*J. Exp. Biol.* (1974), 60, 769–781
*With 2 text-figures*
*Printed in Great Britain*



# NET FLUXES OF WATER IN THE ISOLATED GILLS OF *ANGUILLA DIEFFENBACHII*

BY T. J. SHUTTLEWORTH* AND R. F. H. FREEMAN
*Department of Zoology, University of Otago, Dunedin, New Zealand*

(*Received* 3 *October* 1973)

## INTRODUCTION

Water balance in teleosts, as proposed in the classical theories of osmoregulation originating from the work of Smith (1930), may be summarized as follows. In the marine environment net osmotic water loss from the permeable surfaces of the body, principally the gills, together with the relatively small urinary water loss, is compensated by ingestion of the external medium and the absorption of water from the gut. In the freshwater environment the net osmotic influx of water, again principally at the gills, is balanced by the production of a relatively copious urine. These relationships may be represented in the following manner:

| | Net influx = net outflux |
|---|---|
| In the marine teleost . . . | Drinking = gill + kidney |
| In the freshwater teleost . . . | Gill = kidney |

In virtually all cases published so far the role of the gill in the water balance of teleosts has been deduced indirectly, by first analysing the role of the gut and/or kidney and then applying the suitable 'equation' as given above. Such indirect methods of assessing net water fluxes across the gills can be criticized for many reasons. For example, the 'equations' given above make no allowance for water movements at other permeable surfaces such as the oral membranes. Assessment of urinary water excretion by cannulation of the urinary bladder is complicated by the fact that this structure has been shown to be involved in water regulation (Lahlou, 1967; Hirano, Johnson & Bern, 1971). Also the determination of any parameter from the sum or difference of two independent measurements will obviously produce an increased chance of error in the calculated parameter. This is particularly true when the two measured parameters are as variable and susceptible to numerous shock effects as urine flow and drinking rate (Maetz, 1970).

However, perhaps the most serious criticism of the above approach is that the 'equations' given are not complete. Thus, the relationship for the total water balance of the teleost in sea water takes no account of rectal water losses. Where such losses have been measured it has been shown that they may account for some 20–40 % of the measured drinking rate (Hickman, 1968; Oide & Utida, 1968). Perhaps even more important is that the relationship given for the situation in fresh water takes no account of the drinking rate. Ingestion of the medium when in fresh water is now a well estab-

* Present address: Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster LA1 4YQ, U.K.

lished, though generally poorly accepted, fact for several species of teleost and may make a considerable contribution to the uptake of water in dilute media.

In this paper are presented the results of direct measurements of net water fluxes in the isolated gills of the New Zealand long-finned eel, *Anguilla dieffenbachii*. The results are discussed with reference to the water balance of eels in fresh water and when adapted to sea water.

## METHOD

Eels obtained from a local exporter were kept in a large covered concrete tank supplied with a continuous flow of de-chlorinated Dunedin tap-water. The eels weighed from 0·5 to 3·5 kg with most weighing from 1·5 to 2·8 kg, and only immature yellow eels were used. Adaptation to sea water was accomplished by transferring eels to a similar tank containing aerated sea water obtained from Otago Harbour. A continuous flow of sea water could not be obtained so the water was renewed at frequent intervals. All experiments were carried out on eels obtained in the summer months (October–May).

The technique used for measuring the net fluxes of water in the gill was modified from that of Bellamy (1961) and involves the use of an incubated, isolated gill preparation.

The eels were killed by cutting through the body with a sharp knife at the level of the heart, immediately behind the pectoral fins. The head portion of the eel was pithed to reduce movements, and the operculum was removed. Individual gills were then dissected out as follows. A ligature of silk suture thread was tied around each end of the gill arch and the gill was removed by cutting through the arch outside the ligatures. The gill was then placed on a pad of cotton wool soaked in a Ringer solution (see Table 1). Ringer A was used for all gills removed from freshwater-adapted specimens and Ringer B was used for gills removed from eels adapted to sea water.

Only the first three pairs of gills were used. At least two gills from each eel were used as controls. These control gills were rapidly rinsed in distilled water, gently blotted dry with filter paper and a sample of gill filament was trimmed off the arch. This was analysed for water content by weighing the wet tissue, drying the sample for at least 18 h in an oven at 110 °C, cooling in a desiccator and re-weighing.

The remaining gills (excluding those from the fourth pair of gill arches) were rapidly rinsed in the appropriate external medium and then placed individually in tubes containing 75–100 ml of the desired experimental medium. The various external media used were either dilutions of the normal eel Ringer solution or an artificial 'sea water' solution (see Table 1), thus enabling net water flux to be determined in a series of external solutions with osmotic pressures ranging from those typical of most fresh waters ($< 5$ mOsm) to sea water ($\simeq 1000$ mOsm).

The tubes containing the gills were placed in a water bath maintained at 17 °C, and the solutions were bubbled vigorously with air which provided a source of oxygen and ensured a rapid agitation of the medium over the surface of the gill, preventing the formation of any local osmotic gradients. The gills were left in these tubes for up to 1 h, depending on the osmotic concentration of the external medium (see later), after which they were removed, rapidly rinsed in distilled water and gently blotted dry with filter paper. A sample of gill filaments was then trimmed off the arch and analysed for water content as before.

Table 1. *Compositions of the various solutions used (in g.$l^{-1}$)*

| | NaCl | KCl | $CaCl_2 6H_2O$ | $NaHCO_3$ | $NaH_2PO_4$ |
|---|---|---|---|---|---|
| Ringer A | 7·0 | 0·2 | 0·33 | 1·26 | 0·38 |
| Ringer B | 8·0 | 0·2 | 0·33 | 1·26 | 0·38 |
| 'Artificial sea water' | 28·0 | 0·8 | 1·32 | (pH with Tris = 7·5) | |

The net flux of water, as mg.g dry $wt^{-1}.h^{-1}$, was calculated by taking the difference between the water content of the incubated gill and the mean of the control gills (in mg.g dry $wt^{-1}$) and dividing it by the time (h) of incubation.

The osmotic pressures of the various external media used were measured when the solutions were made up and after each dilution. Measurements were made using a Fiske G 66 Osmometer (Fiske Instrument Co.) reading directly in milliosmoles per kilogram of water (herein designated mOsm). In the case of the more dilute solutions ($< 5$ mOsm) the osmotic pressure could not be determined accurately with the Fiske Osmometer. Therefore the osmotic pressures of these solutions were calculated from the osmotic pressure of the stock solution, divided by the dilution factor.

In this preparation the gill is essentially a tied-off bag (the branchial epithelium) containing a volume of fluid (the blood and extracellular fluid). As such, excessive changes in volume of the internal fluid will be restrained by the physical nature of the epithelial tissue. Also, as water is added or removed from the internal fluid the concentration of this fluid will fall or rise, thus reducing the osmotic gradient across the gill and hence presumably the net flux of water. Therefore initial experiments were carried out to determine a suitable incubation period for the gills when in dilute or concentrated media.

It was found that the net flux of water into the gill in dilute media is linear with time for at least 2 h. However, in concentrated external media the net outflux of water in some gills was linear only over the initial period of incubation and after this the rate of net outflux of water was gradually reduced. The length of this initial linear period appeared to depend on the rate of water loss from the gill. It was decided therefore that the experiments should be terminated before the amount of water lost from the gill exceeded 20 % of the initial water content (i.e. approximately 800 mg.g dry $wt^{-1}$). Preliminary experiments indicated that gills could lose at least this amount of water without the rate of water loss being appreciably affected. In general, therefore, gills were incubated in media equivalent to sea water for 14–45 min and in dilute media for 60 min.

## RESULTS

The results of the measurements of net water fluxes in 39 isolated gills from 16 eels adapted to fresh water and 5 gills from 5 eels adapted to sea water for at least 100 h are shown in Figs. 1 and 2. In Fig. 1 the individual measurements of water flux are plotted against the osmotic concentration of the external incubation medium on a logarithmic scale. The logarithmic scale for the external medium was used merely because of the relatively large number of readings over the lower range of values. From the results obtained a mean value ($\pm$ standard deviation) of net water flux was calculated for each of the eight different external concentrations at which measurements were made. These means and standard deviations are plotted in Fig. 2. It can be seen that a

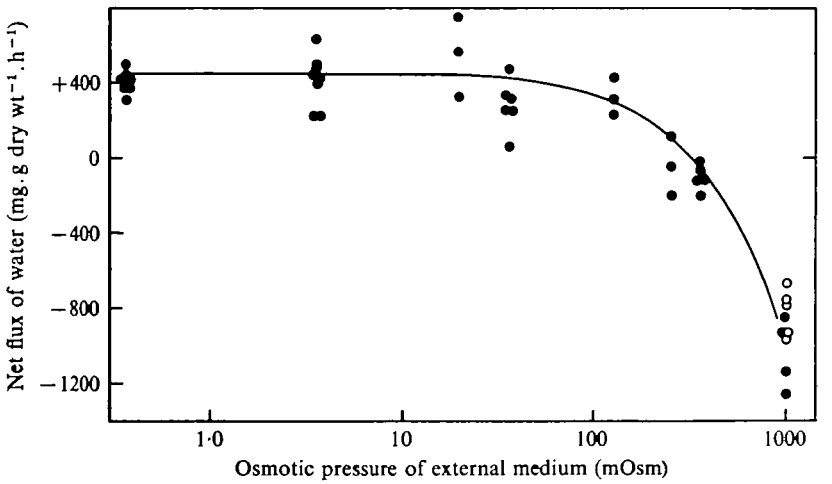


Fig. 1. Net flux of water in different gills plotted against the osmotic pressure of the external incubation medium (external O.P. on a logarithmic scale). ●, Gills taken from freshwater-adapted eels; ○, gills taken from seawater-adapted eels.

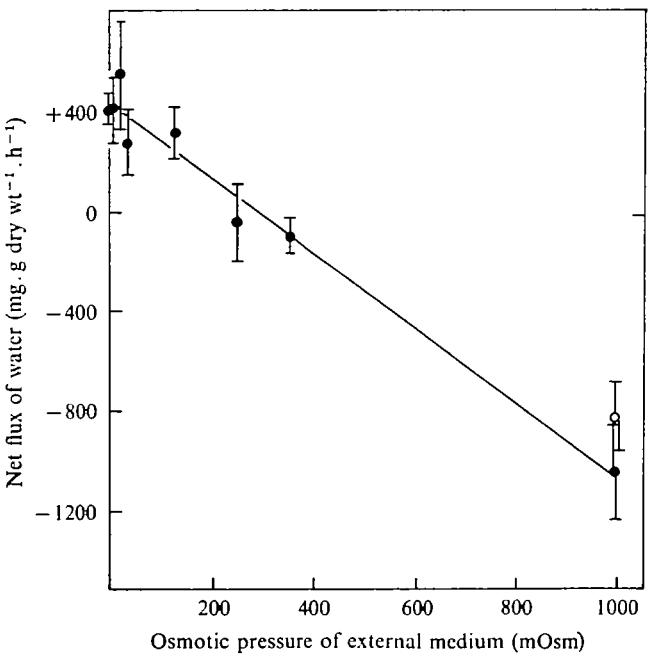


Fig. 2. Mean net flux of water in isolated gills plotted against the osmotic pressure of the external medium. Symbols as in Fig. 1.

straight line can be reasonably drawn through these mean values. This line is, in fact, the same as that drawn in Fig. 1, where its relation to the individual measured fluxes can be seen.

Several features are apparent from these graphs. First, the fact that a straight line can be reasonably drawn through the points indicates that the permeability of the gill to

water is the same for water movement in both directions (i.e. net outflux or net influx of water). Although this is by no means unusual for epithelia, there have been situations in which a differential permeability to water has been demonstrated, for example in the body wall of the sipunculid *Dendrostomum zostericolum* (Gross, 1954).

Secondly, it can be seen from Fig. 2 that the straight line drawn through the mean points passes through the point of zero net flux of water at an external osmotic pressure of about 300 mOsm. This is in good agreement with the value obtained for the osmotic pressure of the serum of freshwater *Anguilla dieffenbachii* (307·7 mOsm) (Shuttleworth & Freeman, 1973). This indicates that the gill is behaving in a purely passive fashion towards water, the net flux of which is determined by the differences between the external and internal (blood) osmotic pressures and when no such differences exist no net movement of water occurs.

The osmotic pressure of most fresh waters is less than 5 mOsm, so by taking the mean water flux of the 15 values obtained in external media of less than 5 mOsm an estimate of the net water influx into the gills in fresh water can be obtained. The value thus obtained is $411 \pm 105$ mg.g dry $wt^{-1}.h^{-1}$. Bellamy (1961) measured the net water influx in gills taken from freshwater eels (*Anguilla anguilla*) and incubated in tap water, and obtained a mean value of 317 mg.g dry $wt^{-1}.h^{-1}$.

A 1 kg specimen of *A. dieffenbachii* has 0·65 g dry weight of filaments (average of 34 observations, S.D. $\pm$ 0·10) (Shuttleworth, 1972), and by using this value results expressed as flux per g dry wt of gill per h can be converted to flux per kg of fish per h. Thus the net influx of water across the gills in fresh water (411 mg.g dry $wt^{-1}.h^{-1}$) amounts to 0·27 ml.$kg^{-1}.h^{-1}$ or 6·4 ml.$kg^{-1}.day^{-1}$.

Similarly the net outflux of water from the gills of a freshwater eel in an external medium of artificial sea water (1000 mOsm) has been measured for the four specimens shown in Fig. 1, and is $1037 \pm 188$ mg.g dry $wt^{-1}.h^{-1}$. Kamiya (1967) studying *A. japonica* obtained a value of 1680 mg.g dry $wt^{-1}.h^{-1}$ for the net outflux of water from the gills of freshwater animals incubated in sea water. Similarly Utida, Oide, Saishu & Kamiya (1967) recorded the following values for the net outflux of water from the gills of freshwater *A. japonica* when incubated in sea water: yellow eels, 1890 mg.g dry $wt^{-1}.h^{-1}$; silver eels, 1000 mg.g dry $wt^{-1}.h^{-1}$; and cultivated (farmed) eels, 1730 mg.g dry $wt^{-1}.h^{-1}$. It appears therefore that the results obtained on *A. japonica* are generally somewhat higher than those obtained with *A. dieffenbachii*. Exact comparison, however, is not possible in the absence of values, in the papers quoted above, for the osmotic pressures of the internal medium or blood of the fish and of the different external media used.

As above, the measured flux for *A. dieffenbachii* can be converted to ml.$kg^{-1}.h^{-1}$ using the factor 0·65 g dry wt.$kg^{-1}$. In this way, a value of 0·67 ml.$kg^{-1}.h^{-1}$ or 16·2 ml.$kg^{-1}.day^{-1}$ is obtained for the net outflux of water across the gills of freshwater animals incubated in artificial sea water.

Also shown in Figs. 1 and 2 are the results of five experiments in which gills were taken from eels which had been adapted to sea water for at least 100 h. This has been shown to be sufficient for the completion of adaptation to sea water in *A. dieffenbachii* (Shuttleworth & Freeman, 1973). These gills were incubated in artificial sea water (1000 mOsm – see Table 1). The mean value for the net outflux of water in these circumstances is $818 \pm 127$ mg.g dry $wt^{-1}.h^{-1}$. This value is not significantly different

from the value of $1037 \pm 188$ mg.g dry wt$^{-1}$.h$^{-1}$ obtained with gills from freshwater animals incubated in artificial sea water (Students $t$ test, $t = 2{\cdot}092$, $P > 0{\cdot}05$).

The mean value recorded here (818 mg.g dry wt$^{-1}$.h$^{-1}$) can be compared with results obtained by other workers using different species of eels. Bellamy (1961) recorded a net outflux of water of 516 mg.g dry wt$^{-1}$.h$^{-1}$ from the gills of seawater-adapted *A. anguilla* incubated in sea water. Kamiya (1967), working on *A. japonica*, reports a net water outflux of 980 mg.g dry wt$^{-1}$.h$^{-1}$ from the gills of seawater-adapted eels incubated in sea water.

As with the results for freshwater-adapted animals, the net outflux of water from the gills of seawater-adapted *A. dieffenbachii* can be converted to ml.kg$^{-1}$.h$^{-1}$. The value obtained is 0·53 ml.kg$^{-1}$.h$^{-1}$ or 12·8 ml.kg$^{-1}$.day$^{-1}$.

From the data presented in Figs. 1 and 2 it is possible to calculate the osmotic permeability coefficient of the gill for both fresh-water-adapted and seawater-adapted eels. The formula used is derived from those of Dainty & House (1966) and of Motais *et al.* (1969) and is represented below:

$$P_{os} = \frac{F_w}{A \times \Delta C \times \sigma},$$

where $P_{os}$ is the osmotic permeability coefficient (cm.sec$^{-1}$), $F_w$ is the net flux of water (mmol.g dry wt$^{-1}$.sec$^{-1}$), $A$ is the area of the gill (cm$^2$.g dry wt$^{-1}$), $\Delta C$ is the osmotic concentration difference between the gill and the external medium (Osm l$^{-1}$ $\equiv$ mOsm cm$^{-3}$), and $\sigma$ is the Staverman or reflexion coefficient which is $\leqslant 1$.

The net flux of water in mg.g dry wt$^{-1}$.h$^{-1}$ can be converted to mmol.g dry wt$^{-1}$.sec$^{-1}$ by dividing by $60 \times 60 \times 18$ (18 being the partial molar volume of water). $A$ is calculated from the data of Gray (1954), who recorded a gill area for *A. rostrata* of 3020 cm$^2$.kg$^{-1}$. If a 1 kg fish has 0·65 g dry wt of gill tissue (see above) then the area in cm$^2$.g dry wt$^{-1}$ will be:

$$3020/0{\cdot}65 = 4646 \text{ cm}^2\text{.g dry wt}^{-1}.$$

$\Delta C$ is taken as the difference between the mean osmotic pressure of the serum (308 mOsm for freshwater-adapted animals and 377 mOsm for seawater-adapted animals – (Shuttleworth & Freeman, 1973) and the known osmotic pressure of the external incubation medium. The Staverman coefficient is effectively a measure of the semipermeability of the membrane or epithelium to the osmolyte in question. Therefore $P_{os}$ should ideally be measured using an impermeant solute, e.g. sucrose, where the Staverman coefficient $\simeq 1$. However, as pointed out by Motais *et al.* (1969), the osmotic flow of water across the skin of amphibians is similar whether measured *in vitro* with sucrose or *in vivo* with sodium chloride solutions, indicating that the Staverman coefficient is nearly equal to 1 for both solutions. Based on evidence indicating that the rates of exchange of sodium and chloride in freshwater teleosts are of the same order of magnitude as those of amphibians, Motais *et al.* (1969) conclude that the Staverman coefficient for the gills of teleosts in solutions which are essentially of sodium chloride, may reasonably be assumed to be nearly equal to 1.

Using the data in Figs. 1 and 2 and the assumption outlined above, the osmotic permeability coefficients for the gills of *A. dieffenbachii* have been calculated and are shown in Table 2.

The mean value for the $P_{os}$ in all freshwater specimens in all external media is

Table 2. *Osmotic permeability coefficients of the gills of* A. dieffenbachii

| O.P. of external medium (mOsm) | N | $P_{os}$ (cm.sec$^{-1}$) |
|---|---|---|
| | Freshwater eels | |
| <5·0 | 15 | $0{\cdot}46 \times 10^{-5}$ |
| 19 | 3 | $0{\cdot}65 \times 10^{-5}$ |
| 35·5 | 6 | $0{\cdot}35 \times 10^{-5}$ |
| 124 | 3 | $0{\cdot}61 \times 10^{-5}$ |
| 252 | 3 | $0{\cdot}28 \times 10^{-5}$ |
| 355 | 5 | $0{\cdot}63 \times 10^{-5}$ |
| 1000 | 4 | $0{\cdot}49 \times 10^{-5}$ |
| | Seawater-adapted eels | |
| 1000 | 5 | $0{\cdot}43 \times 10^{-5}$ |

$0{\cdot}50 \pm 0{\cdot}14 \times 10^{-5}$ cm.sec$^{-1}$, a value that is not significantly different from that recorded for the gills of seawater-adapted specimens incubated in artificial sea water ($0{\cdot}43 \pm 0{\cdot}07 \times 10^{-5}$ cm.sec$^{-1}$ – students $t$ test, $t = 0{\cdot}173$, $P > 0{\cdot}8$). Hence in *A. dieffenbachii* no significant change was found in the osmotic permeability coefficient of the gills on adaptation to sea water.

No values have been published for the osmotic permeability coefficient of the teleost gill determined directly from the net water flux. Those values that have been published have been determined indirectly from consideration of urine flow and drinking rate. There are, however, many disadvantages inherent in such indirect methods. For example, Motais *et al.* (1969) obtained a value for $P_{os}$ in freshwater *A. anguilla* of $7{\cdot}9 \times 10^{-5}$ cm.sec$^{-1}$ or almost 20 times the values obtained above. In their determination they estimated the net water flux across the gills by subtracting the drinking rate (as measured by Maetz & Skadhauge, 1968) from the urine flow. However, the measurement of the urine flow rate was made only 6 h after handling and cannulation of the animals and a value of 538 $\mu$l.h$^{-1}$.100 g$^{-1}$ or 129 ml.kg$^{-1}$.day$^{-1}$ was obtained. This is 3–4 times the normal urine flow rates measured on eels given sufficient time to recover from the effects of handling, etc. (Butler, 1966, 1969; Chester Jones, Chan & Rankin, 1969*a*, *b*). Motais *et al.* (1969) do in fact mention that after only 24 h the urine flow rate in their fish had dropped to 353 $\mu$l.h$^{-1}$.100 g$^{-1}$ or 84·7 ml.kg$^{-1}$.day$^{-1}$. A more normal value for the urine flow of *Anguilla* in fresh water is therefore $\frac{1}{3}$–$\frac{1}{4}$ that used by Motais *et al.* or 135–179 $\mu$l.100 g$^{-1}$.h$^{-1}$. Taking a mean value of 157 $\mu$l.100 g$^{-1}$.h$^{-1}$ and using all the other data as in Motais *et al.* (1969) a value for the $P_{os}$ equal to $0{\cdot}44 \times 10^{-5}$ cm.sec$^{-1}$ is obtained. This is very similar to the value obtained above for the $P_{os}$ of the gills of freshwater *A. dieffenbachii* calculated from the net water flux as measured directly ($0{\cdot}50 \times 10^{-5}$ cm.sec$^{-1}$).

The indirect determinations of $P_{os}$ for a variety of teleosts by Evans (1967*b*, 1969*b*) may also be in error because of the assumption made that the net water flux was equivalent to the measured drinking rate in seawater fish and to the measured urine flow rate in freshwater fish. As pointed out by Evans himself (Evans, 1969*b*) this assumption takes no account of urine production in seawater-adapted fish and drinking in freshwater-adapted fish.

Recently Motais & Isaia (1972) have published further estimations of the $P_{os}$ for *A. anguilla*. The value obtained by these authors for seawater-adapted specimens was

Table 3. *Urine flow rates and drinking rates of various species of* Anguilla *adapted to sea water*

| Species | Urine flow rate ($ml.kg^{-1}.h^{-1}$) | Drinking rate ($ml.kg^{-1}.h^{-1}$) | References |
|---|---|---|---|
| *A. anguilla* | 0·31 | 1·67 | Maetz (1970) |
| | — | 3·15 | Maetz & Skadhauge (1968) |
| | 0·25 | — | Chester Jones, Chan & Rankin (1969*a*) |
| *A. japonica* | 0·39 | 3·70 | Oide & Utida (1968) |
| *A. rostrata* | 0·41 | 2·77 | Smith (1930) |
| | 0·17 | — | Butler (1966) |

(at 15 °C) $0{\cdot}64 \times 10^{-5}$ cm.sec$^{-1}$, which agrees reasonably well with that obtained for *A. dieffenbachii*. The value obtained by Motais & Isaia (1972) for freshwater-adapted specimens was considerably higher ($3{\cdot}8 \times 10^{-5}$ cm.sec$^{-1}$ at 15 °C) but the technique used was the same as described by Motais *et al.* (1969) and consequently is open to the same criticisms.

## DISCUSSION

Considering first the water balance of *A. dieffenbachii* when adapted to sea water, the results obtained showed a net outflux at the gills of 818 mg.g dry wt$^{-1}$.h$^{-1}$ which is equivalent to 0·53 ml.kg$^{-1}$.h$^{-1}$. As discussed earlier, if the fish is to remain in water balance in sea water the net outflux of water at the gills together with that lost in the urine and rectal fluid must equal the drinking rate. In Table 3 values for the urine flow rate of various species of eels adapted to sea water are presented. It can be seen that the values obtained by the various authors on the different species are reasonably similar, and in the absence of any direct measurements it would seem reasonable to assume that urine flow in seawater-adapted *A. dieffenbachii* would not differ greatly from the mean of the values presented in the above table, i.e. 0·31 ml. kg$^{-1}$.h$^{-1}$. Assuming that rectal water loss amounts to 30 % of the drinking rate (Hickman, 1968) the expected drinking rate of *A. dieffenbachii* in sea water can be calculated as follows:

$$\text{Drinking rate} = (0{\cdot}53 + 0{\cdot}31) \times (100/70)\ \text{ml.kg}^{-1}.\text{h}^{-1}$$
$$= 1{\cdot}20\ \text{ml.kg}^{-1}.\text{h}^{-1}.$$

Obviously this value will only be very approximate because of all the assumptions made above. However, when compared with the drinking rate measured by various workers in other species of eels (see Table 3), it can be seen that, although a little low, the estimated value shows reasonable agreement with the measured values especially when species differences and natural variations in the values obtained are considered.

Drinking rates in other species of teleosts adapted to sea water vary from 0·34 ml.kg$^{-1}$.h$^{-1}$ in *Xiphister atropurpureus* (Evans, 1967*a*) to 23 ml.kg$^{-1}$.h$^{-1}$ in *Fundulus heteroclitus* (Potts & Evans, 1967).

With reference to the water balance of *A. dieffenbachii* in fresh water a net influx of water at the gills of 411 mg.g dry wt$^{-1}$.h$^{-1}$ or 0·27 ml.kg$^{-1}$.h$^{-1}$ was found. In Table 4 the urine flow rates of different species of eel in fresh water measured by various workers are presented.

Again, the values obtained appear to be reasonably similar for the various species and

Table 4. *Urine flow rates of various species of* Anguilla *in fresh water*

| Species | Urine flow rate ($ml.kg^{-1}.h^{-1}$) | References |
|---|---|---|
| *A. anguilla* | 1·76 | Chester Jones, Henderson & Butler (1965) |
| | 2·03 | Butler (1966) |
| | 1·10 | Chester Jones, Chan & Rankin (1969*a*) |
| | 1·44 | Chester Jones, Chan & Rankin (1969*b*) |
| *A. rostrata* | 1·45 | Butler (1969) |
| *A. japonica* | 1·95 | Oide and Utida (1968) |

thus, in the absence of any measurements of urine flow in freshwater *A. dieffenbachii*, it is felt reasonable to assume that urine flow would not differ greatly from the mean of the above values, i.e. 1·62 $ml.kg^{-1}.h^{-1}$. This value is obviously much larger than that obtained from the measurement of net water flux across the gills (0·27 $ml.kg^{-1}.h^{-1}$). However, as stated earlier, it has been established that at least several species of teleosts drink when in fresh water. A very approximate estimate of the expected drinking rate in fresh water of *A. dieffenbachii* can be obtained from the above values as follows:

$$\text{Drinking rate} = 1{\cdot}62 - 0{\cdot}27 \text{ ml.kg}^{-1}.\text{h}^{-1}$$
$$= 1{\cdot}35 \text{ ml.kg}^{-1}.\text{h}^{-1}.$$

This value is in fact identical to the drinking rate of fresh water *A. anguilla* as measured by Maetz & Skadhauge (1968).

Ingestion of the medium by freshwater fish was first indicated several years ago by the work of Allee and Frank on the goldfish and on various species of minnow (Allee & Frank, 1948; Frank & Allee, 1950). Since then, drinking in fresh water has been demonstrated in several different species of fish by various workers using many different techniques (Potts *et al.* 1967; Potts & Evans, 1967; Maetz & Skadhauge, 1968; Foster, 1969; Lahlou, Henderson & Sawyer, 1969; Potts & Fleming, 1970; Potts, Foster & Stather, 1970). Of course such demonstrations of ingestion of the medium in several freshwater teleosts poses the immediate question 'why do fresh water fish drink?' It would appear that as the ingested, and presumably absorbed, water can only be eliminated by the kidney, simultaneously incurring the loss of some salts which have to be replaced by active uptake, drinking in fresh water only increases the osmotic and ionic problems of the fish. This apparent enigma perhaps in some measure explains the persistency in the literature of the, by now, obviously fallacious statement that freshwater teleosts do not drink. Of course there have been reports of some freshwater species in which no ingestion of the external medium could be detected. For example, Shehadeh & Gordon (1969) found that *Salmo gairdneri* did not drink in fresh water, but their statement that they consider 'many if not all' reports of drinking by teleosts in fresh water to be 'artifacts' is difficult to reconcile with the published evidence. Nor can it be reasonably stated that the amount ingested by those species studied is insignificant. It has been shown in the eel that ingestion of the medium in fresh water may account for up to 90% of the water excreted in the urine (Maetz & Skadhauge, 1968; Gaitskell & Chester Jones, 1971). In other species the volume of water ingested may be quite considerable – Potts & Fleming (1970) recorded a mean drinking rate for *Fundulus kansae* of 9·4 $ml.kg^{-1}.h^{-1}$. Also in some euryhaline or closely related stenohaline species the

drinking rate measured in sea water, so readily accepted in the literature as an important feature of the total water balance, may be only slightly larger than that recorded in fresh water (Potts & Fleming, 1970; Foster, 1969).

There remains no simple answer to the question posed above, as to why teleosts drink in fresh water. However, it is possible to approach this question in another manner. It was mentioned above that the ingestion of the medium in fresh water leads ultimately to an increase in 'osmoregulatory work' to compensate for the ions lost in the correspondingly larger volume of urine produced. It is possible to calculate the amount of work this entails by considering a hypothetical freshwater teleost that does not drink, and hence to estimate how energetically disadvantageous drinking in fresh water is for the fish. In making this calculation various approaches can be employed. In order to obtain a maximum estimate of the 'energetic expense' to the fish of drinking in fresh water it was decided that the approach used in the following calculations should be that which, of all the possibilities open to such a fish, would result in the greatest saving of osmotic work. On this basis it has therefore been assumed that, in the hypothetical non-drinking fish, the urine concentration remains constant but the urine volume is reduced, thus reducing urinary salt loss. This in turn would reduce the amount of salt required to be actively taken up from the dilute external medium. The equation used in the calculation is that given by Potts (1954) and is represented as follows:

$$\text{work} = RTVU \ln (B/M) \text{ cal.h}^{-1},$$

where $R$ is the universal gas constant, $T$ is the temperature in °K, $V$ is the urine volume in $\text{l.h}^{-1}$, $U$ is the urine concentration in $\text{mol.l}^{-1}$, $B$ is the blood concentration in $\text{mol.l}^{-1}$, and $M$ is the concentration of the external medium in $\text{mol.l}^{-1}$.

Following the approach described above, the only parameter on the right-hand side of the equation that would change in the hypothetical non-drinking fish would be $V$, the volume of urine produced. Thus the equation representing the energetic expense of drinking ($E$) can be represented as follows:

$$E = RTU(V - V^*) \ln (B/M),$$

where $V^*$ is the volume of urine that would be produced if no water was ingested (which is therefore equivalent to the actual urine flow minus the drinking rate, or, in other words, to the net influx of water across the gills). The values to be fitted in the above equation have been taken, as far as possible, from the measurements and estimates made previously for *A. dieffenbachii* which indicate the following values (per kg):

$$V = 0{\cdot}00162 \text{ l.h}^{-1}, \quad V^* = 0{\cdot}00027 \text{ l.h}^{-1}.$$

$B$ (for sodium) = 0·140 $\text{mol.l}^{-1}$ (Shuttleworth & Freeman 1973).

The concentration of the urine in *A. dieffenbachii* has not been determined, but published work on other species of eel would indicate that the sodium concentration is rarely above 0·015 $\text{mol.l}^{-1}$ (Butler, 1966; Oide & Utida, 1968; Chester Jones, Chan & Rankin, 1969*a*, *b*) so this value will be used here. A sodium concentration in the external medium (M) of 0·0003 $\text{mol.l}^{-1}$ and a temperature of 17 °C (290 °K) will be assumed. The equation thus becomes:

$$E = 1{\cdot}99 \times 290 \times 0{\cdot}015 (0{\cdot}00162 - 0{\cdot}00027) \ln 0{\cdot}140/0{\cdot}0003 \text{ cal.kg}^{-1}\text{.h}^{-1}$$
$$= 0{\cdot}07 \text{ cal.kg}^{-1}\text{.h}^{-1}.$$

To account for the active uptake of chloride as well as sodium this value must be approximately doubled. Thus the estimated increase in energy consumption incurred as a result of drinking in a freshwater environment amounts to only some 0·14 cal.$kg^{-1}$.$h^{-1}$, which is approximately equivalent to only 0·05–0·1 % of the total energy consumption of the fish (Bateman & Keys, 1932).

Obviously then, with such an insignificant energetic disadvantage associated with ingestion of the medium by freshwater teleosts it is unlikely that any gross selective pressure would be brought to bear during the evolution of teleosts which would tend to eliminate such a phenomenon. Of course, the question 'why does a freshwater fish drink?' remains unanswered, but the above analysis indicates that energetically it matters little to the fish whether it drinks or not and therefore perhaps explains why such a process should persist once it has been established. Perhaps in a fish that, for the purposes of respiration, is continually taking water into its buccal cavity and forcing it over the gills under pressure, it is energetically more expensive actively to prevent water passing down the gut than it is to make the necessary ionic compensations. Obviously the essential point is that once an animal has evolved the ability to produce a dilute urine, excess water can be eliminated with only minimal salt loss.

The isolated gill technique used in the investigation enabled the osmotic permeability coefficient ($P_{os}$) to be calculated directly from the net fluxes of water across the gills. This is the first time such calculations have been carried out directly and in doing so the inaccuracies and errors inherent in the indirect determinations published to date are avoided. It has thus been calculated that the osmotic permeability coefficient of the gills of *A. dieffenbachii* is equal to $0{\cdot}5 \times 10^{-5}$ cm.$sec^{-1}$.

Krogh (1939) calculated the water permeability of the whole body of the eel using the data of Keys (1933) taken from eels that were prevented from drinking. He obtained a value for the minute number of not less than 5 years (the minute number is defined as the time necessary for 1 $cm^3$ of water to pass through 1 $cm^2$ of membrane under a pressure difference of 1 atm). This corresponds to a $P_{os}$ of not more than $0{\cdot}8 \times 10^{-5}$ cm.$sec^{-1}$, which is very similar to the value found above for the gills alone.

It has been determined in this study that no significant change occurred in the calculated value of $P_{os}$ when *A. dieffenbachii* was adapted to sea water. It is possible that a small, but real reduction in $P_{os}$ occurs on adaptation, as indicated by the mean values obtained but that this is obscured by the relatively large variation in the results obtained with freshwater eels. Motais *et al.* (1969) report that they measured a reduction in $P_{os}$ on adaptation to sea water in *A. anguilla* and *Platichthys flesus*. However, in their study the $P_{os}$ of the gills was calculated indirectly and is therefore susceptible to the numerous errors discussed previously. It is true, however, that a reduction in the diffusional permeability to water ($P_d$), as measured by tritiated water, has been recorded in several teleosts on adaptation to sea water. This was first noted by Potts *et al.* (1967) in *Tilapia mossambica* and was extended to certain other species by Evans (1969*b*). It was concluded by Evans (1969*b*) that, in general, freshwater species had a higher permeability to water than seawater species. He also found that, in the euryhaline species *Platichthys flesus* and *A. anguilla* (yellow form), seawater-adapted specimens had a lower water permeability ($P_d$). However, in *Salmo trutta*, *Gasterosteus aculeatus* and the silver form of *A. anguilla* no such reduction in permeability to water on adaptation to sea water was found, and in fact an increase in permeability may be shown by *Gasterosteus*. Evans

(1967*b*) recorded no change in the water permeability of *Xiphister atropurpureus* in 10% sea water as compared with 100% sea water and no significant change in the water permeability of *Pholis gunnellus* was recorded on adaptation to 20% sea water (normal habitat 100% sea water) by Evans (1969*a*). Evidently then, although a change in permeability to water in different external salinities has been recorded in some species, its occurrence is not universal, and such a phenomenon has been shown to be absent in at least one form of a member of the genus *Anguilla*.

## SUMMARY

1. Measurements of net flux of water have been made on isolated gills removed from freshwater-adapted and seawater-adapted eels and incubated in various media of differing osmotic pressure.

2. From these measurements it has been possible to determine the osmotic permeability coefficient of the gill directly from the net water flux. The values obtained ($0{\cdot}50 \pm 0{\cdot}14 \times 10^{-5}$ cm.sec$^{-1}$ for freshwater eels and $0{\cdot}43 \pm 0{\cdot}07 \times 10^{-5}$ cm.sec$^{-1}$ for seawater-adapted eels) indicate that there was no significant change in this parameter on adaptation of the eels to sea water.

3. The direct measurements made of the net water flux across the isolated gills appear to be compatible with the osmoregulatory pattern of eels as deduced by other workers using different techniques. In particular they illustrate and further emphasize the significance of drinking in the freshwater fish.

4. Calculations indicate that, for a freshwater teleost, the osmotic and ionic problems caused by drinking in fresh water have an insignificant energetic effect and hence, energetically, it matters little to the fish whether it drinks or not.

The authors wish to thank Mrs S. M. Johnston for help in carrying out certain of the analyses, the New Zealand Advisory Committee to the Nuffield Foundation for financial assistance and the Research Committee of the N.Z. University Grants Committee for the purchase of apparatus. This research was carried out during the tenure (by T. J. S.) of a Teaching Fellowship from the University of Otago.

## REFERENCES

ALLEE, W. C. & FRANK, P. (1948). Ingestion of colloidal material and water by goldfish. *Physiol. Zoöl.* **21**, 381–90.

BATEMAN, J. B. & KEYS, A. (1932). Chloride and vapour-pressure relations in the secretory activity of the gills of the eel. *J. Physiol., Lond.* **75**, 226–240.

BELLAMY, D. (1961). Movements of potassium, sodium and chloride in incubated gills from the silver eel. *Comp. Biochem. Physiol.* **3**, 125–135.

BUTLER, D. G. (1966). Effect of hypophysectomy on osmoregulation in the European eel (*Anguilla anguilla* L.). *Comp. Biochem. Physiol.* **18**, 773–781.

BUTLER, D. G. (1969). Corpuscles of Stannius and renal physiology in the eel (*Anguilla rostrata*). *J. Fish Res. Bd Can.* **26**, 639–654.

CHESTER JONES, I., CHAN, D. & RANKIN, J. (1969*a*). Renal function in the European eel (*Anguilla anguilla* L.): Changes in blood pressure and renal function of the freshwater eel transferred to sea water. *J. Endocr.* **43**, 9–19.

CHESTER JONES, I., CHAN, D. & RANKIN, J. (1969*b*). Renal function in the European eel (*Anguilla anguilla* L.): Effects of the caudal neurosecretory system, corpuscles of Stannius, neurohypophysial peptides and vasoactive substances. *J. Endocr.* **43**, 21–31.

CHESTER JONES, I., HENDERSON, I. W. & BUTLER, D. G. (1965). Water and electrolyte flux in the European eel (*Anguilla anguilla* L.). *Archs Anat. microsc. Morph. exp.* **54**, 453–469.

DAINTY, J. & HOUSE, C. R. (1966). An examination of the evidence for membrane pores in frog skin. *J. Physiol., Lond.* **185**, 172–184.

EVANS, D. H. (1967*a*). Sodium, chloride and water balance of the intertidal teleost *Xiphister atropurpureus*. II. Role of the kidney and gut. *J. exp. Biol.* **47**, 519–524.

EVANS, D. H. (1967*b*). Sodium, chloride and water balance of the intertidal teleost *Xiphister atropurpureus*. III. Roles of simple diffusion, exchange diffusion, osmosis and active transfer. *J. exp. Biol.* **47**, 525–34.

EVANS, D. H. (1969*a*). Sodium, chloride and water balance of the intertidal teleost (*Pholis gunellus*). *J. exp. Biol.* **50**, 179–90.

EVANS, D. H. (1969*b*). Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. *J. exp. Biol.* **50**, 689–703.

FOSTER, M. A. (1969). Ionic and osmotic regulation in three species of *Cottus* (Cottidae, Teleost). *Comp. Biochem. Physiol.* **30**, 751–9.

FRANK, P. & ALLEE, W. C. (1950). Ingestion of colloidal thorium dioxide by representative minnows from the Chicago region. *Physiol. Zool.* **23**, 134–9.

GAITSKELL, R. E. & CHESTER JONES, I. (1971). Drinking and urine production in the European eel (*Anguilla anguilla* L.). *Gen. comp. Endocr.* **16**, 478–83.

GRAY, I. E. (1954). Comparative study of the gill area of marine fishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **107**, 219–25.

GROSS, W. J. (1954). Osmotic responses in the sipunculid *Dendrostomum zostericolum*. *J. exp. Biol.* **31**, 402–23.

HICKMAN, C. P. JR. (1968). Ingestion, intestinal absorption, and elimination of seawater and salts in the southern flounder *Paralichthys lethostigma*. *Can. J. Zool.* **46**, 457–66.

HIRANO, T., JOHNSON, D. W. & BERN, H. A. (1971). Control of water movement in flounder urinary bladder by prolactin. *Nature, Lond.* **230**, 469–71.

KAMIYA, M. (1967). Changes in ion and water transport in isolated gills of the cultured eel during the course of salt adaptation. *Annotnes zool. jap.* **40**, 123–129.

KEYS, A. (1933). The mechanism of adaptation to varying salinity in the common eel and the general problem of osmotic regulation in fishes. *Proc. R. Soc. Lond.* B **112**, 184–99.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge University Press.

LAHLOU, B. (1967). Excrétion rénale chez un poisson euryhalin, le flet (*Platichthys flesus* L.): Charactéristiques de l'urine normale en eau douce et en eau de mer et effets des changements de milieu. *Comp. Biochem. Physiol.* **20**, 925–38.

LAHLOU, B., HENDERSON, I. & SAWYER, W. H. (1969). Sodium exchanges in goldfish (*Carassius auratus*) adapted to a hypertonic saline solution. *Comp. Biochem. Physiol.* **28**, 1427–33.

MAETZ, J. (1970). Mechanisms of salt and water transfer across membranes in teleosts in relation to the aquatic environment. *Mem. Soc. Endocr.* **18**, 3–29.

MAETZ, J. & SKADHAUGE, E. (1968). Drinking rates and gill ionic turnover in relation to external salinities in the eel. *Nature, Lond.* **217**, 371–3.

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

MOTAIS, R., ISAIA, J., RANKIN, J. C. & MAETZ, J. (1969). Adaptive changes in the water permeability of the teleostean gill epithelium in relation to external salinity. *J. exp. Biol.* **51**, 529–46.

OIDE, H. & UTIDA, S. (1968). Changes in intestinal absorption and renal excretion of water during adaptation to seawater in the Japanese eel. *Mar. Biol.* **1**, 172–7.

POTTS, W. T. W. (1954). The energetics of osmotic regulation in brackish- and fresh-water animals. *J. exp. Biol.* **31**, 618–30.

POTTS, W. T. W. & EVANS, D. H. (1967). Sodium and chloride balance in the killifish *Fundulus heteroclitus*. *Biol. Bull. mar. biol. Lab., Woods Hole* **133**, 411–425.

POTTS, W. T. W. & FLEMING, W. R. (1970). The effects of prolactin and divalent ions on the permeability to water of *Fundulus kansae*. *J. exp. Biol.* **53**, 317–27.

POTTS, W. T. W., FOSTER, M. A., RUDY, P. P. & PARRY HOWELLS, G. (1967). Sodium and water balance in the cichlid teleost *Tilapia mossambica*. *J. exp. Biol.* **47**, 461–70.

POTTS, W. T. W., FOSTER, M. A. & STATHER, J. W. (1970). Salt and water balance in salmon smolts. *J. exp. Biol.* **52**, 553–64.

SHEHADEH, Z. H. & GORDON, M. S. (1969). The role of the intestine in salinity adaptation of the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **30**, 397–418.

SHUTTLEWORTH, T. J. (1972). A new isolated perfused gill preparation for the study of the mechanisms of ionic regulation in teleosts. *Comp. Biochem. Physiol.* **43** A, 59–64.

SHUTTLEWORTH, T. J. & FREEMAN, R. F. H. (1973). The role of the gills in seawater adaptation in *Anguilla dieffenbachii*. I. Osmotic and ionic composition of the blood and gill tissue. *J. comp. Physiol.* (in the Press).

SMITH, H. W. (1930). The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.* **93**, 480–505.

UTIDA, S., OIDE, M., SAISHU, S. & KAMIYA, M. (1967). Pré-établishement du mécanisme d'adaptation à l'eau de mer dans l'intestin et les branchies isolées de l'anguille argentée au cours de sa migration catadrome. *C. r. Séanc. Soc. Biol.* **161**, 1201–4.