IONIC REGULATION IN THE BROWN TROUT (*SALMO TRUTTA* L.)

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INTRODUCTION

Studies of osmotic and ionic regulation in aquatic organisms may be divided into three major categories:

1. Descriptions of the overall patterns of osmotic and ionic regulation in the internal fluids of the organism when the organism is subjected to various physiologically or experimentally interesting osmotic and ionic stresses. Variations due to sex, time of year, temperature, age and size of organism, etc. should be considered in such descriptions.

2. Studies of the various pathways for movement of water, inorganic ions and other osmotically active substances into and out of the internal fluids of the organism. Such studies may be kinetic, biochemical, pharmacological, etc., and should include any internal movements of ions between the tissues and the internal fluids.

3. Studies of the mechanisms resulting in internal integration of the action of the various pathways (hormones and the central nervous system).

Teleost fishes have been studied to some extent in each of these ways, but a great deal more remains to be learned. Recent reviews of various aspects of the work that has been done are those of Beadle (1957), Black (1957), Fontaine (1954, 1956), Hoar (1953), Hoar, Black & Black (1951), Pickford & Atz (1957), D. C. W. Smith (1956) and von Buddenbrock (1956).

The euryhaline teleosts are of particular interest on account of their ability to survive in environments varying widely in salinity. The adult stages of several such forms have been investigated in some detail, though with greatly varying degrees of completeness.

Fairly complete Category 1 studies investigating the total osmotic pressure and chloride concentration of serum have been carried out on eels (*Anguilla spp.*: Duval, 1925; Boucher-Firly, 1935; Callamand, 1943; Koch, 1949; Fontaine & Koch, 1950, for summary), on the Atlantic salmon (*Salmo salar*: Benditt, Morrison & Irving, 1941; Fontaine, Callamand & Vibert, 1950), on the three-spined stickleback (*Gasterosteus aculeatus*: Gueylard, 1924; Heuts, 1942, 1945; Koch and Heuts, 1942, 1943), and on the ten-spined stickleback (*Pungitius pungitius*: Heuts, 1943).

Much less complete studies of osmotic pressure and/or chloride have been carried

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out on a number of other forms. Most of this work consists simply of measurements of blood concentration in groups of fish acclimatized to fresh water and to salt water, no attention having been given to temperature, season, etc. The forms investigated include brown trout (*Salmo trutta*: Dekhuyzen, 1905; Parry, 1958), rainbow trout (*Salmo gairdneri*: Busnel, 1942; Parry, 1958), chinook salmon (*Oncorhynchus tchawytscha*: Greene, 1904, 1926), various Japanese salmonids (*Oncorhynchus spp.* and *Salvelinus spp.*: Kubo, 1953), Arctic char (*Salvelinus alpinus*: Gordon, 1957), shad (? *Alosa sp.*: Fontaine, 1930) and killifish (*Fundulus heteroclitus*: Burden, 1956; Garrard, 1935).

Eels and Atlantic salmon are the only forms for which any significant amount of information is available on sodium, potassium and phosphorus concentrations in serum—and the data are very sparse even for these. Sodium and potassium were studied in the eel by Drilhon (1943), phosphorus by Fontaine & Firly (1932), Boucher-Firly (1935) and Ichikawa (1955). Sodium in the Atlantic salmon was studied by Fontaine, Callamand & Vibert (1950).

Some additional information available for the brown trout includes: studies of survival in hypertonic environments (D.C.W. Smith, 1956; Parry, 1958); values for the concentrations of most of the major inorganic ions in the serum of trout in fresh water in summer (Phillips & Brockway, 1958); and values for sodium and potassium in the serum of trout in fresh water in winter (Spalding, in Jones, 1956).

Many fewer Category 2 studies have been carried out on fishes, much less on euryhaline forms. Most of the work which has been done is thoroughly discussed in the reviews listed above and elsewhere. It should be noted, however, that with the exception of the somewhat fragmentary work of Mullins (1950) on *Gasterosteus* no work has been done on estimations of ion flux rates along the various pathways, or on the detailed mechanism of salinity adaptations, using modern methods.

A further omission, from the older literature as well as the new, has been a consideration of the possible significance for regulation of blood concentrations of internal ion movements between various tissues, especially the muscles, and the blood. The only references to this topic known to me are in the very partial studies of Drilhon (1937) and Callamand, Fontaine, Olivereau & Raffy (1951) on the eel. Drilhon suggests, and the data of Callamand *et al.* seem to lend some support to her suggestion that the muscles of the eel act as a reservoir of salts when the fish is in salt water.

The reader is referred to the reviews listed above for summaries of the Category 3 work carried out on euryhaline fishes.

The present paper falls into both Categories 1 and 2. The form studied was the brown trout (*Salmo trutta* L.), a euryhaline salmonid fish which is often anadromous. Information on the differences between sea-run and strictly fresh-water brown trout is given by Gordon (1959).

The main purpose of the work was to establish in some detail the overall pattern of normal variation (and the changes occurring in this pattern following osmotic stresses similar to those which would be encountered during migration to the sea)

of the quantitatively most important inorganic constituents of the blood and muscles of brown trout. With this end in view, sexually mature brown trout within a limited size range were acclimatized for varying periods of time to fresh water and to half- and to full-strength sea water. Such acclimatization experiments were repeated three times during the course of a year at one temperature, and once at a different temperature. Blood and muscle samples were taken from groups of fish at various times during the acclimatization process. These were analysed for the following components:

Serum: freezing-point depression, ° C. (Δ); chloride, m-equiv./l. (Cl_s); sodium, m-equiv./l. (Na_s); potassium, m-equiv./l. (K_s); and total phosphorus, mg./l. (P).

Muscles: total solids, g./kg. wet weight; chloride, m-equiv./kg. wet weight (Cl_m); sodium, m-equiv./kg. wet weight (Na_m); and potassium, m-equiv./kg. wet weight (K_m).

The results of this overall (Category 1) survey will be presented first, consideration being given to the effects of season and temperature. The implications of these results will then be considered under the following headings: water and ion movements between the muscles and the blood (a test of Drilhon's (1937) ion store hypothesis—Category 2), and ecological implications.

MATERIAL AND METHODS

Animals

Sexually mature brown trout 18–24 cm. in standard length were obtained from two hatcheries in the states of Connecticut and Massachusetts respectively, the majority coming from the Massachusetts hatchery. All handling necessary was done as gently as possible, usually by means of smooth linen nets. The fish were transported from the hatcheries to the laboratory in 20-gal. galvanized iron garbage cans painted on the inside with black asphaltum varnish. Considerable mortality occurred in preliminary experiments using unpainted cans.* The fish were not fed for several days prior to, and during, the experiments. In order to prevent them from fighting the fish were maintained separately in smaller enclosures within the large tanks used.

Hatchery water temperatures varied seasonally from about 2° to 24° C. Experimental series involving temperatures significantly different from these hatchery temperatures were preceded by a gradual thermal acclimatization of the fish over 12–24 hr. The fish were maintained in aerated hatchery water during this period.

Experimental design

All experimental series followed the same pattern. Control fish were sampled (i) directly from aerated fresh water in the hatchery tanks, (ii) in the laboratory after having undergone the same thermal acclimatization as the experimentals (zero time controls), and (iii) at intervals during the experimental period. They

* Unpainted galvanized tubs were used by Gordon (1957) for handling Arctic char at Churchill, Manitoba. It seems likely that much of the mortality encountered in that work was due to this cause.

were handled and otherwise treated as experimentals. Experimental fish were transferred from fresh water (salinity < 0·2‰) directly to aerated running half-strength sea water (salinity 15 ± 2‰) or to aerated running full-strength sea water (salinity 31·5 ± 0·5‰). Groups of fish were sampled at intervals up to ten days following transfer. In the 20° C. series, where direct transfer to full sea water proved fatal to all fish within 15–30 hr., fish acclimatized to half sea water for 10 days were further transferred to full sea water and samples were taken up to 10 days following the second transfer. The work of Boucher-Firly (1935) on *Anguilla*, that of Gordon (1959) and Parry (1958) on *Salmo trutta*, and the present results indicate that ten days is enough time for at least all major changes in internal concentrations to have taken place.

Experimental series were designed to demonstrate (i) seasonal effects, if any, at constant temperature, and (ii) temperature effects within one season. One preliminary experiment was done using fish from the Connecticut hatchery, but all other experiments were done with Massachusetts fish. Experiments with Massachusetts fish were as follows:

1. Seasonal effects at constant temperature (20 ± 2° C.):

(*a*) controls; half and full sea-water transfers in the breeding season (September–November). (*b*) controls; half and full sea-water transfers in winter and spring (February–April). (*c*) controls; half and full sea-water transfers in summer (June–August).

2. Temperature effects within one season (winter—a few data from the breeding season were also obtained):

(*a*) controls and full sea-water transfers at 10 ± 2° C. (*b*) controls; half and full sea-water transfers at 20 ± 2° C. (Exp. 1 (*b*)).

Details of the experimental protocols are given in Table 1.

The preliminary experiment using Connecticut fish included fresh-water controls and transfers to seven-eighths sea water (salinity 28‰) at 10° C. in February. Its principal purpose was to test technique and it was continued for only three days.

Complete series of serum and muscle analyses were done on all fish in Exps. 1 (*a*), (*b*), 2 (*a*), (*b*), in the fresh-water controls in 1 (*c*) and in the preliminary experiment. Determinations of muscle solids only were done on the half and full sea-water transfers in 1 (*c*).

Photoperiod was uncontrolled in all experiments, but the fish were obtained from outdoor tanks at the hatchery in which they had been exposed to the seasonal variations in light.

Analytical techniques

Blood samples. 1·0–2·0 ml. of whole blood were usually easily obtained by heart puncture with a 1 ml. tuberculin syringe and a fine (no. 22) needle. In cases where difficulties were encountered, samples were taken with the same syringe from the caudal artery and vein dorsal to the anterior edge of the insertion of the anal fin. Grafflin (1935) and Forster (1953) have shown that samples obtained in these two

Table 1. *Protocols of experiments with Massachusetts fish*

(August 1956 to August 1957.)

Starting date	Hatchery water temp. (° C.)	No. of fish	Samples obtained (state of acclimatization, no. of fish in sample)
		1 (*a*) Breeding season series—20 ± 3° C.	
4. ix. 56	16	20	20 in ½ SW: 2 hr. 2; 4 hr. 2; 8 hr. 2; 12 hr. 2; 24 hr. 4; 48 hr. 2; 72 hr. 3. 3 dead.
20. ix. 56	11	22	14 in ½ SW: 8 hr. 2; 48 hr. 4; 240 hr. 7. 1 dead. 8 in FW: 72 hr. 4; 240 hr. 3. 1 dead.
2. x. 56	12	26	6 in FW: 0 hr. 6. 20 in SW: 2 hr. 2; 4 hr. 2; 8 hr. 2; 12 hr. 2; 24–30 hr. 9. 3 dead.
8. x. 56	12	12	9 in ½ SW. 7 survivors after 240 hr. to SW: 12 hr. 2; 48 hr. 5. 3 in SW: 3 hr. 1; 18 hr. 1. 1 dead.
10. x. 56	11	6	Hatchery controls—6.
24. x. 56	13	9	9 in ½ SW. 9 survivors after 240 hr. to SW: 130 hr. 2; 240 hr. 6. 1 dead.
		1 (*b*) Winter and spring series—20 ± 2° C.	
18. ii. 57	3	15	15 in FW: 0 hr. 6; 72 hr. 6. 3 dead.
26. ii. 57	8	9	9 in SW: 8 hr. 4. 5 dead.
4. iii. 57	4	18	18 in SW: 16 hr. 3; 21–27 hr. 6. 9 dead.
8. iii. 57	6	18	18 in ½ SW: 8 hr. 4; 16 hr. 4; 24 hr. 6. 4 dead.
11. iii. 57	4	16	7 in FW: 7 dead. 9 in ½ SW: 72 hr. 6. 3 dead.
18. iii. 57	8	18	9 in FW: 240 hr. 1. 8 dead. 9 in ½ SW: 240 hr. 5. 4 dead.
28. iii. 57	9	18	9 in FW: 9 dead. 9 in ½ SW: 5 survivors after 240 hr. to SW: 240 hr. 1. 4 dead.
29. iii. 57	10	6	Hatchery controls—6.
		1 (*c*) Summer series—20 ± 2° C.	
17. viii. 56	18	6	Hatchery controls—6.
27. v. 57	16	9	9 in ½ SW. 7 survivors after 240 hr. to SW: 240 hr. 3. 4 dead.
5. vi. 57	16	9	9 in ½ SW: 240 hr. 5. 4 others to SW: 4 dead.
21. vi. 57	20	9	9 in ½ SW. 9 survivors after 240 hr. to SW: 9 dead.
25. vi. 57	20	5	Hatchery controls—5.
23. vii. 57	21	10	5 in ½ SW: 24–29 hr. 5. 5 in SW: 5 dead.
5. viii. 57	24	5	5 in SW: 12–16 hr. 5.
		2 (*a*) Winter series—10 ± 2° C.	
11. ii. 57	3	24	6 in FW: 0 hr. 6. 18 in SW: 18 hr. 4; 24 hr. 6; 52 hr. 4 (dying); 72 hr. 4.
13. ii. 57	2	9	9 in SW: 8 hr. 4; 72 hr. 2; 240 hr. 1. 2 dead.
18. ii. 57	3	8	8 in SW: 240 hr. 1. 7 dead.
26. iii. 57	9	12	12 in FW: 72 hr. 6; 240 hr. 6.

ways do not differ significantly with respect to the concentrations being considered here.

Initially, approximately 0·2 mg. of the sodium salt of heparin was added to each sample to prevent clotting. Since this was generally ineffective, most of the samples (> 95 %) were simply allowed to clot. No significant differences were found with

respect to the quantities studied between duplicate series of heparinized and unheparinized samples.

The samples were placed in centrifuge tubes, the clots and corpuscles were centrifuged down, and the clear serum was pipetted off. The serum samples were placed in acid-cleaned vials, stoppered tightly with paraffined corks, and frozen until analysed. Variable amounts of haemolysis occurred in different samples. This will be discussed further below (p. 233).

Muscle samples. Four samples of 150–350 mg. wet weight (usually 200–250 mg.) were excised from the dorsal muscle mass of each fish on the left side somewhat below the dorsal fin. Care was taken to remove all skin and adhering scales. Total muscle solids were determined by placing these samples on tared aluminium pans, weighing them wet, then re-weighing them after 24 hr. in an oven at 105° C. (samples from other parts of single fish gave identical results). It will be seen that there were only small seasonal variations in this total concentration, and for this reason possible fluctuations in fat content are considered insignificant. The dried muscle samples were stored in stoppered Pyrex test tubes (cleaned with acid and detergent) until analysed. Agreement between quadruplicate determinations of total solids was ± 2 g./kg. about the mean.

Chemical methods (only Pyrex glassware used throughout). Serum Δ was determined on approximately 1 mm.^{3} of serum by the method of Kinne (1952), somewhat modified by Werntz (1957). Precision was ± 0·02° C.

All other analyses were carried out on aliquots of serum or dried muscle samples digested at 100° C. for about 30 min. in 0·5–1·0 ml. concentrated nitric acid plus 0·5–1·0 ml. 30% hydrogen peroxide (both Analytical Reagent grade). A drop of caprylic alcohol was added to each muscle sample to prevent foaming; serum samples that foamed were treated similarly. Appropriate standards (both pure and additions to samples) and blanks were run. Duplicate analyses were made for each substance in each tissue of each fish. The deviation of the two observations from their mean value was recorded for many hundreds of such duplicates, and the average deviation is given below as the figure for precision of each analytical method used. Unless noted otherwise serum concentrations are expressed as per litre, muscle concentrations as per kilogram wet weight.

Chloride. Analyses were made by Volhard titration using the method of Van Slyke (1923) as modified by H. W. Smith (1930). Heilbrunn & Hamilton (1942) and Shenk (1954) have criticized this technique as applied to muscle analyses. The data presented by these authors indicate that, for muscle samples, the Van Slyke technique gives incorrect figures for total muscle chloride. However, figures given by Conway (1935, 1957) and others for inorganic muscle chloride, determined in a variety of well-tested ways (including some very similar to that used by Heilbrunn and Hamilton), agree quite well with those obtained by the Van Slyke technique. Primarily for this reason the modified Van Slyke procedure has been used throughout the present work. Serum samples were 0·100 ml., muscle samples were two of the dried pieces described above. Precision for serum analyses was + 2–3 m-equiv./l., for muscle analyses ± 1 m-equiv./kg.

The chloride space (assuming all muscle chloride is extracellular) has been used as an estimate of extracellular volume in the muscles throughout this work. This is known to be somewhat in error, but many studies (cf. Conway, 1957) have shown that this approximation generally results in an error in the volume calculated (compared to inulin space) of only 5–10% in the muscles of cold-blooded animals. The alternative method of calculating the extracellular volume by assuming a Donnan equilibrium across the fibre membranes (Boyle & Conway, 1941; Conway, 1957) gives significantly lower (by 40%) values than the chloride space in the present material. This would be a matter for concern if it were not for the relatively poor precision of this latter method, also for the evidence that has recently been accumulating that the basic assumption may be unjustified—at least in frog muscle (Shaw, Simon & Johnstone, 1956; Shaw, Simon, Johnstone & Holman, 1956; Simon, Shaw, Bennett & Muller, 1957; Stephenson, 1957). No estimate has been made of the blood space within the muscles.

Sodium. Analyses were made at 589 mμ with a Beckman DU quartz spectrophotometer with a flame photometer attachment using an oxygen-acetylene flame. Addition standards showed that no corrections of concentrations determined were needed to compensate for flame background changes due to the influence of the nitric acid or other materials present in the serum samples (compare Berry, Chappell & Barnes, 1946; Parks, Johnson & Lykken, 1948). A correction of −10% for muscle concentrations was indicated by similar standards, however. All figures presented here contain this correction. Excess phosphate (as ammonium hydrogen phosphate) was added to all samples for flame photometry to avoid possible variable effects of low phosphorus concentrations on sodium emission such as were found by Ramsay, Brown & Falloon (1953) (Margoshes & Vallee, 1956, however, found no such effects). Serum samples were 0·200 ml., digested, then diluted quantitatively with glass-distilled water 125 times; muscle samples were aliquots of quantitative dilutions of the nitric acid and peroxide digests of the two dried samples not used for the chloride analyses. Precision for serum analyses was ± 1–2 m-equiv./l., for muscle analyses ± 0·6 m-equiv./kg.

Potassium. Analyses were made at 771 mμ by flame photometer on the same samples as used for sodium. Precision for serum analyses was ± 0·06 m-equiv./l., for muscle samples ± 2 m-equiv./kg. Again, addition standards indicated no correction was needed for serum concentrations determined, but a correction of +5% was necessary for muscle concentrations. All figures presented here contain this correction.

Phosphorus. Analyses were made on aliquots of the quantitative dilutions of the serum samples used for flame photometry. The method of Chen, Toribara & Warner (1956), with ascorbic acid as the reducing agent, was used. Precision was ± 5 mg./l.

Effects of haemolysis

The variable amount of haemolysis occurring in serum samples was mentioned above. It would be expected that rupture of some red corpuscles would influence

serum potassium and phosphorus concentrations. One might expect that higher potassium and phosphorus concentrations in any one group of fish would be correlated with greater amounts of haemolysis. A general correlation of this sort did exist, but moderate degrees of haemolysis had no significant influence on these concentrations. All values for these concentrations in strongly haemolysed samples (these constituted less than 2% of all samples) have been excluded from the figures here presented.

RESULTS

Sexual and seasonal effects

Two general points need consideration before beginning a detailed survey of the results obtained for the specific concentrations studied. These are the questions of sexual differences and seasonal effects.

No consistent sexually correlated differences have been found for any of the concentrations studied in the present work. Comparisons between values for all concentrations studied in all males and all females in both series of experiments between September and April showed no differences statistically significant at the 5% level by '*t*' test. Breeding and non-breeding males and females considered separately also showed no differences. In all the following, therefore, the sexes will be considered together.

No seasonal differences in survival of experimental fish that could not be attributed to one or more of a number of extraneous influences were noted between September and April. A decrease in survival of fish gradually acclimatized to full sea water at 20° C. was noted during the summer, however. This may have been real (see p. 244).

P_s (Table 7) and Na_m (Table 5) were the only quantities measured showing consistent statistically significant seasonal variations between September and April. Excepting these two quantities, therefore, the results of both the breeding-season and the winter–spring series of experiments at 20° C. will be considered together. The seasonal variations in P_s and Na_m will be discussed in later sections.

This absence of seasonal variation was only apparent during the period September to April. The markedly different situation existing during the summer will be described below (p. 241).

Experiments at 20° C., *September to April*

Survival

Table 1 shows that great variations occurred in the mortality due to other than experimental causes in different groups of fish within any one experimental series, as well as between experimental series. As stated above, this was due to many extraneous factors. Allowing for the uncertainty due to these factors, it is probably still accurate to say that trout acclimatized to half-strength sea water, or to half-strength and then to full-strength sea water at 20° C. between September and April survived in the laboratory about as well as did fresh-water controls.

Serum concentrations

Fig. 1 shows the pattern of changes in Δ following isothermal transfers to fresh water, half sea water and full sea water (both directly from fresh water and after ten days' acclimatization to half sea water) at 20° C. between September and April. Cl_s and Na_s varied in almost identical fashions.

The numerical data from these experiments are summarized in Tables 2–4. Zero time controls are separated from other controls in these tables and all following tables. Zero time fish had apparently not completely recovered from the effects of handling, thermal acclimatization, etc. (cf. P, p. 238).

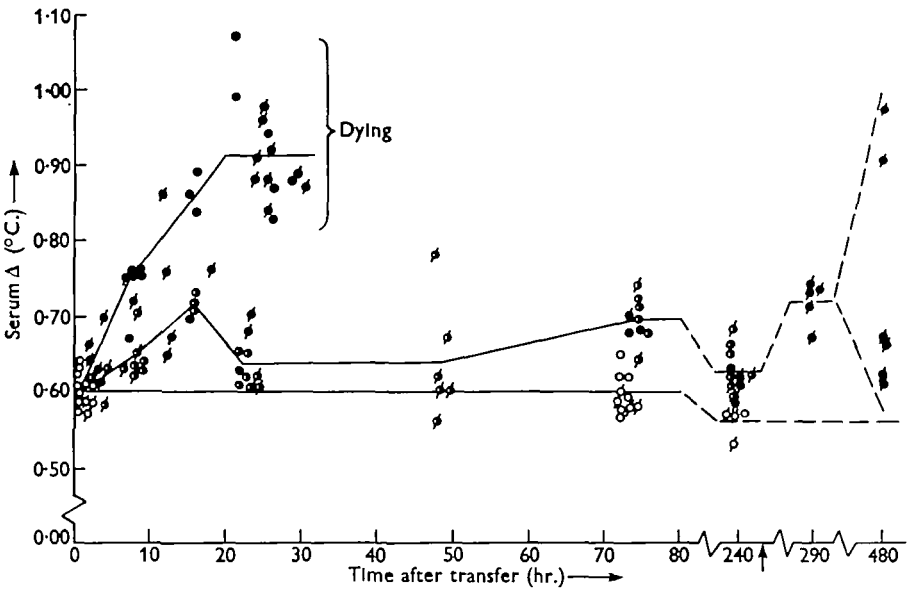

Fig. 1. Time course of changes in serum Δ in Massachusetts hatchery fish following transfers from fresh water to half-strength and to full-strength sea water at 20° C. between September and April. Open circles (○) are fresh-water controls, half-black circles (◑) half sea-water transfers, black circles (●) full sea-water transfers. Symbols with diagonal marks (e.g. ⌀) represent fish sampled during the breeding season. The arrow at 240 hr. indicates transfer from half to full sea water. Ruled lines join the means for the various groups. Unless noted otherwise, the symbols used in the other figures have similar meanings.

In the control fish maintained in fresh water Δ = 0·59° C. and no significant changes occurred (240 hr. controls are insignificantly different from 72 hr. controls at the 1% level by '*t*' test). The shock of handling the fish, and also their being starved for the experimental period, therefore had no effect on their overall osmoregulatory abilities assessed in this way.

In the first few hours following transfer from fresh water to half-strength and to full-strength sea water Δ increased almost linearly with time, the rates of increase varying approximately in proportion to the initial concentration gradients established between the fishes' blood and their external environments.

In half sea water the concentration continued to increase for 10–15 hr., after which period it became more or less stabilized at a new, higher level, $\Delta = 0{\cdot}69°$ C. after 72 hr.

Table 2. *Changes in serum Δ and total muscle solids following application of osmotic stresses*

(September–April.)

State of acclimatization	Serum Δ (° C.) [$\bar{x} \pm$ S.E. (N)]	Muscle solids (g./kg.) [$\bar{x} \pm$ S.E. (N)]
	20° C. experiments	
FW, 0 hr.	0·604 ± 0·005 (12)	210·2 ± 1·2 (12)
FW, 72–240 hr.	0·587 ± 0·008 (14)	209·7 ± 2·3 (14)
¼ SW, 16 hr.	0·715 ± 0·006 (4)	233·2 ± 4·3 (4)
¼ SW, 24 hr.	0·637 ± 0·010 (10)	232·0 ± 2·7 (25)
¼ SW, 48 hr.	0·638 ± 0·032 (6)	
¼ SW, 72 hr.	0·693 ± 0·010 (9)	
¼ SW, 240 hr.	0·625 ± 0·008 (11)	210·1 ± 2·0 (12)
SW, 21–30 hr.	0·914 ± 0·016 (15)	279·7 ± 2·7 (15)
½ SW, 240 hr., SW, 47 hr.	0·716 ± 0·013 (5)	239·2 ± 7·5 (5)
½ SW, 240 hr., SW, 240 hr.	0·738 ± 0·064 (6)	225·1 ± 7·1 (7)
	10° C. experiments	
Hatchery controls	0·563 ± 0·003 (12)	205·2 ± 2·0 (12)
FW, 0 hr.	0·582 ± 0·010 (6)	207·3 ± 4·8 (6)
FW, 72–240 hr.	0·555 ± 0·006 (11)	201·8 ± 3·5 (12)
SW, 24–72 hr.	0·747 ± 0·013 (12)	252·2 ± 4·5 (12)
SW, 240 hr.	0·62, 0·81 (2)	217, 233 (2)

Table 3. *Changes in serum and muscle chloride following application of osmotic stresses*

(September–April.)

State of acclimatization	Cl_s (m-equiv./l.) [$\bar{x} \pm$ S.E. (N)]	Cl_m (m-equiv./kg.) [$\bar{x} \pm$ S.E. (N)]	$Cl_m/Cl_s \times 10^3$
	20° C. experiments		
FW, 0 hr.	129·2 ± 1·3 (12)*	8·2 ± 0·3 (12)	64 (59–68)†
FW, 72–240 hr.	140·5 ± 1·1 (14)*	9·6 ± 0·6 (14)	68 (60–77)
¼ SW, 24 hr.	153·5 ± 3·1 (10)	10·7 ± 0·9 (10)	70 (58–82)
¼ SW, 72 hr.	164·7 ± 2·9 (9)	9·6 ± 0·6 (9)	58 (51–66)
¼ SW, 240 hr.	148·2 ± 2·3 (12)	9·3 ± 0·5 (12)	63 (56–70)
SW, 21–30 hr.	203·6 ± 3·3 (15)	14·0 ± 1·1 (15)	69 (58–80)
½ SW, 240 hr., SW, 47 hr.	170·4 ± 1·6 (5)	13·4 ± 1·0 (5)	79 (67–91)
½ SW, 240 hr., SW, 240 hr.	179·1 ± 11·5 (7)	12·5 ± 1·5 (6)	71 (54–88)
	10° C. experiments		
Hatchery controls	130·0 ± 1·3 (12)	7·8 ± 0·5 (12)	62 (52–72)
FW, 0 hr.	131·3 ± 1·2 (6)	7·5 ± 0·5 (6)	57 (50–65)
FW, 72–240 hr.	128·3 ± 1·2 (11)	8·8 ± 1·5 (6)	69 (45–92)
SW, 24 hr.	171·9 ± 3·2 (12)	10·2 ± 0·6 (6)	60 (52–66)
SW, 72 hr.		8·7 ± 0·7 (6)	50 (42–59)
SW, 240 hr.	146, 189 (2)	9, 17 (2)	62, 90

* The difference in means between these two groups of controls is due to a lag in the response of the fish to temperature change. Temperature of the hatchery water at the times these experiments were carried out was near 10° C. (See Material and Methods section.)

† Approximate 95 % confidence interval. (See p. 241.)

Table 4. *Changes in serum sodium following application of osmotic stresses*

(September–April.)

State of acclimatization	Na_s (m-equiv./l.) [$\bar{x} \pm$ S.E. (N)]
20° C. experiments	
FW, 0 hr.	148·1 ± 1·7 (12)
FW, 72–240 hr.	149·3 ± 1·4 (13)
¼ SW, 24 hr.	165·4 ± 1·9 (10)
½ SW, 72 hr.	179·0 ± 2·3 (9)
¼ SW, 240 hr.	162·1 ± 2·8 (12)
SW, 21–30 hr.	231·1 ± 4·7 (15)
¼ SW, 240 hr., SW, 47 hr.	182·6 ± 1·7 (5)
½ SW, 240 hr., SW, 240 hr.	194·0 ± 15·2 (6)
10° C. experiments	
Hatchery controls	146·9 ± 1·8 (12)
FW, 0 hr.	147·5 ± 1·7 (6)
FW, 72–240 hr.	145·4 ± 1·1 (12)
SW, 24–72 hr.	188·8 ± 4·5 (12)
SW, 240 hr.	154, 190 (2)

Table 5. *Changes in total sodium in the muscles following application of osmotic stresses*

(September–April.)

State of acclimatization	Na_m (m-equiv./kg.) [$\bar{x} \pm$ S.E. (N)]
20° C. experiments—breeding season	
FW, 0 hr.	14·4 ± 1·4 (6)
FW, 72–240 hr.	18·8 ± 1·2 (7)
¼ SW, 24 hr.	16·0 ± 1·4 (4)
½ SW, 72 hr.	13·6 ± 1·6 (3)
¼ SW, 240 hr.	17·9 ± 0·8 (7)
SW, 21–30 hr.	26·6 ± 1·7 (9)
¼ SW, 240 hr., SW, 47 hr.	19·6 ± 1·6 (5)
½ SW, 240 hr., SW, 240 hr.	13·6 ± 1·7 (6)
20° C. experiments—spring	
FW, 0 hr.	8·2 ± 0·2 (6)
FW, 72–240 hr.	12·0 ± 1·3 (7)
¼ SW, 24 hr.	10·7 ± 1·1 (6)
½ SW, 72 hr.	12·1 ± 0·6 (6)
¼ SW, 240 hr.	9·7 ± 0·8 (5)
SW, 21–30 hr.	25·0 ± 1·9 (6)
10° C. experiments—spring unless noted	
Hatchery controls	7·9 ± 0·3 (6)
Hatchery controls, breeding season	11·2 ± 0·8 (6)
FW, 0 hr.	9·0 ± 0·5 (6)
FW, 72 hr.	8·6 ± 2·0 (6)
SW, 24 hr.	13·4 ± 1·0 (6)
SW, 72 hr.	11·6 ± 0·9 (6)
SW, 240 hr.	9·4, 17·1 (2)

In full sea water the concentration continued to increase for approximately 20 hr., after which period it may well have begun to level off at about $\Delta = 0{\cdot}91°$ C., some 56% above the fresh-water level. The fish did not survive long enough for this to be certain, however. Many were already dead after 24 hr. and all were at least moribund after about 30 hr.

The fish in half sea water survived with relatively little mortality for ten days. After this period they had all made some return toward their original fresh-water concentrations, though they still averaged 6% above controls (difference statistically significant at the 1% level). Such acclimatized fish transferred to full sea water again increased in concentration, but only to $\Delta = 0{\cdot}72°$ C., which is about the same value as that reached after transfer from fresh water to half sea water. As far as their ability to regulate blood concentration was concerned, full sea water had essentially become half sea water for these fish.

Ten days in full sea water were now survived with comparatively little difficulty, though the fish often became very emaciated and some died, apparently of starvation and tank injuries. After 10 days in full sea water the relative uniformity of response demonstrated to previous stresses was no longer present. Variability for serum concentration increased by a factor of 4–6. Serum concentrations equal to those found in dying fish 24 hr. after direct transfer from fresh water to full sea water were now encountered in seemingly healthy fish. Other healthy fish had returned all the way to their original fresh-water concentration levels. The significance of these observations for the present work is discussed below (p. 247).

The patterns shown by Cl_s and Na_s were, as noted above, the same as that for Δ. Assuming unit activity for the ions, chloride contributed a quite constant 40–45% of the Δ of the blood, sodium 45–49%. These relations are shown in Fig. 2, in which Cl_s and Na_s are plotted against Δ. The regression lines for these relations, calculated by the method of least squares, are included in Fig. 2. The equations for these lines are: $Cl_s = 211{\cdot}9\,\Delta + 13{\cdot}4$; $Na_s = 234{\cdot}6\,\Delta + 14{\cdot}7$ (using absolute values for Δ). The slopes of the lines are statistically insignificantly different from one another ($P = 0{\cdot}4$, by 't' test). Correlation coefficients for the two sets of data are: Cl_s, Δ: 0·654; Na_s, Δ: 0·704.

Changes in K_s and P following the various transfers were small and virtually all statistically insignificant (Tables 6 and 7). Such variations as did occur were only generally related to the other concentration changes already described. K_s on the average was regulated very well on a low level.

Within the separate experimental series P remained essentially constant. The only variation encountered which appears biologically significant is the seasonal one (see p. 245).

For brief periods following handling or some other sort of shock P often increased somewhat. This effect is illustrated in Table 7 by the higher and more variable phosphorus concentrations found in all groups of zero time controls as compared with controls which had been kept for some time under constant environmental conditions.

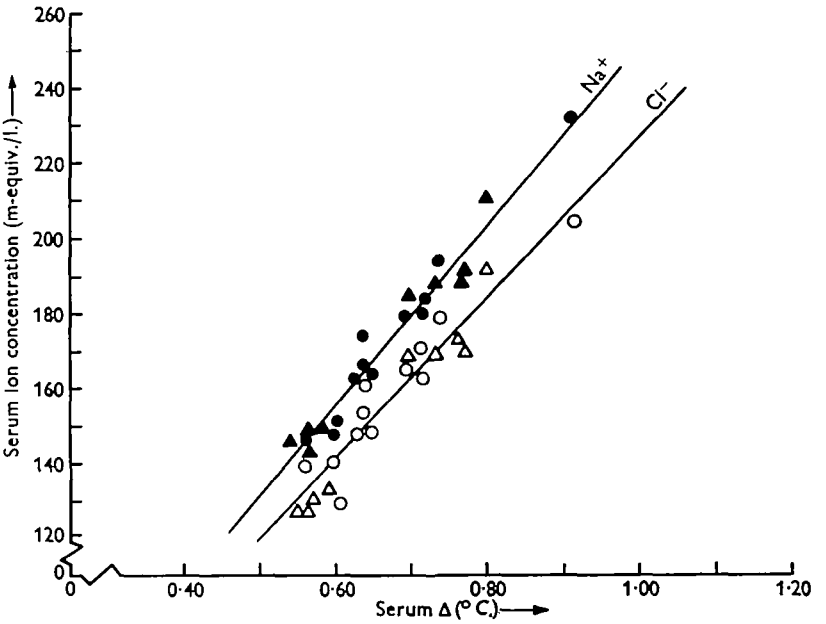

Fig. 2. Relations between Cl_s and Na_s and Δ. For clarity the only points plotted are those representing the mean values for these quantities in the variously acclimatized groups of fish. The straight lines are regression curves calculated from the original data on individual fish using the method of least squares. The equations for these curves are:

$$Cl_s = 211{\cdot}9\,\Delta + 13{\cdot}4$$
$$Na_s = 234{\cdot}6\,\Delta + 14{\cdot}7$$

Chloride concentrations are represented by open symbols, sodium concentrations by solid symbols. Triangles represent experiments at 10° C., circles those at 20° C. Only fish sampled between September and April are included.

Muscle concentrations

The patterns developed for all muscle concentrations were very similar to that described for Δ. The data are presented in Tables 2, 3, 5 and 6. Fig. 3 shows the degree of correlation between muscle total solids and Δ.

There were several quantitative differences in comparison with serum concentrations. The differences which seem worthy of mention are:

(1) The 1- to 3-day stabilization level in half sea water was only 11 % above fresh-water control (18 % for serum concentration after three days), and concentration after 20 hr. in full sea water was only 33 % above control (56 % for serum concentration).

(2) There was a complete return to fresh-water control level after ten days in half sea water (serum concentration still 6 % above control at this time).

(3) There were no significant increases in Cl_m above fresh-water control in fish transferred to half sea water. The means are identical with control from 72 hr. onwards in half sea water on.

(4) Na_m showed a significant seasonal variation; Na_s did not.

Table 6. *Changes in serum and muscle potassium following application of osmotic stresses*

(September–April.)

State of acclimatization	K_s (m-equiv./l.) [$\bar{x}\pm$S.E. (N)]	K_m (m-equiv./kg.) [$\bar{x}\pm$S.E. (N)]
20° C. experiments		
FW, 0 hr.	4·2±0·6 (9)	142·6±1·2 (12)
FW, 72–240 hr.	5·1±0·5 (13)	142·5±1·7 (14)
¼ SW, 24 hr.	4·2±0·7 (9)	153·7±1·5 (25)
¼ SW, 72 hr.	5·3±0·8 (9)	
¼ SW, 240 hr.	3·8±0·7 (11)	141·8±1·0 (12)
SW, 21–30 hr.	6·8±0·6 (11)	180·8±1·2 (15)
¼ SW, 240 hr., SW, 47 hr.	12·4±3·2 (5)	157·6±2·2 (5)
¼ SW, 240 hr., SW, 240 hr.	4·0±0·4 (6)	151·7±6·2 (6)
10° C. experiments		
Hatchery controls	2·4±0·3 (12)	138·2±1·4 (12)
FW, 0 hr.	1·6±0·5 (6)	137·2±1·2 (6)
FW, 72–240 hr.	2·8±0·5 (11)	136·8±2·5 (6)
SW, 24–72 hr.	6·3±0·8 (11)	156·4±2·3 (12)
SW, 240 hr.	4·3, 5·2 (2)	132, 156 (2)

Table 7. *Changes in serum total phosphorus following application of osmotic stresses*

(September–April.)

State of acclimatization	P (mg./l.) [$\bar{x}\pm$S.E. (N)]
20° C. experiments—breeding season	
FW, 0 hr.	120±11 (4)
FW, 72–240 hr.	70± 4 (7)
¼ SW, 24–72 hr.	72± 3 (13)
¼ SW, 240 hr.	101± 9 (7)
SW, 21–30 hr.	128±23 (6)
¼ SW, 240 hr., SW, 47 hr.	159±38 (5)
¼ SW, 240 hr., SW, 240 hr.	100±12 (6)
20° C. experiments—spring	
FW, 0 hr.	131±11 (5)
FW, 72–240 hr.	110± 7 (6)
¼ SW, 24–72 hr.	116± 7 (12)
¼ SW, 240 hr.	120± 2 (4)
SW, 21–30 hr.	178±32 (5)
10° C. experiments—spring unless noted	
Hatchery controls	118± 2 (6)
Hatchery controls, breeding season	81± 4 (6)
FW, 0 hr.	150±14 (6)
FW, 72–240 hr.	109± 8 (11)
SW, 24–72 hr.	150±12 (12)
SW, 240 hr.	95, 40 (2)

A final point is that the data on Cl_s and Cl_m considered together indicate that the volume of extracellular fluid in the muscles is quite independent of the state of salinity acclimatization of the fish. Assuming that all muscle chloride is extracellular, the ratio Cl_m/Cl_s (cf. last column in Table 3) provides an estimate of this quantity. There are no statistically significant variations in this ratio (the figures in parentheses in the last column of Table 3 are approximate 95 % confidence intervals for the ratios listed, calculated from mean $Cl_m \pm 2$ S.E.).

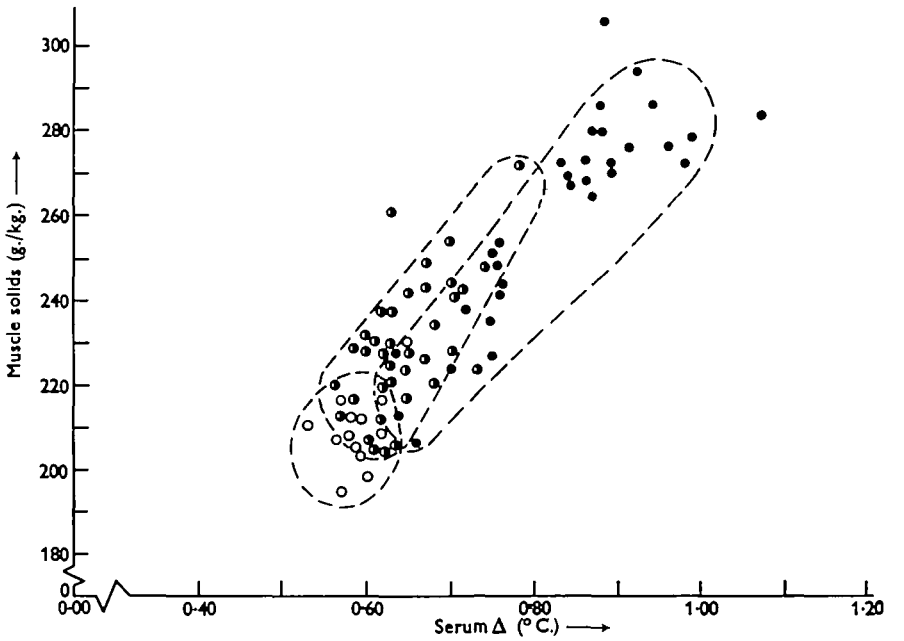

Fig. 3. Relations between muscle solids concentration and serum Δ in Massachusetts fish transferred from fresh water to half-strength and to full-strength sea water at 20° C. between September and April. Dashed lines roughly indicate boundaries of groups.

Experiments at 20° C., June to August

The experiments carried out at 20° C. between June and August were not as complete as those between September and April, but they serve to show that the brown trout in summer is a very different fish, at least with regard to osmotic and ionic regulatory abilities, from the brown trout at other times of year.

Two groups of fresh-water (hatchery) controls were sampled in late June and mid-August. These two series were insignificantly different from one another with respect to Cl_s and total muscle solids (the only analyses done on the June series). The August fish showed significant changes in all serum and muscle concentrations that were invariant between September and April—see Table 8.

The data on variations in total muscle solids following acclimatizations to half-strength and to full-strength sea water during the summer were as follows (figures are means $\pm$ 1 S.E.): Fresh water: $217{\cdot}5 \pm 2{\cdot}8$ (N = 11); half sea water, 24–29 hr.:

Table 8. *Seasonal variability in fresh-water control fish*

(20 ± 2° C.)

Concentration	September–April [$\bar{x}$ ± S.E. (N)]	August [$\bar{x}$ ± S.E. (N)]
Δ (° C.)	0·587 ± 0·008 (14)	0·553 ± 0·009 (6)
Cl_s (m-equiv./l.)	140·5 ± 1·1 (14)	123·2 ± 2·0 (6)
Na_s (m-equiv./l.)	149·3 ± 1·4 (13)	142·2 ± 2·7 (6)
K_s (m-equiv./l.)	5·1 ± 0·5 (13)	3·5 ± 0·3 (6)
P (mg./l.)	70 (Sept.); 110 (April)	165 ± 10 (6)
Muscle solids (g./kg.)	209·7 ± 2·3 (14)	217·2 ± 3·0 (6)
Cl_m (m-equiv./kg.)	9·6 ± 0·6 (14)	11·3 ± 0·9 (6)
Na_m (m-equiv./kg.)	18·8 (Sept.); 12·0 (April)	10·1 ± 0·6 (6)
K_m (m-equiv./kg.)	142·5 ± 1·7 (14)	129·3 ± 1·5 (6)

259·4 ± 4·7 (N = 5); half sea water, 240 hr.: 234·4 ± 5·3 (N = 5); sea water, 12–16 hr.: 297·0 ± 3·2 (N = 5); half sea water, 240 hr., sea water, 240 hr.: 238·0 ± 7·0 (N = 3).

Comparison of these data with Table 2 shows that in all states of acclimatization brown trout in summer were more concentrated (by 7–32 g./kg.) than brown trout during the rest of the year. It can also be seen that summer fish were much poorer regulators than fish during the rest of the year. Long-term acclimatizations to half, or to half and then to full, sea water did not result in as complete returns toward fresh-water levels as occurred between September and April. Furthermore, fish transferred from fresh water to full sea water survived for only about 12–16 hr. (compared to 20–30 hr. during the rest of the year), and became more concentrated. It appears that the brown trout decreases markedly in osmoregulatory ability in the months prior to, but seemingly not during, the breeding season.

Experiments at 10° C., *February*

A lowering of the temperature by 10° C. resulted in a marked improvement in the resistance of brown trout to sudden osmotic stresses. As regards survival, all fish were able to survive direct transfer from fresh water to full sea water for at least 2 days. About one-fifth of the number so transferred and not used at earlier times were able to survive this transition indefinitely (at least for 10 days).

The general pattern of concentration changes at 10° C. was similar to that occurring at 20° C., but with quantitative differences. The fresh-water controls showed the same lack of effect of handling and starvation noted for the 20° C. experiments (all differences in means insignificant at the 1 % level), but Δ and Cl_s were 5 % lower than in fish kept at 20° C. (difference statistically significant at the 1 % level). K_s in fresh water was about 2 m-equiv./l. lower at 10° C. than at 20° C. (also significant at the 1 % level).

The stabilization level reached by Δ after one day in full sea water was 35 % above fresh-water control (compared to 56 % at 20° C.). Total muscle solids were only 20 % above control at the same time (compared to 33 % at 20° C.). The two living fish sampled after 10 days in full sea water were almost as different from one

another as the most extreme fish in the group which had spent 10 days in full sea water at 20° C.

The pattern shown by Δ is presented in Fig. 4. The average curve for the full sea water transfers at 20° C. is included in this figure for reference.

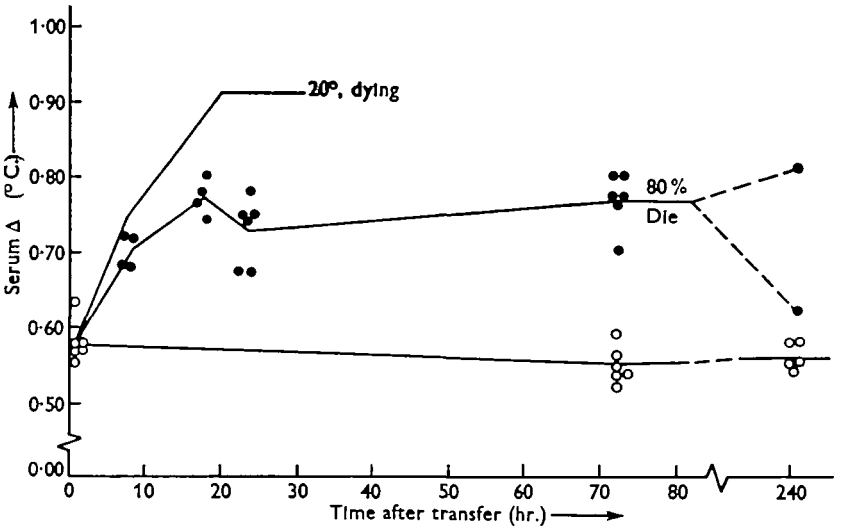

Fig. 4. Time course of changes in serum Δ in Massachusetts hatchery fish following transfers from fresh water to full-strength sea water at 10° C. in February.

The preliminary experiment using fish from the Connecticut hatchery was also done at 10° C. in February. Transfers were from fresh water to sea water of only 28‰ salinity. The general pattern of concentration changes that occurred was the same as for the Massachusetts fish, but blood concentrations in fresh water were significantly higher ($\Delta = 0{\cdot}615 \pm 0{\cdot}015°$ C. (N = 4)), rates of increase of internal concentration following transfer were somewhat lower (they were fish in the same size range), and 1- to 3-day stabilization levels were higher ($\Delta = 0{\cdot}794 \pm 0{\cdot}019°$ C. (N = 12)) even though the external concentration was lower.

The overall pattern of regulation of total serum concentration in Massachusetts *Salmo trutta* between September and April is summarized in the classic form of a plot of total internal *v.* total external concentration in Fig. 5. The variability of the fish acclimatized to full sea water gradually and for a long period at 20° C. (perhaps also at 10° C.) was probably an experimental artefact.

DISCUSSION

The data described above permit a description of osmotic and ionic regulation in the brown trout that is, in some ways, more detailed than for any other fish.

Seasonal effects

Seasonal changes, especially the onset of the breeding season, may or may not be associated with changes in the relation between internal and external media.

In female eels (Fontaine & Koch, 1950, for summary) overall osmoregulatory ability is apparently little affected at this period. Sticklebacks (*Gasterosteus* and *Pungitius*), however, show less osmotic regulation and are less euryhaline (Heuts, 1942, 1943, 1945; Koch & Heuts, 1942, 1943). The seasonal pattern in the brown trout is somewhat different.

D. C. W. Smith (1956) studied seasonal changes in tolerance of brown trout to artificially increased salinities (increased by addition of sodium chloride) above a previously determined 'physiological maximum' (only 24‰!). Results in two successive years differed by over 100% in absolute values, but showed more or

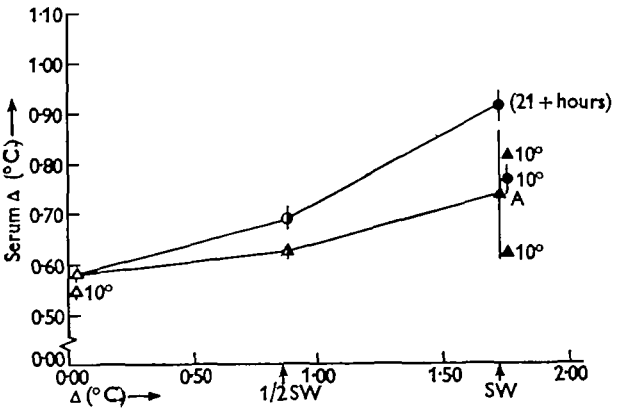

Fig. 5. Relations between freezing-point depressions of serum and external environment in Massachusetts hatchery fish acclimatized to half-strength and to full-strength sea water for varying periods at 20° C. and 10° C. Triangles indicate long-term acclimatizations (240 or more hours), circles short-term acclimatizations (72 hours unless noted otherwise). *A* means acclimatized to half-strength sea water for 240 hours first. 10° C. points are noted as such. The two 10° C. triangles in full-strength sea water are for individual fish. All other symbols indicate means for groups ± two standard errors.

less consistent patterns of seasonal differences. Salinity tolerance was lowest during the summer, went through a peak, was low again from October to December (the breeding season), then went through a broad peak during the spring.

The results here reported agree with Smith's as regards the summer decrease in viability, and show in addition that actual decreases in regulatory abilities are involved; the breeding season, however, was not associated with any changes in these abilities. The only changes correlated with this latter period were in serum phosphorus and total muscle sodium concentrations. Survival and regulatory abilities were seemingly constant over the entire period from autumn to spring.

Reasons for this rather odd pattern of changes in regulatory abilities are not readily apparent. Two environmental correlations which might have affected endocrine functions in some way seem possible, however:

(1) Regulatory abilities may be influenced by photoperiod. The experimental series between September and April were carried out during those parts of the year with nights longer than days. The reverse was true for the summer series.

(2) Regulatory abilities may be influenced by the long-term thermal history of the fish. The September to April series involved fish acclimatized for periods of weeks to hatchery water temperatures in the range 2–14° C. The summer series involved fish acclimatized to temperatures of 18–24° C.

The seasonal changes in muscle sodium are unaccounted for. Serum phosphorus was the only other concentration studied which varied significantly seasonally outside the summer. Its variations are probably due to various seasonal metabolic changes (Ichikawa, 1955; Laskowski, 1936).

Serum phosphorus concentrations during the breeding season were 20–50 mg./l. below the levels measured between February and April and about 90 mg./l. below summer levels. The figures given by Laskowski (1936) for one brown trout during the breeding season and one brown trout outside the breeding season show the opposite effect—breeding is approximately twice non-breeding. The reason for this discrepancy is unknown.

The fact that brown trout in fresh water in summer have different serum ion concentrations from brown trout in fresh water in winter is probably the major reason for the discrepancies existing between serum sodium and potassium concentrations reported for this species by Spalding (in Jones, 1956) and by Phillips & Brockway (1958). Spalding obtained her samples during the winter, Phillips & Brockway obtained theirs during the summer

Water and ion movements between muscles and blood

(*a*) *Water movements*

Studies of total muscle concentration (g. solids/kg. muscle wet weight or Δ of expressed tissue fluid) indicate that this quantity usually varies in a similar fashion to total blood concentration—the muscles are in some sort of osmotic equilibrium with the blood (in salmonids: Busnel, 1942; Greene, 1926; *Anguilla*: Callamand, 1943; Drilhon, 1937, 1943, Koch, 1949; Callamand *et al.* (1951), also working with eels, did not find such parallelism). This is probably true in the brown trout as well. The plot of total muscle concentration against serum Δ for the present experiments at 20° C. shown in Fig. 3 is not incompatible with the view that the muscles are in osmotic equilibrium with the blood (whether or not isotonic is another question—cf. Brodsky, Appleboom, Dennis, Rehm, Miley & Diamond, 1957).

A plot of total muscle concentration *v.* serum Δ for fish transferred to full sea water at 10° C. and 20° C. shows the same relation.

The fact that most of the changes in total muscle solids are due to changes in intracellular water content may also be demonstrated.

Table 3 shows that the extracellular volume in the muscles is small and constant. Changes in water content of the muscles occurred under some conditions which varied from 20–70 g./kg. (Table 2). With constant extracellular volume, this water could have come from no place but the fibres. Even if the statistically insignificant fluctuations in chloride space indicated in Table 3 are real they are insufficient to account for the total water-content changes which occurred.

The data of Callamand *et al.* (1951) on muscle chloride concentrations in hypophysectomized and intact female eels transferred from fresh water to full sea water indicate that a similar situation exists in *Anguilla*.

(*b*) *Ion movements*

A consideration of all the data obtained on ion concentrations in serum and muscle permits a critical test of Drilhon's (1937) hypothesis that the muscles of a fish can act as storage sites for ions.

We have seen that extracellular volume in the muscles of the brown trout is small and quite constant, at least between September and April. Serum sodium and potassium concentrations for these same fish are known, also total muscle sodium and potassium concentrations. Combining these data with those on total muscle solids (hence total muscle water), intracellular concentrations of these two ions can be calculated from the equation:

$$[A]_i = \frac{A_t - [A]_s V_e}{V_t - V_e}.$$

$[A]_i$ = intracellular concentration of ion species A (m-equiv./kg. intracellular water). A_t = total quantity of A (m-equiv.) in 1 kg. of wet muscle. $[A]_s$ = serum concentration of A (use m-equiv./l. as approximation to m-equiv./kg. water). V_t = total weight of water (kg.) in 1 kg. of wet muscle. V_e = weight of extracellular water (kg.), assumed to be present as serum, in 1 kg. of wet muscle.

The results of such calculations for muscle sodium in variously acclimatized brown trout are presented in Table 9. Comparable data for potassium may be taken directly from Table 6, since the extracellular space is small and serum potassium concentrations are low. The muscle sodium data will be considered first.

It should be pointed out that indistinct end-points in the muscle chloride analyses resulted in low precision for the extracellular volume estimations. Small changes in calculated extracellular volume produce large changes in intracellular sodium concentration. By reason of these analytical errors the amount of sodium calculated to be present extracellularly sometimes exceeds the amount of sodium measured in the muscle. Such cases are listed in Table 9 as having zero intracellular sodium. It seems probable that their true intracellular sodium concentrations were low.

Approximate 90% confidence intervals for intracellular sodium concentrations are given in parentheses after the mean values in Table 9. These intervals were calculated in the same manner as the means, but using the following figures. Upper bound: Mean total muscle sodium + 2 S.E., extracellular volume from mean muscle chloride − 2 S.E. Lower bound: Mean total muscle sodium − 2 S.E., extracellular volume from mean muscle chloride + 2 S.E.

Despite the uncertainty just mentioned, the calculated intracellular sodium concentrations bring up several items of interest.

The major point is that, with the exception of fish transferred directly from fresh water to full sea water at 20° C., both during and outside the breeding season,

Table 9. *Changes in intracellular sodium in the muscles following application of osmotic stresses*

(September–April.)

State of acclimatization	Na_i (m-equiv./kg. cell water)
20° C. experiments—breeding season	
FW, 0 hr.	6·7 (2–12)*
FW, 72–240 hr.	12·1 (7–17)
½ SW, 24 hr.	6·4 (0–13)
½ SW, 72 hr.	4·5 (0–11)
½ SW, 240 hr.	10·6 (7–14)
SW, 21–30 hr.	16·3 (7–25)
½ SW, 240 hr.–SW, 47 hr.	6·0 (0–15)
½ SW, 240 hr.–SW, 240 hr.	0 (0–8)
20° C. experiments—spring	
FW, 0 hr.	0 (0)
FW, 72–240 hr.	2·6 (0–8)
½ SW, 24 hr.	0 (0–5)
½ SW, 72 hr.	2·4 (0–6)
½ SW, 240 hr.	0 (0–3)
SW, 21–30 hr.	14·0 (4–24)
10° C. experiments—spring unless noted	
Hatchery controls	0 (0–1)
Hatchery controls, breeding season	2·9 (0–7)
FW, 0 hr.	0·8 (0–4)
FW, 72 hr.	0 (0–8)
SW, 24 hr.	3·0 (0–8)
SW, 72 hr.	3·0 (0–8)
SW, 240 hr.	0, 0

* Approximate 90 % confidence interval. (See p. 246.)

intracellular sodium concentrations during each experimental series were fairly constant. This argues that little, if any, sodium invaded the fibres even during the periods when they were considerably dehydrated and serum concentrations were high. If anything, the fibres excreted sodium during periods of dehydration. Thus, the muscles of the brown trout almost certainly did not act as storage sites for sodium during or after adjustments to non-fatal osmotic stresses.

The data on fish acclimatized to full sea water at 20° C. bring up a point which may bear on the question of why some fish are euryhaline, others not. Many of the fish transferred directly from fresh water to full sea water showed considerable increases in intracellular sodium, these changes being clearest outside the breeding season. But fish acclimatized to half sea water first, while in some cases having serum concentrations as high as those in the direct transfers, seemingly were able to maintain low intracellular sodium concentrations. Perhaps this apparent increase in the ability of acclimatized fish to prevent the invasion of their muscle fibres by large amounts of sodium is one of the factors which enabled them to survive blood concentrations equal to those associated with mortality in the direct transfers.

Stenohaline fishes seem to be unable to keep sodium out of their muscle fibres,

even with long-term acclimatizations (Kaplanski & Boldirewa, 1933; Drilhon, 1937). Steinbach (1954) and others (cf. Glynn, 1957, p. 296) have pointed out the inhibitory effects which low concentrations of sodium have on various enzyme systems important in muscle metabolism. It is tempting to speculate that differences in ability to prevent sodium invasion of muscle fibres are a determining factor in the degree of euryhalinity shown by fishes, and that the mechanism of the effect is that discussed by Steinbach.

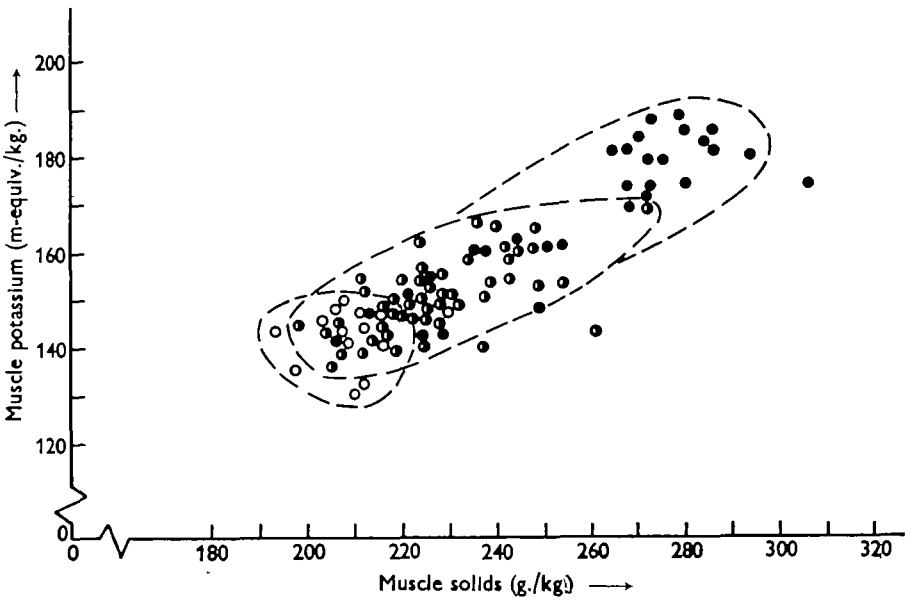

Fig. 6. Relations between muscle potassium, K_m, and muscle solids concentration in Massachusetts hatchery fish transferred from fresh water to half-strength and to full-strength sea water at 20° C. between September and April. Dashed lines roughly indicate boundaries of the three groups.

That temperature, as well as acclimatization time, may be significant in keeping sodium out of muscle fibres is illustrated by the fish transferred directly from fresh water to full sea water at 10° C. and 20° C. in the spring. The 20° C. fish, as noted, considerably increased their intracellular sodium concentrations—the 10° C. fish seemingly did not. Serum concentrations at 10° C. never reached the high levels noted at 20° C., which in itself may explain the greater survival noted at 10° C. However, the difference in behaviour of muscle sodium concentration may also have been significant.

The potassium data indicate that the muscles did not act as reservoirs for this ion, either. Small differences in large quantities are involved here, however, so it is difficult to be certain. The close correlation existing between muscle potassium and total muscle concentrations is shown in Fig. 6.

Ecological implications

Gordon (1959) has shown that responses of Scottish brown and sea trout to osmotic stresses are similar to those described for Massachusetts fish in the present paper. The many generations of genetic isolation that separate European and American populations of *Salmo trutta* have resulted in only quantitative differences in osmotic and ionic regulatory mechanisms.

In the same regard it is interesting that serum Na:Cl ratios in all groups of American fish are in the range 1·06–1·13, which is the range found in Scottish fresh water *S. trutta*. Scottish sea trout have serum Na:Cl ratios in the range 1·18–1·28. Perhaps this indicates that at least the great majority of the ancestors of present-day American *S. trutta* were brown trout, only a few, if any, having been sea trout.

Finally, the experiment at 10° C. seems to present an interesting possibility for explaining a zoogeographic problem in the distribution of the anadromous habit in at least some fishes. Brook trout (*Salvelinus fontinalis*), lake whitefish (*Coregonus clupeaformis*), various ciscoes (*Coregonus* (*Leucichthys*) *spp.*), and many other fishes are commonly anadromous in the far northern parts of their ranges (e.g. Hudson Bay) but seldom or never so near their more southern limits (Walters, 1955; Wohlschlag, 1957). Assuming that these fish make their transitions from fresh water to salt water fairly rapidly, one might postulate that, at the higher temperatures encountered in the southern parts of their ranges, they are unable to maintain their blood and muscle concentrations on a level compatible with survival. At lower temperatures in the more northerly parts of their ranges they can maintain lower blood and muscle concentrations and thus survive.

There would seem to be considerable adaptive advantage in going to sea, primarily from the standpoint of increased food supply. Some counter-selection is therefore probably operating to prevent such behaviour in the southern parts of the ranges of these fish. Perhaps the temperature effect on short-term levels of regulation of blood and muscle concentrations is part of this counter-selection.

SUMMARY

1. The osmotic and ionic regulatory abilities of adults of the euryhaline brown trout (*Salmo trutta*) have been studied in experiments roughly duplicating the stresses of migration from fresh water to the sea. Brown trout will survive indefinitely in full sea water if acclimatized to it at rates inversely related to the temperature.

2. Blood serum samples have been analysed for Na, K, Cl, total P and Δ; muscle samples for Na, K, Cl and total solids. Changes in the concentrations of these constituents following transfers from fresh water to 50% and 100% sea water have been studied. Transfers were made throughout the year and at temperatures of 10° C. and 20° C.

3. Following transfers to 50% sea water at 20° C. blood concentrations rose

significantly above fresh-water levels, but returned very nearly to these levels after about a week. Transfer from 50% sea water to 100% sea water at 20° C. caused the same sequence of changes. Transfer to 100% sea water at 20° C. was fatal, and associated with very high serum concentrations. Many fish survived transfer to 100% sea water at 10° C., however, and showed evidence of internal concentrations returning to fresh-water levels after 10 days. The brown trout is strongly homoiosmotic on a long-term basis.

4. Both survival and regulatory ability were lower during the summer. There were also seasonal variations in the blood and muscle concentrations of fish in a given state of acclimatization.

5. Muscle concentration changes closely paralleled blood changes. Extracellular volume remained constant, so muscle concentration changes were attributable to changes in intracellular water. The muscles did not act as storage sites for sodium and potassium.

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