J. Exp. Biol. (1969), 50, 179–190
With 1 text-figure
Printed in Great Britain

SODIUM, CHLORIDE AND WATER BALANCE OF THE INTERTIDAL TELEOST, *PHOLIS GUNNELLUS*

BY DAVID H. EVANS*

Department of Biological Sciences, University of Lancaster, Lancaster, England

(*Received* 18 *June* 1968)

INTRODUCTION

Studies of the sodium, chloride and water balance of *Xiphister atropurpureus* (Evans, 1967*a–c*) showed that this intertidal teleost is relatively impermeable to these ions and to water, has an exchange-diffusion system for sodium only, and apparently lowers its permeability to sodium but not to chloride or water when acclimated to a lower salinity. An investigation of the intertidal teleost, *Pholis gunnellus*, was undertaken to determine whether the sodium, chloride and water balance of this species exhibit the same characteristics and to explore further the problem of the sodium, chloride and water balance of teleosts in general.

MATERIALS AND METHODS

Specimens of *Pholis gunnellus* were collected at Menai Bridge, Anglesey, Wales. Individuals weighed from 1 to 12 g. and were kept in 10 l. Perspex aquaria in a constant-temperature room (10 ± 1° C.) in which most experiments were performed. Fish were fed *Gammarus* sp. or *Nereis* sp. only if kept in the laboratory longer than 2 weeks. 100% sea water contained 410 mM-Na/l. and was made up by adding quantities of a sea–salt mixture (Pantin, 1959) to dilute sea water obtained from Morecambe Bay. It was observed that 20% sea water was the lowest salinity tolerated by *Pholis*, and animals were acclimated to this salinity for at least 5 days before experiments were performed. Weighings of individual fish were performed on a Mettler balance to 0·05 g. after drying with paper towelling; MS 222 (0·01%) was used for anaesthetization. Experiments were performed without regard to sex or reproductive state. All experimental values are expressed as mean ± S.E. (number of samples).

The influx of sodium (^{22}Na or ^{24}Na) was studied by loading fish in a radioactive bath of 100–200 ml. of either 100% or 20% sea water containing approximately 1 μC./ml. of radio-sodium. After 1 hr. the fish were removed and washed for from 2 to 3 min. The amount of radioactivity in the fish and in samples of the medium was determined using a Nuclear Enterprises whole-body counter. In some experiments the efflux of sodium was followed by placing fish that had been loaded in this way (for 1 hr.) into 100 ml. of the desired salinity and counting the radioactivity in the fish and in the medium after 1 hr. The 'compartmentalization' of the sodium efflux from fish acclimated to 100% sea water was studied by loading four fish to isotopic equilibrium

* Present address: Department of Biology, University of Miami, Coral Gables, Florida 33124, U.S.A.

(9 days), washing in non-radioactive medium for approximately 2 min. and then monitoring the decrease in radioactivity in the fish (using the whole-body counter) for 48 hr. The efflux of sodium from fish injected intraperitoneally with the isotope was also studied by determining the radioactivity in the fish and the efflux bath 1 hr. after injection. The injection solution consisted of 50 μl. of saline containing variable amounts of ^{22}Na or ^{24}Na, and in one series of experiments the fish were anaesthetized before injection.

The total body sodium content was determined by dissolving weighed fish in concentrated nitric acid and measuring the sodium concentration of a diluted sample on an EEL flame photometer. The method of blood collection and plasma separation has been described previously (Evans, 1967*a*). The sodium concentration of 2 μl. samples of plasma, diluted 1/500 with distilled water, was determined on an EEL flame photometer. The chloride concentration of 2 μl samples of plasma, diluted 1/2500 with distilled water, was determined on a Cotlove chloride titrator.

The fluxes of chloride (^{36}Cl) across the fish were studied using methods similar to those used for the sodium fluxes. The influx of ^{36}Cl was determined by loading the fish in a radioactive bath (100% or 20% S.W.) for 1 hr. and then killing and weighing the fish. The fish was then washed, dried and placed in a few ml. of distilled water in a glass jar and cooked for 5 min. at 15 p.s.i. in a pressure cooker. The cooked fish was then homogenized in a blender and made up to 100 ml. with distilled water. An aliquot was centrifuged and a 0·5 ml. sample of the supernatant was added to 3 ml. of NE240 scintillation fluid and counted in a Nuclear Enterprises liquid scintillation counter. An 0·5 ml. sample of the loading solution was added to the homogenate of a non-radioactive fish and made up to 100 ml. with distilled water. An aliquot was centrifuged and an 0·5 ml. sample of the supernatant was added to 3 ml. of the scintillation fluid and also counted. Comparison of this standard with a standard made by making 0·5 ml. of the loading solution up to 100 ml. with distilled water showed that there was no detectable quenching of the scintillation fluid by the presence of small quantities of fish homogenate. The total body chloride was found by titrating a sample of the supernatant from these experimental fish on a Cotlove chloride titrator. The efflux of ^{36}Cl was followed only from fish injected with the isotope. The fish were always anaesthetized before injection, and the amount injected was determined from triplicate samples of the injection solution. After 1 hr. 0·5 ml. samples of the efflux bath (100 ml.) were added to 5 ml. of a modified Bray's solution (Bray, 1960; White, 1967) and counted as before.

The influx of water across *Pholis* was studied by adding tritiated water (to a specific activity of 1 μC./ml.) to aquaria containing fish fully acclimated either to sea water or to 20% sea water. The fish were not disturbed for at least 48 hr. before the start of an experiment. At the end of 1 hr. the fish were removed, washed in non-radioactive medium for 15–30 sec. and frozen. Later, the fish were thawed, dried, weighed and a sample of water was extracted by the procedure described by Rudy (1967). Water was extracted from cut-up fish for approximately 5 min. because it was found that the specific activity of the water extracted did not vary with prolonged extraction. The radioactivity in an 0·1 ml. sample of water from the fish was compared with the radioactivity in an 0·1 ml. sample of water extracted in the same way from the loading bath.

The rate constants (K; fraction of the exchangeable sodium or chloride or water com-

partment exchanged per hour) of the influx and efflux of both ions and water were calculated using standard formulae (Evans, 1967*c*; Potts & Evans, 1967; Potts *et al.* 1967).

The effect of rapid changes in the external salinity on the sodium efflux ('exchange-diffusion effect'—Motais, Garcia Romeu & Maetz, 1966) was studied by a system designed to give immediate information of a change in the rate of efflux from the fish. The apparatus consisted of a glass cylinder large enough (50 ml.) to contain the fish, connected by a circuit of rubber tubing to an aluminium chamber fitted to a Nuclear Enterprises crystal scintillation well. The volume of the system was approximately 200 ml. and the water in the system was circulated by a peristaltic pump. The glass cylinder containing the fish was submerged in a constant temperature bath maintained at approximately 8° C. The warming of the circulating water in the system of tubing outside the constant temperature bath increased the temperature around the fish to approximately 10° C. The volume of water in the aluminium chamber was approximately 50% of the volume of the system and the change in the radioactivity passing through the chamber was recorded graphically by a Beckman Pen Recorder connected through a Nuclear Enterprises Ratemeter. The solution in the system could be changed within 30 sec. by opening the circuit of rubber tubing and pumping out the original solution while simultaneously drawing in another solution. 500 ml. of solution were flushed through when the salinity was changed and in every case the radioactivity registered in the aluminium chamber had declined to background. The speed of the pen recorder was 2·5 cm./min. and a measurable slope could be recorded two minutes after an experiment was begun. Experiments consisted of placing fish containing ^{22}Na or ^{24}Na (either loaded or injected) in the glass cylinder, filling the system with sea water and recording the slope of the increase of radioactivity in the system for from 5 to 10 min. The medium was then rapidly changed to fresh water (*ca.* 0·2 mM-Na/l.) and the slope of increase in activity was recorded for a further 10–20 min. In most cases the medium was again changed back to sea water and another slope was recorded. In other experiments the effect of anaesthetization on the efflux of sodium was examined by injecting MS222 (to make an 0·01% solution) into the system after the last transfer to sea water and recording the slope until the fish was fully anaesthetized (5–10 min.).

Experiments designed to measure the exchange-diffusion component of the flux of sodium across animals acclimated to 20% sea water and of the flux of chloride across animals acclimated to 100% sea water were performed as described previously (Evans, 1967*c*) except that fresh water was used rather than iso-osmotic mannitol solutions. A sample of the freshwater bath was taken 30 min after transfer.

The potential between the body fluids and the external medium was determined using glass capillary micro-electrodes inserted into the peritoneal cavity. The fish were anaesthetized in the usual manner and then placed ventral side up in a V-shaped holder submerged in a solution of either 100% or 20% sea water containing 0·003% MS222. The micro-electrodes were filled with 3M-KCl and had an impedence of from 5 to 15 MΩ. Two silver, silver-chloride electrodes (one connected to the glass micro-electrode and the other in the bath) were fed through a conventional cathode follower imput stage to a Techtronic 502A dual-beam oscilloscope. At least three readings of potential were recorded from each animal on 35 mm. film and the magnitude was read using a film enlarger. Potentials could be determined by this method to an accuracy of ± 1 mV.

RESULTS AND DISCUSSION

The regulation of the sodium and chloride content of the whole body and the plasma in 100% sea water and 20% sea water is shown in Table 1. It is clear that *Pholis* does not regulate its plasma concentration in lower salinities to the degree that *Xiphister* does. This is probably why *Pholis* cannot tolerate salinities below 80 mM-Na/l. while *Xiphister* lives well in 50 mM/l (Evans, 1967*a*). The difference between the decline in the total body chloride and the decline of the plasma chloride must be due to loss of this ion from the intracellular spaces.

Table 1. *Body and plasma ion content in* 100% *and* 20% *sea water*

	Total body content (mM/kg. fish)		Plasma concentration (mM/l.)	
	Sodium	Chloride	Sodium	Chloride
100% sea water	43 ± 1 (9)*	42 ± 1 (12)	176 ± 2 (8)	156 ± 2 (5)
20% sea water	27 ± 4 (7)	20 ± 1 (7)	104 ± 4 (7)	112 ± 4 (5)

* Mean ± S.E. (number of samples).

Table 2. *The rates of influx* (K_i) *and efflux* (K_e) *of Na, Cl and water across* Pholis *in* 100% *and* 20% *sea water*

	K_i	K_{e-1}	K_{e-2}	K_{e-3}
		100% sea water		
Na	0·13 ± 0·01* (28)	0·20 ± 0·01 (18)	0·36 ± 0·03 (8)	0·20 ± 0·01 (7)
Cl	0·042 ± 0·006 (11)	—	—	0·084 ± 0·015 (8)
H_2O	0·13 ± 0·01 (22)	—	—	—
		20% sea water		
Na	0·012 ± 0·004 (12)	0·114 ± 0·004 (9)	—	0·012 ± 0·001 (4)
Cl	0·012 ± 0·003 (7)	—	—	0·026 ± 0·005 (6)
H_2O	0·11 ± 0·01 (21)	—	—	—

* All values are rate constants, hr^{-1}. Mean ± S.E. (number of fish). $e-1$ is the efflux from fish loaded with the isotope for 1 hr. $e-2$ is the efflux from fish injected with the isotope *without* prior anaesthetization. $e-3$ is the efflux from fish injected with the isotope *with* prior anaesthetization.

The rate constants of the sodium, chloride and water-influxes (K_i) and effluxes (K_e) across *Pholis* are presented in Table 2. It is obvious that the value for K depends to a great extent on how it was measured. K_e for sodium and chloride in 100% sea water is always greater than K_i. This has also been found in other species of teleosts (*Platichthys flesus* (Motais, 1967), *Fundulus heteroclitus* (Potts & Evans, 1967)). Potts & Evans (1967) have described why K_i for sodium best approximates to the flux expressed as a fraction of the total body sodium, while K_e for sodium probably approximates to the flux expressed as a fraction of the fast sodium compartment alone (except in cases where the animal has been loaded to isotopic equilibrium).

It appears that injection of the radio-sodium will give a K_e equivalent to that from animals loaded with the isotope only if the animal is anaesthetized before the injection, i.e. $K_{e-2} \gg (K_{e-1} = K_{e-3})$. K_{e-3} for sodium is nearly identical to the K_e of *Xiphister* (Evans, 1967*c*). The flux of chloride in 100% sea water is much below that of sodium. In this respect *Pholis* resembles *Xiphister* (Evans, 1967*c*) and *Platichthys*

(Motais, 1967), but is different from *Fundulus* (Potts & Evans, 1967) and the elasmobranch *Scyliorhinus* (Maetz & Lahlou, 1966), where the chloride flux is greater than the sodium flux. K_{e-3} for chloride in 100% sea water is approximately half the K_e from *Xiphister*.

In fish acclimated to 20% sea water the extremely high K_{e-1} for sodium (from animals loaded with the isotope) is almost certainly due to surface contamination. Small quantities of isotope adhering to the fish after washing would alter greatly a very small K_e. This explanation is borne out by the low efflux from injected fish (K_{e-3}), which is very similar to the K_e from *Xiphister* (Evans, 1967*c*). The similarity between K_{e-3} and K_i of sodium in 20% sea water is probably due to the fact that the flux across the surface of the animal is so small that incomplete mixing of the isotope within the fish does not affect the apparent efflux. The K_i of chloride in 20% sea water is the same as the K_i of sodium; while K_{e-3} of chloride is greater than the K_{e-3} of sodium. This difference between K_e for Na and Cl was also found in *Xiphister* (Evans, 1967) and *Fundulus* (Potts & Evans, 1967) and may be due to differences in the 'compartmentalisation' of the ions in the body. It is interesting that even at this relatively high salinity (82 mM-Na/l.) the K_i (approximately 0·01) of sodium and chloride is equivalent to that found for other euryhaline teleosts in fresh water (Motais, Garcia Romeu & Maetz, 1966; Potts & Evans, 1967; Potts *et al.* 1967).

It might be argued that the K_e from fish injected with the isotope (with prior anaesthetization) is limited in the first hour after injection by uptake of the isotope from the peritoneum. This possibility can be safely ruled out because the efflux from injected fish (as long as they are anaesthetized first) is linear for from 4 to 5 hr. In addition, 15 min. after injection only from 15 to 30% (3 animals) of the injected radioactivity can be washed out of the peritoneum. After 60 min. only 7–10% (3 animals) of the injected radioactivity could be found in the peritoneal fluid.

The flux of tritiated water across *Pholis* is very similar to the water flux across *Xiphister* (Evans, 1967*c*). The apparent decline in 20% sea water is probably not significant ($P < 0{\cdot}05 > 0{\cdot}02$). Since it can be shown (Evans, 1967*c*) that approximately 99% of the gross water flux is due to osmosis it appears that *Pholis* does not change its permeability to water when acclimated to a lower salinity. This is also true for *Xiphister* (Evans, 1967*c*) but Potts *et al.* (1967) found that the water permeability of *Tilapia mossambica* was highest in fresh water; J. Maetz (personal communication) has reported similar results for the water flux across *Anguilla*.*

The efflux of fish loaded to isotopic equilibrium is plotted in Fig. 1. Like similar data from other animals (Rudy, 1966, 1967; Dunson, 1967; Motais, 1967; Potts & Evans, 1967; Potts *et al.* 1967) the curve can be analysed graphically. The following equation describes the curve:

$$A_t = A_0 (0{\cdot}65e^{-0{\cdot}154t} + 0{\cdot}35e^{-0{\cdot}056t})$$

where A_t is the activity in the fish at any time t and A_0 is the initial activity in the fish.

* *Note added in proof.* It has recently been found (Evans, D. H. (1969). Studies on the permeability to water of selected marine, fresh water and euryhaline teleosts. *J. exp. Biol.* (in the press)) that fresh water teleosts are generally more permeable to water than marine teleosts and that while the flounder and the yellow eel increase their permeability to water when acclimated to fresh water, the silver eel does not and the three-spined stickleback decreases its water permeability when acclimated to fresh water.

This equation simply states that 65% of the total body sodium exchanges at a rate of 15% per hour while 35% of the total body sodium exchanges at a rate of 5·6% per hour. It appears that the fast phase is kinetically external to the slow phase and is the compartment that has the highest specific activity after a short-term load or injection of isotope.

It is not known where the fast and slow compartments of sodium are in the fish. The sodium content of the fast compartment (65% of 42 mM/kg. fish, or 27 mM/kg. fish) is 30% greater than the size of the extracellular pool of sodium (20 mM/kg. fish) of *Xiphister* (Evans, 1967*a*). Potts *et al.* (1967) postulate that it may be tissue differences rather than cellular membranes that result in a two-compartment sodium system within the fish. They feel that the slow sodium pool is contained in the somatic muscles that have a poor blood supply.

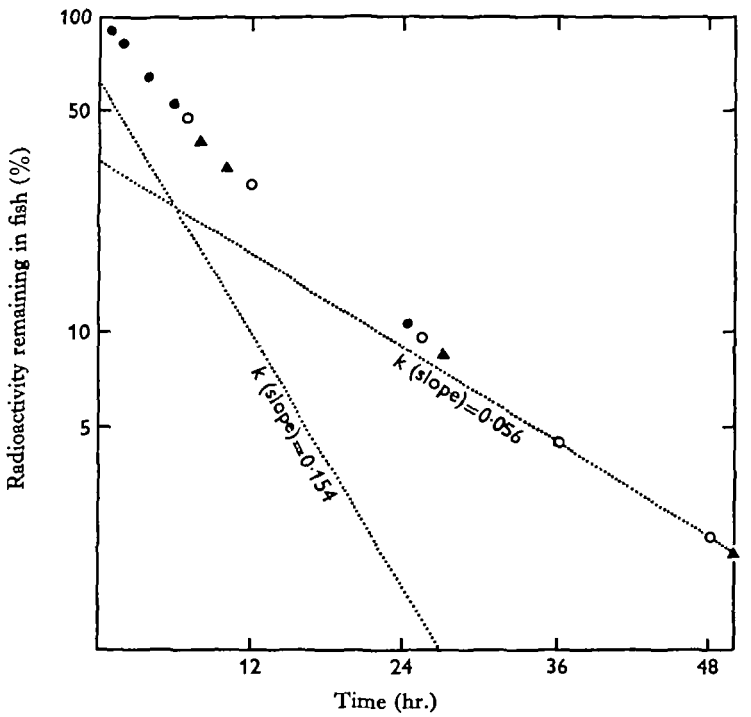

Fig. 1. The efflux of Na from fish loaded to isotopic equilibrium. ● is the mean of four fish, ○ is the mean of three fish, and ▲ are the data from a single fish.

The efflux from the total body sodium is the sum of the products of the K's and the pool sizes (0·65) (0·15) + (0·35) (0·06) and in *Pholis* amounts to 0·120 per hour which is certainly very close to K_t for sodium in Table 2. This supports the assumption (Potts & Evans, 1967) that the K_t of a short-term influx best approximates to the K of the total body sodium.

The sodium efflux from fish acclimated to 100% sea water, loaded with the isotope and placed in the exchange-diffusion system described, was reduced to 39 ± 1% (13) of the original flux when the medium was changed to fresh water. This is below the value found for *Xiphister* (53%; Evans, 1967*c*). (If the isotope was injected intra-

peritoneally without prior anaesthetisation the flux declined to $93 \pm 6\%$ (12) of the sea water level. It appears therefore that the shock of injection without prior anaesthetization increases the efflux of sodium due to diffusion and/or active transport without increasing the exchange-diffusion component to the same degree.) When the medium was changed back to sea water the flux returned to only $80 \pm 1\%$ (13) of the original flux in sea water. Thus, it seems that even after only 10–20 min. in fresh water a relatively irreversible change has taken place. It is not known whether this change is due to an actual reduction of the flux as a result of a decline in permeability or active efflux or to a breakdown of some part of the exchange-diffusion system. The latter is possible because it has been shown that *Platichthys* acclimated to fresh water (Motais, 1967) and *Xiphister* acclimated to 10% sea water (Evans, 1967*c*) have lost the ability to 'turn on' an exchange-diffusion component when placed in sea water. Addition of MS222 to the sea-water system flowing past the fish reduced the efflux to $95 \pm 2\%$ (5) of the flux across unaesthetized animals. It therefore appears that short-term anaesthetisation has little immediate effect on the flux of sodium across the fish. Middler, Kleeman & Edwards (1968) have found that MS222 does not affect the sodium balance of *Bufo marinus*.

The flux of sodium across animals acclimated to 20% sea water does not contain an exchange-diffusion component. When the medium was changed to fresh water the flux remained at normal levels ($113 \pm 8\%$ s.w. level, 8 animals). This corroborates the lack of an exchange-diffusion component of the sodium flux across *Xiphister* acclimated to 10% sea water (Evans, 1967*c*). These data indicate that, at least in two intertidal blennies, the exchange-diffusion component requires an appreciable sodium content in the medium before it begins to function.

The chloride flux across fish acclimated to 100% sea water does not decline appreciably ($93 \pm 13\%$ sea water level, 9 fish) when the medium surrounding the fish is changed to fresh water. It appears, therefore, that like *Xiphister* (Evans, 1967) *Pholis* does not possess a chloride exchange-diffusion system.

Since the sodium and chloride concentrations of both the plasma and the medium are known in both salinities one can calculate the electrical potential that would be found across *Pholis* if it is assumed that the fish is permeable only to the single ion species in question (Na or Cl) and that this ion species has distributed itself in passive equilibrium on both sides of the membrane. This is done by means of the Nernst equation. If the membrane (in this case the fish) is impermeable to both ions, or both ions are actively transported against the concentration gradient then the measured potential will be close to zero. On the other hand, if one ion is in passive equilibrium and the other ion is either actively transported or does not pass through the membrane then the measured potential will be close to the Nernst potential for the first ion.

Table 3 compares the electrical potential expected from the Nernst equation (E_{Na} and E_{Cl}) with the potential actually measured across *Pholis*. The magnitude of the measured potential and the presence of a sign change corroborates the only other data on the electrical potential across teleosts (House, 1963; Maetz & Campanini, 1966). Sodium appears to be nearly in passive equilibrium; the fact that the measured potential for sodium in 100% sea water is slightly less than the Nernst potential for sodium may indicate that there is some active extrusion of this ion. Chloride certainly appears to be actively pumped out in 100% sea water and pumped inward in 20% sea

water. The alternative hypothesis, that the fish is impermeable to chloride, is ruled out from the flux data in Table 2. The presence of a diffusion potential caused by the more rapid diffusional influx of sodium than of chloride in 100% sea water and the more rapid diffusional efflux of sodium than of chloride in 20% sea water cannot be ruled out with the available data. The calculations discussed subsequently (see Table 4) do indicate that some of the measured potential across *Pholis* may be due to a diffusion potential.

Table 3. *Comparison of the Nernst potentials for sodium and chloride with the potential measured across* Pholis

E_{Na} (mV.)	E_{Cl} (mV.)	$E_{measured}$ (mV.)
	100% sea water	
+20·5*	−27·3	+18 ± 1 (5)†
	20% sea water	
−4·41	+4·86	−6 ± 1 (5)

* Sign convention—inside of fish with respect to outside medium.
† Mean ± S.E. (number of samples).

An active chloride pump has also been described for the squid giant axon (Keynes, 1962), the muscle fibres of the locust, cockroach (Wood, 1965; Usherwood, 1967) and moth (Huddart, 1967), the skin of the frog *Leptodactylus* (Fischbarg, Zadunaisky & De Fisch, 1967), the gastric mucosa, (Rehm, 1966), the frog cornea (Zadunaisky, 1966), the turtle bladder (Gonzalez, Shamoo & Brodsky, 1967) and the gill of the crayfish (Croghan, Curra & Lockwood, 1965). It has been shown that at least in fresh-water goldfish (Garcia Romeu & Maetz, 1964) and in *Leptodactylus* (Salibian, Pezzani-Hernandez & Garcia Romeu, 1968) chloride is exchanged for bicarbonate ions while sodium is exchanged for ammonium ions. Since the chloride pump in *Pholis* appears to be electrogenic it is obvious that the exchange in this species is not one-for-one.

The preceding data can be used to construct a model for the sodium and chloride balance of *Pholis*. The calculations are similar to those described for *Xiphister* (Evans, 1967*c*) except that in this case a correction can be applied for the effect of the electrical potential on the ion fluxes across the fish. This correction factor is calculated from the graph of the relative flux against potential from Potts & Parry (1964). Enteric losses of sodium and chloride are considered to be minimal and therefore are not included in the calculations. The drinking rate in 100% sea water (12·3 ml/kg fish/day) is taken from earlier data (Evans, 1968). Urine losses of sodium and chloride in both salinities and oral influx in 20% sea water are taken from the data on *Xiphister* because the part played by these routes is small and the other components of the fluxes across *Pholis* are so similar to those across *Xiphister* that transfer of data seems reasonable. The computation of the sodium balance in 100% sea water will suffice to outline the method used. If the total influx of sodium in 100% sea water is 52·7 mM/kg. fish/day (K_1 × non-exchange-diffusion flux × total body sodium × 24 hr.) and the drinking influx is 5·1 mM/kg. fish/day (12·3 ml./kg. fish/day × 410 μM/ml. sea water), the 'diffusional' influx of sodium is 47·6 mM/kg. fish/day. This 'diffusional' influx is actually less than the true diffusional influx because there is an electrical potential of 18 mV. (inside

positive) across the fish tending to retard the passive entry of sodium ions. The correction factor from Potts & Parry (1964) is 1·35 so the true diffusional influx of sodium would be equal to 64·3 mM/kg. fish/day if there were no potential present. The ratio of the true diffusional efflux to the true diffusional influx is assumed to be equal to the ratio of the sodium concentration of the blood to the sodium concentration of the sea-water bath. The diffusional efflux (in the absence of an electrical potential) is therefore 176/410 × 64·3 or 27·7 mM/kg. fish/day. An 18 mV. potential (inside positive) increases the diffusional efflux to 37·4 mM/kg. fish/day. The efflux of sodium via the urine is taken (from *Xiphister*) to be only 0·5 mM/kg. fish/day. The remainder of the efflux is therefore 14·8 mM/kg. fish/day, and must be active. The components of the sodium and chloride balance in both salinities are presented in Table 4.

Table 4. *The components of the Na and Cl balance of* Pholis gunnellus

Influx			Efflux	
Na	Cl		Na	Cl
		100% s.w.		
52·7*	42·3	Total flux†	52·7	42·3
5·1	5·9	Drinking	—	—
—	—	Urine	0·5	0·5
47·6	36·4	Diffusion and potential‡	37·4	5·6
64·3	25·1	Diffusion§	27·7	8·1
—	—	Active	14·8	36·2
		20% s.w.		
8·07	5·76	Total flux	8·07	5·76
0·14	0·16	Drinking	—	—
—	—	Urine	0·50	0·50
7·22	3·73	Diffusion and potential‡	7·57	5·26
6·56	4·10	Diffusion§	8·32	4·78
0·69	1·87	Active	—	—

* All values are in mM/kg. fish/day.
† Total flux is that amount not due to exchange-diffusion.
‡ The 'diffusional flux' when no correction is made for an electrical potential.
§ The true diffusional flux calculated by correcting for an electrical potential.

Examination of the various components shows that in both salinities the diffusional flux of sodium is greater than the diffusional flux of chloride. One would expect the reverse from consideration of the size of the hydrated ions. If the permeability to sodium or chloride is taken as being proportional to the ratio of the diffusional efflux or influx to the concentration of the ion in the blood or medium respectively, it can be calculated that in 20% sea water the permeability to sodium is only 51% of the sea-water level while the chloride permeability is 82% of the sea-water level. A lowering of the body's permeability to sodium has now been described for four species of teleosts (*Xiphister atropurpureus*, Evans (1967); *Fundulus heteroclitus*, Potts & Evans (1967); *Tilapia mossambica*, Potts *et al.* (1967); and *Pholis gunnellus* (present investigation)). *Xiphister* did not seem to show a decline in chloride permeability, but if it is assumed that there is a small potential across *Xiphister* in 10% sea water (for instance, 6 mV, inside negative) then the calculated chloride permeability does decline and is 92% of that expected.

It has already been shown that in *Pholis* the permeability to water probably does not decline with salinity. It is interesting to note that if the water fluxes are considered to be significantly different in the two salinities (see Table 2) then the permeability to water declines to 85% of the permeability of fish acclimated to 100% sea water. This decline in water permeability is very similar to the decline in chloride permeability but much below that for sodium.

If the total body water is assumed to be 75% of the body weight then the water flux in 100% sea water amounts to 2340 ml. H_2O/kg. fish/day (750 ml. H_2O/kg. fish × 0·13 × 24 hr.). If the fish is assumed to have a total ionic concentration of 0·3 osmoles/kg. water and sea water is taken as 1·0 osmoles/kg. water then the mole fractions of water in the fish and the medium are 0·994 and 0·982 respectively. The net flux of water will therefore be equivalent to the difference between the mole fractions, or 0·012 of the gross water flux. Thus, the net flux of water (outward) in fish acclimated to 100% sea water will be 28·1 ml./kg. fish/day. This is more than twice the drinking rate of *Pholis* (Evans, 1968). Similar calculations of the net flux of water across *Pholis* acclimated to 20% sea water indicate that it is approximately equal to the urine flow that would be expected in this salinity (4–6 ml./kg. fish/day, calculated from the data on *Xiphister*). Since the drinking rate in 100% sea water and the urine flow in 20% sea water are measures of the osmotic permeability it appears that like *Xiphister* (Evans, 1967*c*), *Tilapia* (Potts *et al.* 1967) and the marine alga *Valonia* (Gutknecht, 1967) but unlike some decapod crustacea (Rudy, 1967) and frog skin (Dainty & House, 1966) the osmotic permeability is approximately equal to the diffusional permeability as measured by the flux of tritiated water. It therefore seems that the flux of water across *Pholis* is not affected by bulk flow of water through pores or by an unstirred layer.

The simplest explanation for the somewhat anomalous data on the sodium, chloride and water balance of *Pholis* is that the passive movements of sodium, chloride and water are independent of one another and do not take place through uncharged, water-filled pores. This conclusion is supported by three independent lines of evidence: (1) The osmotic permeability to water is approximately equal to the diffusional permeability. (2) Sodium ions 'diffuse' across *Pholis* faster than chloride ions. (3) The permeability changes in lowered salinity are not equivalent. The independence of permeability to ions and water has also been described for hormonal and detergent effects on amphibian skin and bladder (Bourguet & Maetz, 1961; Tercafs & Schoffeniels, 1961; Köver, Tercafs & Schoffeniels, 1963; Maetz, 1963; Bentley & Heller, 1964; Zadunaisky & de Fisch, 1964).

SUMMARY

1. Measurements were made of the regulation of the body ionic content, the fluxes of Na, Cl and water and the electrical potential across the intertidal teleost, *Pholis gunnellus* in 100% (410 mM-Na/l.) and 20% sea water.

2. The rates of the ion flux depended on the method of measurement. The flux of Cl is much below the flux of Na in 100% sea water. In 20% sea water the fluxes of Na and Cl are the same.

3. In 100% sea water only the Na flux has an exchange-diffusion component. There is no exchange-diffusion component of the flux of either ion in 20% sea water.

4. The electrical potential across *Pholis* changes from 18 mV. (inside positive) in 100% sea water to 6 mV (inside negative) in 100% sea water. Comparison with the Nernst potentials indicate that Cl is actively transported in both salinities while Na may possibly be actively transported in 100% sea water.

5. In 20% sea water the permeability to Na decreases to 51% of the permeability in 100% sea water, while the Cl permeability decreases to 82% and the water permeability remains at the sea-water level.

6. In both salinities the rate of diffusion of Na ions is greater than the rate of diffusion of Cl ions.

7. The osmotic permeability to water is approximately equal to the diffusional permeability to water in both salinities.

8. It is concluded that the passive movements of Na, Cl and water must be independent of each other and not via uncharged, water-filled pores.

This work was supported by a Public Health Service fellowship, 5-F2-GM-28, 367-02, from the National Institute of General Medical Sciences. Appreciation is expressed to Professor W. T. W. Potts for reading and discussing the manuscript. *Pholis gunnellus* were collected by Roger Smith and the electrical potential measurements were made in collaboration with Dr H. Huddart.

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