OSMOTIC AND IONIC REGULATION IN SCOTTISH BROWN TROUT AND SEA TROUT (*SALMO TRUTTA* L.)

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(*Received* 11 *July* 1958)

The brown trout (*Salmo trutta* L.) was originally a Palearctic fish, widely distributed in Europe and western Asia from the Mediterranean to the Arctic. Many river drainage systems in this area contained, and still contain, two or even three more or less morphologically and behaviourally recognizable forms of this species. These forms are the 'lake trout', living in fresh-water lakes, the 'brown trout', living in fresh-water streams, and the 'sea trout', an anadromous form. The question of whether or not these forms, especially the brown trout and the sea trout, are 'really' (equals genetically) different from one another has been debated by fishermen and others for centuries.

Trewavas (1953) reviewed the most significant portions of the voluminous literature on this subject. She concluded that average differences in some characters (meristic characters, age at maturity and frequency of spawning) do separate some populations of the two types, but these do not separate all brown trout from all sea trout. Trewavas favoured the view that the forms are ecotypes of a single species, although there are difficulties attendant on the application of even this generalized term.

The results of hatchery breeding experiments carried out under fairly uniform conditions by Skrochowska (1951) were unknown to Trewavas at the time she wrote her paper, but confirm her view. This also is the case with tagging experiments reported by Skrochowska (1953), though these latter data are not as numerous as one might wish. Svärdson (1955) further supports Trewavas and presents an ingenious hypothesis explaining the existence of the two forms on the basis of responses of individual fish to environmental changes.

The morphological, behavioural and genetic evidence thus seems to indicate very strongly that brown trout and sea trout are at most morphotypes of a single species. Alm (1949), however, described morphological differences between the brown trout and the lake trout which seem to be genetically determined. Further, a very closely related form, the rainbow trout (*Salmo gairdneri*), shows what appear to be genetically determined differences in lateral line scale-counts between sea-run and fresh-water populations (Neave, 1944).

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† Contribution number 927 from the Woods Hole Oceanographic Institution.

That a polymorphic species differentiated into three more or less equally morphologically distinct forms, say *A*, *B* and *C*, would have *A* and *B* genetically different from one another, but *B* and *C* not so, would seem unlikely. It also seems doubtful that the rainbow trout is significantly older than the brown trout in an evolutionary sense. It would therefore be surprising if the brown trout, faced with environmental stresses identical to those faced by the rainbow trout, has not made genetic rearrangements similar to those made by the rainbow. One might also expect that such rearrangements would be reflected in the osmotic and ionic regulatory abilities of the fish.

During the course of a detailed study of osmotic and ionic regulation in an American hatchery population of *Salmo trutta* the writer observed several instances of variable behaviour between individual fish subjected to the same strong osmotic stress for long periods of time (Gordon, 1959). American brown trout are derived from European brown trout, and no one knows whether or not, or how many, European sea trout were included in the original *Salmo trutta* shipments sent to the United States. This fact, combined with the considerations mentioned above, made it seem reasonable to interpret the variability noted in the American fish as being a result of their being hybrid fish descended from physiologically distinct ancestors.

To test this hypothesis arrangements were made to obtain blood serum samples from known Scottish sea and brown trout subjected to osmotic stresses similar to those used on the American fish. The results of the analyses for freezing-point depression, chloride, sodium, and potassium that were carried out on these samples, the implications of these analyses for the brown trout or sea trout problem, and some comparisons of these data with material obtained from American fish form the basis for this paper.

MATERIALS AND METHODS

Known Scottish sea trout and brown trout were obtained from the area around Aberdeen between November 1956 and May 1957 by Dr Isabel W. Smith of the Marine Laboratory, Aberdeen. The fish were all sexually mature and varied in size from 18 to 55 cm. standard length. Temperature throughout was $10 \pm 2°$ C. Fish were sampled as follows:

Sea trout. In November 1956 three sea trout were sampled after unknown, but perhaps quite short, periods in fresh water while on the upstream phase of their spawning migration. Two sea trout caught similarly were sampled 24 hr. after having been transferred to full sea water (salinity 32·6‰). Five more sea trout were sampled after having been maintained in full sea water for 5 months at the Marine Laboratory aquarium. In May 1957 seven sea trout were sampled after having been captured in commercial nets in an estuary containing water varying tidally in salinity from about 2–32‰.

Brown trout. In March 1957 a number of fish locally considered to be *Salmo levenensis*, or fresh-water brown trout, were removed from a reservoir near Aberdeen in which they had been stocked either 12 or 20 years earlier. Eleven of these were sampled while still in fresh water (some of them twice), then were

transferred to one-half sea water (salinity 16‰) where they were kept for 10 days. Several of them were then sampled and all were transferred to full sea water. Additional samples were taken from varying numbers of these fish after 2 days in sea water, 10 days in sea water and 64 days in sea water (three survivors).

Blood samples were taken from the fish by Dr Smith by either heart or caudal artery puncture using a syringe with a fine needle. The samples were allowed to clot and the serum was pipetted off and placed in acid-cleaned vials. The vials were then tightly stoppered with paraffined corks, the samples frozen on dry ice and shipped in the frozen state by air express to the United States. Analyses for freezing-point depression (Δ), chloride (Cl), sodium (Na) and potassium (K) were then carried out by the author using methods described elsewhere (Gordon, 1959).

The multiple sampling of single brown trout in the present work is similar to the procedure used with Arctic char (*Salvelinus alpinus*) by Gordon (1957). It did not seem to cause any shock effects in the char.

Table 1. *Serum* Δ *in Scottish brown and sea trout*

State of acclimatization	N	Serum Δ (° C.) ($\bar{x} \pm$ S.E.)
Brown trout		
F.W.	11	0·607 ± 0·011
½ S.W., 240 hr.	5	0·622 ± 0·038
½ S.W., 240 hr.–S.W., 48 hr.	4	0·748 ± 0·030
½ S.W., 240 hr.–S.W., 240 hr.	8	0·674 ± 0·021
½ S.W., 240 hr.–S.W., 64 days	3	0·637 ± 0·022
Sea trout		
F.W.	3	0·653 ± 0·045
S.W., 24 hr.	2	0·79, 0·81
S.W., 5 months	5	0·662 ± 0·014
Brackish, indefinite	7	0·666 ± 0·015

RESULTS

The data on serum Δ, Cl, Na and K in the sea and brown trout are presented in Tables 1–4. Gordon (1959) has shown that neither sex of the fish nor season (during the sampling periods involved here) have any significant effect on these concentrations.

The main point demonstrated by these data is that, with only one exception, the concentrations studied were insignificantly different (at the $P = 0{\cdot}05$ level by 't' test) in the brown trout and the sea trout when the fish were acclimatized to full sea water for long periods (10 days to 5 months). The one exception was serum Cl in brown trout acclimatized to sea water for 10 days as compared with sea trout in sea water for 5 months. These two means are highly significantly different ($P \ll 0{\cdot}01$). This exception is discussed below.

Similar agreement between the two forms occurred for their fresh-water concentrations as well, though the sample size of only two fish for most of the sea trout concentrations makes this point less certain.

Table 2. *Serum chloride in Scottish brown and sea trout*

State of acclimatization	N	Serum Cl (m-equiv./l.) ($\bar{x} \pm$ S.E.)
Brown trout		
F.W.	11	133·3 ± 3·1
½ S.W., 240 hr.	5	131·4 ± 7·8
½ S.W., 240 hr.–S.W., 48 hr.	4	185·2 ± 4·5
½ S.W., 240 hr.–S.W., 240 hr.	8	168·9 ± 6·1
½ S.W., 240 hr.–S.W., 64 days	3	150·0 ± 9·5
Sea trout		
F.W.	2	110, 137
S.W., 24 hr.	2	186, 196
S.W., 5 months	5	138·4 ± 0·8
Brackish, indefinite	7	121·7 ± 1·6

Table 3. *Serum sodium in Scottish brown and sea trout*

State of acclimatization	N	Serum Na (m-equiv./l.) ($\bar{x} \pm$ S.E.)
Brown trout		
F.W.	5	149·8 ± 3·1
½ S.W., 240 hr.	5	139·0 ± 10·7
½ S.W., 240 hr.–S.W., 240 hr.	6	179·7 ± 6·9
½ S.W., 240 hr.–S.W., 64 days	3	163·0 ± 6·5
Sea trout		
F.W.	2	141, 168
S.W., 24 hr.	2	203, 206
S.W., 5 months	5	165·8 ± 2·2
Brackish, indefinite	1	144

Table 4. *Serum potassium in Scottish brown and sea trout*

State of acclimatization	N	Serum K (m-equiv./l.) ($\bar{x} \pm$ S.E.)
Brown trout		
F.W.	5	2·7 ± 0·5
½ S.W., 240 hr.	5	5·1 ± 0·8
½ S.W., 240 hr.–S.W., 240 hr.	6	1·5 ± 0·2
½ S.W., 240 hr.–S.W., 64 days	3	3·1 ± 0·3
Sea trout		
F.W.	2	4·0, 6·6
S.W., 24 hr.	2	0·5, 1·3
S.W., 5 months	5	3·5 ± 1·5
Brackish, indefinite	1	> 10

DISCUSSION

It seems reasonable to conclude from this that, at least for the populations of sea and brown trout near Aberdeen and for the concentrations measured, there are no significant osmotic or ionic regulatory differences between the two forms. However, the implications of the somewhat anomalous behaviour of serum chloride concentration complicate the situation.

The serum Na:Cl ratios for all groups of brown trout are in the range 1·07–1·13. Excepting the two fish sampled after only 24 hr. in sea water (and therefore probably not in states of internal ionic equilibrium) all groups of sea trout had Na:Cl ratios of 1·18–1·28. This difference in ratios is perhaps indicative of a larger contribution of alkaline reserve to total serum concentration in the sea trout than in the brown trout. Such a difference in alkaline reserve could account for the statistically significant difference found between the mean serum chloride concentrations of brown trout in sea water for 10 days as compared with sea trout in sea water for 5 months. It also might indicate a real difference between sea and brown trout with respect to mechanisms for control of blood acid-base balance.

If correct, this interpretation would perhaps not be in agreement with the results of work on the eel (*Anguilla*) done by Boucher-Firly (1935). Boucher-Firly found consistent decreases of alkaline reserve (to about 40% of mean fresh-water levels) in all life-history stages of the eel following long-term acclimatizations to sea water (more than 12 days). The relative constancy of the Na:Cl ratios in brown trout and sea trout in both fresh and sea water argues against the occurrence of such changes in *Salmo trutta*. However, the actual concentration changes involved would be small (on the order of 10 m-equiv./l.) and may have been masked in the present material by other effects. Direct measurements of alkaline reserve in brown and sea trout are obviously needed.

The only previously published data on osmotic and ionic concentrations in *Salmo trutta* that the writer is aware of are given by Dekhuyzen (1905), Spalding, in Jones (1956), and Phillips & Brockway (1958). Dekhuyzen lists serum Δ for one brown trout in fresh water in the Amsterdam aquarium (−0·57° C.), and a mean for six dead, or nearly dead, sea trout from sea water at Bergen, Norway (−0·78° C.). Spalding lists mean serum Na and K concentrations for brown trout in fresh water during the winter (Na: 144·2 m-equiv./l.; K: 8·9 m-equiv./l.). Phillips & Brockway (1958) present figures for the means and ranges of Cl, Na and K concentrations (also concentrations of several other ions) in the serum of brown trout in fresh water in summer. These values are: Cl: 119 m-equiv./l. (range: 118–124 m-equiv./l.); Na: 156 m-equiv./l. (range: 151–162 m-equiv./l.); K: 5·2 m-equiv./l. (range: 4·3–6·6 m-equiv./l.).

The present data on serum Δ and Na in fresh water agree well with the figures of Dekhuyzen and Spalding, the K data do not. The cause of the discrepancy is unknown. The Δ from dying or dead sea trout is probably unreliable.

The figures given by Phillips & Brockway (1958) are different from both the present data and those of Spalding, probably primarily as a result of having been obtained from summer fish. Brown trout in summer are quite different from brown trout during the rest of the year (Gordon, 1959, for details).

The patterns of variation in serum concentrations indicated here for Scottish brown trout are similar to those found in American brown trout. The latter are discussed in detail elsewhere (Gordon, 1959). However, there are several points which should be made here concerning comparisons between the two groups of fish.

As noted, the overall patterns of variation of the different serum components are very similar in the two groups, though there are small quantitative differences. This may be seen for serum Cl by comparing Table 5 with Table 2. Table 5 presents serum Cl data for brown trout from a hatchery in Massachusetts acclimatized in the ways noted. The experiments were carried out during the same times of year as the experiments with Scottish fish. The only significant difference between the two series is that the American experiments were done at 20° C., the Scottish at 10° C. Additional data on the American fish show that this temperature difference results in the American figures averaging 10 m-equiv./l. higher than they would have been if the experiments had been carried out at 10° C.

Table 5. *Serum chloride in American brown trout*

State of acclimatization	N	Serum Cl (m-equiv./l.) ($\bar{x} \pm$ S.E.)
F.W.	14	140·5 ± 1·1
½ S.W., 240 hr.	12	148·2 ± 2·3
S.W., 21–30 hr.	15	203·6 ± 3·3
½ S.W., 240 hr.–S.W., 47 hr.	5	170·4 ± 1·6
½ S.W., 240 hr.–S.W., 240 hr.	7	179·1 ± 11·5

This agreement in overall patterns of regulation indicates that conclusions concerning mechanisms of ionic regulation in the brown trout reached on the basis of the American experiments are very likely valid for the species as a whole. The quantitative differences between the two groups indicate that their many generations of genetic isolation from one another have resulted in small amounts of divergence, but not in any basic changes.

The two groups of data also demonstrate that the brown trout is an almost perfect osmotic and ionic regulator. With long-term acclimatizations over the range of salinities from fresh water to full sea water (again excepting serum Cl after 10 days in sea water) all internal concentrations were either statistically insignificantly different from the fresh-water levels or were within 10% of the fresh-water levels. Many individual trout were completely homoiosmotic. *Salmo trutta* is the first salmonid known to possess such excellent regulatory abilities (cf. Gordon, 1957).

Finally, there is a small but interesting point of difference between American and Scottish fish with regard to the degree of parallelism shown between changes in serum Δ and changes in serum Cl and Na. In American brown trout under all the conditions studied serum Cl contributed 40–45%, serum Na 45–49% of the Δ (assuming unit activities for the ions). Much the same was true for the Scottish fish, but with one clear exception. This was the group of sea trout sampled from brackish water in an estuary. In these fish ion concentrations were significantly below the range to be expected from the Δ measurements (only enough material for one Na determination was available). Cl made up only 35%, Na only about 41% of the total concentration.

It might be worthwhile investigating the possibility that, in fish subjected to continually and widely varying salinities, some blood component other than Na and Cl becomes important in regulation of total osmotic pressure. The one serum K value from a fish in brackish water was well above the calibration range of the analyses. Perhaps K is the other component varied (though this would seem unlikely on the basis of known depressant effects of high external K concentrations on nerve and muscle function). This situation may be similar to the differential behaviour of serum Δ and serum Cl noted in shallow-water fishes in Hebron Fjord, Labrador, in winter by Scholander *et al.* (1957).

SUMMARY

1. Adult brown trout (*Salmo trutta* L.) of both sea-run (sea trout) and fresh-water stream (brown trout) forms were captured in the vicinity of Aberdeen and acclimatized to full-strength sea water for periods of up to 5 months.

2. Blood serum samples from these fish were analysed for freezing-point depression, chloride, sodium and potassium concentrations.

3. The patterns of regulation of these concentrations are very nearly the same in both forms. Brown trout and sea trout, at least in eastern Scotland, thus appear to be virtually identical in osmotic and ionic regulatory abilities. However, there is a possibility that there is a difference between the two forms with respect to mechanisms controlling blood acid-base balance.

4. The patterns of regulation shown by Scottish fish are the same as those shown by American hatchery fish treated similarly. The different populations of the species seem not to have diverged significantly from one another in this regard after many generations of more or less complete genetic isolation.

5. The species *Salmo trutta* is strongly homoiosmotic. Internal concentrations are either unchanged or increase by less than 10% above fresh-water levels with long-term acclimatizations to half and full sea water. The brown trout is the first salmonid species known to regulate so well.

These studies were aided by National Science Foundation Predoctoral Fellowships for 1954–57 and a grant from the Higgins Fluid Research Fund, Yale University. This paper is a portion of a dissertation presented to the faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy. Contribution number 927 from the Woods Hole Oceanographic Institution.

The author's thanks for aid and advice are due to the following: Drs E. J. Boell, T. H. Waterman, G. E. Pickford and L. M. Passano, Yale University; Drs C. O'D. Iselin, B. H. Ketchum and A. C. Redfield, Woods Hole Oceanographic Institution; Dr H. Barnes, Marine Laboratory, Millport, Scotland; Mr K. A. Pyefinch, Brown Trout Research Laboratory, Pitlochry, Scotland; Dr I. W. Smith, Marine Laboratory, Aberdeen, Scotland; Dr H. O. Werntz, Harvard University; and Mr H. Robbins, Wareham, Mass.

REFERENCES

ALM, G. (1949). Influence of heredity and environment on various forms of trout. *Rep. Inst. Freshw. Res. Drottning.* **29**, 29–34.

BOUCHER-FIRLY, S. (1935). Recherches biochimiques sur les teleostéens apodes (Anguille, Congre, Murène). *Ann. Inst. oceanogr. Monaco*, **15**, 217–327.

DEKHUYZEN, M. C. (1905). Sur la pression osmotique dans le sang et dans l'urine des poissons. *Arch. néerland. Sci.* **10**, 121–36.

GORDON, M. S. (1957). Observations on osmoregulation in the Arctic Char (*Salvelinus alpinus* L.). *Biol. Bull., Woods Hole*, **112**, 28–33.

GORDON, M. S. (1959). Ionic regulation in the brown trout (*Salmo trutta* L.). *J. Exp. Biol.* **36**, 227–52.

JONES, I. C. (1956). The role of the adrenal cortex in the control of water and salt-electrolyte metabolism in vertebrates. *Mem. Soc. Endocrin.* no. 5, 102–20.

NEAVE, F. (1944). Racial characteristics and migratory habits in *Salmo gairdneri*. *J. Fish. Res. Bd., Can.* **6**, 245–51.

PHILLIPS, A. M., JR. & BROCKWAY, D. R. (1958). The inorganic composition of brown trout blood. *Progr. Fish Cult.* **20**, 58–61.

SCHOLANDER, P. F., VAN DAM, L., KANWISHER, J., HAMMEL, T. & GORDON, M. S. (1957). Supercooling and osmoregulation in Arctic fish. *J. Cell. Comp. Physiol.* **49**, 5–24.

SKROCHOWSKA, S. (1951). The rearing of sea trout, *Salmo trutta* L., in artificial ponds. *Bull. Int. Acad. Polon. Sci. Lett., Classe Sci. Math. Nat.* Ser. B (II), no. 4–6, 179–226.

SKROCHOWSKA, S. (1953). The migrations of sea trout (*Salmo trutta*) and other salmon fishes bred in ponds. *Polsk. Arch. Hydrobiol.* **1**, 89–135 (in Polish, English summary).

SVÄRDSON, G. (1955). Salmon stock fluctuations in the Baltic Sea. *Rep. Inst. Freshw. Res. Drottning.* **36**, 226–62.

TREWAVAS, E. (1953). Sea-trout and brown trout. *Salmon and Trout Mag.* **139**, 199–215.