ionized water increased sharply, due to the production of urine isotonic with the rising blood concentration (Sutcliffe, 1967c). This section examines sodium loss in the urine of animals from a freshwater locality.

A batch of about 150 animals was successively acclimatized to a range of concentrations increasing from 0.5 to 530 mM/l. NaCl (undiluted sea water) at 10° C. The loss rates into de-ionized water at 10° C. are shown in Fig. 7, together with loss rates into slightly hyper-osmotic sugar solutions. These experiments were carried out before those described in the previous paper, and a solution of dextrose was used to determine loss rates in animals acclimatized to 2 and 220 mM/l. NaCl. From Fig. 7 it can be seen that the loss into dextrose was slightly faster than the loss from the same animals into de-ionized water. The reason for this is still uncertain, but the same effect was observed (at the time of these experiments) with G. pulex, where it appeared to be due to the low pH of sugar solutions following extraction of sodium on Amberlite resin in the hydrogen form (Sutcliffe, 1967b). The dextrose solutions

![Fig. 7. Sodium loss into de-ionized water and sugar solutions at 10° C. in Gammarus duebeni from Lough Melvin acclimatized to a series of increasing external concentrations. Loss rates from six or twelve groups of animals into de-ionized water (•), dextrose (Δ) and sucrose (O). Vertical lines indicate extent of standard deviations where these are greater than ±0.01.](image-url)
used here were also passed over Amberlite resin, and the one used to determine the loss rate in animals acclimatized to 220 mM/l NaCl had a pH value below 4.0. It was thought that the results might be influenced by (1) the low pH, (2) the relatively small molecule of dextrose or (3) a combination of both, so all other loss-rate determinations were made into sucrose solutions passed over a double exchange resin bed providing an effluent with a pH of about 7.0 (Sutcliffe, 1967b). However, if pH or dextrose do have any real effect on sodium loss then it must be a very subtle one. In six groups of animals acclimatized to 270 mM/l NaCl the mean loss rate into dextrose with a pH of 5.8 was 0.13 \( \mu M/\text{animal/hr.} \), standard deviation 0.04. This was not significantly different from the mean loss of 0.12 ± 0.03 \( \mu M/\text{hr.} \) into sucrose with a pH of 6.9 (shown in Fig. 7). Also, the mean loss rate in the same animals in very dilute hydrochloric acid (pH = 4.1) was 0.22 ± 0.04 \( \mu M/\text{hr.} \), and this was not significantly different from the mean loss rate of 0.18 ± 0.05 \( \mu M/\text{hr.} \) in de-ionized water with a pH of about 5.9 (Fig. 7).

Returning to Fig. 7, the loss rate into de-ionized water remained constant in animals acclimatized to the range 0.5–220 mM/l. NaCl and then increased rapidly in animals acclimatized to higher external concentrations. On the other hand, the loss rates into sucrose remained constant until the animals were acclimatized to undiluted sea water. These closely resemble the previous results obtained with *G. duebeni* from Britain. The rapidly increasing loss rate into de-ionized water at the higher external concentrations may also be attributed to sodium loss in urine isotonic with the blood if it is assumed that the blood concentration increased as in the animals from brackish water (Sutcliffe, 1967c). To account for the observed sodium loss the urine flow rate would need to be roughly equivalent to 35% body weight/day at 10° C. when the osmotic gradient between the blood and medium is equivalent to 300 mM/l. NaCl. In this particular experiment the loss rate into sucrose from animals acclimatized to 530 mM/l. NaCl was double the loss rate from animals kept in lower external concentrations, and this increase in the rate of outward diffusion might also be associated with an increase in the blood sodium concentration.

**Estimation of the sodium concentration in the urine**

Unfortunately, the acclimatization of animals to the lower range of external concentrations and the determination of the loss rates into sucrose solutions have yet to be carried out. This would provide a firm estimate of the sodium concentration in the urine of animals acclimatized to concentrations below 270 mM/l. NaCl. However, the following considerations allow a fairly reliable estimate of the sodium concentration in the urine to be made.

From the results of loss rate determinations in de-ionized water, presented in Table 7 and Fig. 7, it is clear that the sodium loss rate remained constant in animals acclimatized to the range 0.5–220 mM/l. NaCl. This is quite different from the situation found in *G. duebeni* from brackish water in Britain, where the sodium loss into de-ionized water gradually increased in animals acclimatized to the range 0.5–55 mM/l. NaCl. This was apparently due to an increase in the urine sodium concentration from about 40 to 190 mM/l. The loss rate then remained constant until the external concentration was raised to more than 270 mM/l. NaCl (Sutcliffe, 1967c). Now since *G. duebeni* from brackish-water localities can produce a dilute
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urine at very low external concentrations (see also Lockwood, 1961), there is no reason why *G. duebeni* from fresh water should not also produce a dilute urine with a sodium concentration of, say, 40 mM/l. in animals acclimatized to 0.5 mM/l. NaCl. In this case the urine sodium concentration must have remained at this low level in animals acclimatized to external concentrations as high as 220 mM/l. NaCl. If it is assumed that the urine flow in de-ionized water at a temperature of 10° C. was equivalent to about 28% body weight/day (Lockwood, 1961; Sutcliffe, 1967c), then sodium loss in urine with a sodium concentration of 40 mM/l. would account for approximately 0.02 μM/animal/hr. Since the observed sodium loss rate in de-ionized water was 0.11–0.14 μM/hr., from 0.09 to 0.12 μM/hr. of this loss would then represent sodium loss across the body surface, and this estimated rate agrees well with the observed loss rate rate into sucrose from animals acclimatized to 270 mM/l. NaCl (Fig. 7). If the above considerations are extended to the results obtained with animals acclimatized to 0.07 mM/l. NaCl where the sodium loss into de-ionized water was reduced to only about 0.04 μM/hr. (Table 7), then it appears that, as in *G. pulex* (Sutcliffe, 1967b), the urine sodium concentration was probably reduced to well below 40 mM/l.

**DISCUSSION**

Certain features of the sodium regulatory mechanism are practically identical in *G. duebeni* from the freshwater localities in Ireland. The most characteristic feature is that the sodium transporting system is half-saturated at an external concentration of about 0.5 mM/l. sodium, and fully saturated at 1–2 mM/l. sodium. These properties clearly distinguish the animals living in fresh water in Ireland from the animals living in brackish-water localities in Britain and from the animals living in fresh water on the Kintyre Peninsula, Argyll, and on the Isle of Man (Fig. 8). In all of these cases the sodium transporting system is only about half saturated at external concentrations where the system is fully saturated in *G. duebeni* from Ireland.

A second feature which may also be characteristic of animals from Ireland is the manner in which sodium movements into and out of the animal are regulated over the range of external sodium concentrations usually encountered in fresh waters.
Thus the sodium-transporting system is activated to increase the influx only when the external concentration is reduced to between 0.5 and 0.25 mM/L sodium and, at the same time, the biggest reductions in sodium loss rates occur at external concentrations below about 0.25 mM/L NaCl. These changes in the rates of sodium influx and loss are probably associated with only a slight fall in the blood sodium level as, from the few measurements made so far, it appears that the blood sodium concentration is maintained at a fairly constant level until the external concentration falls below 0.25 mM/L. Again this clearly distinguishes *G. duebeni* in Irish fresh waters from *G. duebeni* in both brackish and fresh waters in Britain. In these latter, large changes in the influx and loss rates occur when the external concentration is reduced below about 10 mM/L, and these adjustments are associated with a steady fall in blood sodium concentration (Shaw & Sutcliffe, 1961; Sutcliffe, 1967c).

A third distinctive feature is the production of urine with a low sodium concentration, roughly 40 mM/L, when *G. duebeni* from Ireland is kept at concentrations below 40% sea water and, in particular, the possible production of an even more dilute urine at the lowest external concentrations which can be tolerated. This is uncommonly like the situation found in *G. pulex* (Sutcliffe, 1967b) and it would be interesting to see if the animals living in very dilute fresh water on the Kintyre Peninsula can also produce a very dilute urine.

It is clear that *G. duebeni* from fresh water in Ireland is physiologically distinct from *G. duebeni* living in brackish-water localities in Britain and, rather surprisingly, it is also distinct from *G. duebeni* living in freshwater localities outside Ireland. The fact that animals from fresh water on the Isle of Man and the Kintyre Peninsula can achieve sodium balance at external concentrations as low as those achieved by animals from Ireland, i.e. at 0.07–0.1 mM/L sodium, might lead one to suppose that animals from the former localities are as well adapted for living in fresh water as the animals from Ireland. Even the animals from brackish-water localities can achieve sodium balance at an external concentration of 0.17–0.21 mM/L sodium (Sutcliffe, 1967c) and again it might be inferred that the sodium regulatory mechanism in these brackish-water animals is adequate for widespread colonization of fresh water. But these minimum balance concentrations can only be maintained for short periods, and they represent the extreme limits of the sodium regulatory system under experimental conditions. Even in the case of *G. duebeni* from Ireland the animals begin to die off quickly after a few days exposure to concentrations lower than 0.12 mM/L NaCl, and it was found that, to keep them alive and healthy for a period of several weeks in the laboratory the minimum external concentration required was about 0.25 mM/L NaCl. Moreover, at this concentration survival was much better at a temperature of 10° C. than it was at 20° C., and it may be significant that the lowest sodium concentration found in a stream containing *G. duebeni* in Ireland was 0.27 mM/L (Sutcliffe, 1967a). In point of fact, consideration of the factors involved in maintaining sodium balance shows that the relatively small differences in the regulatory mechanisms found in *G. duebeni* from different localities within the British Isles are extremely important with regard to the successful colonization of fresh waters containing differing concentrations of sodium ions.

Now in the case of the freshwater species *G. pulex* and *G. lacustris* the sodium regulatory mechanism appears to be geared so that external concentrations greater
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than 0.2–0.3 mM/l. present optimum conditions, and the blood sodium concentration is maintained at a constant level by balancing the amount of sodium lost with uptake of an equivalent amount when the sodium transporting system is (a) operating at a low rate and (b) is more than half-saturated. Any reduction in body sodium content, such as may occur following injury or during a moult, can be quickly replaced by increasing the influx (Sutcliffe, 1967a). A rather similar system operates in the freshwater crayfish, Astacus pallipes (Shaw, 1959a, 1964; Bryan, 1960) and in the freshwater crab Potamon niloticus (Shaw, 1959b). On the other hand the same criteria indicate that for G. duebeni from brackish-water localities external concentrations greater than 2–10 mM/l. present optimum operating conditions for the sodium regulatory system. Certainly at concentrations below 1–2 mM/l. the blood concentration begins to fall rapidly, the sodium transporting system is less than half saturated, and the minimum rate of sodium uptake is insufficient to balance sodium loss (Shaw & Sutcliffe, 1961; Sutcliffe, 1967b). The precise nature of this relationship between the external concentration and the movements of sodium into and out of the animal is illustrated in Fig. 9. This shows the minimum A and maximum B sodium influxes in G. duebeni from brackish water in Britain, and the minimum C and maximum D influxes in G. duebeni from fresh water in Ireland at external concentrations below 1 mM/l. These animals were roughly equal in size and weight, so the influxes are closely comparable. Observations indicate that in animals from both areas the minimum rate of sodium loss, L1, is about 0.15 μM/hr. In Fig. 9 L2 and L3 indicate sodium loss rates in animals acclimatized to various external concentrations (see legend to Fig. 9). From Fig. 9 it is clear that when G. duebeni from brackish water is placed in a medium containing 1 mM/l. sodium, L1 exceeds

Fig. 9. The relationship between sodium influx and loss in Gammarus duebeni at external concentrations below 1 mM/l. A, Minimum and B, maximum influx in animals from brackish water in Britain; C minimum, D maximum influx in animals from the River Boyne in Ireland. L1, Minimum loss rates; L2, loss rates in animals acclimatized to 0.25 mM/l. NaCl (River Boyne animals) and 0.5 mM/l. NaCl (brackish water animals); L3, loss rates in animals acclimatized to 1 mM/l. NaCl.

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the minimum influx \( A \) and, in order to maintain balance, the influx must be increased. At an external concentration of 0.5 mM/l. sodium, the influx must be increased almost to its maximum \( B \) in order to balance the loss rate \( L_2 \). This means that there is little or no capacity for a further increase in the influx if this is required to make good any extra sodium loss. To achieve sodium balance at lower external concentrations requires the maximum influx \( B \) and a further reduction in loss rate to \( L_1 \), when temporary balance is achieved at about 0.2 mM/l. sodium.

The maximum rate, \( B \), in the brackish-water animals is slightly less than the minimum rate, \( C \), found in animals from the River Boyne, and \( C \) is two to three times greater than the minimum rate, \( A \), in animals from brackish water. The considerable advantage gained by this increase in the minimum rate in the Irish animals is seen in Fig. 9, where \( C \) is sufficient to balance all loss rates \( (L_2, L_3) \) at external concentrations greater than 0.25 mM/l. sodium, with a large reserve in hand \( D \). Thus, in G. duebeni from fresh water in Ireland temporary balance can be maintained for a period of several days at an external sodium concentration of 0.07 mM/l. when \( D = L_1 \). G. duebeni from the Isle of Man and the Kintyre peninsula can also achieve temporary balance at 0.07-0.1 mM/l. sodium, but balance is seldom maintained for more than about 24 hr. at these very low concentrations. In these animals from fresh water in Britain the maximum influx is greater than the maximum influx \( B \) in the brackish-water animals (see Figs. 8, 9) and, in fact, the maximum influx appears to be slightly greater than the minimum influx \( C \) in G. duebeni from fresh water in Ireland. But in considering these differences in the sodium influxes it must also be noted that in G. duebeni from Britain the sodium uptake systems are less than half saturated at all external concentrations below 1 mM/l. sodium (Fig. 8).

Concentrations between 0.2 and 0.6 mM/l. sodium represent the range found in most of the inland fresh waters in the British Isles, and over this range the maximum influx in brackish-water animals from Britain represents only some 10-20% of the saturation rate. Comparable influxes in the freshwater animals from coastal areas in Britain represent about 15-35% of the saturation rate, whereas in the freshwater animals from inland areas in Ireland the influxes range from 30 to 80% of the saturation rate. In this respect sodium influx in G. duebeni from Ireland is much closer to that found in other animals regarded as fully adapted to fresh water. For example, in G. pulex and G. lacustris the sodium uptake systems are half saturated at an external concentration of 0.11 mM/l., 70% saturated at 0.3 mM/l. and 90% saturated at 0.5 mM/l. sodium (Sutcliffe, 1967a; Sutcliffe & Shaw, 1967) and in the crayfish Astacus pallipes the system is half-saturated at about 0.25 mM/l. (Shaw, 1959a, 1960). Thus in most fresh waters these uptake systems are operating close to the saturation point, which is probably the most efficient position.

It was suggested that the higher influxes in populations of G. duebeni living in fresh water in Britain are simply the result of natural selection of individuals in which the sodium uptake rate is higher than the average rate of uptake found in populations of G. duebeni living in brackish water (Sutcliffe, 1967c). But this simple argument is not sufficient to account for the characteristic features of the sodium regulatory mechanism found in G. duebeni living in fresh water in Ireland, unless it is postulated that at least some individuals with these particular features are also present in the populations living in brackish water round the coast of Ireland, but not round the
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coasts of Britain. Otherwise natural selection acting on the animals living on the Kintyre peninsula, where the sodium concentration is nearly as low as in many Irish fresh waters, might be expected to select a population of individuals with the same features characteristic of the Irish animals. These problems of population physiology are currently under investigation.

It is suggested that G. duebeni living in fresh water in Ireland constitutes a physiological and geographical race distinct from G. duebeni in Britain, and perhaps also distinct from G. duebeni in fresh waters in other coastal areas of north-west Europe. With the retention of a high blood concentration when in fresh water, and its ability to survive in full-strength sea water, the Irish race of G. duebeni resembles the freshwater race of the isopod Saduria (= Mesidotea) entomon in Swedish lakes (Lockwood & Croghan, 1957). In view of the low chloride concentrations in these lakes, 0.2 mM/l. in Lake Vättern and 0.5 mM/l. in Lake Mälaren, the sodium concentrations in these waters are probably also low, and it would therefore be of great interest to make a comparative study of sodium uptake in S. entomon and G. duebeni. The isopod almost certainly entered fresh water in post-glacial times (Segerstråle, 1957) and G. duebeni probably also invaded fresh water very recently (Sutcliffe, 1967a). Perhaps both species are at present adapting to the fresh-water environment. In the case of the Irish race of G. duebeni the sodium uptake system might be improved to operate even closer to its saturation rate at the very low sodium concentrations found in many of the fresh waters in Ireland. In this event, the differences between the sodium uptake systems in the Irish and British races of G. duebeni would then resemble the differences found between the sodium uptake systems in the African freshwater crab Potamon niloticus (Shaw, 1959b) and the European brackish and freshwater crab Eriocheir sinensis (Shaw, 1961).

Finally, it seems likely that G. duebeni from Ireland can, like G. pulex, produce a very dilute urine, and this would improve the efficiency of the regulatory mechanism. However, the production of urine with a sodium concentration of 40 mM/l. represents only about 16% of the total sodium loss when in fresh water, so the elaboration of an even more dilute urine would not provide anything like the advantage gained by increasing the sodium influx. But it could be important in G. duebeni living in streams on the Kintyre peninsula, where the differential between the maximum sodium influx and the minimum sodium loss rate is very small at the sodium concentrations encountered in these streams.

SUMMARY

1. A quantitative study of sodium influx and loss was made on populations of Gammarus duebeni obtained from four freshwater localities in Ireland.

2. Characteristic features of sodium regulation in animals from the four localities were as follows. (a) The sodium influx increases gradually with increasing external sodium concentrations, but a maximum (saturation) level is abruptly reached at an external concentration of 1-2 mM/l. and the transporting system is half saturated at about 0.5 mM/l. sodium. (b) Over the range of sodium concentrations found in fresh waters a low rate of sodium uptake is sufficient to balance sodium losses at concentrations down to between 0.5 and 0.25 mM/l. At lower concentrations the influx is increased and the loss rate is reduced. (c) Calculations suggest that hypotonic
urine containing approximately 40 mM/l. sodium is produced at external concentrations ranging from fresh water to 40% sea water. At external concentrations below 0.25 mM/l. sodium the urine concentration is probably reduced to well below 40 mM/l. sodium.

3. A detailed comparison is made of sodium regulation at external concentrations ranging between 0.07 and 1 mM/l. sodium in *G. duebeni* from fresh water in Ireland and from fresh water and brackish water in Britain. It is suggested that *G. duebeni* in Ireland constitutes a distinct physiological race adapted for living in fresh waters with relatively low sodium concentrations.

We wish to thank Dr W. E. Frost for advice and assistance given when first obtaining *G. duebeni* from Ireland. D. W. Sutcliffe gratefully acknowledges support for this investigation from D.S.I.R. through the award of a Research Fellowship (1960-61), followed by a grant for equipment and field-work.

REFERENCES


