

STUDIES ON THE PERMEABILITY TO WATER OF SELECTED MARINE, FRESHWATER AND EURYHALINE TELEOSTS

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INTRODUCTION

Recent studies of the fluxes of sodium and chloride across euryhaline teleost fish have shown that acclimation to fresh water involves a sharp reduction in the flux of both ions (Evans, 1967*c*, 1969; Motais, 1967; Maetz *et al.* 1967*a, b*; Potts & Evans, 1967; Potts *et al.* 1967). It has been shown, at least in *Fundulus heteroclitus* (Potts & Evans, 1967), *Tilapia mossambica* (Potts *et al.* 1967), *Xiphister atropurpureus* (Evans, 1967*c*) and *Pholis gunnellus* (Evans, 1969), that part of the reduction of the sodium flux is due to a change in the body's permeability to this ion. The permeability to chloride also declines in the latter two species, but to a lesser degree. On the other hand, it appears that the flux of tritiated water either does not change with salinity (Evans, 1967*c*, 1969) or actually increases when the fish is acclimated to fresh water (Potts *et al.* 1967). Since it can be calculated (Evans, 1967*c*) that drinking and urine flow amount to only 1% of the gross flux of tritiated water, these data indicate that in euryhaline teleosts the permeability changes to sodium, chloride and water do not parallel each other.

Since the body fluid concentration of fish is approximately 350 m-osmoles/l. while the concentration of sea water and fresh water is approximately 1000 and 1 m-osmole/l. respectively, in sea water there is a net loss of water from the fish and in fresh water there is a net gain of water. If the osmotic flow occurs entirely by diffusion, the net flux (computed from the gross flux of tritiated water) will be a function of the diffusion permeability (P_d) and will be equal to the osmotic permeability (P_{os} : e.g. Gutknecht, 1967). In the case of teleosts, the net flux (P_d) in any salinity can be calculated if the gross flux of tritiated water and the relative mole fractions of water in the fish and the various media are known. According to the accepted model for teleost osmoregulation the drinking rate in sea water and the urine flow in fresh water are functions of the osmotic permeability or P_{os} . If water is moving by simple diffusion across the permeable surfaces of the fish then the drinking rate should equal the calculated net flux outward in sea water and the urine flow should equal the net influx in fresh water. The rate of ingestion of the medium in sea water will in fact be somewhat higher than the calculated efflux because renal loss of water will add to the passive loss of water. In addition, the urine flow in fresh water will also be somewhat higher than the calculated net influx because of some drinking of the medium (Evans, 1967*b*;

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Potts & Evans, 1967; Potts *et al.* 1967; Maetz & Skadhauge, 1968). Further, the rectal loss of water must also be considered. It is obvious that it would add to the net passive loss (P_d) in sea water and add to the osmotic loss (P_{os}) in fresh water.

Gross discrepancies between P_{os} and P_d are common in biological systems and both the bulk flow of water through pores (Koefoed-Johnsen & Ussing, 1953; Rich *et al.* 1967) and the presence of an unstirred layer (Dainty & House, 1966) have been hypothesized to account for an osmotic permeability several times greater than the calculated diffusional permeability. It has been shown that $P_{os} \gg P_d$ in erythrocytes (Rich *et al.* 1967), frog skin (Koefoed-Johnsen & Ussing, 1953; Dainty & House, 1966) and decapod crustacea (Rudy, 1967). On the other hand, P_{os} approximates P_d in the marine alga *Valonia* (Gutknecht, 1967) and various teleosts: *Tilapia* (Potts *et al.* 1967), *Xiphister* (Evans, 1967*c*) and *Pholis* (Evans, 1968*a*). It would seem, therefore, that the presence of an unstirred layer or pores in the membrane has little effect on the movement of water across these organisms.

Studies were therefore undertaken to measure the permeability to water of various marine, freshwater and euryhaline teleosts and to study the effects of temperature, body size, species differences, salinity, stress and anaesthetization on these fluxes. Further, calculations were made to determine whether the net flux approximates to the published drinking rates or the urine flows of the species studied.

MATERIALS AND METHODS

Table 1 presents a systematic list of the species used in this study as well as the site of capture and the laboratory conditions of the experiments. In all cases acclimation to temperature was for at least 24 hr. Salinity acclimation was for at least 48 hr. if the fish was transferred from brackish water to sea water and for at least 4 days if the salinity change was over a greater range (i.e. from sea water to fresh water). Experiments were either performed in a 10° C. constant-temperature room at Lancaster, England, or in a large bath of circulating 13° C. sea water at the Danmarks Akvarium, Charlottenlund, Denmark. In all cases 2–10 fish (depending on size) were placed in either glass aquaria or plastic buckets and left undisturbed for at least 12 hr. before an experiment was started. The volume of the fish was never more than 3% of the volume of the bath. Trout were found to be extremely sensitive to external stimuli; experiments on these species were therefore performed in closed opaque containers. They were acclimated to these containers for at least 3 days before experiments were performed. Tritiated water (to a concentration of from 0.5 to 1 $\mu\text{C.}/\text{ml}$. water in the bath) was added by pipette and 1 hr. later the fish were removed, washed for a few seconds in non-radioactive medium and then deep frozen (–25° C.). A sample of the loading bath was also taken. Water was extracted from thawed fish by a method previously described (Rudy, 1967). If the fish weighed less than 20 g. the whole animal was cut up for water extraction. The water from larger fish was either extracted from cross-sectional slices from the gill, gut and tail region or from tissue samples from the gut, liver and tail musculature. It was found that the specific activity of the extracted water did not vary with the length of time of extraction or the tissue used. The rate constant (K) of the influx of water was calculated using a standard formula (Rudy, 1967).

Measurements of the ingestion of the medium in sea water were performed only on the plaice, *Platichthys platessa*. ¹²⁵I-labelled PVP was used as an inert marker and the method used has already been described (Evans, 1968).

Table 1. *Systematic list of the species used in this study together with their site of capture and maintenance*

Order	Site and date of capture	Experimental conditions
Anguilliformes		
<i>Anguilla anguilla</i> (yellow form), eel	Freshwater ponds and Øresund,* Sealand, Denmark, Feb. and June 1968	f.w.† > 5 days, 13° C., 48 hr. s.w.‡ > 4 days; 13° C., 48 hr.
(silver form), eel	Øresund, Denmark, Oct. 1967 (starved from date of capture)	f.w. 5 days, 13° C., 36 hr.
Salmoniformes		
<i>Salmo trutta</i> , brown trout	Commercial hatchery, Lancashire, England, Mar. 1968	f.w.§, 10° C., > 48 hr. s.w. , 10° C., 6 days 70% s.w., 10° C., 4 days
<i>S. gairdneri</i> , rainbow trout	Commercial hatchery, Yorkshire, England, June 1968	f.w., 10° C., 3 days
Cypriniformes		
<i>Carassius auratus</i> , goldfish	Pet shop, Lancaster, England, Apr. 1968	f.w., 10° C., 3 days f.w., 20° C., 3 days
<i>Rutilus rutilus</i> , roach	Windermere, England, Aug. 1968	f.w., 10° C., 3 days
<i>Phoxinus phoxinus</i> , minnow	Windermere, England, May 1968	f.w., 10° C., 3 days f.w., 20° C., 4 days
Gasterosteiformes		
<i>Gasterosteus aculeatus</i> , 3-spined stickleback	Brackish ponds, Sealand, Denmark, June 1968	s.w., 13° C., > 4 days f.w., 13° C., > 4 days
Scorpaeniformes		
<i>Cottus morio</i> , miller's thumb	Windermere, England, Mar. 1968	f.w., 10° C., 48 hr.
Perciformes		
<i>Zoarces viviparus</i> , viviparus blenny	Øresund, Denmark, June 1968	s.w., 13° C. 48 hr.
<i>Ctenolabrus rupestris</i> , gold-sinny wrasse	Øresund, Denmark, June 1968	s.w., 13° C., 3 days
<i>Lumpenus lampretaeformis</i>	Kattegat, Denmark, May 1968 (brackish)	s.w. 1 month, 13° C., 24 hr.
<i>Gobius niger</i> , black goby	Øresund, Denmark, June 1968	s.w., 13° C. 48 hr.
<i>Trachinus vipera</i> , lesser weaver	Morecambe Bay, England, July 1968	s.w., 10° C., 3 days
Pleuronectiformes		
<i>Platichthys flesus</i> , flounder	Morecambe Bay, England, Apr. 1968	s.w., 10° C. > 4 days f.w., 10° C. > 1 week¶
<i>Pl. platessa</i> , plaice	Morecambe Bay, England, Apr. 1968	s.w., 10° C. > 4 days s.w., 21° C. > 3 days

* Øresund has a sodium concentration of approximately 200 mm/l.

† Fresh water in Charlottenlund was 0.9 mm Na/l.

‡ Sea water in Charlottenlund was 410 mm Na/l.

§ Fresh water in Lancaster was 0.2 mm Na/l. || Sea water in Lancaster was 410 mm Na/l.

¶ This fresh water was 1.0 mm Na/l.

RESULTS AND DISCUSSION

The effect of temperature

Because experiments were performed at two temperatures three species were acclimated to both 10 and 20° C. (21° C.) to determine the Q_{10} of the influx of water. The average Q_{10} was 1.90 (Table 2), which is approximately what would be expected from such a system (Giese, 1963). All the data from fish acclimated to 13° C. was therefore corrected to enable comparison with data from fish acclimated to 10° C.

Table 2. *The effect of temperature on the flux of water across three species of teleosts*

Species	10° C.	20° C.	Q_{10}
<i>Carassius auratus</i>	0.429 ± 0.015 (8)*	0.916 ± 0.037 (13)	2.12 ± 0.10
<i>Phoxinus phoxinus</i>	0.785 ± 0.048 (14)	1.390 ± 0.075 (8)	1.77 ± 0.15
<i>Platichthys platessa</i>	0.101 ± 0.001 (7)	0.194 ± 0.005 (6)†	1.81 ± 0.01

* All values are rate constants (K , hr.⁻¹) ± s.e. (number of fish).

† These fish were acclimated to 21° C.

The effect of body weight

Because the various species used in this study varied considerably in their weight it was necessary to correct for any differences between species that may be due solely to differences in body weight. Five species were chosen for this study and ranged in general body shape from the fusiform trout, *Salmo trutta*, the laterally compressed stickleback, *Gasterosteus aculeatus*, the elongate yellow eel, *Anguilla anguilla*, to the flounder, *Platichthys flesus*, and plaice, *Platichthys platessa*. The water flux across these species was converted to ml. water/fish/hr., on the assumption that the fish have a total body water content of 750 ml./kg. fish (Evans, 1967*a*). Fairly large errors in this assumption will have little effect on the following considerations. The data from the flounder and the plaice were combined because the fluxes across these two species were so similar (Fig. 1). This certainly indicates that it is not differences in permeability to water that cause the euryhalinity of the flounder and the stenohalinity of the plaice. The water flux was plotted (Fig. 1, 2) against body weight (in g.) in a manner analogous to the comparison between the metabolic rate and the body size (Hemmingen, 1960; Prosser & Brown, 1962). A line drawn through the resulting data points could be expressed by the equation $m = aw^x$, where m is the water flux in ml. water/fish/hr., a is the Y intercept, w is the body weight in g. and x is the slope of the line (Potts *et al.* 1967). The consistency of the data and the use of a range of sizes of approximately two orders of magnitude enabled very accurate lines to be drawn by eye.

If the flux is directly related to the body weight, x will equal 1.0, while if the flux is related to the surface:volume ratio x will equal 0.67. The values for x for the data shown in Figs. 1 and 2 were: *Salmo trutta*, 0.85; *Gasterosteus aculeatus*, 0.94; *Anguilla anguilla*, 0.92; *Platichthys flesus* (*platessa*), 0.81. The average slope was 0.88 and a line with this slope is drawn for reference in all of the figures. The exact placement of this line (i.e. the value of a) is the same in every graph to simplify comparison of the data from different figures and was determined from the data in Fig. 3. Potts *et al.* (1967) have reported a value for x of 0.83 for *Tilapia*. A simple calculation shows what effect a slope of this sort will have on the apparent rate constant of the flux of water across

a fish weighing 1 g. compared with other individuals of the same species weighing 10 or 100 g. If one arbitrarily takes the reference line to be the line drawn through the data of some species of fish, then the flux of water across a 1 g. fish of this species would have a rate constant of 0.27. This value is calculated by noting where a vertical line drawn from the point for 1 g. meets the reference line. The corresponding value

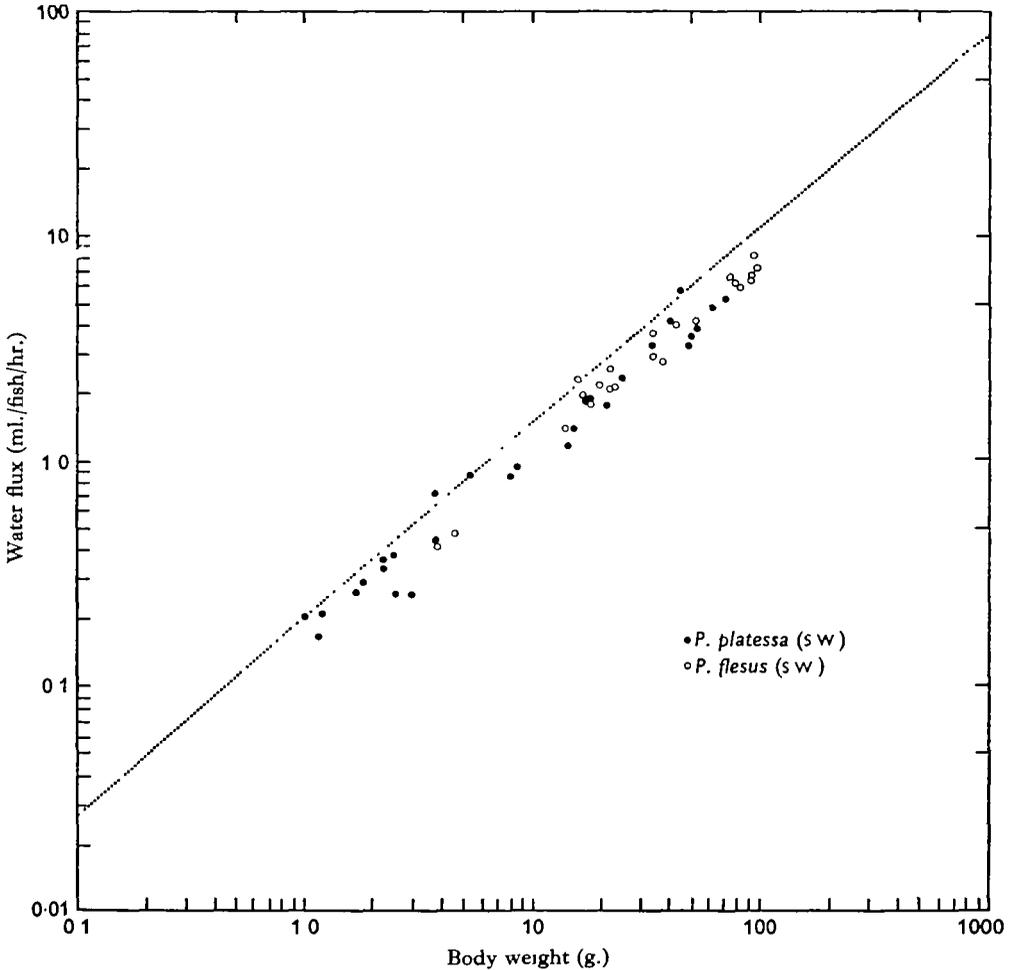


Fig. 1. The relationship between the body weight and the flux of tritiated water across the plaice, *Platichthys platessa* (s.w.) and the flounder, *Platichthys flesus* (s.w.). Each point represents a single fish. The dotted line is for reference (see text).

on the ordinate, divided by the weight (in this case 1 g.), times 0.75 ml. water/g. fish gives the rate constant. Similar calculations for 10 and 100 g. fish along the same reference line give rate constants of 0.20 and 0.15, respectively. Consequently, if the water flux across teleosts increases as the 0.88 power of the weight, then the apparent rate constant will decline by one-quarter over a weight increase of one order of magnitude and will nearly halve over a weight increase of two orders of magnitude. This certainly

shows the importance of considering weight when studying the water relations of various species.

It seems most reasonable to assume that the slope of these data should be correlated with the changes in the gill surface area with increase in size. If the gill surface area of the freshwater bass *Micropterus dolomieu* (Price, 1931; Hughes, 1966) is plotted against

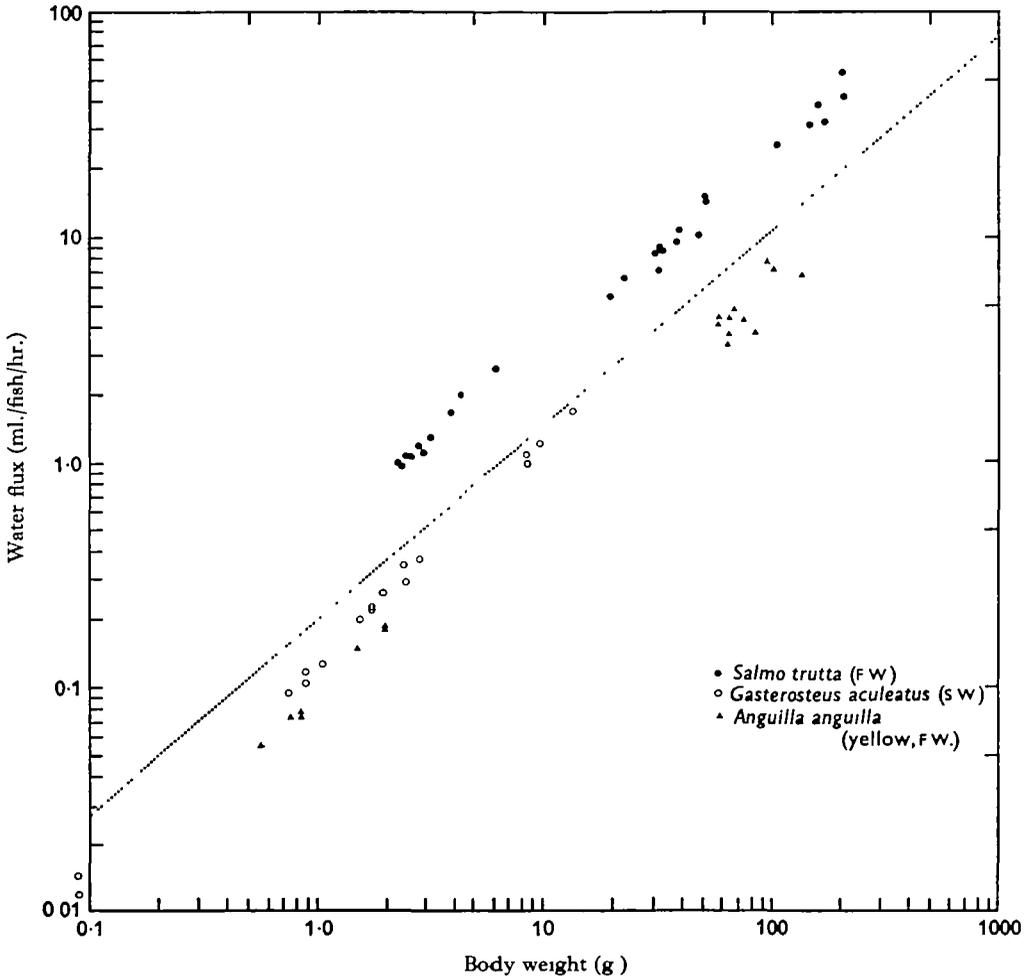


Fig. 2. The relationship between the body weight and the flux of tritiated water across the brown trout, *Salmo trutta* (F.W.); the 3-spined stickleback, *Gasterosteus aculeatus* (s.w.), and the yellow form of the eel, *Anguilla anguilla* (F.W.). Each point represents a single fish except the two points in the region of 0.1 g.; they represent the mean of 10 fish. The dotted line is for reference (see text).

the weight in a manner analogous to that of Figs. 1 and 2 the resulting value for x is 0.83, which is certainly close to the average slope of the variation of water flux with weight. It appears, therefore, that the variation of gill surface area with weight is the major factor determining the variation of the water flux with weight, i.e. the flux of water is mainly across the gills. This is further supported when one considers the wide

variation in shapes used in this study. It is difficult to believe that the surface area of the eel changes with weight variation in approximately the same manner as the surface area of the flounder.

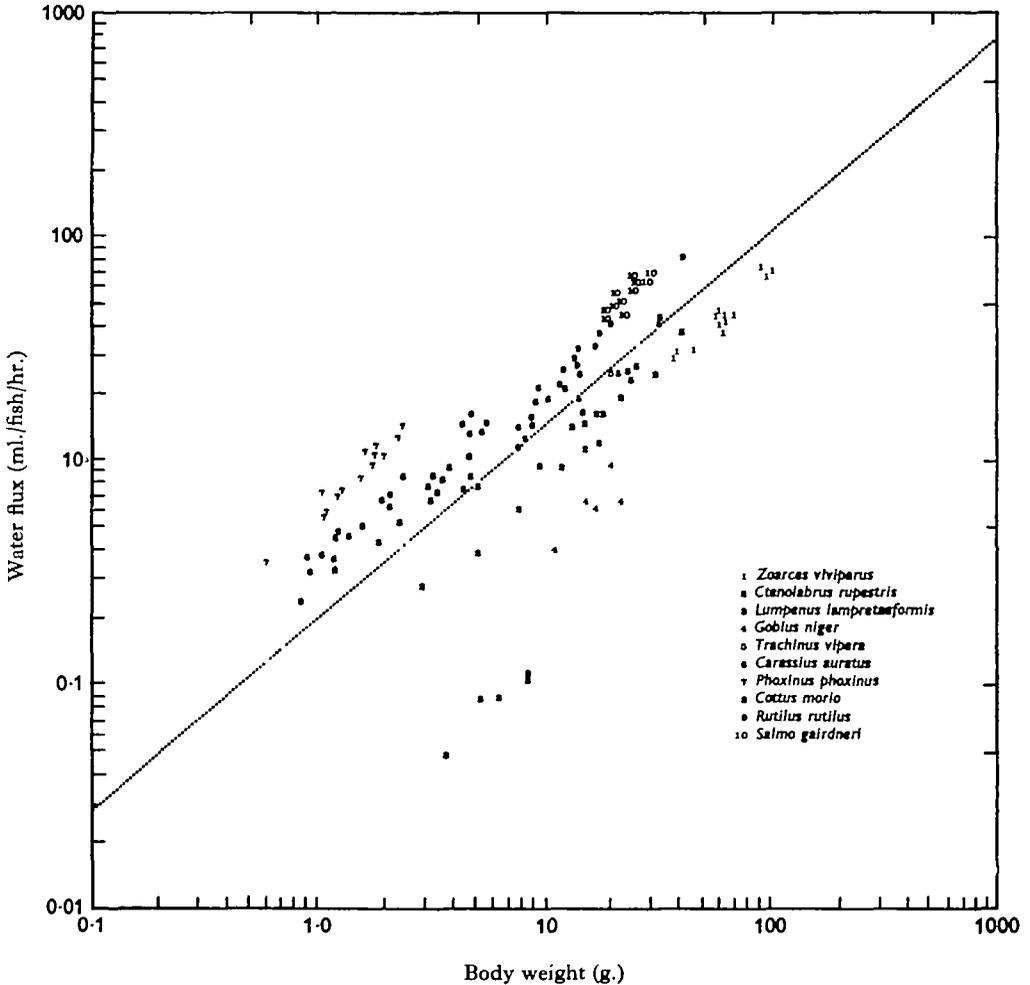


Fig. 3. The flux of tritiated water across five species of marine teleosts and five species of freshwater teleosts. Each point represents a single fish. The dotted line is for reference (see text).

The effect of species differences

Figure 3 displays the data from the tritiated water influx experiments performed on five species of marine teleosts and five species of freshwater teleosts. One of the most striking features is that all of the data seems to lie approximately parallel to the reference line calculated from Figs. 1 and 2. This certainly supports the flux against weight data from the trout, stickleback, eel, flounder and plaice. The first five fish listed in the legend are marine species while the second five are freshwater species. It is obvious that all of the freshwater species are considerably (approximately 2 times) more permeable to water than the marine species. It should be added that this differ-

ence is seen even if no correction for Q_{10} is applied to the species kept at 13° C. The reference line was placed to best separate the two groups of data. It therefore appears that (at least in these 10 species) evolutionary adaptation to fresh water has involved a higher permeability to water than adaptation to sea water. If the data on *Tilapia* (Potts *et al.* 1967), *Xiphister* (Evans, 1967c) and *Pholis* (Evans, 1968a) is corrected for size and temperature, the flux of water across *Tilapia* (in both fresh water and sea water) is in the 'freshwater fish range' while the flux across both *Xiphister* and *Pholis* is in the 'sea-water fish range'. It appears, therefore, that euryhaline fish do not, as a group, have a low or high permeability but merely are as permeable as their closest relatives, i.e. marine euryhaline teleosts are in the 'sea-water fish range' while freshwater euryhaline teleosts are in the 'freshwater fish range'. The data on the brown trout, stickleback, eel, flounder and plaice support this. Both the eel and the stickleback are common in fresh water but the orders to which they belong (Anguilliformes and Gasterosteiformes) are predominantly marine.

If one considers the metabolic activity of the various species mentioned in Fig. 3 an alternative hypothesis is possible. Most of the marine fish used in this study are bottom-dwelling, sluggish forms while the freshwater species used are free-swimming. Since it has been shown that both the gill surface area (Hughes, 1966) and the respiratory rate (Marshall, 1965) are directly correlated with the level of activity in the animal it could be that the more active freshwater species of this study appear to be more permeable because more tritiated water enters the fish due to their greater gill surface area and respiratory rate. But various exceptions to this general trend tend to rule out this hypothesis. Both the stickleback, *Gasterosteus*, and the labrid, *Ctenolabrus*, are active marine forms and they are both in the 'sea-water fish range'. They are certainly just as active as many of the freshwater species used in this study. Moreover, Hughes (1966) reports that the closely related labrid *Crenilabrus melops* has approximately the same gill surface area (mm.²/g.) as the brown trout. Further, the bottom-dwelling lesser weaver *Trachinus vipera* is at the top of the 'sea-water fish range', and the sluggish miller's thumb, *Cottus morio*, is in the 'freshwater fish range'. It therefore appears that the best explanation for the data in Fig. 3 is that (at least for the species involved in this study) freshwater teleosts are more permeable to water than marine teleosts. This is especially surprising when one considers that it is fairly well established (see Introduction) that freshwater teleosts are less permeable to sodium (and chloride) than marine teleosts.

The effect of salinity

Because it appears that freshwater teleosts are more permeable to water than marine teleosts various euryhaline teleosts were tested to determine if they increased their permeability to water when acclimated to fresh water. The euryhaline teleosts, *Platichthys flesus*, *Gasterosteus aculeatus* and *Anguilla anguilla*, were used. Both the yellow and silver forms of *Anguilla* were studied. Figure 4 and Table 3 present the results. While the flounder and the yellow form of *Anguilla* are more permeable to water when they are acclimated to fresh water, the permeability of the silver forms of *Anguilla* does not change and the stickleback may even decrease its permeability when it is acclimated to fresh water. Three brown trout acclimated to 70% sea water and one acclimated to 100% sea water had the same permeability to water as other brown

trout acclimated to fresh water. Thus, it seems that while some euryhaline teleosts have the ability to change their permeability in accordance with salinity other species have not.

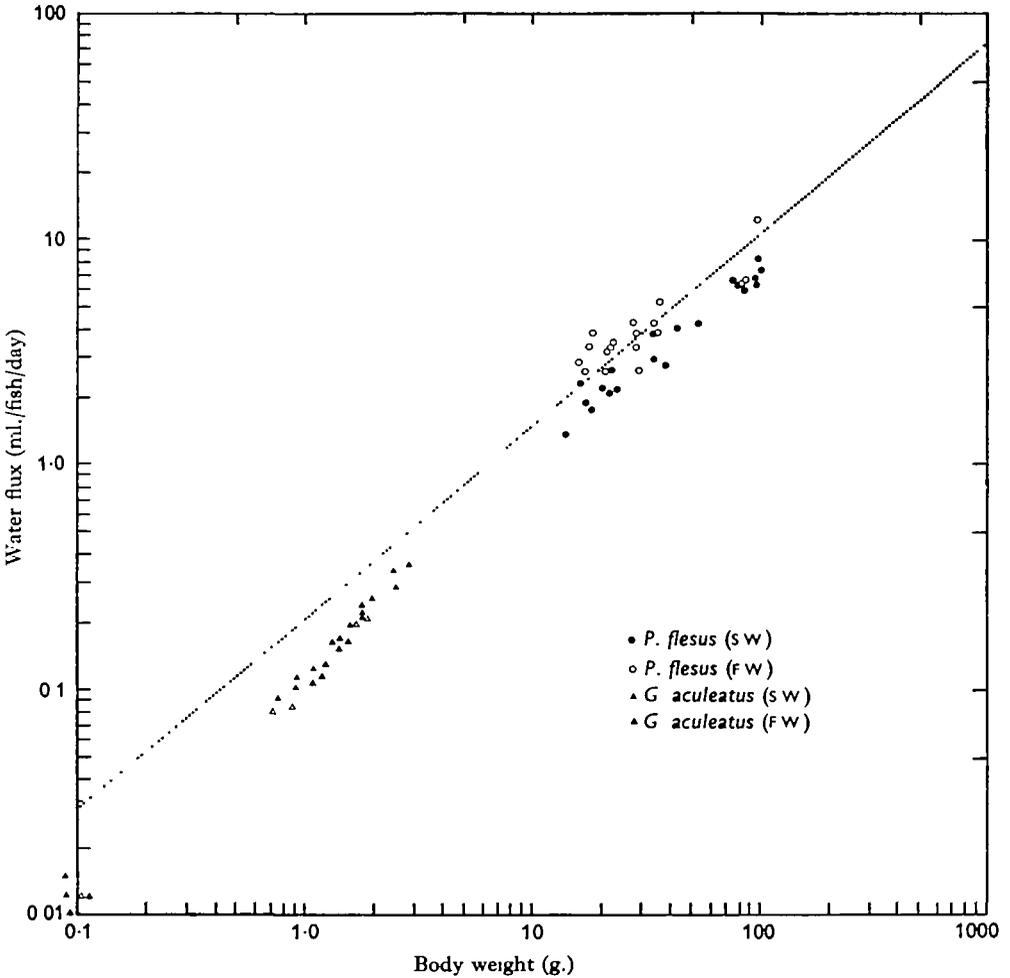


Fig. 4. The effect of salinity on the flux of tritiated water across the flounder and the 3-spined stickleback. Each point represents a single fish except the five points in the region of 0.1 g.; they are the mean of 10–20 fish. The dotted line is for reference (see text).

The effect of stress

As mentioned earlier, it was soon found that trout were very susceptible to external stimuli and were therefore kept in closed, opaque containers. To test the actual effects that stress may have on the flux of water across the species a few trout were kept in glass tanks and then transferred with a net to another tank immediately before the tritiated water was added (termed 'stressed' in Fig. 5). In other experiments trout were transferred to an intermediate tank and then immediately into another tank to which the tritiated water was added (termed 'stressed' in Fig. 5). To test the effect of stress on a more sluggish species similar experiments were performed on the plaice. In this case

fish were merely transferred to a container immediately before the tritiated water was added. It is obvious from Fig. 5 that while stress had a marked effect on larger trout, there is no effect on small trout. The cause of the differences between large and small individuals is unknown. It is also clear that the greater the stress the greater the increase in the permeability to water. Since the animals were presumably becoming less stressed with time it would appear that the actual rate of exchange during the first

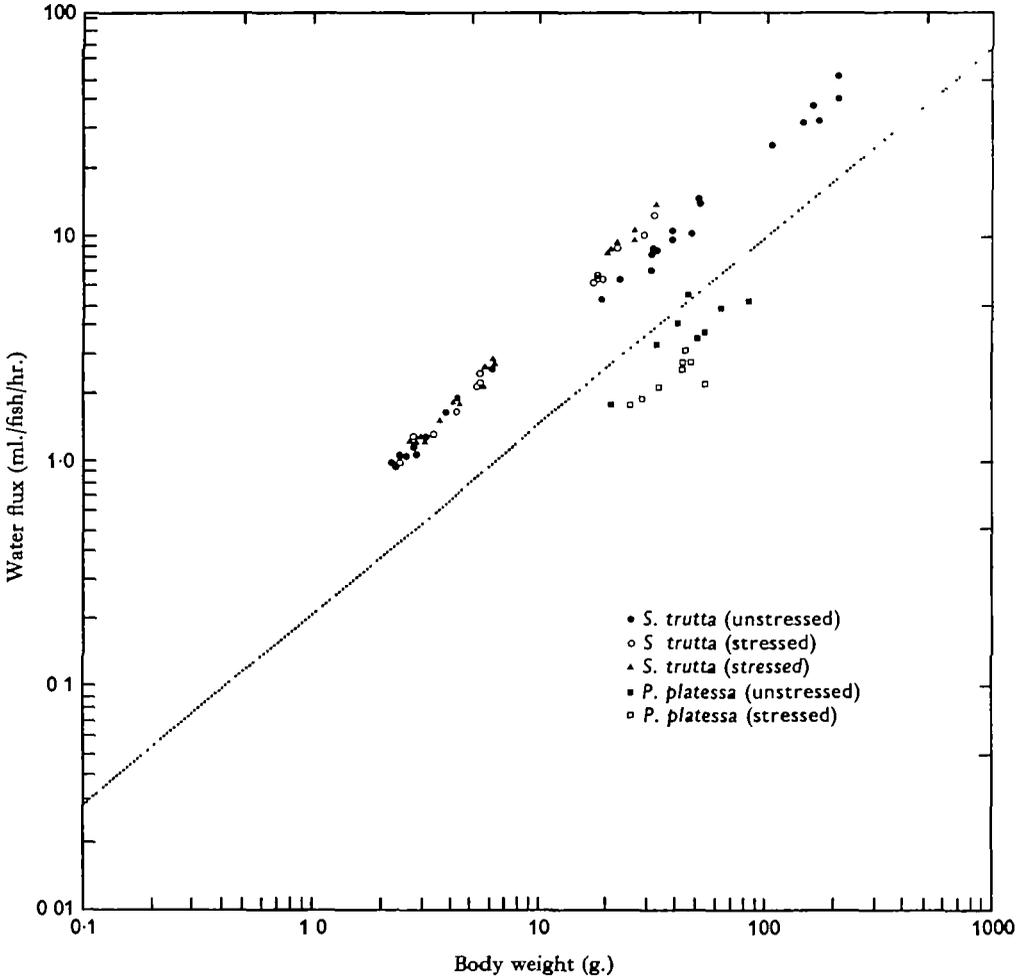


Fig. 5. The effect of stress on the flux of tritiated water across the brown trout and the plaice. Each point represents a single fish. The dotted line is for reference (see text).

Table 3. *The effect of salinity on the flux of water across the eel, Anguilla anguilla*

	100% s.w.	Fresh water
Yellow eel	0.057 ± 0.005 (10)*	0.086 ± 0.006 (11)
Silver eel	0.080 ± 0.002 (12)	0.080 ± 0.003 (6)

* All values are rate constants (K , hr.^{-1}) \pm S.E. (number of fish).

few minutes after transfer was considerably higher than the rate averaged over one hour. Experiments on the rainbow trout also showed that stress markedly increased the permeability to water. These results show that the cause of laboratory diuresis in trout (Holmes, 1961) is an immediate increase in water permeability after handling. Previously it was not known whether this was the cause or whether the increases in urine flow after handling was merely due to kidney malfunction followed by a loss of body weight. In the plaice, in contrast to the trout, stress appears to actually decrease the permeability to water.

Table 4. *The effect of anaesthetization on the flux of water across two species of teleosts*

Species	Unanaesthetized	Anaesthetized
<i>Carassius auratus</i>	0.429 ± 0.015 (8)*	0.426 ± 0.013 (7)
<i>Anguilla anguilla</i> (silver)	0.080 ± 0.002 (12)	0.124 ± 0.007 (5)

* All values are rate constants (K , hr.⁻¹) ± s.e. (number of fish).

The effect of anaesthetization

Because MS 222 (Sandoz) and urethane are commonly used as anaesthetic agents for teleosts, experiments were performed to test the effects of these drugs on the influx of water. A small group of goldfish were tested in the normal manner except that 30 min. before the end of the experiment MS 222 was added to a concentration of 0.01%. Within 15 min. the fish were anaesthetized and 15 min. later the fish were killed in the normal way, i.e. after a total of 60 min., 30 min. of which was under anaesthetic. Five silver eels were anaesthetized in a 1% solution of urethane before placing them in a loading tank and adding the tritiated water. The eels were just recovering at the end of the 1 hr. loading period. The rate constants of the anaesthetized fish are compared with the rate constant from control fish of the same weight range in Table 4. It is obvious that while MS 222 has no effect on the water balance of goldfish, urethane considerably increases the movement of water across silver eels. It has been shown that MS 222 has little effect on the sodium flux across the marine teleost, *Pholis* (Evans, 1969).

The relationship between P_{0a} and P_d

Table 5 compares the net diffusional permeability (P_d) calculated from the preceding data with the published values for the drinking rates or urine flows of the same or closely related species. The net diffusional flux was corrected to the temperature and body weight of the fish used in the drinking and urine-flow studies. Only data from experiments which included the size of the fish and the experimental temperature were used because it has already been shown that variation in these two parameters can cause great variation in the apparent flux of the water across the fish. The drinking rate of the plaice, *Platichthys platessa*, has not been published before and was found to be 37 ± 5 ml. water/kg. fish/day (\bar{x} ± s.e., 13 fish).

The net diffusional flux was calculated under the assumption that the concentration of the body fluids of all the fish examined was approximately 350 m-osmoles/l. and the sea water and fresh water used was approximately 1000 m-osmoles/l. and 1 m-osmole/l., respectively. The net flux outward in sea-water fish would therefore

be 1.2% of the gross flux (Evans, 1967c) and the net flux inward in fresh water would be 0.6% of the gross flux (Potts *et al.* 1967). *Paralichthys lethostigma* was assumed to have the same water permeability as the flounder and the plaice.

Examination of the data in Table 5 shows that with the exception of *Platichthys flesus* in sea water and *Anguilla anguilla* in fresh water the ratio of P_{os}/P_d is fairly consistent and of the same order as that found for erythrocytes (Rich *et al.* 1967). However, it must be noted that various errors may affect this estimate greatly and it is therefore difficult to say that these data necessarily enable one to conclude that there are either pores in the permeable membrane of the fish or an unstirred layer present.

Table 5. *The comparison of published drinking rates or urine flows (P_{os}) with the calculated net diffusional flux of water (P_d)*

Sea water						
Species	Weight (g.)	Temperature (° C.)	Drinking rate (P_{os})	Net flux (P_d)	P_{os}/P_d	Source
<i>Anguilla anguilla</i>	80-225	20	78*	34*	2.3	Maetz & Skadhauge (1968)
<i>Platichthys flesus stigma</i>	70-305	15-18	250	36	7.0	Motais & Maetz (1965)
<i>P. platessa</i>	30-70	10	37	25	1.5	Present study
<i>Paralichthys lethostigma</i>	1000	20	109	33	3.3	Hickman (1968)
Fresh water						
Species	Weight (g.)	Temperature (° C.)	Urine flow (P_{os})	Net flux (P_d)	P_{os}/P_d	Source
<i>Anguilla anguilla</i>	70-300	18	85*	13*	6.5	Sharratt <i>et al.</i> (1964)
<i>Salmo gairdnerii</i>	150-280	10	83	28	3.1	Holmes (1961)
	163	4-10	110	28	3.9	Holmes & Stanier (1966)
<i>Platichthys flesus</i>	80-330	13-22	69	20	3.5	Motais (1967)

* ml./kg. fish/day.

As mentioned earlier, both the renal and rectal losses in sea water will add to the net outward passive flux and thereby increase the drinking rate relative to the net diffusional flux in sea water (i.e. increase P_{os}/P_d). To take an example, Hickman (1968) found that in sea water the urine flow of *Paralichthys lethostigma* was approximately 4.3 ml./kg. fish/day and the rectal loss was 26.6 ml./kg. fish/day. The net loss calculated in Table 5 should therefore be 33 + 4.3 + 26.6 or 64 ml./kg. fish/day. The P_{os}/P_d ratio is then approximately 1.71, which is half that calculated without taking rectal and renal losses into consideration. Addition of similar quantities of water loss to the net flux calculated for *Anguilla* and *Platichthys platessa* would reduce the P_{os}/P_d ratio to approximately 1.0. It is not clear why the drinking rate published for the flounder is so large (Motais & Maetz, 1965). The drinking rate was estimated using the colorimetric determination of phenol red first used by Smith (1930) and

could well have been in error. The similarity between species of the other drinking rates published and the water influxes of this study seem to support this.*

Similar arguments can be applied to the P_{os}/P_d ratios calculated for the freshwater fish studied. While addition of the water influx due to drinking in fresh water will tend to increase the urine flow relative to the net calculated diffusional flux, addition of rectal water loss will decrease it. No data are available on the rectal loss of water in fresh water but it is now well established that teleosts drink substantial quantities of the medium in fresh water or other hypo-osmotic media (Evans, 1967*b*; Potts & Evans, 1967; Potts *et al.* 1967; Maetz & Skadhauge, 1968). Maetz & Skadhauge (1968) found that *Anguilla* drank approximately 33 ml./kg. fish/day. in fresh water at 20° C. while Potts *et al.* (1967) found a rate of 63 ml./kg. fish/day for the much smaller *Tilapia* at approximately 20° C. It is obvious that addition of drinking rates of this order to the data on *Anguilla* and other teleosts in fresh water would reduce the apparent P_{os}/P_d considerably and may reduce it to 1.0 in the case of the trout and the flounder. The P_{os}/P_d ratio of *Anguilla* is higher than that of the other species probably because of diuresis during the urine flow studies. Sharratt, Chester Jones & Bellamy (1964) mentioned that rates of only 20 ml./kg. fish/day were found with other techniques and Butler (1966) found a rate of 49 ml./kg. fish/day at 15° C. The weight of his experimental animals was not given but it is unlikely that they differed considerably in weight from the eels used by Sharratt, Chester Jones & Bellamy. If one assumes that a urine flow of 20–40 ml./kg. fish/day is a more reasonable figure for the eel, than addition of the drinking rate found by Maetz & Skadhauge to the net influx would reduce the apparent P_{os}/P_d ratio of *Anguilla* to approximately 1.0.

It is obvious that while consideration of all the factors may tend to reduce the apparent P_{os}/P_d ratio of water movement across fish to near unity, the system is far too complex to treat like a single cell system, such as *Valonia* or the erythrocyte. Until all of the routes of water movement across a single species are studied or an isolated gill or skin system is proved feasible one cannot make any definite statements (from the relationship between P_{os} and P_d) about the membrane structure of the permeable surfaces of teleosts. The lack of parallelism between the permeability changes to sodium (chloride) and water tend to rule out simple, water-filled pores and the data presented here tends to substantiate this, but further studies are certainly warranted.

SUMMARY

1. Measurements were made of the flux of tritiated water across various marine, freshwater and euryhaline teleosts. The effects of temperature, body size, species differences, salinity, stress and anaesthetization were studied.

2. The Q_{10} of the flux of water across teleosts is approximately 1.90 and the flux is related to the 0.88 power of the body weight.

* N.B. Since this manuscript was completed J. Maetz (personal communication) has reported that use of colloidal gold (^{198}Au) to measure drinking rates gives a rate for *Platichthys flesus* of 51 ml./kg. fish/day. This value is certainly more in line with what would be expected from the water flux studies reported here. Maetz has also undertaken water flux studies and reports somewhat higher rate constants for *Anguilla*, *Platichthys flesus* and *Carassius*. However, if the difference in temperature (16–20° C.), body size and experimental technique is considered, the data are comparable to those reported in the present work. He also found that both the flounder and the eel are more permeable to water when acclimated to fresh water than when acclimated to sea water.

3. All of the freshwater species studied were more permeable to water than the marine species. Euryhaline teleosts appear to have about the same permeability as species to which they are most closely related.

4. While the flounder and the yellow eel are more permeable to water in fresh water than in sea water, the silver eel and the brown trout do not change their permeability and the 3-spined stickleback is less permeable to water in fresh water than in sea water.

5. While stress markedly increases the permeability to water of large brown trout, it has no effect on small brown trout and seems to decrease the water permeability of the plaice.

6. Anaesthetization has no effect on the water permeability of the goldfish but markedly increases the permeability to water of the silver eel.

7. The relationship between the flux of water and either the drinking rate in sea water or the urine flow in fresh water is discussed.

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