SALT AND WATER BALANCE IN RAINBOW TROUT (SALMO GAIRDNERI) RAPIDLY TRANSFERRED FROM FRESH WATER TO SEA WATER

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(Received 4 January 1979)

SUMMARY

Physiological responses of rainbow trout (mean weight 13.3 g) to sudden changes in salinity were investigated. An initial period lasting about 8 h was characterized by increased drinking and an increase in plasma and body ions. Fish failed to survive more than 2 days in full strength sea water but in two-thirds sea water there were few mortalities and adaptation was complete in 7–10 days. During this period there were gradual physiological changes resulting in normal plasma ion concentrations but significantly increased body ionic content. The intracellular concentrations of muscle chloride showed the greatest increase.

INTRODUCTION

Ionic regulation in euryhaline fish has been extensively studied but the physiological processes involved in rapid transfer from fresh water to sea water remain relatively unknown. For salmonids some of the most relevant work is that of Parry (1958, 1960, 1961, 1966) and Potts, Foster & Stather (1970). It was shown that survival of rainbow trout transferred directly from fresh water to sea water was related mainly to size, i.e. only fish larger than about 80 g were likely to survive direct transfer. On the other hand salmon smolts weighing around 20 g could withstand direct transfer.

Much work has been directed towards understanding the nature of unidirectional Na⁺ and Cl⁻ fluxes in various marine fish, transferred from sea water to fresh water or occasionally the reverse, and this is reviewed by Maetz (1971), Maetz & Bornancin (1975) and Evans (1975).

This paper describes physiological changes which occur when rainbow trout are subjected to an abrupt increase in salinity. Previous studies, e.g. Houston (1959) have monitored physiological parameters at daily intervals; this study concentrates also on the period immediately after transfer.

MATERIALS AND METHODS

Rainbow trout (weight 13.3 ± 0.4 g, length 11.0 ± 1.1 cm) were obtained from a local hatchery and kept in dechlorinated water for several weeks before experiments.
Sea water was collected from the North Sea and diluted with dechlorinated aquarium water. Salinity was measured using an Electronic Switchgear Ltd, Salinometer, full strength seawater being 33 °/oo Cl-. 

(a) Survival after abrupt transfer to sea water
Groups of 20 fish were transferred directly from holding tanks to 40 l of either one-, two- or three-thirds sea water and times of death noted.

(b) Efflux rates of Na+ and Cl−
The efflux rate constant (K_{eff}) for Na+ and Cl− was measured using radioisotopes as previously described by Eddy & Bath (1979). Fish were taken from their fresh-water stock tanks, injected with radioisotope contained in saline and then immediately transferred to one-third, two-thirds or full-strength sea water. Samples of the medium were taken at known intervals and K_{eff} calculated for each point.

(c) Time course of adaptation to two-thirds sea water
Approximately 100 fish were transferred from fresh water to two-thirds sea water and groups of six fish were sampled at 2, 4, 6, 8, 11, 18, 24, 30, 48, and 72 h for (i) body and muscle ionic content and water content; (ii) blood haematocrit; and (iii) plasma ions and water.

Collection of blood was as described in the previous paper.
Samples of muscle were then taken from the dorsal block anterior to the dorsal fin, weighed to the nearest milligram and water content determined by drying in an oven at 108 °C to constant weight. The rest of the body was treated in the same way to give body water content. The dried tissue was ground to a fine powder using a pestle and mortar and inorganic ions eluted from a weighed sample in 0.1 N-HNO₃ at 4 °C for 1 week. Following centrifugation the supernatant was diluted with distilled water for ion analysis. It should be noted that compared to the homogenizing and sonicating technique employed in the preceding paper (Eddy & Bath, 1979) nitric acid elutes rather more Na+ but is less effective in eluting Cl−, especially in muscle samples (Hickman et al. 1963; R. N. Bath, unpublished results).

Plasma water was measured by drying a known weight in an oven at 108 °C overnight. Ion content of plasma was determined by diluting with distilled water; Na+ was determined using an El 100 flame photometer and Cl− using a Buchler-Cotlove ampiometric titrator. Drinking was determined at 2, 4, 6, 8 and 24 h after transfer using the method of Evans (1968).

(d) Transbranchial potentials
These were measured as described in the preceding paper (Bath & Eddy, 1979) in fish transferred to sea water. Fish were allowed to recover for 24 h after insertion of the peritoneal cannula and to acclimate to the holding chamber. Potentials were measured in fresh water for a few hours before transfer which was carried out as follows. The holding chamber (250 ml) was sealed while the fresh water in the reservoir (8 l) was replaced by sea water. Upon unsealing the holding chamber rapid mixing was achieved, aided by pumping, giving a transfer time of less than 30 s, without handling the fish.

(e) Calculations
Ion spaces and intracellular ion contents were calculated as in a previous paper (Eddy & Bath, 1979) and all values are in m-mole/kg water.

**RESULTS**

**Survival in sea water.** There were no mortalities in fish transferred to one- or two-thirds sea water during the experimental period of 200 h. However no fish survived abrupt transfer to sea water, deaths occurring between 14 and 50 h with a mean survival of 26 h.

**Na+ and Cl− effluxes** (Fig. 1). In fish transferred to one-third sea water effluxes remained near the fresh water value for about 12 h and then gradually increased over the next 2 days to the adapted value of around 3-4%/h.

On abrupt transfer to two-thirds sea water Cl− efflux increased immediately whereas Na+ efflux remained at the fresh water value. During the next 24 h Na+ efflux increased and regression analysis suggested a change of rate starting at 11-12 h. After 48 h both Na+ and Cl− effuxes were still below the rates for fully adapted fish and presumably over the next 7 days there was a gradual build up to these levels. Fish transferred to sea water responded with much greater effluxes but still showed the
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For legend see p. 198.
same differential between Na\(^+\) and Cl\(^-\) as in two-thirds sea water. After 3–4 h there were further significant increases in the efflux rates of both ions, but these were not sustained. However, effluxes for a surviving fish showed a rapid increase to 24 h, when death occurred.

It is of interest to note that fish transferred to sea water are unable to increase efflux rates above those observed in two-thirds sea water indicating a possible maximum efflux rate for any given time after transfer.

**Transfer to two-thirds sea water.** The results indicate an initial period lasting about 8 h where rapid physiological changes occur followed by a second stabilising period (Fig. 2).

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(a) Blood and plasma measurements

Both plasma Na\(^+\) and Cl\(^-\) concentrations showed immediate increases, that for Cl\(^-\) being much greater. After 10 h the values had steadied and then gradually decreased to the adapted value (240 h). K\(^+\) concentration showed no great changes.

Plasma water showed an initial decrease with a low point at 8 h, then slowly returned over the next 24 h to the fresh water value. Blood haematocrit showed a similar trend but with a low point at 6 h. There is an apparent conflict in these findings because a decrease in plasma water might be expected to lead to an increase in haematocrit. This is discussed at a later point.

(b) Body ions, ion spaces and water

Body Na\(^+\) showed an initial small increase and then became relatively steady while Na\(^+\) space remained nearly constant. Cl\(^-\) concentration, Cl\(^-\) space and (Cl\(^-\)–K\(^+\)) space showed a much greater initial increase which steadied after about 12 h. Body K\(^+\) concentration was irregular but overall showed a gradual increase over the experimental period.

Body water showed a slow dehydration to around 30 h, then a gradual rehydration to approximately the fresh water value at 72 h.

(c) Muscle ions, ion spaces and water

For the first 3 days these showed similar trends to those for whole body, though muscle K\(^+\) showed an initial increase which was maintained to the time of full adaptation. Fully adapted fish had significantly increased muscle Na\(^+\) and Cl\(^-\) content, compared to fresh water fish. Muscle had a significantly higher water content than whole body and showed the same trends as whole body after transfer.

(d) Intracellular ion content

Both Na\(^+\) and Cl\(^-\) content of whole body cells increased for 8 h, but remained stable over the next 48 h. Intracellular muscle contents showed a similar trend.

Intracellular K\(^+\) content of the whole body showed a gradual increase to 30 h followed by a decrease before full adaptation. Intracellular K\(^+\) content of muscle tissue showed a more rapid increase, but values were rather irregular.
Fig. 4. Diagram outlining the main physiological events which occur when rainbow trout are transferred from fresh water to sea water. The period immediately after transfer and lasting about 8 h is characterized by major physiological changes and is referred to as the 'crisis' period.

This is followed by a period lasting 7-10 days when slower changes occur, resulting finally in completed adaptation, and is called the 'stabilization' period.

(e) Drinking

Upon entering two-thirds sea water the fish showed a large increase in drinking, reaching a peak around 8 h, then rapidly declining to levels characteristic of two-thirds sea water adapted fish. This behaviour is similar to that described for eels entering sea water (Kirsch & Mayer-Gostan, 1973).

(f) Potentials

In fresh water the potential was about -0.36 mV and upon transfer to sea water increased briefly to +1.13 mV, then rapidly decreased to around zero followed by a steady rise over 10-15 h to +2 mV (Fig. 3). This value is close to values obtained in two-thirds sea water adapted trout (Eddy & Bath, 1979).

**DISCUSSION**

The results show that small rainbow trout survive direct transfer to dilute sea water, up to at least two-thirds sea water, but are unlikely to survive more that 2 days in full strength sea water. Survival in sea water, after preadaptation to two-thirds sea water, was slightly prolonged to 4-5 days (Eddy & Bath, 1979). Parry (1958, 1960) experimented with rainbow trout, as well as a variety of other salmonids, and noted that salmon parr and smolts, of similar size to the fish used in the present work, survived direct transfer to sea water. Clearly there are important physiological differences between the two species in this respect. This difference is borne out by comparing efflux rates for Na⁺ and Cl⁻ of sea water adapted smolts (Potts et al. 1970), and values obtained for two-thirds sea water adapted rainbow trout, i.e. for smolts turnover are...
with an increase in levels of Na\textsuperscript{+}-K\textsuperscript{+} ATPase (Giles & Vanstone, 1976; Thomson & Sargent, 1977). Also there are likely to be changes in intestinal and renal function, though as yet little is known of these.

In conclusion the results show some of the physiological responses by rainbow trout to a sudden increase in salinity. An initial period of increased drinking, together with diffusion of salts into the gills, results in an increased salt load, reflected in increased plasma and body salt content. Fish fail to adapt to full strength sea water but after 8 h in two-thirds sea water a stabilization period begins, lasting some 7-10 days and eventually resulting in fully adapted fish which show normal plasma ion concentration, but increased cellular content of Na\textsuperscript{+} and Cl\textsuperscript{−}, especially in muscle tissue.

This work was supported by NERC grant GR3/2942.

REFERENCES


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