

OSMOREGULATION IN SALMON AND SEA TROUT ALEVINS

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SUMMARY

Changes in survival, body-water content, body electrolyte concentration and Na^+ turnover rates were studied in alevins of Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) at different developmental stages following exposure to various dilutions of sea water. In 100% sea water, salmon alevins at 9 days post-hatch survived approximately 6 h, and sea trout alevins 13 days post-hatch survived approximately 4 h. In both species, survival in hyperosmotic media decreased and rates of dehydration and Na^+ turnover increased with age and development. The progressive change in resistance to osmotic stress is associated with a decrease in body surface area occupied by the relatively impermeable vitelline membrane and to the development of functional gills, leading to an increase in permeability to water and salt.

INTRODUCTION

Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.) spend two periods of their life in fresh water (as juveniles up to the smolt stage and as spawning adults) and the remainder in sea water. Considerable knowledge has been acquired concerning osmoregulatory processes associated with the migration between waters of different salinities (Parry, 1958, 1960, 1966; Potts, Forster & Stather, 1970; Hoar, 1976). In contrast, the osmoregulatory ability of alevins (yolk-sac embryos) is poorly understood. This stage in the life-history appears particularly interesting as it has been shown by Parry (1960) that newly hatched Atlantic salmon and trout alevins are capable of surviving for unexpectedly long periods in hyperosmotic media. As the alevin stage progresses, this ability is gradually lost so that fry and parr up to about 4 cm show virtually no tolerance of salt water. Indeed, the survival times of newly hatched alevins in sea water are not matched until the fish reach 7–10 cm in length and are some 100 times the body mass of alevins. Parry (1960) suggested that the change in apparent osmoregulatory ability of alevins may be related to permeability changes.

The aim of this study was to investigate further the mechanisms that enable early salmon and trout alevins to survive in hyperosmotic media, and to establish why this ability is lost as the alevin develops into the juvenile fish.

MATERIALS AND METHODS

Salmon and sea trout alevins were obtained from the Department of Agriculture and Fisheries hatchery at Almondbank, Perthshire. These were obtained at three stages of development: salmon at 9, 29 and 62 days post-hatching and sea trout at 13, 25 and 58 days post-hatching. The mean wet weights (\pm standard error) of five salmon and five sea trout at each stage of development were 103.4 ± 1.7 , 140.1 ± 2.6 and 167.0 ± 5.3 mg for the salmon and 56.2 ± 1.5 , 58.5 ± 1.3 and 125.3 ± 6.3 mg for the sea trout respectively. Alevins were transported to the aquarium at Dundee University, where they were held in fresh running water at 8–10 °C, the experimental temperature.

Body water and ionic content. Twenty-five salmon and sea-trout alevins were transferred from the freshwater holding tanks into beakers containing 500 ml of constantly aerated media of different salinity. The media used were 100% sea water (32‰), 50‰ s.w., 25% s.w. and fresh water (Na^+ , 0.22 mM; Cl^- , 0.3 mM; Ca^{2+} , 0.1 mM). At intervals, five alevins were removed from each of the test media, rinsed in fresh water, blotted dry, and transferred to preweighed squares of aluminium foil for wet weight determination. Body water content was determined by drying the alevins in an oven at 90–100 °C for at least 12 h. The dried tissue was then dissolved in a minimal volume of concentrated nitric acid (normally 0.5 ml) for subsequent determination of Na^+ and K^+ , using an EEL 100 emission flame photometer.

Survival. Batches of at least six alevins were placed in conical flasks containing 250 ml of each of the media described above. The flasks were examined at intervals and mortalities recorded.

Na^+ efflux rate. This was determined using ^{22}Na , a gamma emitter obtained from the Radiochemical Centre, Amersham. Alevins were placed in 200 ml fresh water to which 20 μCi ^{22}Na was added, giving a specific activity of 0.5 $\mu\text{Ci } \mu\text{M}^{-1}$ Na, and were 'loaded' in this medium for about 17 h before being rinsed and transferred to individual plastic bottles containing 50 ml medium, either fresh water, 25% s.w., 50% s.w. or 100% s.w., which was constantly aerated. Six alevins were used at each salinity. At intervals, each alevin was carefully removed, rinsed, and counted for 40 s in a test tube of water placed in the well of a Panax iodide crystal connected to a Panax Reigate counter. Rate of Na^+ efflux was calculated from

$$K = \frac{1}{T} \ln \frac{A_0}{A_T},$$

where K is the rate constant, T the time, A_0 the initial activity of the fish, and A_T the activity at time T .

RESULTS

Survival. The earliest salmon alevins (9 days post-hatch) were the most resistant to hyperosmotic media, surviving about 6 h in sea water and more than 50 h in 50% s.w. With increasing age both salmon and sea trout survived for progressively shorter periods in 50 and 100% s.w., such that 62-day post-hatch salmon alevins survived only 1–2 h in 100% s.w. (Fig. 1). Similar results were noted by Parry (1960) for salmon alevins. Sea trout survived slightly less well than salmon in the various

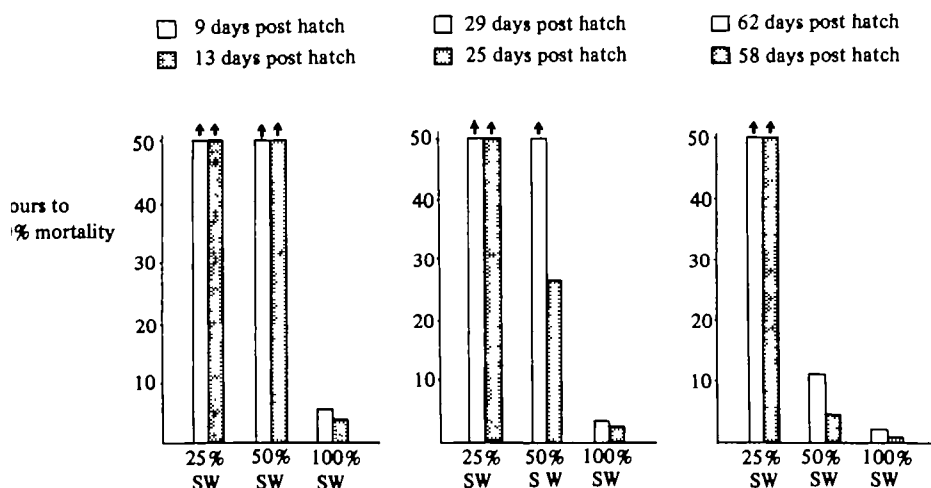


Fig. 1. Survival of salmon (open bars) and sea trout (hatched bars) alevins in 32‰ sea water (100% s.w.), 50% s.w. and 25% s.w. Arrows above the bars indicate that alevins survived indefinitely.

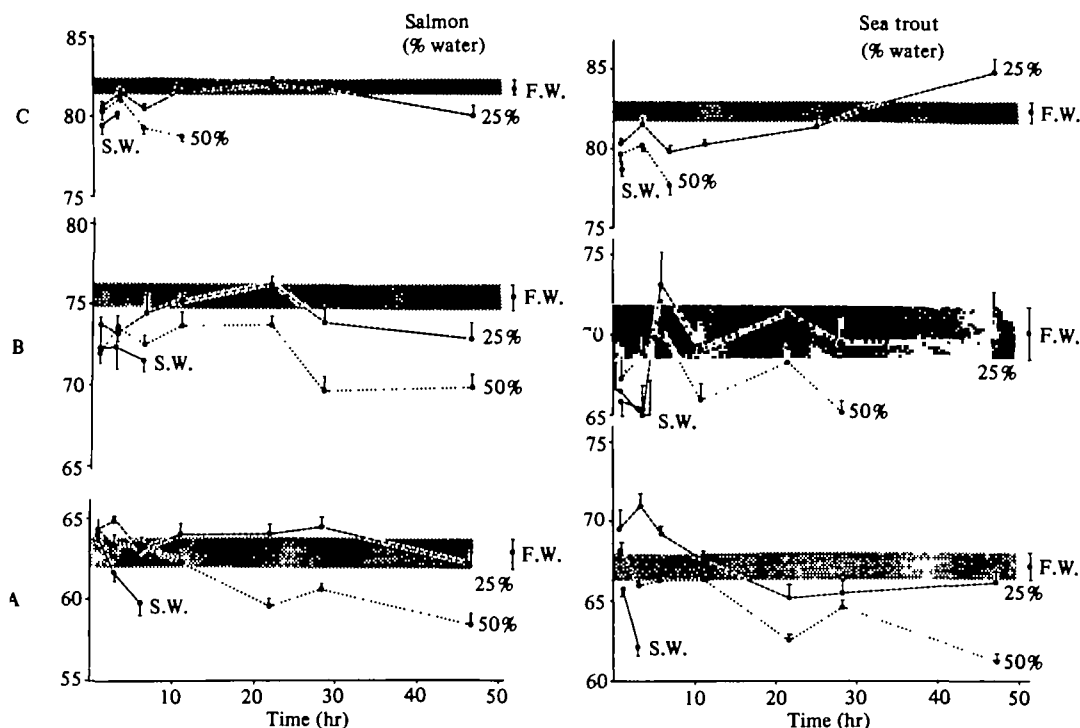


Fig. 2. Water content of salmon and sea trout alevins placed in 100% s.w., 50% s.w., 25% s.w. and fresh water. The freshwater value (± standard error) is indicated by the horizontal shaded bar. Salmon: (A) 9 days post-hatch; (B) 29 days post-hatch; (C) 62 days post-hatch. Sea trout: (A) 13 days post-hatch; (B) 25 days post-hatch; (C) 58 days post-hatch.

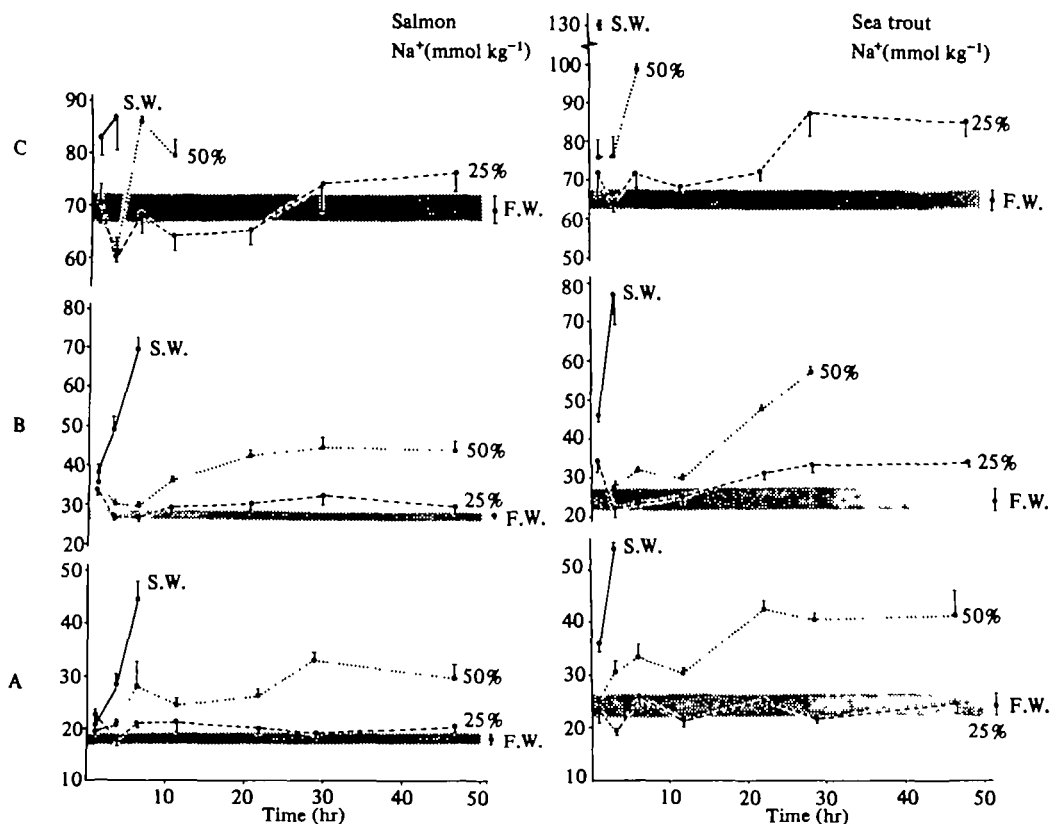


Fig. 3. Sodium content (mmol kg^{-1} body weight) of salmon and sea trout alevins placed in 100 % s.w., 50 % s.w., 25 % s.w. and fresh water. The freshwater value (\pm standard error) is indicated as a horizontal bar. (A, B and C) days post-hatching as for Fig. 2.

salinities. At all stages of development both salmon and sea trout alevins survived indefinitely in 25 % s.w.

Water content. The earliest alevins of both species contained relatively little water, approximately 65 % body weight, reflecting the low water content of the lipid-rich yolk sac which is prominent at this stage (Fig. 2). As the alevins developed their yolk sacs gradually disappeared and body-water content of both species increased to approximately 82 %. Hayes (1930) found that the water content of salmon embryos and yolk remained constant at 84 % and 67 % respectively, whereas the water content of the alevin increased steadily after hatching as the embryo to yolk ratio increased. At all stages of development studied, a marked dehydration occurred in both species in 50 % and 100 % s.w. Neither species showed any appreciable dehydration in 25 % s.w. In 100 % s.w., salmon alevins died when the water loss was equivalent to 5.6, 6.1 and 3.3 % of the total body water at 9, 29 and 62 days after hatching, respectively. These water losses represent 0.9, 1.0, and 1.1 % h^{-1} over the period of survival in 100 % s.w. Corresponding values for sea trout are 7.5 % (2.5 % h^{-1}), 7.9 % (2.6 % h^{-1}) and 4.2 % (4.3 % h^{-1}). In 50 % s.w., salmon alevins 9 and 29 days post-hatch and sea trout 13 days post-hatch survived levels of dehydration which proved fatal to alevins in 100 %

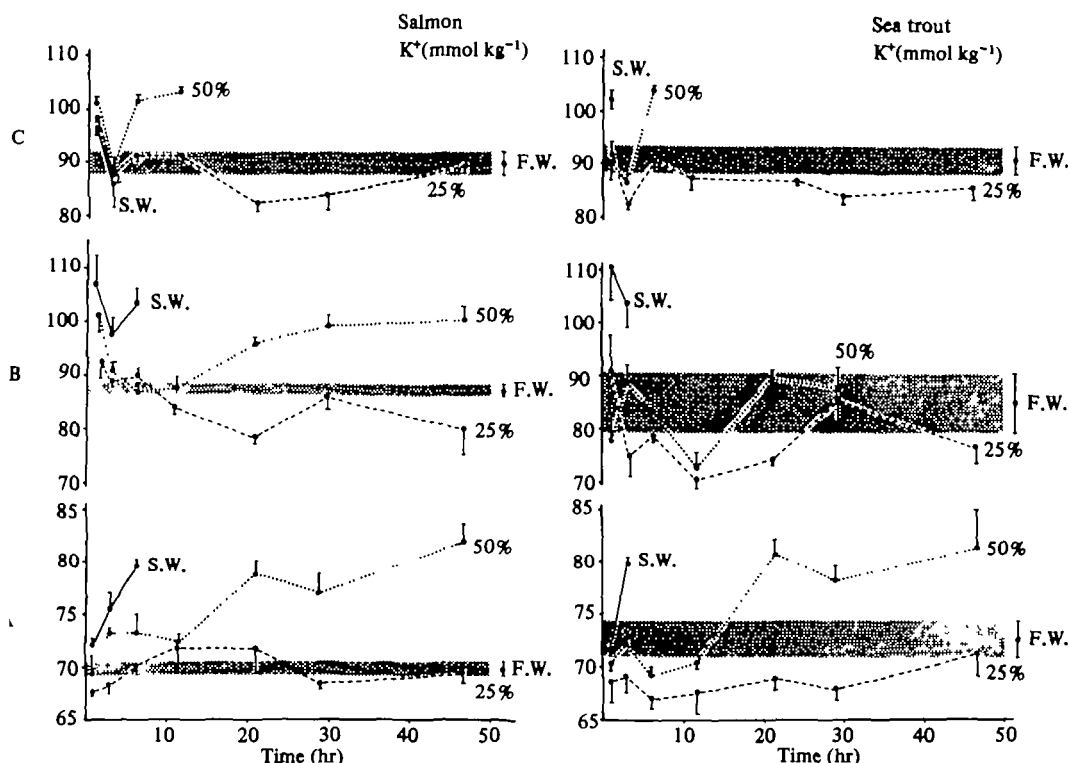


Fig. 4. Potassium content (mmol kg^{-1} body weight) of salmon and sea trout alevins placed in 100% s.w., 50% s.w., 25% s.w. and fresh water. The freshwater value (\pm standard error) is indicated by the horizontal bar. (A, B and C) days post-hatching as in Fig. 2.

s.w. At the other stages of development of the two species, death usually occurred in 50% s.w. at levels of dehydration somewhat greater than those found in alevins exposed to 100% s.w.

Na^+ and K^+ content. During the first 4 weeks following hatching, freshwater-adapted alevins of both species contained approximately 20–25 $\text{mmol Na}^+ \text{kg}^{-1}$ body weight, whereas by 9 weeks after hatching, total body Na^+ content had increased in both species to approximately 70 mmol kg^{-1} (Fig. 3). Rudy & Potts (1969) found that alevins 10 days after hatching contained 34 $\text{mmol Na}^+ \text{kg}^{-1}$ body weight. Busnel, Drilhon & Raffy (1946) reported that the freezing-point depression (Δ , $^{\circ}\text{C}$) of blood in hatching salmon eggs was 0.49°C , rising as the alevin developed to 0.59°C after 31 days.

In 25% s.w., the body Na^+ content of salmon alevins at all stages of development remained virtually unchanged from the freshwater values. Early sea trout alevins (13 days post-hatch) showed little change in body Na^+ content when exposed to 25% s.w. but in later stages some increase was evident, particularly in the 58-day post-hatch animals. Throughout the alevin stage of both salmon and sea trout the body Na^+ content increased markedly in the two higher salinities. In 100% s.w. the body Na^+ content increased by factors of 2.3, 2.4 and 1.3 for salmon and 2.3, 3.4 and 2.1 for sea

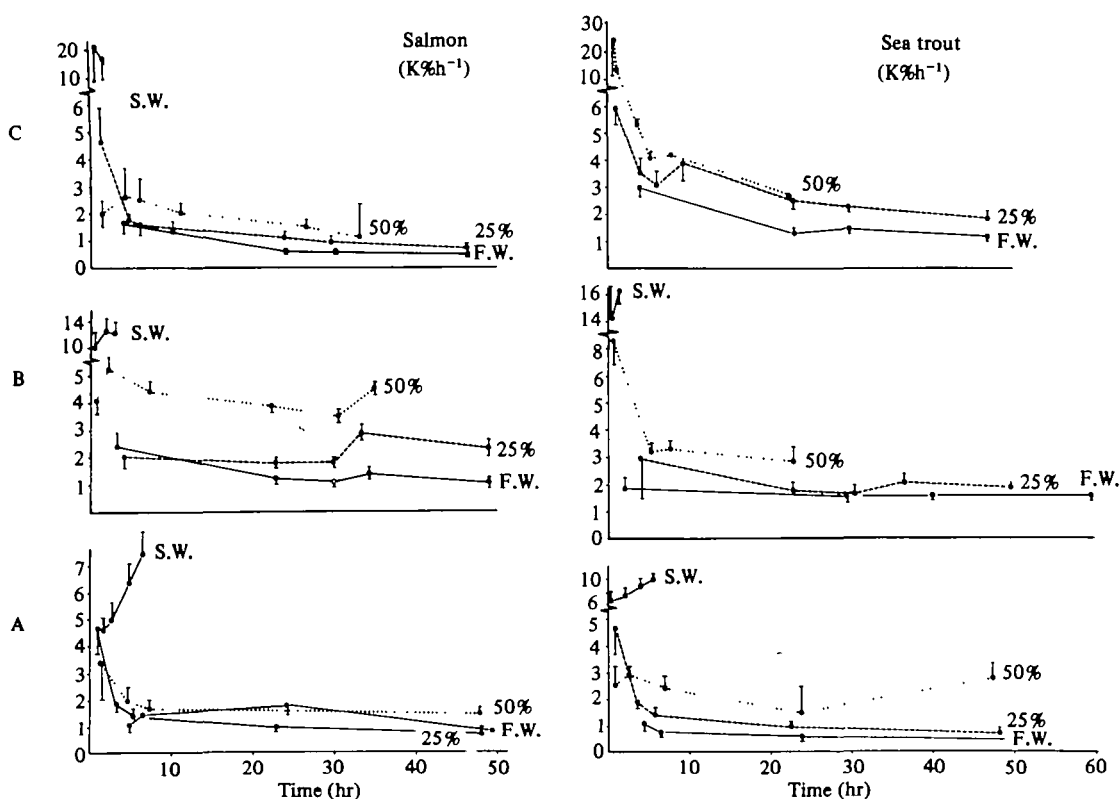


Fig. 5. Sodium turnover rates, % h^{-1} , in salmon and sea trout alevins placed in 100% s.w., 50% s.w., 25% s.w. and fresh water. (A–C). Days post-hatching as in Fig. 2.

trout at the three stages studied. When alevins were exposed to hyperosmotic media the rate of increase in the body Na^+ content was greater in late alevins of both species compared to the earliest stage studied. During the period of survival in 100% s.w., salmon alevins gained Na^+ at a rate of 4.5, 6.6 and 6.0 $\text{mmol kg}^{-1} \text{h}^{-1}$ at the three stages of development. Corresponding rates for sea trout were 10.3, 20.7 and 65.0 $\text{mmol kg}^{-1} \text{h}^{-1}$.

Approximately 2 weeks after hatching, the K^+ content of freshwater-adapted salmon and sea trout alevins was about 70 mmol kg^{-1} , increasing to around 90 mmol kg^{-1} in both species at the two later stages (Fig. 4). At all stages of development the body K^+ content of salmon and sea trout alevins in 25% s.w. remained close to the freshwater values, although there appeared to be some tendency (especially in sea trout) for the body K^+ content to decrease slightly. Throughout the alevin stage, exposure to 50 and 100% s.w. caused the body K^+ content of both species to increase, although often in a rather erratic fashion.

Sodium efflux. In freshwater-adapted alevins of both salmon and sea trout, Na^+ efflux (i.e. percentage body Na^+ exchanged each hour) was usually 2% h^{-1} or less, similar to values reported in other freshwater salmonids (Eddy & Bath 1979) (Fig. 5).

For any given salinity, the Na^+ efflux rate tended to increase with age in both salmon and sea trout alevins. In addition, at all stages of development of both species, Na^+

Flux rates increased as the concentration of sea water increased. For example, when placed in 100% s.w., the Na^+ efflux rate of salmon alevins 62 days post-hatch ($15\text{--}20\% \text{ h}^{-1}$) is some 3 times higher than in alevins 9 days post-hatch at the same salinity, and approximately 15 times higher than in fresh water. An increase in Na^+ efflux in freshwater-adapted fish transferred to s.w. is a general phenomenon. Bath & Eddy (1979) showed that rainbow trout transferred abruptly to sea water underwent a slow increase in Na^+ efflux from 2% to $9\% \text{ h}^{-1}$ over 30 h. Potts *et al.* (1970) found that when salmon smolts were transferred from fresh water to sea water, the Na^+ efflux remained constant at $3\% \text{ h}^{-1}$ for the first 6 h, reaching $10\% \text{ h}^{-1}$ 20 h later. Alevins did not survive long enough for any long term trends in Na^+ efflux to be observed, but it is apparent that a large increase takes place almost immediately following exposure to 100% s.w. and this increase becomes progressively greater as the alevin develops.

DISCUSSION

The results of this study are broadly in agreement with those reported by Parry (1960). Immediately after hatching, salmon and sea trout alevins are capable of surviving for relatively long periods in hyperosmotic media but as the alevin develops this ability is gradually lost. Salmon alevins consistently survived for longer periods in hyperosmotic media than did sea trout. At each of the stages studied, salmon alevins were heavier than sea trout alevins and probably had a lower surface-area-to-volume ratio; this may account for the better survival times seen for salmon alevins.

The rate of water loss from alevins exposed to hyperosmotic media will be partly dependent on the magnitude of the osmotic gradient across the body surface. It was found for both species that the rate of water loss was slower in 50% s.w. than in 100% s.w. and that levels of dehydration were reached and tolerated in 50% s.w. which proved fatal in 100% s.w. The rate of water loss, as well as the absolute loss, is obviously important in determining the survival, and alevins may withstand osmotic stresses better if they are acclimatized slowly to hyperosmotic media. At all stages of development the rate of water loss was higher in sea trout than in salmon alevins in 100% s.w., and in both species, particularly sea trout, the rate increased as the alevins developed. The rate of water loss from the two species and the changes with alevin age may account in part for the relative differences in survival in hyperosmotic media. In addition, there appeared to be a lower tolerance to the effects of exposure to hyperosmotic media in late alevins. When exposed to 100% s.w., death occurred in late alevins of both species after a smaller absolute loss of body water than was found for the earliest alevins. The changes in body Na^+ similarly indicate a reduced tolerance to osmotic stress. In 100% s.w., late alevins of both species could only survive a relatively small increase in body Na^+ compared with alevins at the two earlier stages studied. It is possible that any decrease in the tolerance of older alevins may be related to an increase in complexity and specialization of tissues and organs, which may be more easily disrupted by changes in body water and electrolyte concentrations.

Following exposure to 50% s.w., the increase in body Na^+ content was usually considerably less than that found for alevins exposed to 100% s.w. On the other hand,

the body K^+ and water contents in alevins exposed to 50% s.w. usually reach levels similar to those found at the end of the respective periods of survival of alevins exposed to 100% s.w. It would appear that changes in body Na^+ are largely brought about by net Na^+ gain, whereas the body K^+ concentration is determined by the body water content. It is also apparent from the relative changes in body water and Na^+ contents in alevins in 50% and 100% s.w. that survival in hyperosmotic media is determined more by the effects of dehydration than by a rise in body Na^+ concentration.

In both species, body Na^+ content, and to a lesser extent K^+ content, increased as the alevin developed. The increase in body Na^+ at around 9 weeks post-hatch is particularly marked, being approximately twice the concentration found in alevins 4–5 weeks post-hatch. The increase in body electrolyte concentration may reflect the fact that by 9 weeks post-hatch, alevins were taking exogenous food and not relying entirely on yolk for nutrition, as was the case with the two earlier samples. It is possible that food contributes a significant source of electrolytes at the end of the alevin stage.

Potts *et al.* (1970) reported a Na^+ efflux rate of 3 % h^{-1} in late salmon parr, 11 % h^{-1} in early smolts and 24 % h^{-1} in late smolts when adapted to sea water, and suggested that body Na^+ regulation and survival in sea water is related to the ability to excrete salt. It has been shown by various authors (e.g. Giles & Vanstone, 1976; Zaugg & Wagner, 1973) that smolts of several salmonid species show increased levels of gill Na^+/K^+ ATPase, which is thought to be the enzyme responsible for active sodium excretion. In the present work, however, Na^+ efflux rates tended to be higher in the later alevins, which survived less well in hyperosmotic media. The increased Na^+ efflux rates seen in older alevins following transfer to hyperosmotic media, particularly 100% s.w., do not appear to be evidence for the development of an active sodium excretion mechanism but rather an indication that passive diffusion had increased due to the body surface becoming more permeable. There does not appear to be any indication from the observed changes in body Na^+ content that the high Na^+ efflux rates seen for older alevins in hyperosmotic media are part of an effective ionic regulatory mechanism. The rapid increase in Na^+ efflux when alevins are placed in hyperosmotic media is interpreted as indicating a largely passive diffusion process across a permeable body surface, rather than the relatively slow stimulation of an active process as seen in smolts. In the absence of active sodium excretion, the high Na^+ efflux rates seen in late alevins in hyperosmotic media may be due to some osmotic damage to epithelia at the body surface such as shrinkage of cells causing rupture of the intercellular junctions.

The lack of hypo-osmoregulatory ability seen in late alevins and fry of *S. salar* and *S. trutta* is not universal among salmonid species. Conte *et al.* (1966) found that *Oncorhynchus kisutch* develops an ability to survive high salinities soon after the yolk sac is absorbed, and at a similar stage of development Baggerman (1960) found that *O. keta* and *O. gorbuscha* show seawater preference.

These data would appear to support the suggestion made by Parry (1960) that the early alevin is relatively impermeable to ions and water and that body surface permeability increases as the alevin develops. It is thought that the progressively increasing permeability of alevins with age is primarily related to changes in gross morphology. On hatching, the embryo in our experiments accounted for approximately 10% of the

evin's weight, with the yolk sac accounting for the remainder. In contrast, at 9 weeks post-hatch the embryo constituted 90–95 % of the alevin. Thus, as the alevin develops there is a progressive change in the nature of the body surface from one which contains a large proportion of vitelline membrane to one which comprises normal epithelium. It has been shown (Potts & Rudy, 1969; Rudy & Potts, 1969; Potts & Eddy, 1973) that the vitelline membrane is relatively impermeable to both ions and water and therefore the exchange of the vitelline membrane for normal epithelium will increase the permeability of the body surface.

No specific reference could be found in the literature on the ontogenesis of the gills of *S. salar* and *S. trutta*, but Ballard (1973) referring to *S. gairdneri* states that after hatching 'gill filaments make their appearance while the yolk mass is elongating and shrinking'. The development of gill filaments in the alevin will greatly increase the body surface area and this will also contribute to the observed progressive increase in permeability to water and ions.

Salmon and sea trout alevins would seem to be truly freshwater forms with no active hypo-osmoregulatory powers at any stage of development. The relatively high survival of alevins in hyperosmotic media during the first weeks after hatching appears to be mainly a consequence of their anatomy and to some extent to a slightly higher tolerance. A relatively low permeability to water and ions is probably a general characteristic of most fish embryos immediately after hatching.

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