J. exp. Biol. (1980), **88**, 259–267
With 3 figures
Printed in Great Britain

EFFECTS OF pH ON SODIUM UPTAKE IN NORWEGIAN BROWN TROUT (*SALMO TRUTTA*) FROM AN ACID RIVER

BY PHILIP G. McWILLIAMS

Department of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ, U.K.

(*Received* 18 *February* 1980)

SUMMARY

Sodium uptake rates were measured in wild and hatchery reared Norwegian brown trout (*Salmo trutta*) exposed to media of a range of pH. Sodium uptake was strongly dependent on external acidity, being reduced in media of low pH. Wild fish from the naturally acid R. Tovdal (S. Norway) were more tolerant of acid media than hatchery reared fish. The effects of increasing external sodium concentrations were strongly influenced by low external pH. The results are discussed with respect to brown trout population decline in certain areas of S. Norway.

INTRODUCTION

The physiological problems faced by freshwater fish in acid waters have been the subject of a number of recent papers. These problems include detrimental changes in the gas-carrying capacity of the blood (Vaala & Mitchell, 1970; Dively *et al.* 1977) and a failure to maintain a balanced salt regulation (Packer & Dunson, 1970; 1972, McWilliams & Potts, 1978). Survival time of fish in acid waters is invariably reduced (Dunson & Martin, 1973; Kwain, 1975; Falk & Dunson, 1977; Daye & Garside, 1977; Dunson, Swarts & Silvestri, 1977), but the cause of death cannot be ascribed to any one factor. Many of the experiments reported have been carried out using fish not previously exposed to acid water. It is known that exposure of fish to water of sublethal acidity can modify the effects of subsequent exposures on sodium regulation (McWilliams, 1979). An increase in survival time has also been recorded following pre-treatment with acid water (Dunson *et al.* 1977; Trojnar, 1977). Similar experiments performed by other investigators have not provided consistent results (Lloyd & Jordan, 1964; Robinson *et al.* 1976; Falk & Dunson, 1977).

The problem faced by brown trout populations in Southern Norway is one of survival in low conductivity, acid water subject to substantial seasonal pH fluctuations associated with snow melt or heavy autumn rainfall. It is claimed (Leivestad & Muniz, 1976; Wright & Gjessing, 1976) that there is a progressive decrease in lake and river pH which began several decades ago and is still continuing. This is considered to be the result of acid rainfall into poorly buffered catchments. Seasonal

changes in river acidity are reputed to cause massive fish mortalities (Leivestad & Muniz, 1976). Fish examined following these periods of acid conditions have been shown to have reduced plasma sodium and chloride levels (Leivestad & Muniz, 1976). Other authors have also reported changes in plasma electrolyte concentrations in acid exposed fish (Mudge & Neff, 1971; McWilliams, 1979). The ionic concentrations of natural waters of low pH have been shown to be important in the survival of fish (Brown, 1980) and calcium in particular is important with respect to salt balance (McWilliams & Potts, 1978).

This paper reports the results of a series of experiments carried out in conjunction with the Norwegian SNSF-project (an interdisciplinary research programme 'Acid Precipitation–Effects on Forest and Fish), during the spring of 1977, designed to measure the sodium uptake rates of wild brown trout from the Tovdal River, and hatchery reared fish from water of similar quality exposed to step changes in pH of similar magnitude to those which occur in the R. Tovdal during the period of the spring snow melt.

MATERIALS AND METHODS

Sodium uptake rates were measured using ^{22}Na isotope (Amersham, England) in fish reared at the Lyngdal Hatchery (R. Lygna, S. Norway) in 'limed' water (pH approx. 6·5) and also in wild fish from the R. Tovdal, collected from sites of various pH prior to, and during, the period of the spring snow-melt.

The fish used in the experiments were brown trout weighing 30–40 g (hatchery strain) and, where possible, fish of this size were selected from the natural Tovdal population. However, because of the low numbers of fish resident at some of the sites sampled, the size of Tovdal fish used varied.

During the uptake experiments fish were kept in plastic tanks (8 fish per 25 l) containing recirculated water of controlled pH and Na content from a R. Tovdal feeder stream (Ramse Brook). pH was controlled by a Radiometer Titrator Unit ($\pm$ 0·05 pH units) dispensing 0·1 N-H_2SO_4 or 0·1 N-KOH and the water recirculated using Watson-Marlow Peristaltic pumps. NaCl was added where necessary to give final concentrations of either 30 or 450 μequiv Na/l. Fish transferred to the tanks were left overnight to settle down and the tanks covered with black plastic sheeting to avoid disturbance. ^{22}Na isotope was added to the external medium at the onset of each influx period to give a specific activity of approx. $3 \cdot 10^3$ cpm/ml. After 4 h the fish were removed from the loading medium, placed in fresh Ramse Brook water for about 15 min to remove any superficial isotope, and then killed by a sharp blow to the head. Each fish was macerated and a weighed sample removed and counted, using an ECKO Type N597 Na-iodide crystal connected to a Nuclear Enterprises Scaler Ratemeter Type SR3. Each sample was counted for 10 min and correction applied for counting efficiency and background count. A 5 ml sample of the loading medium was taken at the start of the influx period and counted to determine specific activity. A known specific activity (cpm/μg Na^+) allows a simple calculation of the amount of non-radioactive sodium taken up. Following initial uptake experiments an estimation of possible backflux of isotope was made but this was found to be negligible

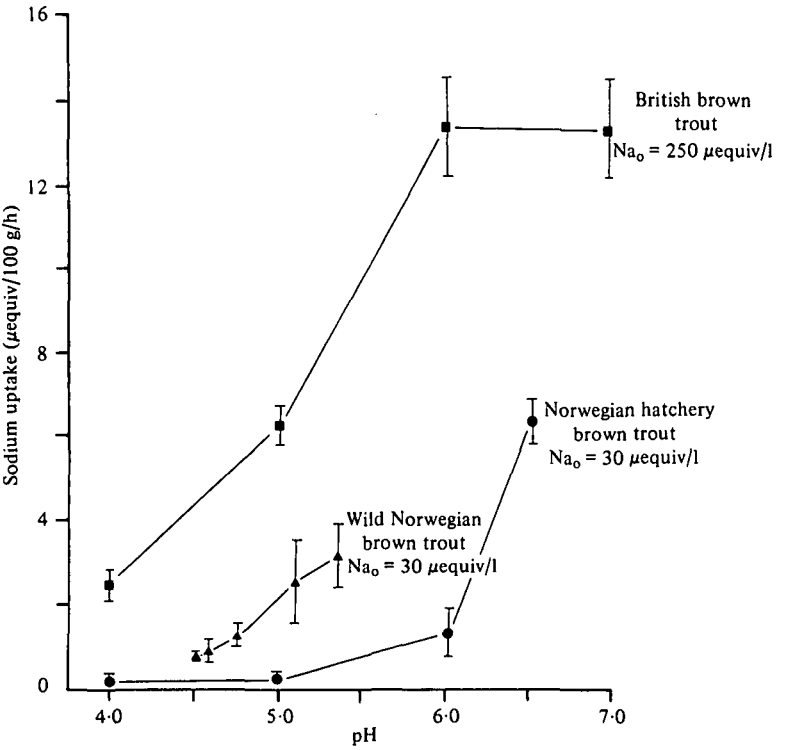

Fig. 1. Sodium ion uptake in brown trout in media of a variety of pH values. (■—■ from McWilliams & Potts, 1978) Means ± 1 × S.E. (n = 7). Ca^{2+} = 25 μequiv/l for Norwegian fish. Ca^{2+} = 250 μequiv/l for British fish.

(< 0·5 %) of total uptake rate, and so corrections were not subsequently applied. All uptake rates are expressed in μequiv Na/100 g. h.

Fish collected from the hatchery were maintained in Ramse Brook water at a pH of 6·5 for at least 1 week before being used for uptake experiments. Wild fish were kept in Ramse Brook water at its natural pH of around 4·6. Uptake rates were also measured in wild fish in water taken from the site of capture on the R. Tovdal.

RESULTS

Transfer of Norwegian hatchery trout from pH 6·5 to media of a range of pH shows that the rate of sodium uptake is strongly dependent on external pH (Fig. 1), being reduced from 6·4 μequiv Na/100 g.h in a medium of pH 6·5 to a value close to zero at pH 4·0. This effect of pH on sodium uptake closely matches that found by McWilliams & Potts (1978) for British hatchery *S. trutta* (Fig. 1) exposed to a similar pH range. The relatively low uptake rates reported here for Tovdal fish compared to those reported by McWilliams & Potts for British fish are probably a reflection of the comparatively low sodium content to which the Norwegian fish are adapted. Moreover, the Norwegian trout have a lower tissue sodium content compared to the

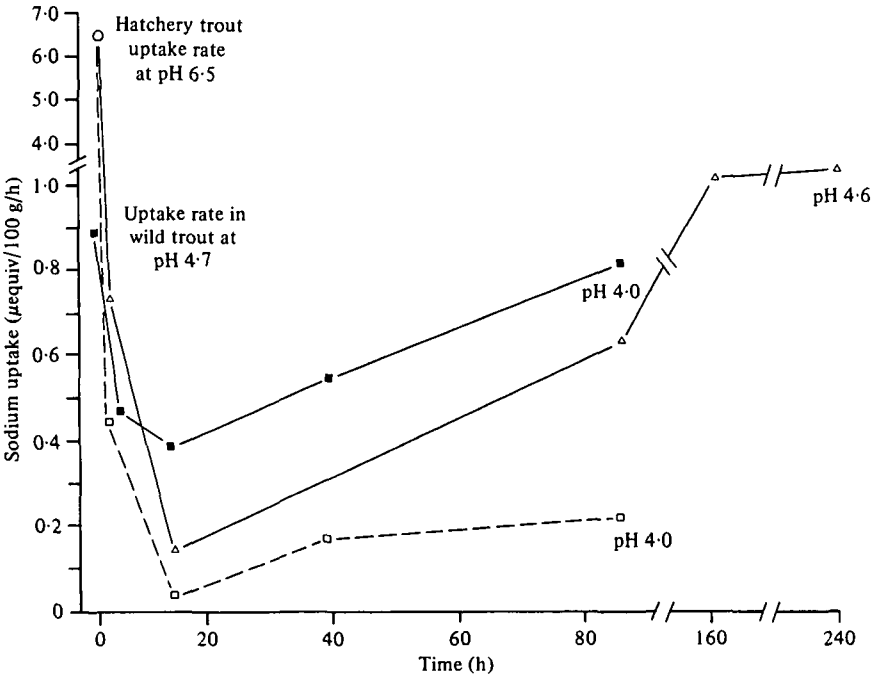

Fig. 2. Effects of a step change in pH on sodium uptake in Norwegian brown trout. ■—■ Wild Tovdal fish transferred from pH 4·7 to pH 4·0. △—△ Hatchery fish transferred from pH 6·5 to pH 4·6. □—□ Hatchery fish transferred from pH 6·5 to pH 4·0. External sodium concentration = 20 μequiv/l. Ca^{2+} = 25 μequiv/l, n = 7.

British trout (av. 17·5 μequiv Na/g wet wt. (Leivestad, personal communication) compared to 32·5 μequiv Na/g wet wt. respectively) which may also contribute to the comparatively low uptake rates in Norwegian trout.

McWilliams (1980) reports a 20% reduction in total body sodium content in brown trout exposed to an acid medium of pH 6·0 for 6 weeks. This is associated with a lowering of the overall sodium turnover rate to approximately half of that shown by fish adapted to neutral media of the same quality. Sodium uptake rates in natural Tovdal water in wild fish collected from various sites along the R. Tovdal (Fig. 1) again demonstrates a rate of uptake dependent on external pH, the lowest uptake rates being found in fish taken from sites where the water is most acid. At certain sites pH may have been artificially high as a result of 'liming' by local landowners.

The influence of continued acid exposure on the ability to take up sodium from media of acid pH was tested in Norwegian hatchery fish and wild fish from the R. Tovdal (Fig. 2). The wild fish, which have a significantly lower uptake rate than the hatchery fish in media of similar sodium content but different pH, showed a rapid, temporary decrease in uptake rate when exposed to media of pH 4·0, but after 4 days exposure, uptake rates were back to near-normal values (Fig. 2). Hatchery fish also showed a reduction in uptake rate on exposure but their ability to restore normal uptake values was dependent on external pH (Fig. 2). On exposure to media of pH 4·0 these fish showed very little increase in uptake rate following the initial decrease and 100% mortality was recorded after 4 days exposure. On exposure to media o

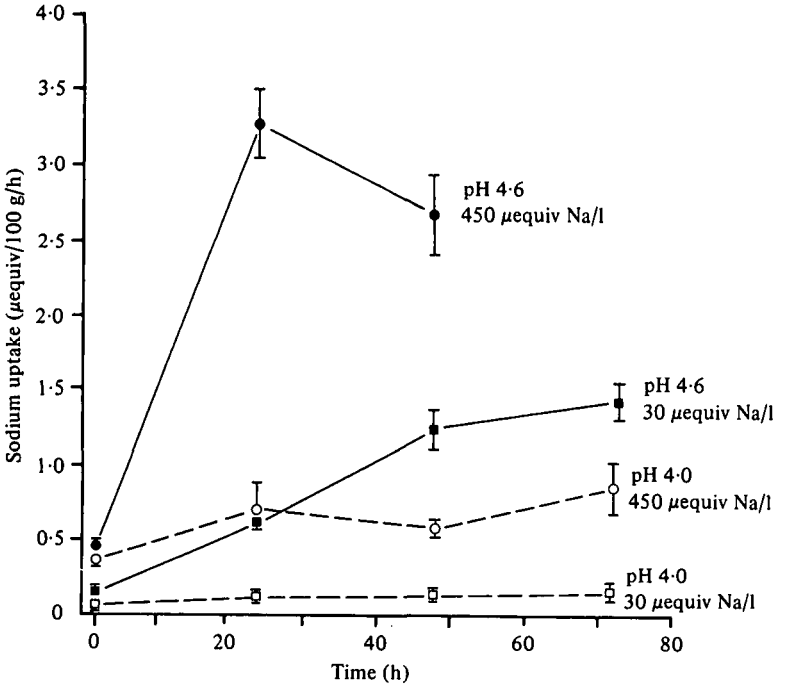

Fig. 3. Effects of transfer to acid media of high (450 μequiv/l) or low (30 μequiv/*l*) sodium content on uptake rates in Norwegian hatchery trout. Ca^{2+} = 25 μequiv/l. Means ± 1 × S.E. (n = 7).

pH 4·6 they did show a significant increase in uptake rate following the initial decrease and after 10 days exposure all of the fish were alive but with an uptake rate of only 18% of the value in water of pH 6·5.

The influence of external Na concentration on the ability to take up sodium from acid media was also tested using Norwegian hatchery fish. Two groups of 30 fish were transferred from water of pH 6·5 containing 30 μequiv Na/l and subsequently exposed to water of pH 4·0 containing 30 or 450 μequiv Na/l respectively. A further two groups were exposed to water of pH 4·6 with similar sodium concentrations. Water quality was changed (0 h) following the usual settling down period and sodium uptake rates measured at suitable intervals (Fig. 3). Initially, (0 h), the rate of sodium uptake in media of pH 4·0 and 4·6 was similar, although the uptake rate was higher in fish at the higher Na concentration (Fig. 3). As the acid exposure continued the uptake rates in pH 4·0 media showed little change at both sodium concentrations (Fig. 3) but in media of pH 4·6, uptake rates increased steadily over the duration of the experiment (Fig. 3). Surprisingly, in the high Na media at pH 4·6 there were insufficient fish left alive after 48 h exposure to make reliable measurements of uptake rates (Fig. 3).

DISCUSSION

The results presented in this paper confirm the findings of other workers that, in freshwater teleosts, sodium uptake is strongly dependent on the acidity of the external

medium, and that an inability to regulate ionic balance is an important factor contributing to the death of fish in acid waters. The experiments carried out here were designed to be as representative of natural conditions as possible and so calcium concentrations were not altered. Calcium concentrations in natural Norwegian waters range between 20 to 120 μequiv/l (Leivestad & Muniz, 1976). Values for Ramse Brook water were steady at about 25 μequiv Ca/l at the time of study. Calcium has been shown to be essential in the maintenance of ionic balance in freshwater teleosts (Cuthbert & Maetz, 1972; Oduleye, 1973; Eddy, 1975) and this is considered to be a result of its influence on the permeability of the gills to certain ions (Isaia & Masoni, 1976; McWilliams & Potts, 1978). However, in the few freshwater teleosts examined, sodium uptake is seen to be relatively insensitive to external calcium concentrations (Cuthbert & Maetz, 1972; McWilliams, 1979), whereas passive sodium losses can be significantly altered. In this respect, the results presented in Fig. 1 for wild river trout are considered to be pH effects only and confirm the strong dependence of sodium uptake rate on external pH in natural waters.

In water of similar sodium concentration, the significantly higher sodium uptake rate of wild fish compared to hatchery fish over the pH range 5·35 to 4·55 (Fig. 1), suggest that by virtue of their continued exposure to water of low pH, the wild fish may be more tolerant of their acid environment compared to the hatchery fish. Brown (1980) found that Norwegian hatchery trout did not survive as long in acid media as trout from the acid Tovdal river, suggesting that the previous history of the fish was important in determining sensitivity to low pH. Edwards & Gjedrem (1979) examined a large number of Norwegian brown trout strains from different localities in S. Norway and demonstrated that tolerance to acidity is an heritable trait. It is likely that the differences shown here between hatchery and wild fish may contain a genetic component since they are derived from different stocks but this was not tested.

Step changes in pH to a more acid medium result in an initial rapid decrease in uptake rate to a few per cent of the normal operating value in Norwegian hatchery fish (Fig. 2) but the magnitude of the step change is important in determining the subsequent increase in uptake rates on continued exposure. Exposure of hatchery fish to water of pH 4·0 strongly inhibited sodium uptake until death after 4 days exposure. Exposure to water of pH 4·6 did not permanently inhibit uptake which then showed a significant increase, but which was still well below normal even after 10 days. In a similar experiment with wild river fish (Fig. 2), a step change in pH from pH 4·7 to pH 4·0, a pH change of the magnitude likely to be encountered in the R. Tovdal, also decreased uptake rates but after a relatively short interval (4 days) uptake rates were back to normal. This suggests that the wild trout population could cope with natural pH changes in terms of maintaining sodium uptake rates. Sodium loss rates were not measured in these experiments so it is not possible to say whether the fish were in sodium balance following such pH changes, but sodium loss rate would be expected to increase on transfer to acid or lower calcium media (Packer & Dunson, 1970; McWilliams & Potts, 1978).

The fish used in this study were taken from waters of low sodium content (30–40 μequiv/l) and would be expected to possess a sodium uptake mechanism with a high affinity for sodium, compared to fish from waters of higher sodium concentrations.

This has recently been shown to be the case for Crustacea (Potts & Fryer, 1979) and, as the sodium uptake mechanisms in many freshwater organisms tested have essentially similar properties (Shaw, 1959; Potts & Parry, 1967; Kerstetter, Kirschner & Rafuse, 1970; Potts & Fryer, 1979), the same is assumed to be so for brown trout. The advantages of a high affinity for sodium are more obvious at lower external concentrations – an organism with a high affinity would be operating at a higher percentage saturation rate than one with a low affinity.

Under normal conditions, the sodium uptake mechanism in most freshwater animals operates at about 40–60% of the maximum saturation rate. At a pH of 6·5 the highest measured sodium uptake rate for Norwegian hatchery fish was 6·4 μequiv Na/100 g.h (Fig. 2), and so the maximal uptake rate would be expected to be significantly higher than this. A fifteen-fold increase in external sodium concentration should increase sodium uptake by a high affinity mechanism to near maximal values but, as shown in Fig. 3, at low pH the increase is quite small. In the high sodium media sodium uptake at pH 4·0 averages 0·6 μequiv Na/100 g.h and at pH 4·6 uptake reaches a maximum of about 3 μequiv Na/100 g.h. Both these values are well below the expected maximal rate. This clearly illustrates that low pH can affect the sodium uptake mechanism over a large range of external sodium concentrations. It is not known whether exposure to acid media can affect the affinity of the sodium uptake mechanisms. Preliminary calculations suggest that there is no difference in affinity in media of pH 4·6 and 4·0, but exposure time is relatively short.

A recent analysis of the factors influencing the fishery status of lakes in Sorlandet, S. Norway (Wright & Snekvik, 1978) has shown that most of the variation can be explained in terms of calcium concentration and pH (in that order of importance) and that sodium concentration is low on the list of important factors. However, in the experiments reported here the survival of fish in high sodium acid media does not appear to be as good as in low sodium acid media. This is an unexpected observation, but agrees with the findings of Brown (1980).

The mechanism by which external H^+ affects sodium uptake in these fish is not clear. In very low external sodium concentrations it is possible that competition between Na^+ and H^+ ions for carrier sites on the gill may occur, but this is unlikely to be significant at high sodium concentrations. Gill potentials become more positive (inside) in acid-exposed brown trout (McWilliams & Potts, 1978) increasing the work load on the sodium pump and, if the uptake mechanism involves an exchange of Na^+ for H^+ (Maetz, 1973), an increase in external H^+ will increase the concentration gradient against which the pump must expel H^+ ions. The means by which variations in acid tolerance between populations of trout can be achieved remains to be determined.

The reported decline in the numbers of resident brown trout in the lakes and rivers of S. Norway, which has been correlated with water pH (SNSF Report, 1976), suggests that the rate of adaptation (genetic or otherwise) may be taking place more slowly than changes in lake water pH. Although adult wild fish are more tolerant of prevailing acid conditions than hatchery reared fish it is likely that further increases in acidity will result in further decreases in population numbers, probably through recruitment failure. This has been shown to be the mechanism of fish population decline in Ontario lakes (Beamish & Harvey, 1972).

With present practices, Tovdal hatchery reared fish are clearly unsuitable for local restocking although pre-treatment of these fish with acid water before release may be of limited value.

The author wishes to thank Professor W. T. W. Potts and Dr G. D. Howells for helpful criticism of the manuscript. The trip to Tovdal, S. Norway where this research was carried out was funded by C.E.R.L. (Leatherhead) and accommodation and laboratory facilities kindly arranged by the SNSF-project.

REFERENCES

BEAMISH, R. J. & HARVEY, H. H. (1972). Acidification of the La Cloche mountain lakes, Ontario, and resulting fish mortalities. *J. Fish Res. Bd Can.* **29**, 1131–1143.

BROWN, D. J. A. (1980). The effects of various cations on the survival of brown trout (*Salmo trutta*) at low pHs. *J. Fish Biol.* (In the Press.)

CUTHBERT, A. W. & MAETZ, J. (1972). The effects of calcium and magnesium on sodium fluxes through gills of *Carassius auratus*, L. *J. Physiol., Lond.* **221**, 633–643.

DAYE, P. G. & GARSIDE, E. T. (1977). Lower lethal levels of pH for embryos and alevins of Atlantic Salmon (*Salmo salar*). *Can. J. Zool.* **55**, 1504–1508.

DIVELY, J. L., MUDGE, J. E., NEFF, W. H. & ANTHONY, A. (1977). Blood PO_2, PCO_2 and pH changes in brook trout (*Salvelinus fontinalis*) exposed to sublethal levels of acidity. *Comp. Biochem. Physiol.*, **57**, 347–351.

DUNSON, W. A. & MARTIN, R. R. (1973). Survival of brook trout in a bog-derived acidity gradient. *Ecology* **54**, 1370–1376.

DUNSON, W. A., SWARTS, F. & SILVESTRI, M. (1977). Exceptional tolerance to low pH of some tropical blackwater fish. *J. exp. Zool.* **201**, 157–162.

EDDY, F. B. (1975). The effect of calcium on gill potentials and on sodium and chloride fluxes in the goldfish, *Carassius auratus*. *J. comp. Physiol.* **96**, 131–142.

EDWARDS, D. & GJEDREM, T. (1979). Genetic variation in survival of brown trout eggs, fry and fingerlings in acidic water. SNSF Research Report 16.

FALK, D. L. & DUNSON, W. A. (1977). The effects of season and acute sub-lethal exposure on survival times of brook trout at low pH. *Water Res.* **11**, 13–15.

HARGIS, J. (1975). Ventilation and metabolic rate of young rainbow trout (*Salmo gairdneri*) exposed to sublethal environmental pH. *J. exp. Zool.* **196**, 39–44.

ISAIA, J. & MASONI, A. (1976). The effects of calcium and magnesium on water and ionic permeabilities in the sea water adapted eel, *Anguilla anguilla*, L. *J. comp. Physiol.* **109**, 221–233.

KERSTETTER, T. H., KIRSCHNER, L. B. & RAFUSE, D. D. (1970). On the mechanism of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.* **56**, 342–359.

KWAIN, W. (1975). Effects of temperature on development and survival of rainbow trout (*Salmo gairdneri*), in acid waters. *J. Fish Res. Bd Can.* **32**, 493–497.

LEIVESTAD, H. & MUNIZ, I. P. (1976). Fish kill at low pH in a Norwegian River. *Nature, Lond.* **259**, 391–392.

LLOYD, R. & JORDAN, D. H. M. (1964). Some factors affecting the resistance of rainbow trout (*Salmo gairdneri*) to acid waters. *Int. J. Air Wat. Pollut.* **8**, 393–403.

MCWILLIAMS, P. G. (1979). The effects of external acidity and calcium concentration on sodium regulation in the brown trout (*Salmo trutta*, L.). Ph.D. thesis, University of Lancaster.

MCWILLIAMS, P. G. (1980). Acclimation to an acid medium in the brown trout, *Salmo trutta*. *J. exp. Biol.* (In press.)

MCWILLIAMS, P. G. & POTTS, W. T. W. (1978). The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta*. *J. comp. Physiol.* **126**, 277–286.

MAETZ, J. (1973). Na^+/NH^+_4, Na^+/H^+ exchanges and NH_3 movement across the gills of *Carassius auratus*. *J. exp. Biol.* **58**, 255–275.

MUDGE, J. E. & NEFF, W. H. (1971). Sodium and potassium levels in serum of acid exposed brook trout, *Salvelinus fontinalis*. *Proc. Pa Acad. Sci.* **45**, 101–103.

ODULEYE, S. O. (1973). The effects of calcium and prolactin on water and electrolyte regulation in the brown trout. (*Salmo trutta*). Ph.D. thesis, University of Lancaster.

PACKER, R. K. & DUNSON, W. A. (1970). Effects of low environmental pH on blood pH and sodium balance of brook trout. *J. exp. Zool.* **174**, 65–72.

PACKER, R. K. & DUNSON, W. A. (1972). Anoxia and sodium loss associated with the death of brook trout at low pH. *Comp. Biochem. Physiol.* **41A**, 17–26.

POTTS, W. T. W. & FRYER, G. (1979). The effects of pH and salt content on sodium balance in *Daphnia magna* and *Acantholeberis curvirostris* (Crustacea: Cladocera). *J. comp. Physiol.* **129**, 189–294.

POTTS, W. T. W. & PARRY, G. (1967). Sodium balance in two fish from Lake Garda. *Memorie. Ist. ital. Idrobiol.* **22**, 53–60.

ROBINSON, G. D., DUNSON, W. A., WRIGHT, J. E. & MAMOLITO, G. E. (1976). Differences in low pH tolerance among strains of brook trout (*Salvelinus fontinalis*). *J. exp. Zool.* **204**, 33–42.

SHAW, J. (1959). The absorption of sodium ions by the crayfish *Astacus pallipes* (L). I. The effect of external and internal sodium concentrations. *J. exp. Biol.* **36**, 126–144.

TROJNAR, J. R. (1977). Egg hatchability and tolerance of brook trout (*Salvelinus fontinalis*) fry at low pH. *J. Fish. Res. Bd Can.* **34**, 574–579.

VAALA, S. S. & MITCHELL, R. B. (1970). Blood pO_2 changes in acid exposed brown trout. *Proc. Pa Acad. Sci.* **44**, 41–44.

WRIGHT, R. F. & GJESSING, E. T. (1976). Acid precipitation: changes in the chemical composition of lakes. *Ambio* **5**, 219–223.

WRIGHT, R. F. & SNEKVIK, E. (1978). Chemistry and fish populations in 700 lakes in southernmost Norway. *Verh. Internat. Verin. theor-angew. Limnol.* **20**, 765–775.