THE DEVELOPMENT OF SALINITY TOLERANCE IN THE SALMON, *SALMO SALAR* (L.) AND SOME RELATED SPECIES

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INTRODUCTION

The freshwater life of a young salmon begins with the hatching of the egg, and after a variable number of years culminates in the seaward migration of the smolt. During this time the young salmon changes in size and behaviour, from fry to parr and from parr to smolt. This latter transformation is identified with very marked changes in appearance and behaviour. This change has been studied intensively in both the genus *Salmo* and the genus *Oncorhynchus*, especially with regard to the extrinsic factors, to behaviour, and to the hormonal balance (Hoar, 1953; Fontaine, 1954). While there are many outward manifestations of the parr–smolt change known, and a good deal about the behavioural changes and probably related hormonal changes associated with the transformation, there are many unexplained phenomena in the physiology of such fish.

One of the most spectacular changestaking place during the juvenile life of the salmon is the development of mechanisms for osmoregulation in both fresh water and sea water. The ability to tolerate environmental changes is a faculty which may have developed over the whole period of juvenile life, or it may have appeared as a sudden change prior to the parr–smolt transformation and the seaward migration. That is, some ability to tolerate environmental changes may always be present, increasing with age; or it may be latent until a 'triggering-off' by some unknown stimulus sends the smolt downstream to the sea. Thus it seemed that a study of the 'salinity tolerance' of young salmon would provide information relevant to a study of the seaward migration. Three principal questions arose:

(1) What is the degree of tolerance to different salinities at each stage in the life history and how does it compare with that of other fish?

(2) If there is a difference in the tolerance of parr and smolts, does this come about suddenly with the other manifestations of the parr–smolt change, or does it develop continuously during the juvenile life of the salmon?

(3) Is there any pre-migratory adaptation, in 'pre-smolts' or smolts, for marine life?

The following study was undertaken in an attempt to provide information on these questions.

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METHODS

Three species of the genus *Salmo* have been used: *salar* (L.), *trutta* (L.) and *gairdnerii* (Richardson), all hatchery-reared fish of different ages and sizes. Although coming originally from both hard and soft waters, these experimental fish had been maintained for some weeks in a hard unchlorinated water in standard laboratory conditions. Smolts of *Salmo salar* were an exception to this; these fish were naturally reared, caught on or just prior to their downstream migration and were investigated in the field by the same methods as were used in the laboratory.

For each experiment starved fish were used in batches of ten, or duplicate batches of five for larger fish, in 40 l. static aerated tanks at constant temperature (10–12° C.) and oxygen (8–9 p.p.m.). The water was changed frequently to avoid accumulation of waste products and to ensure that the oxygen level was constant.

Salinities used were appropriate to full strength Bay of Biscay sea water (designated 100% sea water = 33·9‰ sal.; Oliver, 1957) and dilutions of this sea water were made with unchlorinated hard water (75, 50 and 25% sea water). The control fish were kept in 40 l. tanks of fresh water. Experiments with the newly hatched alevins were made with batches of 20 in litre beakers suspended in a water bath to maintain the standard conditions of temperature.

Tolerance to salinity changes was measured in two ways. First, by observing the experimental fish and recording the times of their deaths, so that survival times could be considered in relation to salinity. In this case the figures of survival of the fish are expressed in terms of the median survival time, the median being a useful parameter especially where all the fish did not die, or where some survived for much longer periods than the others. Secondly, much more precise information was obtained by measuring the freezing-point depression of the blood of the experimental fish. The blood samples were taken by cardiac puncture, without anaesthetic. Very small quantities of blood were taken from the fish (less than 10^{-3} ml.) and samples for freezing-point (10^{-6} ml.) used with Ramsay's cryoscopic apparatus (Ramsay, 1949; Ramsay & Brown, 1955). The freezing-point depression (Δ° C.) of the blood of individual fish was recorded 1, 2, 4, 8 and 24 hr. after the beginning of each experiment, and then on subsequent days. These measurements indicated whether the fish was able to regulate in the experimental medium, or only able to tolerate the increased salinity, and they also gave some measure of the time required by each group of fish to accommodate to the conditions of the experiment.

RESULTS

Considering first the survival of young *Salmo salar* in the experimental salinities (Table 1 and Fig. 1), it can be seen that in all the batches of fish survival is complete in control conditions (static fresh water) and that it is reduced as the salinity of the experimental medium is increased. Taking fry (3 months old) as an example: these fish were all able to survive a salinity of 25% sea water for the duration of the experiment, but when the salinity of the medium was increased to 50% sea water (and thus hypertonic) the median survival time was only 7·0 hr. The median survival time in 75% sea water was reduced further to 4·0 hr., and in 100% sea water was only 2·0 hr. The same decline in survival with increasing salinity can be seen in the figures for parr and smolts. An exception to the general trend is shown by the figures for newly hatched alevins. These fish showed very similar survivals in 25 and 50% sea water (with a median survival time of 45 hr. in each case), with

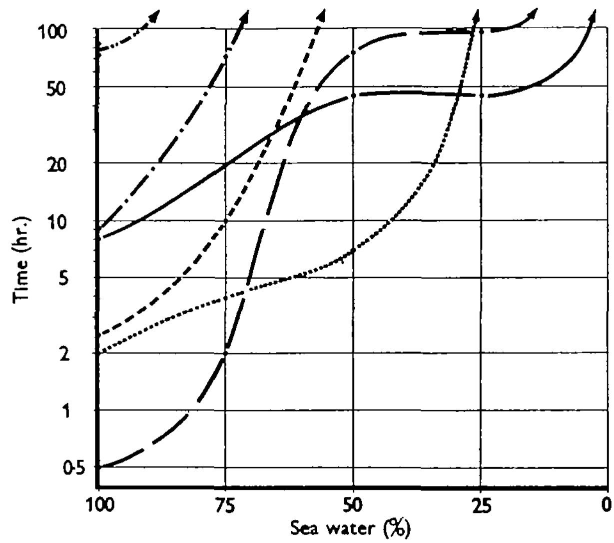

Fig. 1. *Salmo salar* relative survival in sea-water dilutions at different ages and sizes. Alevins, 1 week old, ——; alevins, 6 weeks old, — —; fry, 3 months old, ····; parr (1), 7 months old, - - -; parr (2), 7 months old, — · —; smolts, 2+ years old, - · · -.

Table 1. *Relative survival of* Salmo salar *at different ages*

Stage in life-history	Size range (cm.)	Age	Median survival time (hr.): 100% sea water	75% sea water	50% sea water	25% sea water	Control
Alevins	—	1 week post-hatch	8·0	19·0	45	45	∞
Alevins	—	6 weeks post-hatch	0·5	2·0	76	96	∞
Fry	1·5–2	3 months	2·0	4·0	7·0	∞	∞
Parr	3–4	9 months	2·5	10	∞	∞	∞
Parr	7–10	9 months	9·0	72	∞	∞	∞
Smolts ex R. Lledr	12–15	2+ years	72	∞	∞	∞	∞
ex R. Coquet (migrating)	12–15	2+ years	84	∞	∞	∞	∞

declining survival times in the higher salinities. The greater survival time of the 1-week post-hatch alevins is notable compared with that of fry in all the experimental salinities, and a similar increase in survival in 25 and 50% sea water in the 6-week post-hatch alevins. Whether this increased survival of the alevins represents a tolerance of internal salinity changes or a resistance to external ones will be discussed further below.

In the stages of fish chosen, from fry to migrating smolts, there is a continuous increase in survival time in the experimental media, as the fish gets older. There is some indication that this increase in survival is related to an increase in the size of the fish. Two groups of *Salmo salar* parr, one containing fish of 3–4 cm. length, the other fish of 7–10 cm. length, show clear differences of survival time, the larger fish having the higher values. These two groups of fish were of the same age and had been reared in a single tank in a hatchery, the size groups being separated only a week or two before the experiment. Migrating smolts (from two natural fresh-water environments) showed a greatly increased tolerance to sea-water dilutions. Only in full strength sea water was there any mortality, and while a figure for median survival (and thus a 50% kill) could be obtained in some experiments, in others more than 50% of the fish survived the direct transfer.

Table 2. *Relative survival of* Salmo trutta *and* S. gairdnerii *at different ages*

			Median survival time (hr.)				
Species	Size range (cm.)	Age	100% sea water	75% sea water	50% sea water	25% sea water	Control
S. trutta	8–10	9 months	7·5	76·0	∞	∞	∞
S. trutta	12–15	2 years	11·5	76·0	∞	∞	∞
S. trutta	19–20	> 3 years	36·0	∞	∞	∞	∞
S. gairdnerii	8–10	9 months	7·5	18·7	∞	∞	∞
S. gairdnerii	15–20	> 2 years	120·0	∞	∞	∞	∞

Similar experiments were made with different groups of the related species, *S. trutta* and *S. gairdnerii* (Table 2). In these species, the same conclusion could be reached, that salinity tolerance increases with increasing size of the fish. Thus *S. trutta* of 8–10 cm. size survived less well than those of 19–20 cm. size; and similarly for the two groups of *S. gairdnerii*. In comparing the results for all three species, it will be seen that within any one age group (with slightly differing size range) there is some indication of a species difference. Thus, in parr of the three species (all about 9 months old) the survival order is

S. salar > *S. gairdnerii* > *S. trutta*

and in 'smolts' of the 2-year age group,

S. gairdnerii > *S. salar* > *S. trutta*.

The relative change in the position of *S. gairdnerii* in this series is interesting; it should be noted that the fish of this species used in the experiments are larger in size than those of the other two (although reared in very similar hatchery conditions), and that this size difference is more marked in the 2-year age group than it is in the earlier one. This observation thus confirms the conclusion drawn from the comparison of survival of the two groups of salmon parr.

A diagram illustrating the relative survival of all these groups of fish, together with comparative results for two stenohaline freshwater fish (Herbert & Mann, 1958), is shown in Fig. 2. Generally speaking, the survivals can be plotted as a series of parallel curves, with *S. gairdnerii* (15–20 cm.) on the extreme left with the greatest survival time, and freshwater roach and perch on the right with the lowest survival. While *S. salar* smolts show a high relative survival, there is very little to distinguish *S. trutta* (8–10 cm.) and *S. salar* (7–10 cm.) at the parr stage, while *S. trutta* of larger size and 2-year age group, seem to be more stenohaline in behaviour. The curve for the alevins shows quite a different pattern, with a sharp break in the curve, indicating a relatively good survival in the lower salinities, but very limited survival in 75 and 100% sea water.

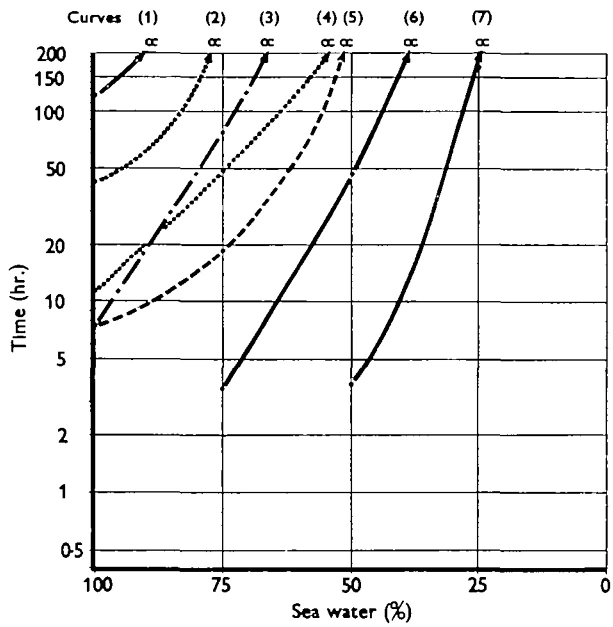

Fig. 2. Relative survival in sea-water dilutions of some teleost fishes. Curves (1) *Salmo gairdnerii* (15–20 cm.), (2) *S. trutta* (19–20 cm.); (3) *S. trutta* (12–15 cm.); (4) *S. trutta* (8–10 cm), (5) *S. gairdnerii* (8–10 cm.); (6) *Perca* (8 cm.); (7) *Rutilus* (9 cm.),

In addition to observations on survival, freezing-point measurements of the blood of individual fish were made and these give some indication of the degree of osmotic control of which the fish is capable. A summary of these results is given in Table 3, where is tabulated the time taken for fish to return to the 'normal' range of blood concentration.

Measurements of the freezing-point depression of the blood of the three species of salmonids in freshwater conditions show a limited range of concentration. Figures for 1- to 2-year-old fish are shown below:

Salmo salar Δ 0·60 ± 0·04° C. (n = 24). Range 0·50–0·65° C.

S. trutta Δ 0·57 ± 0·02° C. (n = 33). Range 0·53–0·61° C.

S. gairdnerii Δ 0·55 ± 0·03° C. (n = 30). Range 0·49–0·62° C.

When these fish are acclimatized to sea water the blood concentration (measured by freezing-point depression) rises by about 10% of the freshwater values.

Table 3. *Time required for blood regulation*

Species	Size range (cm.)	Age	Time (hr.) to return to normal blood concentration in 100% sea water	75% sea water	50% sea water
S. salar	3–4	9 months	*	*	200
S. salar	7–8	9 months	*	900	200
S. salar	12–15	> 2 years	24	24	4
S. trutta	8–10	9 months	*	300	300
S. trutta	12–15	> 2 years	*	150	100
S. trutta	19–20	> 3 years	*	150	80
S. gairdnerii	8–10	9 months	*	400	150
S. gairdnerii	15–20	> 2 years	150	250	25

* All fish in experiment died.

After introducing the fish into a salinity higher than the fresh water in which it had been living the initial response was a rise in the blood concentration. In the lower experimental salinities the fish usually controlled this rise quite quickly and brought the blood concentration back to the normal level. In the higher external salinities this regulation took longer, and in the case of the smaller size-groups did not occur at all. Thus the time taken to regain control (as expressed in Table 3) gives some measure of the osmoregulatory ability of the fish. These estimates of osmoregulatory ability in general confirm the conclusions drawn from a study of the median survival times, but there are some interesting differences. For instance, the two most 'successful' groups, *S. salar* and *S. gairdnerii* (about 2 years old), both regain control of the blood concentration when in a medium of full strength sea water, but although the median survival time for *S. gairdnerii* is greater than that shown for *S. salar*, the young salmon require the shorter time to control the level of blood concentration in these conditions. Thus the order of osmoregulatory ability for the three species would be

S. salar > *S. gairdnerii* > *S. trutta*.

This difference could indicate that the increased survival time in the rainbow trout depends upon tolerance or resistance to the external conditions, rather than on the ability or activity of the osmoregulatory mechanisms. The order of osmoregulatory ability in the smaller 9-month-old fish of the three species is not so clear.

The internal changes which take place after the fish are put into sea water, or its dilutions, can be seen expressed graphically in Fig. 3. Here the blood concentration changes in individual salmon of different age groups, after immersion in 100% sea water, are expressed in terms of the freezing-point depression of the blood. It can be seen that the blood concentration rose in all groups, within 30 min. of the transfer. In 6-week alevins or fry this rise continued unchecked, until within 2 hr. the blood concentration rose to more than Δ 1·00° C. After such a rise the fish always died within a variable period. Parr (7 months old) and the younger group of alevins showed a somewhat lower rise in blood concentration but reached the lethal limit of blood concentration in about 5 hr. Only smolts could survive the direct transfer to sea water, and after an initial rise in 2 hr. to a level greater than the normal, the level of concentration was reduced and relatively constant within 4 hr.

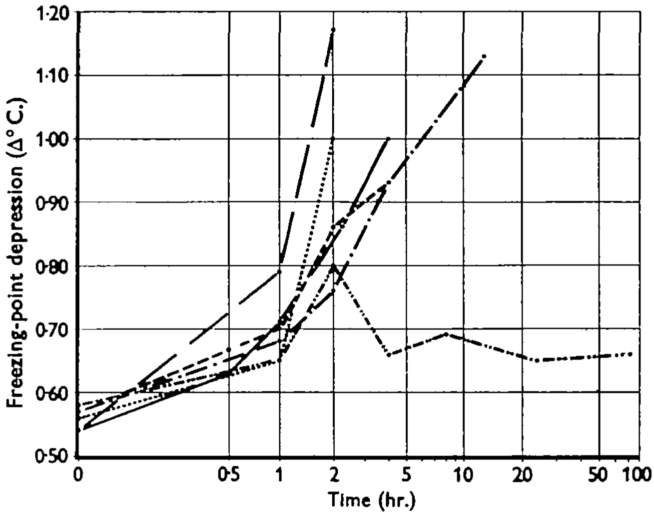

FIG. 3. *Salmo salar*: changes in blood concentration after immersion in full strength sea water. Key as in Fig. 1. Each point refers to an individual fish.

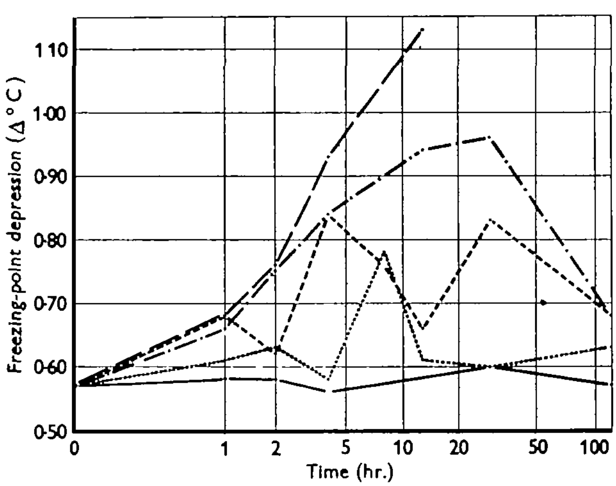

Fig. 4. *Salmo salar* parr: changes in blood concentration after immersion in sea water dilutions. Control, ——; 25 % sea water, ····; 50 % sea water, - - -; 75 % sea water, — · —; 100 % sea water, — —. Each point refers to an individual fish.

A comparison of the osmoregulatory ability of salmon parr and smolts may be made by comparing the results expressed in Figs. 4 and 5, where the blood concentration changes following immersion in the various media are shown. Parr in 100 % sea water lost control of the blood concentration immediately, and in 1 hr.

the concentration of the blood rose to its usual sea-water level (Δ 0·67° C.); but in 2 hr. it had risen still further and so on, until in 10 hr. it was greater than 1·10° C. In 75% sea water there is a similar but not quite so steep rise in blood concentration, and the fish was able, after about 24 hr. and a rise to Δ 0·95° C., to bring sufficient regulatory processes into action to reduce the blood concentration to the sea-water level. In 50% sea water the rise in blood concentration is not nearly so great, although the variability of the results does indicate that the fish are in considerable stress. In 25% sea water there is again some variability in blood concentration, even though this medium is hypo-osmotic to the blood. Determination of freezing-point of the blood of control fish taken at the same time show that the individual variations do not go beyond the usual range for these fish living in fresh water. In Fig. 5 similar results for salmon smolts are shown. These fish were 1 year

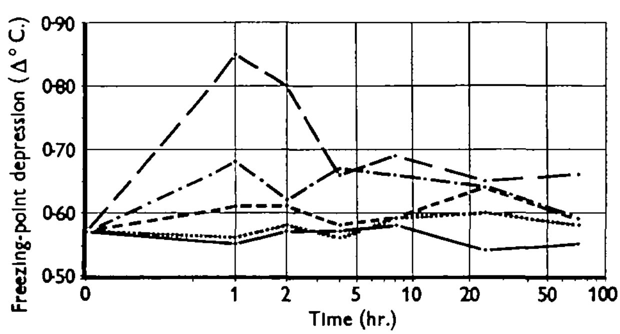

Fig. 5. *Salmo salar* smolts: changes in blood concentration after immersion in sea water dilutions. Key as in Fig. 4. Each point refers to an individual fish.

older and about 4–5 cm. larger than those of the parr group just discussed. It is immediately clear from these figures (drawn to the same scale) that the regulation shown by the smolts is very much better than that shown by the parr. Not only did these fish survive the transfer to 100% sea water, but the rise in blood concentration following immersion in sea water was not so high, and was relatively quickly reduced (after about 4 hr.) to the normal level for marine fish. The fish in 75% sea water did not even show the initial rise beyond the normal level but only a rise to about the normal level for marine fish. The variation of the blood concentration in these fish in 50 and 25% sea water is scarcely evident, and much less than that shown by the parr.

DISCUSSION

The results of the experiments described above allow some conclusions to be drawn about the ability of these salmonid fish to survive a sudden transfer to sea water. Both survival and the ability to osmoregulate in different salinities depends upon the size and age of the fish, as well as on the species. It may be concluded from the evidence put forward here that survival and osmoregulation are better in larger fish, or in older fish, although if these two factors can be distinguished size appears to be the more important. Within the three salmonid species used for these experiments the salmon seems to survive salinity changes better than the other two species, and the rainbow trout better than the brown trout.

The behaviour of the two age groups of salmon alevins described in this paper is very different from that for the older fry. It is possible that osmoregulation is quite different in these larvae. The epithelium of the alevin is singularly well supplied with mucous cells (Parry, 1958). While the mucus produced cannot by itself maintain an osmotic barrier, is it possible that the epithelium as a whole can maintain some degree of impermeability? The general pattern of the results obtained with alevins could indicate such an impermeability, which breaks down after a time interval almost irrespective of the external salinity.

The increase in survival in the groups of fish, discussed above, could be the result of several factors. First, the effect of an increase in size could indicate that the surface:volume ratio is important, a larger fish being subjected to a lower osmotic stress. Secondly, as the fish gets older it is possible that the skin becomes thicker and less permeable, although osmotic exchanges through the thin and vastly convoluted respiratory epithelium of the gills must completely overshadow any exchanges via the rest of the surface. Thirdly, as the fish grows older there is also the possibility that some salt-regulatory mechanism becomes more efficient. If an increase in the number of acidophil ('Keys-Willmer') cells in the gills could be associated with an increased efficiency in osmoregulation, this would be valuable evidence that this factor was important. However, although there is evidence that salt-loading induces the development of such cells in goldfish and guppies (Liu, 1942; Vickers, 1958), and that injections of thyroid will also induce their development, along with the silvering characteristic of the smolt change (Hoar, Black & Black, 1951), there is no clear and unequivocal evidence that these cells do, in fact, salt-regulate (Parry, Holliday & Blaxter, 1959). Acidophil cells are present from a very early stage (in 4-week alevins); they are more numerous in larger and older fish, but what is needed is to know if they increase out of proportion to the osmotic work required in fresh water.

The growth of a tolerance to salinity is of great importance in the ecology of salmonids, and especially in the case of *S. salar*. It would appear justified from the evidence of these experiments to conclude that those fish which grow quickly are those which develop more quickly a tolerance to sea water. In a population of young salmon the fastest growing parr are thought to be the first to become smolts and to be first to the sea (Pyefinch, 1955). These fish will best be able to withstand such a salinity transfer. If rapid freshwater growth is related to rapid marine development, these fish will complete the life cycle in a shorter time and thus provide a quicker return to a river for spawning. The establishment of this hypothesis by further experimentation could have far-reaching ecological implications.

SUMMARY

1. A study has been made of the survival and osmotic regulation of young salmonid fishes following transfer from fresh water to various dilutions of sea water.

2. Survival is in the order:

Salmo salar > *S. gairdnerii* > *S. trutta*

and is generally better the larger the fish.

3. The survival pattern of alevins differs from that of the older stages.

4. Hypo-osmotic regulation is first seen in parr and becomes fully effective in smolts.

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