J. exp. Biol. (1979), 83, 181–192
With 4 figures
Printed in Great Britain

IONIC REGULATION IN RAINBOW TROUT (*SALMO GAIRDNERI*) ADAPTED TO FRESH WATER AND DILUTE SEA WATER

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(*Received 4 January* 1979)

SUMMARY

Small rainbow trout (5–20 g) adapt to salinities of up to at least 22‰ but not to full strength sea water. In adapted fish plasma ions are regulated near the fresh water values, but there is an ionic invasion of the tissues, particularly by Cl^- in muscle cells. Analysis of ionic regulation in adapted fish indicates that balance is maintained mainly by expending energy on Cl^- regulation. Fish in full strength sea water can no longer regulate Na^+ or Cl^- in plasma or tissues, which results in high tissue concentrations of these ions and eventual death.

INTRODUCTION

Like other salmonids, mature rainbow trout are euryhaline and readily adapt to sea water. The physiology of sea water adaptation has been discussed by Hoar (1976) and Holmes & Donaldson (1969) while the earlier literature has been reviewed by Parry (1966). Three important points emerge for rainbow trout: (i) Larger individuals adapt more easily to sea water than smaller ones. (ii) Survival is greatly improved if the fish are first preadapted to dilute sea water. (iii) Adaptation to sea water is accompanied by an initial adjustment period lasting 3–4 days when the fish has to tolerate increased plasma and body ion concentrations (Houston, 1959).

Much of the work on sea water adaptation has been upon various salmon species, many of which, unlike rainbow trout, metamorphose from parr to smolt before entering sea water. Atlantic salmon parr and rainbow trout of similar size (10–20 g) are unable to withstand direct transfer to sea water, while smolts survive the transition (Parry, 1958). Ionic regulation in Atlantic salmon parr and smolts was studied in some detail by Potts, Foster & Stather (1970). Smolts in fresh water had low Na^+ and Cl^- turnover rates which increased tenfold after 1 day in sea water. Parr and early smolts showed smaller increases and, as was shown earlier by Parry (1960), they were less tolerant of full strength sea water.

In the present work ionic regulation of small rainbow trout adapted to various dilutions of sea water was studied by measuring a variety of parameters, including salt turnover, drinking rate, and ion concentrations.

MATERIALS AND METHODS

Rainbow trout (average weight 10·3 g) were obtained from a local hatchery and kept in dechlorinated water at 10 ± 1 °C. The were allowed to settle for several weeks and were fed commercial trout pellets except during experiments.

Sea water (SW) of 33‰ salinity was collected from the North Sea and diluted with dechlorinated water to give $\frac{1}{3}$ SW (11‰) and $\frac{2}{3}$ SW (22‰). Fish were adapted to $\frac{1}{3}$ SW for 10 days, then transferred to $\frac{2}{3}$ SW for a further 10 days and finally to full strength SW. Weight; length; haematocrit; plasma Na^+, K^+ and Cl^- concentrations; body and muscle water; and Na^+, K^+ and Cl^- contents of body and muscle were measured for fish in fresh water (FW), $\frac{1}{3}$ SW, $\frac{2}{3}$ SW and SW.

Fish were killed by a blow to the head and the tail amputated so that blood could be collected from the caudal artery into heparinized capillary tubes which were centrifuged to determine haematocrit and then deep frozen for subsequent analysis of plasma ions. A muscle sample was taken from the dorsal block anterior to the dorsal fin, scraped clean of skin and weighed to the nearest milligram, as was the remainder of the fish. At this stage the fish were divided into two groups; the first was dried in an oven at 108 °C to constant weight for determination of water content while the second was homogenized and disrupted ultrasonically in a known volume of distilled water to liberate readily exchangeable ions. These were eluted for 1 week at 4 °C, when the sample was centrifuged for ionic analysis of the supernatant.

Plasma and supernatant were diluted with distilled water to measure Na^+ and K^+ concentrations using an Eel 100 flame photometer and Cl^- was determined with a Buchler–Cotlove ampiometric titrator.

Potentials were determined as described by Potts & Eddy (1973) and Bath & Eddy (1979). After inserting the peritoneal cannula the fish were allowed to recover for at least 20 h before continuous recordings were made using a Bryans–Southern 28000 pen recorder.

Na^+ and Cl^- effluxes. ^{22}Na and ^{36}Cl were obtained from the Radiochemical Centre, Amersham. The radio-isotopes were mixed with trout saline to give a solution with a specific activity of approximately 20 μCi ml^{-1} of each. Fifty μl was injected parenterally into each fish which was then placed in 750 ml of appropriate constantly aerated medium. Two ml samples were removed at known intervals. ^{22}Na activity was determined using a Packard 5250 automatic γ spectrometer and total β activity with a Tricarb Corumatic liquid scintillation counter. ^{36}Cl activity was obtained by subtracting the ^{22}Na β activity previously derived by γ counting. A sample of the injection mixture was similarly assayed to determine the exact activity injected.

The rate constant, K_{eff}, was determined graphically using the equation

$$K_{eff} \cdot t = \ln \left[\frac{C_{eq} - C_t}{C_{eq}} \right],$$

where t is time, C_{eq} counts in the medium at time t (Motais, 1967). K_{eff} is the slope of the line, here fitted by regression analysis, obtained when $(C_{eq} - C_t/C_{eq})$ is plotted against t.

Drinking rate was determined by the method of Evans (1968) using ^{125}I-labelled PVP.

Inulin space. This was measured by injecting ^{14}C-labelled inulin into the peritoneal

cavity, allowing 24 h for equilibration throughout the body and then sampling plasma, muscle and whole body (as described earlier). The ^{14}C activity of the samples was determined by liquid scintillation counting. Inulin space, In_{sp}, can be calculated by the equation

$$In_{sp} = \frac{^{14}C \text{ per ml sample water}}{^{14}C \text{ per ml plasma water}} \times 100.$$

Ion spaces. Na^+_{sp}, Cl^-_{sp}, and chloride–potassium space ($Cl\text{–}K_{sp}$) were calculated using the mean values shown in Table 1 via equations given by Holmes & Donaldson (1969) and Conway (1957).

Intracellular water and ions. IC_{H_2O} and IC_{ion} respectively, were calculated from the mean values in Table 1 using the equations:

(i) IC_{H_2O} = total tissue − inulin space water.

(ii) $$IC_{ion} = \frac{\text{total tissue ion} - \text{extracellular ion content}}{1 - \text{extracellular water content}}.$$

Extracellular ion concentration was taken as:

$$In_{sp} \times \text{plasma ion} \times \text{Donnan* equilibrium concentration factor.}$$

RESULTS

Rainbow trout used in the experiments survived indefinitely in FW, $\frac{1}{3}$ SW, and $\frac{2}{3}$SW but survival in SW averaged 5 days. Ionic composition of trout adapted to various salinities is shown in Table 1; SW values refer to fish which had been in this medium for 4 days. Full adaptation to $\frac{1}{3}$ and $\frac{2}{3}$ SW is indicated by constant plasma ion concentrations and by well regulated body and muscle water content. Fish in SW show breakdown of osmotic and ionic regulation; there being large increases in plasma ion concentrations and significant dehydration of both body and muscle.

Whole body ions showed a modest increase in concentration in salinities up to $\frac{2}{3}$ SW, while muscle ions, especially Cl^-, showed rather larger increases. Further evidence of failure to maintain ionic balance in SW fish is shown by massive increases of all ions in both whole body and muscle.

A similar situation was observed for body and muscle ion spaces, where the greatest increases were observed in muscle Cl^- and ($Cl\text{–}K_{sp}$), especially at the highest salinity. In contrast inulin space showed a slight decrease with salinity.

Ion contents calculated for the intracellular compartment are also shown in Table 1. With increasing salinity the values for muscle increase rather more than those for total body and the greatest increase was observed for Cl^- content of muscle cells.

Blood haematocrit was variable and there was no significant difference between the values recorded in the various salinities.

Drinking rates are shown in Fig. 1. FW fish drank approximately 0·5 ml kg^{-1} h^{-1}, rather lower than 1·3 ml kg^{-1} h^{-1} recorded for salmon smolts (Potts *et al.* 1970), but in contrast to the results of Shehedah & Gordon (1969) where no drinking was observed in FW rainbow trout. Drinking rate increased to 2·5 ml kg^{-1} h^{-1} in $\frac{1}{3}$ SW and 4 ml kg^{-1} h^{-1} in $\frac{2}{3}$ SW which are similar to the values reported by Shehedah & Gordon

* As indicated by Manery (1954).

Table 1

Parameter	Units	FW adapted	⅓ SW adapted	⅔ SW adapted	SW adapted
Salinity					
Na^+	mM/l	0·22	148	296	444
Cl^-	mM/l	0·30	189	378	567
Plasma					
Na^+	mM/l	137·78 ± 3·21 (20)	134·78 ± 5·49 (9)	139·30 ± 4·27 (16)	191·11, 200·99, 157·66
Cl^-	mM/l	111·52 ± 3·19 (20)	109·48 ± 2·83 (17)	108·25 ± 3·36 (16)	158·08, 160·95, 135·31
K^+	mM/l	11·26 ± 0·76 (12)	12·56 ± 0·91 (12)	12·90 ± 1·61 (8)	40·65, 44·38, 24·4
Water					
Total body	g/100 g wet wt	77·3 ± 0·31 (12)	75·60 ± 0·39 (6)	78·68 ± 0·47 (6)	73·58 ± 1·23 (4)
Muscle	g/100 g wet wt	78·50 ± 0·78 (12)	76·76 ± 0·97 (6)	78·15 ± 0·69 (6)	70·57 ± 1·07 (4)
Plasma	g/100 g plasma	92·63 ± 0·97 (6)	94·33 ± 0·97 (6)	93·44 ± 0·97 (6)	(94)*
Ions					
Total body Na^+	m-mole/kg body H_2O	52·16 ± 2·04 (12)	59·14 ± 3·86 (6)	69·01 ± 4·44 (6)	115·05 ± 10·93 (4)
Total body Cl^-	m-mole/kg body H_2O	40·39 ± 1·93 (12)	49·73 ± 1·23 (6)	53·94 ± 2·02 (6)	101·35 ± 10·92 (4)
Total body K^+	m-mole/kg body H_2O	107·39 ± 6·05 (12)	112·28 ± 10·89 (6)	118·61 ± 3·57 (6)	159·25 ± 16·07 (4)
Muscle Na^+	m-mole/kg muscle H_2O	28·96 ± 1·45 (12)	48·62 ± 6·57 (4)	49·35 ± 3·96 (6)	106·36 ± 7·04 (4)
Muscle Cl^-	m-mole/kg muscle H_2O	22·91 ± 2·91 (12)	43·11 ± 6·89 (4)	49·92 ± 3·23 (6)	164·75 ± 3·59 (4)
Muscle K^+	m-mole/kg muscle H_2O	123·41 ± 10·79 (12)	121·03 ± 11·05 (6)	149·12 ± 1·90 (6)	169·51 ± 12·49 (4)
Compartments					
(i) Total body					
Inulin space	ml/100 ml muscle H_2O	25·94 ± 1·035 (5)	21·46 ± 1·081 (5)	22·68 ± 1·334 (6)	(20)*
Na space	ml/100 ml muscle H_2O	37·23	43·94	49·14	62·65
Cl space	ml/100 ml muscle H_2O	32·78	41·86	45·49	61·38
([redacted])	ml/100 ml muscle H_2O	28·01	37·52	41·84	66·53

[redacted]Muscle					
Inulin space	ml/100 ml body H_2O	9·33 ± 0·386 (5)	7·98 ± 1·186 (5)	7·36 ± 0·634 (6)	(7)*
Na space	ml/100 ml body H_2O	20·69	36·12	35·14	57·92
Cl space	ml/100 ml body H_2O	18·59	36·29	42·10	99·81
(Cl–K_{ep})	ml/100 ml body H_2O	13·58	33·16	39·56	105·1
Cellular Compartment					
(i)Total body					
Cellular water	% wet wt.	57·25	59·38	60·84	(58·9)*
Cellular water	% body water	74·06	78·54	77·32	(80)*
Cellular Na	m-mole/kg cellular H_2O	18·33	36·26	45·52	95·14
Cellular Cl	m-mole/kg cellular H_2O	12·37	31·61	35·78	86·24
Cellular K	m-mole/kg cellular H_2O	140·75	139·31	149·39	189·36
(ii) Muscle					
Cellular water	% wet wt	71·18	70·64	72·40	(65·6)*
Cellular water	% muscle water	90·67	92·02	92·64	(93)*
Cellular Na	m-mole/kg cellular H_2O	16·64	40·45	41·54	99·71
Cellular Cl	m-mole/kg cellular H_2O	13·10	36·79	44·77	165·01
Cellular K	m-mole/kg cellular H_2O	134·86	130·37	159·86	179·35
Transbranchial potential	mV	−0·364 ± 0·73 (9)	+2·38 ± 0·21 (7)	+1·075 ± 0·75 (4)	+1·78 ± 1·094 (6)
Blood haematocrit	%	25·29 ± 1·72 (24)	31·95 ± 1·35 (22)	24·39 ± 1·33 (24)	—

Ionic content of plasma, total body and muscle of rainbow trout adapted to FW and various salinities. Data relating to the distribution of these ions in the tissues is included together with inulin space, blood haematocrit and transbranchial potential.

(*a*) * values in the SW column are not measured but are estimates.
(*b*) values for ion spaces and intracellular ion content have been calculated using the mean values for ion content of the appropriate tissue.
(*c*) transbranchial potentials for SW adapted fish refer to fish which had been in SW for 15 h.

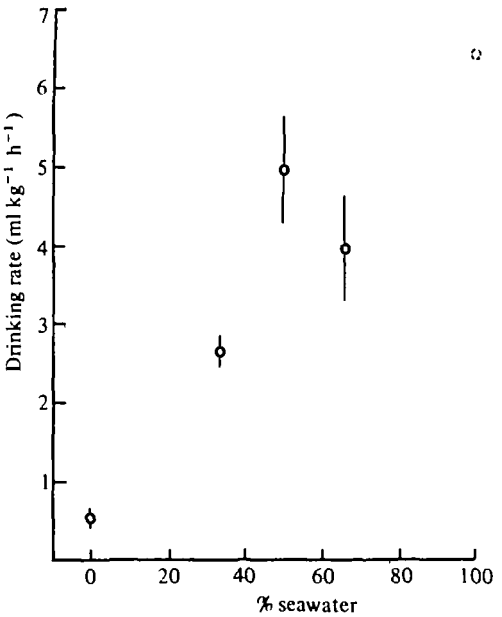

Fig. 1. Drinking rates for rainbow trout adapted to fresh water, ¼ SW, ½ SW and ¾ SW. The SW value is an estimate based on the values given by Shehedah & Gordon (1969). Mean values ± 1 S.E. At least six fish were used at each salinity.

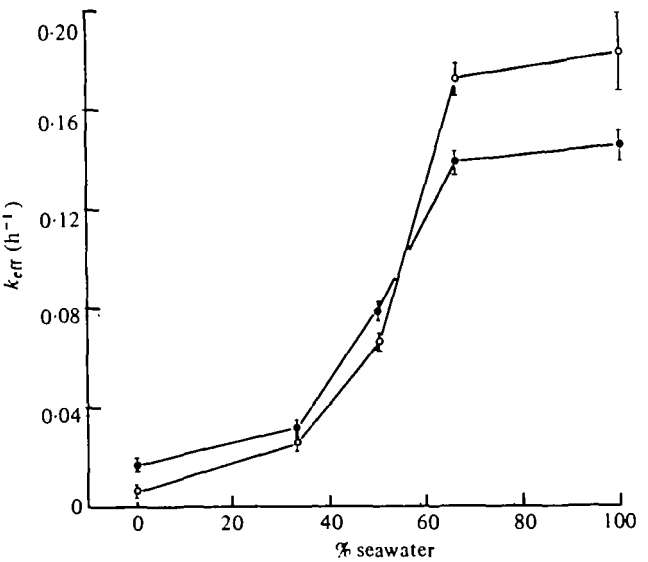

Fig. 2. Na^+ (●) and Cl^- (○) efflux constants (K h^{-1}) for fish adapted to FW, ¼ SW, ½ SW, ¾ SW and SW. Mean and standard error are indicated. For each salinity at least six fish were used.

Table 2. *Salt balance in rainbow trout adapted to various salinities*

It has been assumed that branchial efflux is balanced by branchial influx plus drinking, and faecal and urinary losses are negligible. Values in m-mole kg^{-1} h^{-1}.

	Na^+			Cl^-		
Medium	Efflux	Drinking	Influx	Efflux	Drinking	Influx
FW	0·6910	0·0001	0·6909	0·2135	0·0001	0·2134
⅓SW	1·5569	0·264	1·2929	1·004	0·337	0·667
⅔SW	7·619	0·95	6·67	7·566	1·21	6·353
SW	12·73	2·31	10·42	14·01	2·95	11·06

Salt absorption by the gut is based on values given by Shehedah & Gordon (1969), 80% in FW, 66% in ⅓ SW, 80% in ⅔ SW and 80% inSW.

(1969). It is of interest to note that drinking increases in proportion to salinity and therefore the osmotic gradient between body fluids and the external medium.

In FW fish the potential was slightly negative at 0·4 mV (Table 1) (the polarity refers to the inside of the fish). In ⅓ and ⅔ SW potentials remained steady at around +2 mV during the measurement period which in some cases continued for up to 24 h.

Fig. 2 shows Na^+ and Cl^- efflux constants. In FW the rate for Cl^- is 0·68% h^{-1} while that for Na^+ is larger at 1·7% h^{-1}. The rates increase slightly in ⅓ SW and then rapidly up to ⅔ SW reaching 14% h^{-1} for Na^+ and 18% h^{-1} for Cl^-. In SW the rates increased little above the ⅔ SW values indicating a possible saturation of the efflux mechanism. These data indicate that entry to hyperosmotic media stimulates the efflux mechanisms.

Ionic balance of trout adapted to various salinities is shown in Table 2. In these calculations it was assumed that:
branchial efflux = branchial influx + drinking, and that urinary and faecal salt losses are negligible.

DISCUSSION

The results show that small (5–20 g) rainbow trout are well able to adapt to dilute sea waters, up to at least 22‰ but survive only a few days in full strength SW even after lengthy adaptation to ⅔ SW. Failure to survive SW appears to be associated with an inability to control plasma ion concentrations and with an invasion of the tissue cells by inorganic ions, especially Cl^-. This point is discussed in a subsequent section.

Tissue and body ion contents

The results indicate that adaptation to salinity is associated with increased cellular salt concentration, particularly in muscle (Table 1). Body and muscle ion spaces increased with salinity, particularly the Cl^- and (Cl–K_{sp}) spaces which are sometimes used to give an estimate of extracellular space. However, it is unlikely that they reflect an increase in extracellular space where even in dilute SW the values become very high, e.g. 45% for Cl^- space in ⅔ SW and also the inulin space remained relatively constant at various salinities (Table 1). The Cl^- space of FW trout (32·78 ml/100 ml body water) agrees well with the values reported for various FW fish (Lutz, 1972).

Thus adaptation to dilute sea waters involves a number of mechanisms. First, the salt load is not distributed evenly throughout the body but is concentrated mainly

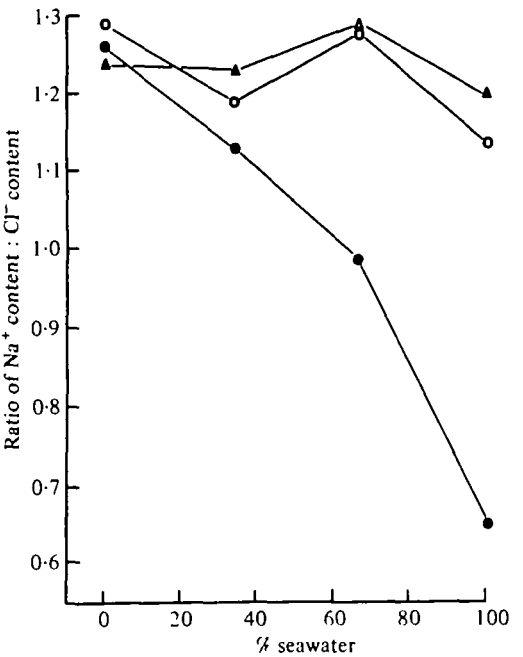

Fig. 3. Relative Na^+ and Cl^- permeability of whole body, muscle and plasma in rainbow trout. Values calculated from mean values in Table 1. △, Plasma; ○, whole body; ●, muscle.

in the cellular compartment. However, to obtain a complete picture of the salt load distribution, it would be necessary to analyse the principal body tissues. The results indicate that muscle is relatively more permeable to Cl^- (Fig. 3). Since the whole body Na^+:Cl^- ratio remains steady with increasing salinity, this suggests that other tissues may be relatively more permeable to Na^+ as has been indicated in liver cells of perch adapting to ½ SW (Lutz, 1972). Secondly, the uneven distribution of the salt load between extracellular and intracellular compartments allows the former to remain relatively constant, thus protecting delicate tissues such as the brain from osmotic stress. In SW these mechanisms are insufficient and the tissues are exposed to osmotic stress.

Salt and water balance

The basic principles of salt and water balance in marine fish were originally outlined by Smith (1930) and later by Maetz (1971). Trout entering a hyperosmotic environment suffer an osmotic water loss which is replaced by drinking the medium. The imbibed salt is excreted via the gills and there is strong evidence to suggest that in euryhaline and marine species salt output is associated with branchial 'chloride' or mitochondrion rich cells (Maetz & Bornancin, 1975, Thomson & Sargent, 1977). In addition to the active salt output there are also bidirectional passive movements of both Na^+ and Cl^- across the gills whose exact nature is unknown, but may contain diffusional and exchange diffusional components (Potts *et al.* 1970, Maetz & Bornanum, 1975).

While the data in Table 1 give steady-state information on ionic regulation in rainbow trout, they give less guidance as to how this state is achieved than do drinking

Table 3. *Sodium and chloride balance in rainbow trout adapted to $\frac{1}{3}$ and $\frac{2}{3}$ SW and in full strength SW*

				Na^+			Cl^-				
Medium	Measured potential	Equilibrium Potential Na^+	Equilibrium Potential Cl^-	Total efflux	Passive influx	Calculated passive efflux	Active efflux	Total efflux	Passive influx	Calculated passive efflux	Active efflux
FW	−0·36	−157	+144	0·6910	—	—	—	0·2135	—	—	—
$\frac{1}{3}$ SW	+2·4	+2·2	−13·4	1·5569	1·2929	1·3006	0·2563 (16·46 %)	1·004	0·667	0·4242	0·5798 (57·75 %)
$\frac{2}{3}$ SW	+1·1	+18·4	−30·5	7·619	6·669	3·273	4·347 (57·04 %)	7·566	6·353	1·897	5·669 (74·93 %)
SW	+1·78	—	—	12·73	10·424	4·597	8·133 (63·89 %)	14·01	11·06	3·273	10·73 (76·64 %)

(Equilibrium potentials were calculated using the Nernst equation

$$E = \frac{RT}{zF} . ln\left[\frac{C_i}{C_e}\right],$$

where R is the universal gas constant, F the Faraday, z the ionic charge, C_i the internal concentration, C_e the external concentration and E is the potential. Passive efflux was calculated from the equation

$$\frac{\text{Pass. efflux}}{\text{Pass. influx}} = \frac{C_i}{C_e} \exp \frac{EF}{RT}.$$

Fluxes are in m-mole/kg^{-1} h^{-1}. The 'active' efflux is also expressed as a percentage of the total efflux. Potentials are given in mV, polarity referring to inside of fish. In fresh water the passive influx is negligible.)

rates and fluxes (Figs. 1 and 2, and Table 2). For a fish to be in ionic balance salt uptake must equal salt output:

$$\text{branchial efflux} + \text{urine} + \text{faeces} = \text{drinking} + \text{branchial influx}.$$

In SW fish salt losses via urine and faeces are negligible. The branchial efflux may be considered to consist of two components, the active and the passive effluxes:

$$\text{branchial influx} = \text{active efflux} + \text{passive efflux} - \text{drinking}.$$

Assuming that the branchial influx is entirely passive, then using the potentials data the passive efflux can be calculated (Table 3), i.e. exchange diffusion and active influx are equal to zero.

In $\frac{1}{3}$ SW Na^+ concentrations in blood and water are almost equal and the equilibrium potential for Na^+, calculated from the Nernst equation (Table 3) is close to the measured potential of $+2{\cdot}3$ mV. Thus little energy is needed to maintain Na^+ balance and the active component of the efflux is relatively small. However the external Cl^- concentration (174 mM) is higher than the plasma value (109 mM), the Nernst potential is about -12 mV, and thus Cl^- tends to enter the fish down both concentration and electrical gradients. This necessitates energy expenditure for Cl^- regulation and the 'active' component is correspondingly high at about 58% of the total Cl^- efflux.

Similar arguments can be applied to fish adapted to $\frac{2}{3}$ SW; in this case both Na^+ and Cl^- enter down concentration and electrical gradients and energy must be expended to maintain ionic balance, hence the large 'active' components of the Na^+ and Cl^- effluxes (Table 3), Cl^- being the greater. This analysis has also been extended to SW fish; in this case the 'active' components are similar to those for $\frac{2}{3}$ SW adapted fish, suggesting an inability to increase this portion of the efflux. However, in these fish ionic balance cannot be assumed since they continually gain salt up to the time of death.

It is probably incorrect however to assume that the influx is entirely diffusional (potential dependent), i.e. there may well be an exchange diffusion component. An exchange diffusion component for Na^+ efflux for the intertidal fish *Xiphister* in 100% SW was proposed by Evans (1967) while a similar component of the passive Na^+ fluxes in the SW smolt was suggested by Potts *et al.* (1970). Part of the passive Na^+ and Cl^- fluxes in some marine fish such as the mullet (Maetz & Pic, 1975) may be exchange diffusion.

From the present data there is no way of assessing this component but if an exchange diffusion component is included in the calculations for passive efflux (Fig. 4) one sees the following effects. As this component is increased the active efflux is steadily decreased, the values for Na^+ being approximately 10% lower than those for Cl^- throughout, except for fish adapted to $\frac{1}{3}$ SW where the 'active' component for Na^+ remains almost constant. Thus it appears that as the hypothetical exchange diffusion component is increased the energy required for ionic balance would be reduced.

In conclusion small rainbow trout are well able to adapt to salinities up to at least $\frac{2}{3}$ SW but not to full strength SW. In adapted fish plasma ions remain near the FW values, but there is ionic invasion of the tissue cells, e.g. Cl^- into muscle cells. Analysis of ionic balance in adapted fish shows that ionic equilibrium is maintained mainly by

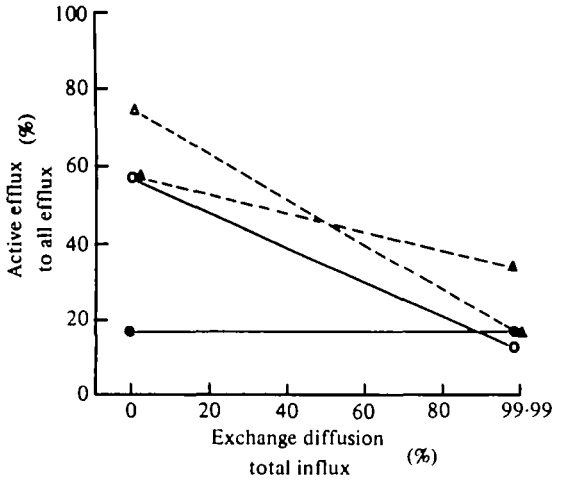

Fig. 4. Percentage active Na^+ and Cl^- efflux in rainbow trout adapted to ¼ and ⅓ SW as a function of increasing percentage exchange diffusion. Values were calculated using the data and equations shown in Table 3. ●, Na^+, ¼ SW; ○, Na^+, ⅓ SW; ▲, Cl^-, ¼ SW; △, Cl^-, ⅓ SW.

greater energy expenditure, especially in Cl^- regulation. Fish in full strength SW can no longer maintain ionic equilibrium and Na^+ and Cl^- invade both tissues and plasma, resulting in eventual death.

This work was supported by NERC grant GR3/2942.

REFERENCES

BATH, R. N. & EDDY, F. B. (1979). Measurement of transbranchial potentials in fish. *J. Physiol., Lond.*

CONWAY, E. J. (1957). Nature and significance of concentration relations of K^+ and Na^+ in skeletal muscle. *Physiol. Rev.* **37**, 84–132.

EVANS, D. H. (1967). Sodium, chloride and water balance of the intertidal teleost, *Xiphister atropurpureus*. III. The roles of simple diffusion, exchange diffusion and active transport. *J. exp. Biol.* **47**, 525–534.

EVANS, D. H. (1968). Measurement of drinking rates in fish. *Comp. Biochem. Physiol.* **25**, 751–753.

HOAR, W. S. (1976). Smoult transformation: evolution, behaviour and physiology. *J. Fish. Res. Bd Can.* **33**, 1234–1252.

HOLMES, W. N. & DONALSON, E. M. (1969). The body compartments and the distribution of electrolytes. In *Fish Physiology*, vol. 1, (ed. W. S. Hoar and D. J. Randall), pp. 1–89. Academic Press.

HOUSTON, A. H. (1959). Osmoregulatory adaptation of steelhead trout (*Salmo gairdneri* Richardson) to sea water. *Can. J. Zool.* **37**, 729–748.

LUTZ, P. (1972). Ionic and body compartment responses to increasing salinity in the Perch *Perca fluviatilis*. *Comp. Biochem. Physiol.* **42**A, 711–717.

MAETZ, J. (1971). Fish gills: mechanisms of salt transfer in freshwater and seawater. *Phil. Trans. R. Soc. Lond.* B **262**, 209–249.

MAETZ, J. & BORNANCIN, M. (1975). Biochemical and biophysical aspects of salt excretion by chloride cells in teleosts. *Fortschrite de Zoologie.* **23**, 323–362.

MAETZ, J. & PIC, P. (1975). New evidence for Na^+/K^+ and Na^+/Na^+ exchange carrier linked with Cl^- pump in the gill of *Mugil capito* in sea water. *J. comp. Physiol.* **102**, 85–100.

MANERY, J. F. (1954). Water and electrolyte metabolism. *Physiol. Rev.* **34**, 334–417.

MOTAIS, R. (1967). Les mechanismes d'exchanges ioniques branchiaux chez les teleosteens. *Annals. Inst. Oceanog. Monaco* **45**, 1–84.

PARRY, G. (1958). Size and osmoregulation in salmonid fishes. *Nature, Lond.* **181**, 1218.

PARRY, G. (1960). The development of salinity tolerance in the salmon, *Salmo salar* L., and some related species. *J. exp. Biol.* **37**, 425–434.

PARRY, G. (1966). Osmotic adaptation in fishes. *Biol. Rev.* **41**, 392–444.

POTTS, W. T. W. & EDDY, F. B. (1973). Gill potentials and sodium fluxes in the flounder, *Platichthys flesus*. *J. comp. Physiol.* **87**, 29–48.

POTTS, W. T. W., FOSTER, M. A. & STATHER, J. W. (1970). Salt and water balance in salmon smoults. *J. exp. Biol.* **52**, 553–564.

SHEHEDEH, 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.

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

THOMSON, A. J. & SARGENT, J. R. (1977). Changes in the levels of chloride cells and ($Na^+ + K^+$) dependant ATPase in the gills of yellow and silver eels adapting to sea water. *J. exp. Zool.* **200**, 33–40.