ACCLIMATISATION OF RAINBOW TROUT

TO SEA WATER

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Abstract

The intention of this work was to investigate the problems concerned with transferring rainbow trout Salmo gairdneri directly from fresh water into sea water with reference to this procedure on a marine trout farm. A laboratory study of some of the physiological changes that occur following direct transfer of rainbow trout to sea water was undertaken. The effect of size and salinity on the osmoregulatory ability of the fish was investigated. Field trials involving sea cages were also performed with fish of different sizes and varying salinities. The results obtained from the field were used to produce a nomogram relating size and salinity with expected levels of mortality; this nomogram is thought to have considerable applications in the marine trout farming industry. The results from the field and the laboratory were compared and they indicated a considerable detrimental effect of prior transportation.

Two methods for alleviating the osmotic shock following transfer were investigated in the hope of lowering mortalities. The first method investigated was the feeding of a wet diet containing 50% fresh water following transfer with the intention of reducing the dehydration and high plasma concentrations. This method was shown to be ineffective and possible reasons for this are discussed.

The second method examined in an attempt to reduce mortalities was the prior feeding of a high salt diet. It was found that the feeding of a diet containing 10% NaCl for a period prior to seawater transfer significantly reduced mortalities during the first few days in the marine environment. The optimum period of prior feeding of the high salt diet was found to be about 2 weeks as longer periods did

not improve the beneficial results and resulted in poorer growth rates. Those fish fed on the high salt diet were found to have significantly lower plasma osmotic concentrations following transfer and this finding is discussed in relation to known mechanisms of salt excretion. A study of the "chloride cells" in the gills was undertaken but no attributable effect was found following the feeding of the high salt diet.

CONTENTS

INTRODUCTION

Rainbow trout culture is becoming increasingly common in many regions of the world. However, one of the factors which is limiting its expansion is the number of suitable freshwater sites. In several countries, notably Norway and Scotland, this has led to the on-growing of rainbow trout in sea water.

The trout which are to be on-grown in the sea are transferred from the freshwater hatchery either to seawater tanks or floating cages. Floating cages are at present more widely used since they require about half the capital expenditure and also have considerably lower operating costs than seawater tank systems requiring pumped water (Varley, 1977). The indented coastline of both Norway and Scotland also provides a large number of suitable sheltered sites for the mooring of cages.

One major disadvantage of the cage system is that the fish may have to be transferred directly from the fresh water to the sea with no possibility of acclimatisation to intermediate salinities. Most of the freshwater hatcheries are chosen for good water quality, and are often some distance from the sea. Thus the use of acclimatisation tanks, where the salinity could be raised over a period of weeks, becomes impractical. Almost all the trout farmers therefore transfer their trout directly from the hatchery to the floating cages, having learned from experience that fish of above 100g will transfer to full-strength sea water with few mortalities. Trout normally reach this weight during their second summer, but it is during summer that a freshwater shortage is most likely to occur and as the flow rates decrease so the water temperatures increase. It would therefore be much more convenient to transfer them earlier.

There is not only the problem of decreasing water availability but

also the fact that as the fish grow, more water is required. For every 1000kg of fish held there must be a flow rate of 10-15 l/sec. In practice this means that a small farm producing 50,000kg of 250g trout will require 100 l/sec in the spring when the fish are 50g and 250 l/sec by the summer, when they are 100g, if they continue to be held in fresh water.

The problems of holding trout in fresh water until they are over 100g, sometime in their second summer, has meant that most farmers are obliged to transfer their fish in the second spring when the fish are about 60g. At this size the osmotic shock caused by transferring the trout straight from fresh water into the sea can often result in mortalities as high as 10%. At present farmers accept a certain level of mortality to obtain the advantages of getting their fish into the sea before the freshwater temperatures increase and the flow rates decrease.

Another advantage in transferring trout at the smallest possible size is that sea water temperatures on the west coasts of Scotland and Norway rarely fall below 7°C in winter: since trout will grow till the temperature falls below 5°C growth can thus continue throughout the winter. This contrasts with fresh water where no growth occurs for about 3 months during December, January and February. For this reason it is an advantage to transfer trout in the autumn of their first year. Normally the fish are only 20 to 30g at this time and cannot tolerate sea water transfer, however, if a marine site is used where the salinity is reduced by freshwater run-off, it is possible to effect a transfer with acceptable levels of mortality (Landless, 1976).

The number of sites where the salinity is reduced sufficiently to allow autumn transfer is small and for this reason several farms particularly in Norway, have developed an alternative cycle of production (Edwards, 1978). By holding brood fish in spring water with a temperature

of 7°C all winter they are able to induce spawning in January, instead of April. Then by heating the water to 10°C they accelerate hatching and fry development. The fish then grow rapidly during the spring and summer and by their first autumn they are 50-70g and large enough for some of them to tolerate sea-water transfer.

Early trials on-growing trout in sea water involved the steelhead race of Salmo gairdneri which is naturally anadromous and undergoes smolting in spring after one or two years in fresh water. Although it naturally adapts to a seawater environment, there are a number of disadvantages. It is only possible to effect a safe transfer during the period of smolting, March and April; at other times mortalities tend to be high (Conte and Wagner, 1965). Within a stock of trout not all the fish will smolt after one year; many have to be held in fresh water for another year before they will change. The whole stock therefore has to be examined, the smolts removed and the remaining fish held over for another 12 months. For these reasons the steelhead trout have been discarded and almost all trout now on-grown in the sea are of rainbow trout stock.

The purpose of this research was to examine the problem of transferring rainbow trout directly from fresh water into sea water. At present farmers accept a mortality of up to 10% during the 2 weeks following transfer. On a farm producing 50,000kg per annum of 250g trout a 10% mortality is a loss of 20,000 fish. This represents a direct economic loss of about £1500 at 1978 prices, but more importantly it reduces the total annual production by about 5000kg (with a current value of £6000). The factor which limits annual production on most seawater farms is the number of fry which can be reared in the available fresh water at the hatchery. The seawater site is rarely the limiting factor as additional cages can be constructed to accommodate any expansion in

the output of the hatchery. Therefore any reduction in the number of mortalities at transfer is reflected by a proportional increase in total annual production.

Before methods can be devised to reduce the mortality at transfer, it is important to understand the physiological changes that occur during acclimatisation. A study of some of these changes was undertaken including the effect on them of fish size, salinity and temperature difference. Field trials were completed with trout of different sizes and water of different salinities with the intention of developing a means by which, knowing the salinity and the size of the fish, an estimation of the expected mortality could be made.

With a knowledge of the physiological changes which trout experience following transfer, attempts were made to reduce the osmotic stress and so lower the mortality. Two methods were evaluated, one involving the feeding of a wet diet, the other feeding a diet containing a high level of NaCl. Landless (1976) suggested that the feeding of a wet diet following transfer might prove beneficial by reducing the osmotic stress on the trout, so this possibility was examined. Zaugg and McLain (1969) and Basulto (1976), both working with salmon species, reported that prior feeding of a diet containing a high level of NaCl reduced mortalities following transfer to sea water; perhaps by stimulating the salt secretory mechanisms required for a marine environment. It was therefore decided to test this technique using rainbow trout.

MATERIALS AND METHODS

1. Holding facilities

Sea cages in Dunstaffnage Bay

The cages used for both the holding of stock and acclimatisation trials were 4m square and made to the design of Landless (1974). Five of these cages were moored 50m from the shore in the sheltered waters of Dunstaffnage Bay in a minimum of 5m of water. The cages consisted of three basic components, the framework, the flotation and the net. The framework was constructed using galvanised scaffolding tubing and standard scaffolding fittings. The inner square formed by the tubing was 4m x 4m and along two of the sides of this square were the floats. Each float was 4m long and was constructed of expanded polystyrene encased in glass reinforced concrete; two 6m lengths of scaffolding passed through the middle of each float and provided strengthening. The floats being 0.6m wide also provided a walkway which eased access to the nets.

Another three cages were constructed in late 1975 to increase the holding capacity for the trials required in this study. A modification as suggested by Landless (1974) was included in the new cages. In the old design cracks had appeared in the concrete skin in the middle of the 4m floats. This was thought to be due to slight flexing during rough weather. The modification consisted of replacing each 4m float by two 1.85m floats, but retaining the two 6m tubes. This allowed the tubes to bend in the middle without cracking the concrete. These new cages have now been in the water for three years and there is no evidence of the concrete skin cracking.

The nets hung from the scaffolding framework were of the knotless

variety, as this was seen to cause less damage to the fish. For the holding of stock, the full cage was used and a 4m cube net suspended from the scaffolding with a weight in each of the four lower corners. When using small numbers of fish for acclimation trials the surface of one of the cages was divided into four by means of two 4m scaffolding tubes and four 2m cube nets hung from them. The nets were changed whenever fouling became heavy and started seriously to reduce water interchange. The time between changes depended upon the mesh size and the time of year.

The temperature of the water in Dunstaffnage Bay varied seasonally, reaching a maximum in September of 16°C and a minimum in February of about 5°C. The salinity also varied from month to month depending on the freshwater run-off into the adjacent Loch Etive. This fresh water is mixed with coastal sea water by the tidal race at the mouth of Loch Etive about 2km from the experimental site. The salinity can reach as low as 12°/oo after a continuous period of heavy rain and at times of summer drought can reach 32 to 33°/oo, only just lower than the coastal water.

The salinity and to a lesser extent, the temperature of the water in Dunstaffnage Bay fluctuates with the tidal cycle. The ebb tide brings low salinity water from the inner basin into the bay, while a flood tide brings in coastal water of higher salinity. The lowest salinities are therefore recorded at low tide while the highest are recorded at high tide. The fluctuations are most marked when the salinity from the inner basin is greatly reduced. Salinities can then range from 15°/oo at low tide to 28°/oo at high tide. The temperature fluctuations during a tidal cycle are much smaller and are due to the differences in temperature between the coastal water and the water of the inner basin of Loch Etive.

Sea cages at Kames Fish Farm.

The sea cages at Kames Fish Farm are moored in a sheltered bay in

Loch Melford, 40km south of Oban. Unlike the Landless cage the frame is a wooden construction, but expanded polystyrene is used for flotation. The cages are moored in 7m of water, close to the shore. Access is by means of a floating pontoon alongside a pier. The cages are of two different sizes, one 6m x 6m, designed to hold three tons of fish, the other 4m x 4m, holding one ton. When transferring fish, both under normal circumstances and during experiments in this study, fish are washed down a chute attached to the transporter tank positioned at the end of the pier. The fish then fall about 5m (depending on the state of the tide), into the cage below.

The water of Loch Melford, unlike that in Dunstaffnage Bay, has an almost constant salinity of 32-33°/oo throughout the year with no tidal fluctuations. The temperature of the water reaches a maximun of 16°C in September and a minimum of about 7°C in February.

Freshwater tanks at the Laboratory

In some of the early experiments fish which had been transported from Stirling were given a period in fresh water to recover before the acclimation trials started. The fresh water was collected from Lusragan Burn near Connel and placed in two large circular tanks of 1100 l capacity and used as required. This proved rather unsatisfactory due to the problems of collection and also, the fish could only be held for a limited period of time in the still water.

It was decided to try and hold fish in the Laboratory's tap water supply. Earlier work which involved holding trout in the Laboratory's tap water had led to problems with copper poisoning, (Landless Ph.D. thesis). A group of fish was however held in the tap water for three months with no visible lethal or sub-lethal effects. This indicated that

after several years the level of copper in the water had apparently fallen to a level which had no noticeable effect on the fish.

As part of a new Water Board Scheme, chlorination of the water was started in March 1977. The residual level of chlorine was of the order of 0.05 p.p.m. Dandy (1972) showed that with brown trout Salvelinus fontinalis. survival time in 0.04 p.p.m. was only 48h and that levels of 0.005 p.p.m. caused depression in general activity. Several deaths were recorded at the time chlorination started, so all freshwater experiments were terminated. Since many of the planned experiments involved the holding of several thousand fish for several months in fresh water prior to transfer, it was thought essential to find a site where such experiments could be performed.

There were other experiments planned which required fewer than a hundred fish involved in physiological studies. Most of these laboratory experiments were carried out in an air-conditioned room at Dunstaffnage. The room was equipped with freshwater, and seawater taps from the Laboratory's own supply. To remove any residual chlorine the fresh water was passed through an activated charcoal filter (see Appendix 1). Once the filter had been installed the fish showed no sub-lethal effects and feeding and behaviour appeared normal.

The temperature of the tap water ranged from 3°C in January to 17°C in August. The air-conditioning unit in the room was set at 14°C, but the fast flow rates of water required resulted in there being only a small change of temperature in the tanks.

The sea water was pumped to the aquarium and air-conditioned rooms at 10 psi. Early experiments of holding trout in sea water led to some unexpected deaths which may have been due to air supersaturation caused by entrainment of air in the pumps. Measurements with an oxygen meter

showed levels sometimes exceeding 120% saturated. To reduce the supersaturation the sea water was first passed into a header tank where it was vigorously aerated, forcing the excess gas out of solution.

The temperature of the pumped sea water ranged from 7°C in winter to 16°C in summer. The salinity also fluctuated, but it was not as marked as the salinity changes out on the rafts and rarely fell below 30°/oo. The smaller fluctuation was due to the position of the intake pipe while the large storage tanks at the Laboratory smoothed out the tidal fluctuations.

There were normally three tanks installed in the air-conditioned room, two circular black tanks with a capacity of 225 l and one large white glassfibre tank with a capacity of 1100 l. The salinity into one of the 225 l tanks could be adjusted by controlled mixing of the sea and fresh water. Since the pressure in both water systems varied considerably a constant head device was built (Fig. 1), which ensured a constant predetermined salinity. Once adjusted a given salinity could be maintained throughout an experiment, unless the salinity of the incoming sea water changed. Regular check was therefore made by a hydrometer specially calibrated in °/oo, a temperature correction being made for water of more or less than 10°C.

For most of the experiments no attempt was made to control the temperature as such large flow rates were required that either heating or cooling would prove impractical. The exception to this was the experiment which was designed to test whether the degree of thermal shock between the fresh water and sea water effected the ability of trout to acclimatise to a rapid change in salinity (Section 3).

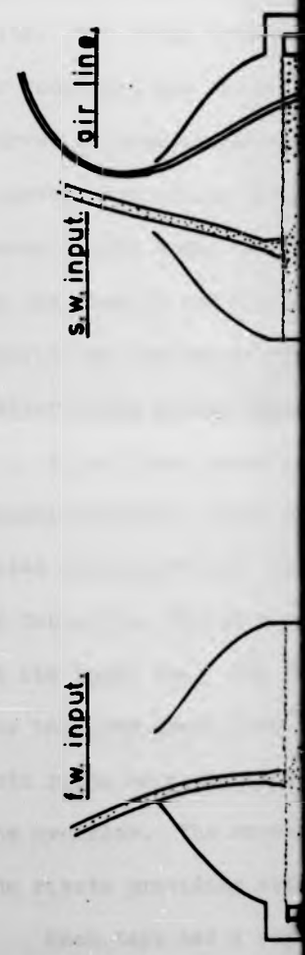
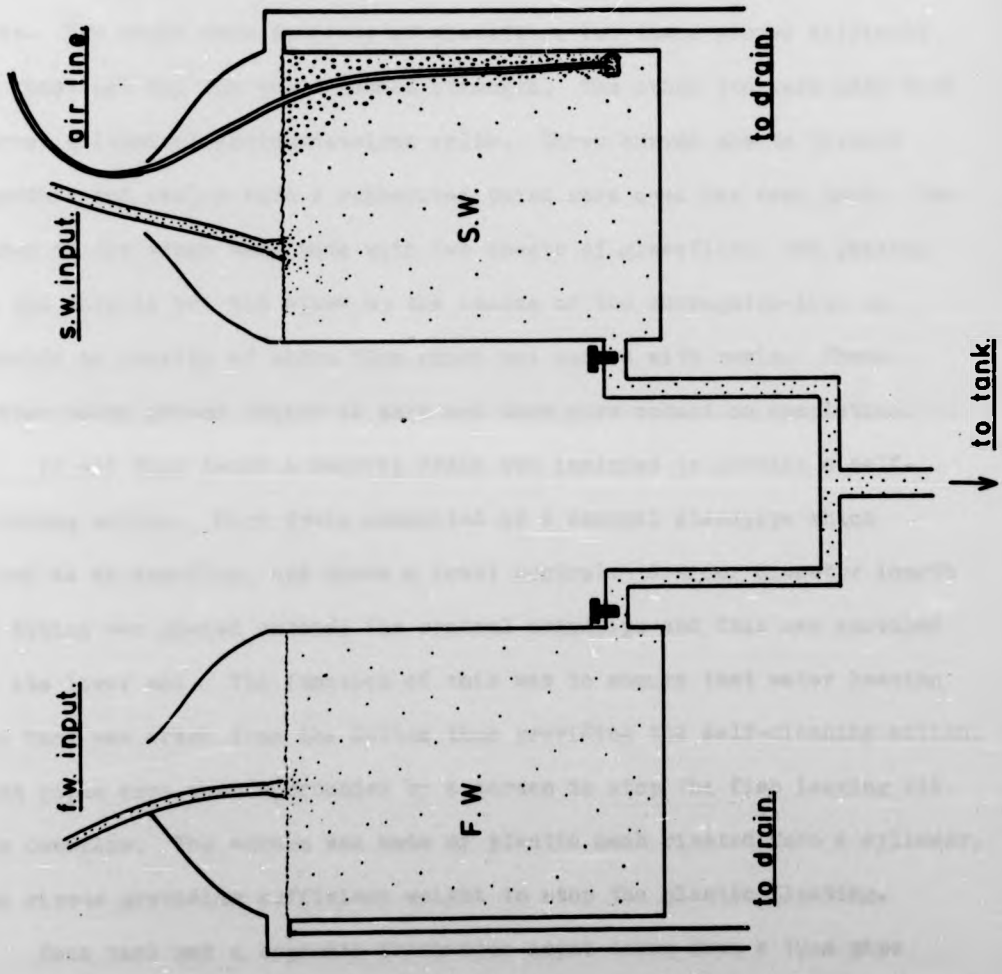


Figure 1. Constant-level mixing device.

A constant level was maintained in the two 10 l vessels by means of an input and an overflow, this ensured a fixed pressure of water at the two taps. The salinity was set by means of adjusting the taps until the required value was obtained, measured using a hydrometer. The value remained constant unless the salinity of the incoming sea water changed. Aeration was present in the seawater vessel to reduce any supersaturation.


air line.
s.w. input.
S.W.
to drain.
to tank.
f.w. input.
F.W.
to drain.

Freshwater tanks at Kilmore

A freshwater site was required where large numbers of fish could be held for extended periods. A freshwater spring with a reliable flow of 2 l/sec and a 50cm head was available at Kilmore, 5km south of Oban. Four tanks each 150cm in diameter and 50cm deep were constructed on this site. Two tanks were entirely of glassfibre but these proved difficult to construct and the walls lacked strength. The other two were made with curved galvanised corrugated-iron walls. Three curved sheets riveted together and sealed with a rubberised paint were used for each tank. The bases of the tanks were made with two sheets of glassfibre, one passing up the outside and the other up the inside of the corrugated-iron to provide an overlap of about 10cm which was sealed with resin. These latter tanks proved easier to make and were more robust on completion.

In all four tanks a central drain was included to provide a self-cleaning action. Each drain consisted of a central standpipe which acted as an overflow, and hence a level control. A wider diameter length of tubing was placed outside the central standpipe and this was knotched at its lower end. The function of this was to ensure that water leaving the tank was drawn from the bottom thus providing the self-cleaning action. Both pipes were then surrounded by a screen to stop the fish leaving via the overflow. The screen was made of plastic mesh riveted into a cylinder, the rivets providing sufficient weight to stop the plastic floating.

Each tank had a separate freshwater input taken from a 10cm pipe coming from the spring. The water input was arranged to produce a circular motion in the tank, thus ensuring a complete exchange of water as well as creating a vortex action which washed any detritus down the drain.

The spring water remained at a temperature of 9°C throughout the

year, like much other spring water the oxygen saturation was only about 80%. The oxygen level of the incoming water was therefore only about 9.2 mg/l instead of 11.6 mg/l. Rainbow trout cannot tolerate oxygen levels much below 5 mg/l so very careful attention was paid to water flows and stocking densities.

Freshwater tanks at Kames Fish Farm

During the freshwater phase the fish at Kames were held in large 4m diameter corrugated-iron tanks with a concrete base, situated within an old factory building with no ar tificial light. A central drain and circular water movement ensured good water exchange and a self-cleaning action in each tank. Each tank had a separate input of water collected from two nearby streams. There was a seasonal fluctuation in temperature reaching a maximum of 18°C in August and a minimum of 1°C in January. The water flow was subject to drought and in times of water shortage a partial recirculating system could be brought into operation, although this was not required during the course of any of the reported experiments.

2. Fish stocks

The fish used in all the experiments were *Salmo gairdneri* of rainbow trout stock as opposed to the smolting steelhead trout. They were purchased mainly from Cloan Hatcheries at Auchterarder while smaller numbers were acquired from Howieton and Northern Fisheries near Stirling. The fish at Kames had originally been purchased from Cloan as fry. No difference was noticed between stocks bought from different hatcheries.

3. Fish food and feeding

Commercial trout pellets purchased from Edward Baker Ltd. of Bathgate

were used for all feeding. The percentage composition of the normal diets according to the manufacturers were as follows:

	Floating pellet		High density pellet
Size of pellet	No. 4	No. 5	No. 5
Oil	8	5	8
Protein	47	41	47
Fibre	4.5	4.5	4.5
Ash	10	10.5	10
Moisture	8	9	11
Carbohydrate	22.5	30	19.5

The level of NaCl salt varied according to the source of the fish meal used to form the pellet, but was always below 1%. The High Salt Diet (H.S.D.) which was used for pre-transfer feeding was made specially to order by Edward Baker and had the same composition as the control diet (High density No. 5), except the salt level was artificially raised to 10%.

The wet diet used was produced by soaking pellets (Floating No. 4 or 5) in fresh water for 30 min, any longer and the pellets lost their form. The pellets took up almost exactly their own weight in water, e.g., 100g of a No. 4 floating pellet absorbed 106g of water. A floating pellet was normally used as this held more water than the sinking high-density pellets.

The levels of feeding varied. The stock fish and many of the experimental fish were fed according to the table provided by the manufacturers (Appendix III). Following transfer in the acclimation trials fish were fed to satiation, the amount consumed depending on their

capacity to adjust. A scale was devised to help compare feeding activity in different trials.

N - None, no feeding activity.

VL - Very light, a few pellets taken as they sank.

L - Light, pellets taken as they sank, a few from the surface.

A - Active, a normal level of feeding with most fish taking the food at the surface.

VA - Very active, all the fish at the surface thrashing to obtain food, as seen with healthy fish after one day of starvation.

Feeding of stock fish was usually by automatic feeder, either of commercial design or made at the Laboratory (Landless pers. comm.). All feeders composed an electrically driven vibrating plate or worm screw with a photocell control to ensure daytime feeding only. Since the fish being used in most of the experiments were below 100g short feeds at half-hourly or hourly intervals were used. Control and experimental fish were fed to the same feeding regime.

Fish being used for the acclimation trials were all hand fed for the first 10 to 14 days so that a note could be made of their feeding activity. Fish held in the tanks in the air-conditioned room were always hand fed, as were the fish at Kames where it was felt that hand feeding was a good husbandry practice and gave an idea as to the state of health of the fish.

4. Blood sampling

Blood samples of about 0.4ml were required for the determinations of plasma osmolarity. To obtain this volume from fish of the sizes used

necessitated killing them. The method used was adapted from Conte and Wagner (1965). The fish were first anaesthetized in a 1:10,000 solution of MS 222 (Tricaine methansulfonate, Sandoz); they were then blotted by wrapping in a paper towel. Blood was obtained through severance of the caudal peduncle. Blood flowing from the caudal artery was bled onto a stretched piece of cold paraffin-wax film and immediately transferred via a glass capillary tube, into a heparinised 0.5ml polypropylene centrifuge tube. The disposable tubes were then sealed and placed in a Hawksley Micro-Haematocrit centrifuge fitted with the Micro-Chemistry head which develops 10,000g. The tubes were then spun for 3 min and the supernatant plasma removed by glass capillary and placed in polypropylene tubes. The plasma was then deep frozen until required for analysis on the osmometer.

A Fiske osmometer was used for determinations of freezing-point depression. It was adjusted to take the smallest sample size of 0.2ml and then calibrated using three standards of 100, 300 and 900 mOsm/l. The accuracy of the osmometer was stated to be $\pm$ 1 mOsm/l.

The haematocrit values were obtained at the same time as the plasma samples by drawing a small volume of blood into a heparinised haematocrit tube. The tubes were then sealed with Cristaseal, spun for 5 min on the microcentrifuge using the haematocrit head which develops 12,000g. The packed cell volume was then determined using the Hawksley micro-haematocrit reader. Occasionally clotting occurred before separation and these samples were discarded.

5. Determinations of water content

The measurements of water content of whole fish were obtained using a method similar to Black (1951). The fish were first killed and weighed

and then placed in pre-weighed dry open-necked flasks and dried to constant weight in an oven at 105°C.

6. Fish transportation

The methods used for transportation varied on the number of fish to be moved as well as the distance they had to be transported. Large numbers of fish were always transported in a commercial 2000 l fish transporter tank on the back of a lorry. Bottled oxygen was passed into the water through a diffuser on the bottom of the tank, this ensured they could survive many hours in the tank.

Smaller numbers of fish were transported over long distances in polythene bags, holding about 25 l of water. In summer blocks of ice were added to keep the temperature as low as possible. The bags were then inflated with oxygen and tied. In very hot weather the bags were then placed in 100 l tanks filled with crushed ice.

Small numbers of fish were transported over short distances, e.g., from Kilmore to the Laboratory, in plastic dustbins. The dustbins were half filled with water and the number of fish added to each bin was dependent upon their weight. Each bin contained a diffuser stone connected to an oxygen cylinder.

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SECTION 1. ACCLIMATISATION - LABORATORY EXPERIMENTS

Introduction

Previous trials of transferring rainbow trout from fresh water to sea water have always used percentage mortality as a measure of the successfulness of the transfer (Landless,1976; Landrein,1973). This method has disadvantages in that a large number of fish are required if a reliable result is to be obtained. Another disadvantage of using mortality as a criterion of successful transfer from fresh water to sea water is that one is simply measuring the number of fish which have failed to transfer and not how rapidly the rest of the population adjusted. For these reasons it was felt that another parameter was required which would give an indication as to how a population was adjusting to the rapid change in the environment.

This parameter had to be easily measured since large numbers of samples would have to be taken both in the laboratory and in the field. The most obvious physiological changes that occur in the blood after transfer from fresh water to sea water are a rise in plasma levels of CL^-, Na^+ and K^+, (Houston, 1959; Gordon, 1959; Parry, 1960) and a reduction in water content (Black, 1951). Conte and Wagner (1965) found that measuring the overall osmotic concentration of the plasma during direct transfer of steelhead trout was very successful in monitoring their ability to adapt. They also found that the rise of the individual ions during transfer was closely matched by an increase in the overall osmotic concentration of the plasma. Both Gordon (1959) and Parry (1960) found that freezing point depression measurements of the blood plasma were a very useful way of assessing the ability of salmonids to regulate after transferring to varying salinities.

Black (1951) showed that the water content of small Coho salmon decreased from 82% to about 76% 20h after transfer to 30°/oo; death occurred shortly afterwards. While in small Chum salmon, which seemed to have no trouble in adapting, the water content remained at 82% after transfer. The method used by Black was easily applicable both in the field and in the Laboratory, so it was decided to evaluate this technique also.

Most reported trials involving transfer of salmonids from fresh to sea water have been concerned with smolting species. Parry (1960 & 1961), Houston (1960), Knutsson & Grav (1976) and Farmer et al (1978), all used Atlantic salmon; while Houston (1959) and Conte & Wagner (1965) used Steelhead trout. Conte et al (1966) and Clarke & Blackburn (1977) worked with Coho salmon and Black (1951) and Houston (1961) with Chum salmon. Gordon (1959) carried out some transfer trials with non-migratory Salmo trutta and used plasma freezing point depression determinations to assess their regulatory ability. It was therefore felt that the effects of size and salinity should be examined in relation to the physiology of rainbow trout during adjustment to a rapid change in salinity similar to the abrupt change experienced by trout at seawater transfer on a trout farm.

Experiment 1. Equilibrium levels in fresh and sea water.

Fish which had been held in fresh water and sea water for at least 1 month were sacrificed to obtain a measure of the equilibrium points of the different parameters in the two different media. Fish of varying sizes were used and from different holding conditions: freshwater-adapted fish from tanks at the Laboratory and tanks at Kames; saltwater-adapted fish from tanks in the Laboratory and sea cages at Kames. Samples were

also taken from groups of fish which were showing signs that they had not fully adjusted to the marine environment.

The mean osmotic concentration of the plasma of fish in fresh water was 315 mOsm/l (S.D. = 6, n = 25). No correlation between size and osmotic concentration was observed although different populations of fish in different holding conditions seemed to have different equilibrium points ranging from 305 to 320 mOsm/l. Haematocrit values in fresh water had a mean P.C.V. (Packed Cell Volume) of 41% (S.D. = 4, n = 13).

Trout sampled from the sea had an equilibrium point of 341 mOsm/l (S.D. = 12, n = 40), which is significantly different from the level in fresh water ($t = 9.6$, $p = < 0.001$). Samples taken from different populations again varied. Some fish which were not feeding well and were dark in colour with several recently recorded deaths were sampled and shown to have a mean of 351 mOsm/l (S.D. = 13). A few fish which were showing very obvious signs of stress and were close to death were sampled giving a level of 422 mOsm/l (S.D. = 11). Haematocrits of these fish showed a mean P.C.V. of 23% (S.D. = 7), as opposed to 43% (S.D. = 4) for healthy fish in sea water. Another group of unhealthy fish had an osmotic concentration of 381 mOsm/l (S.D. = 8) and a P.C.V. of 33% (S.D. = 3).

The water contents of the fish appeared to be size-dependent, the larger the fish the lower the water content. Fig. 2 shows this as a regression of percentage water content against weight in grams. There was no significant difference between the level in fresh water and the level in sea water. Fish showing signs of osmotic stress and with plasma concentrations of 380 mOsm/l had water contents 3-4% below those of healthy fish with plasma concentrations of 340 mOsm/l.

Figure 2. A regression line of % water content of the fish against weight in grams.

Equation

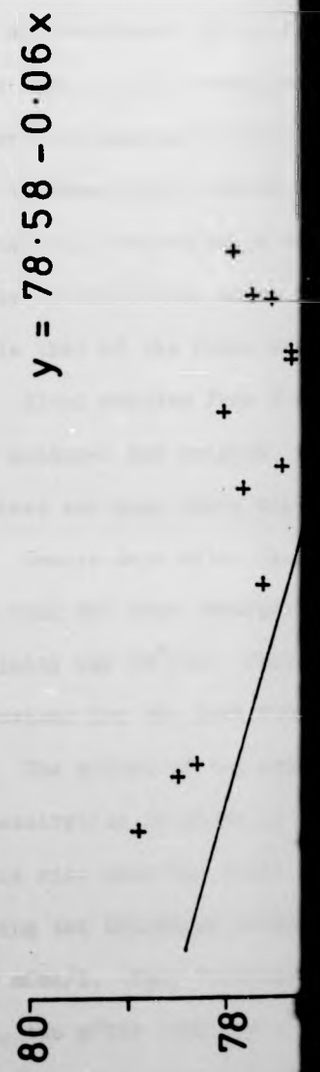
$$y = 78.58 - 0.06\,x.$$

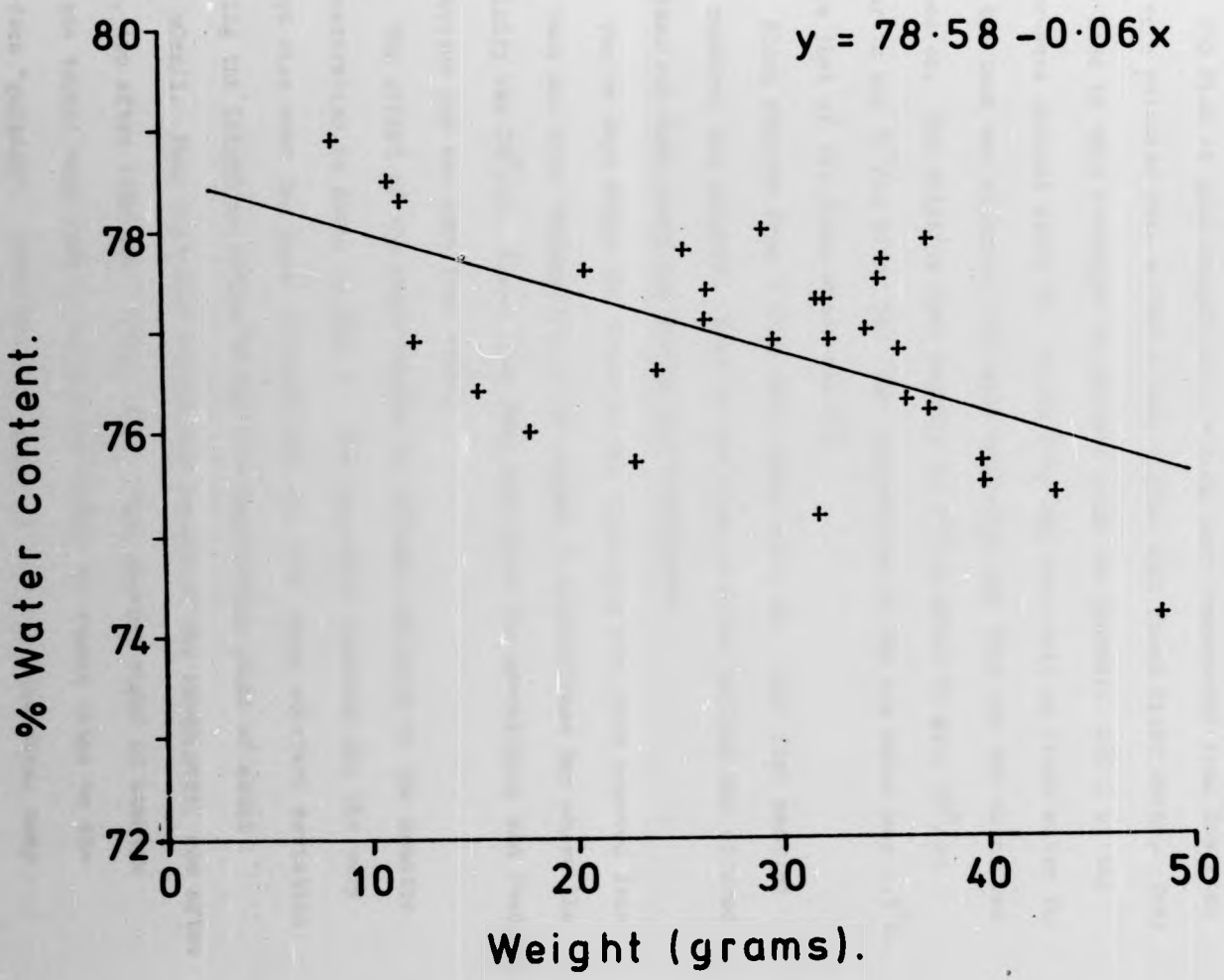
Test of significance for regression.

Source of variation	df.	SS	MS	Fs.
Explained - dueto linear regression	1	10.7	10.7	13.03
Unexplained - error around regression line.	27	22.16	0.821	

P = <0.005.

A significant negative regression is present.




y = 78·58 – 0·06x
80
78
76
74
72
% Water content.
0
10
20
30
40
50
Weight (grams).

Experiment 2. Effect of direct transfer on the osmotic concentration of the plasma.

250 Fish of mean weight 32.9g ± 5.2g were transported from Howieton and were unloaded into a 1100 l tank filled with fresh river water. They were held in this overnight to recover from the journey. 250 l of the water were changed every 3h. After they had been held in fresh water for 20h the tank was siphoned till only half-full and then the sea water was turned on. The salinity rose rapidly to 27°/oo after 30 min, 28°/oo after 1h and 30°/oo after 3h. The temperature of the sea water was 9.5°C, while that of the fresh water was 6°C.

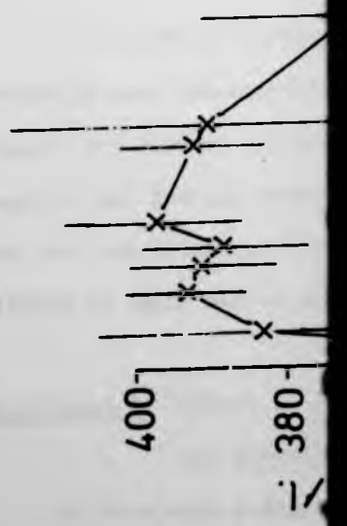
Blood samples from 3 fish were taken every 4h. Each fish sampled was measured and weighed. Food in the form of dried pellets was offered at dawn and dusk every day during the experiment.

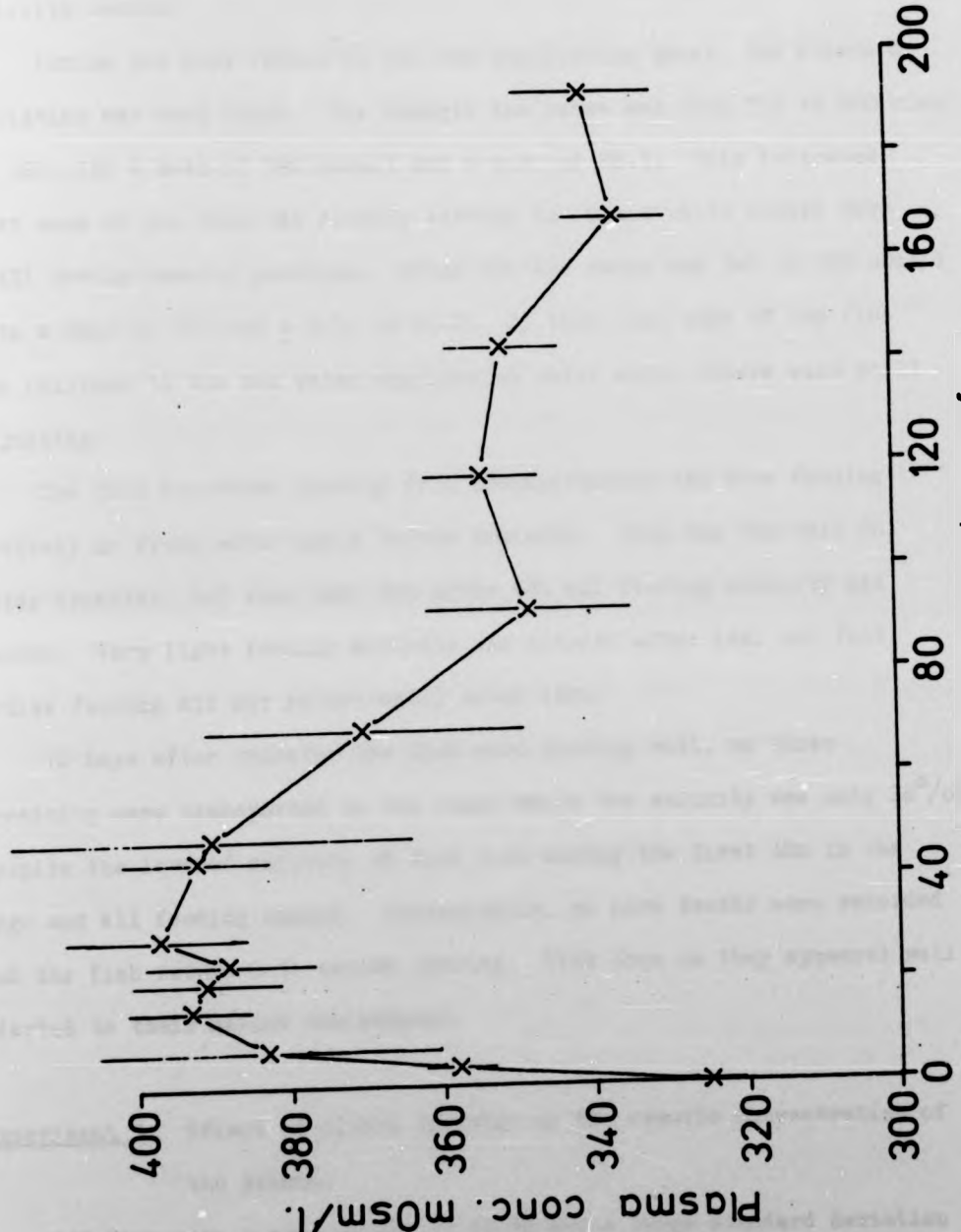
Twelve days after the transfer the remaining fish were removed from the tank and were transported to the cages in Dunstaffnage Bay where the salinity was 28°/oo. There they were monitored for mortalities and feeding behaviour for the next four weeks.

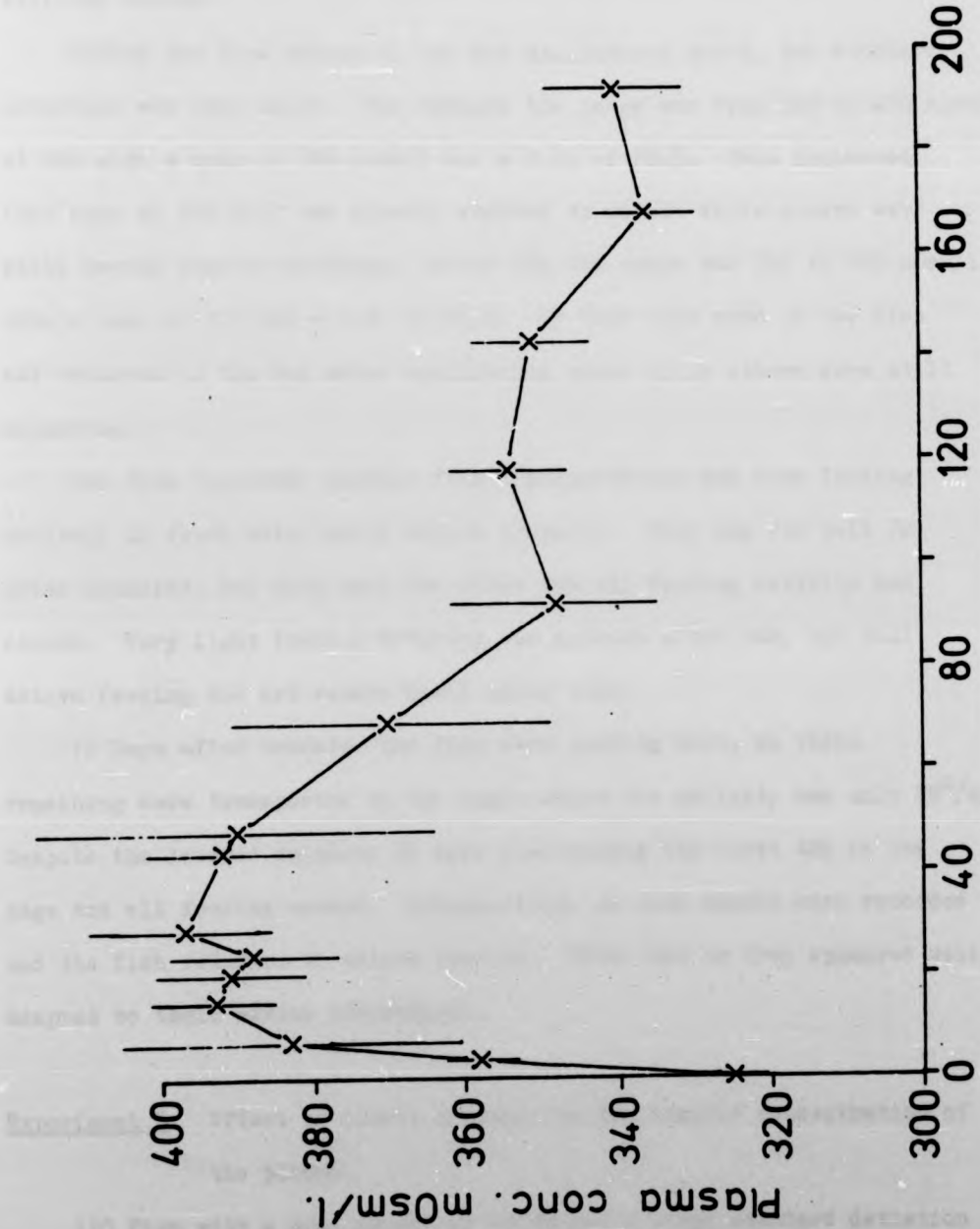
The effect of the rapid change in external salinity on the osmotic concentration is shown in Fig. 3. The important features are the very rapid rise over the first 20 hours and the very large standard deviation during the lengthier return to the new equilibrium point of about 340 mOsm/l. Four fish died during the course of the experiment, one after 92h, two after 118h and 1 after 143h. Fish showing signs of osmotic stress turned very dark in colour and tended to remain close to the surface "gulping". Later as they began to lose co-ordination, they started "tailing", that is lying in the water almost vertically, with the tail down. Occasionally fish under stress propelled themselves across the surface of the water in a rapid motion. Eventually the fish

Figure 3. Change in the osmotic concentration of the plasma following seawater transfer (30°/oo) of fish mean weight 32.9 g.

Three fish were sampled for each point, the bars represent the standard deviation within each sample. Note the very large standard deviations during the return to an equilibrium level of 340 mOsm/l 40 to 100 h after transfer.




Plasma conc. mOsm/l.
400
380
360
340
320
300
0
40
80
120
160
200
Hours after transfer.


Plasma conc. mOsm/l.
400
380
360
340
320
300
0
40
80
120
160
200
Hours after transfer.

turned over and lay upside down; shortly after this stage breathing activity ceased.

During the slow return to the new equilibrium point, the standard deviation was very large. For example the range was from 358 to 408 mOsm/l at 48h with a mean of 390 mOsm/l and a S.D. of 28.3. This indicated that some of the fish had already started to adjust while others were still having osmotic problems. After 69h the range was 345 to 385 mOsm/l with a mean of 370 and a S.D. of 22.2. By this time some of the fish had returned to the sea water equilibrium point while others were still adjusting.

The fish recovered quickly from transportation and were feeding actively in fresh water again before transfer. They had fed well 2h after transfer, but when next fed after 18h all feeding activity had ceased. Very light feeding activity was noticed after 64h, but full active feeding did not return until after 160h.

12 Days after transfer the fish were feeding well, so those remaining were transported to the cages where the salinity was only $28^{o}/oo$. Despite the lowered salinity 25 fish died during the first 48h in the cage and all feeding ceased. Subsequently, no more deaths were recorded and the fish returned to active feeding. From then on they appeared well adapted to their marine environment.

Experiment 3. Effect of direct transfer on the osmotic concentration of the plasma.

110 Fish with a mean weight of 52.7g and a large standard deviation of 11.2g were transported from Howieton and divided equally between two 225 l black tanks with a strong flow-through of tap water at $5.5^{o}C$. They were held in tap water for three days until their feeding activity

returned after the stress of transport.

When the fish had fully recovered the sea water was turned on and the salinity rose rapidly in the tanks to 30°/oo after 1h and 33°/oo after 3h. Blood samples were taken at intervals and occasionally fish showing obvious signs of osmotic stress were removed for sampling. Three or four fish were sampled on each occasion, except for the final point where six fish were sampled. After five days only 21 fish remained and the mortality was still continuing, so the experiment was terminated and the remaining fish returned to fresh water.

The changes in plasma concentration during this experiment are given in Fig. 4. Compared with the previous experiment the initial rise over the first 20h was considerably less. The gradient for the first 20h in experiment 2 was 3.3 mOsm/l/h, while in this trial it was only 1.75 mOsm/l/h. But the rise, instead of levelling after 20h, continued for about 100h and the mean never returned to an equilibrium point of 340 mOsm/l. The deaths during this experiment were as follows:

Hours after transfer	No. of deaths
24	0
48	3
52	8
72	28
98	16
120	5

In addition to these, 2 fish which were on the point of death were sampled at 48h, another at 52h and another at 72h. The flow of blood was greatly reduced from these fish and it proved difficult to obtain

Figure 4. Change in the osmotic concentration of the plasma following seawater transfer (33°/oo) of fish with a mean weight of 52.7 g.

The number of fish in each sample varied (see text); the bars represent the standard deviation within each sample. The triangles indicate the plasma concentrations of moribund fish selectively sampled to determine the lethal limit. Note the very large standard deviation in the final sample indicating that some fish had obtained the seawater equilibrium level of about 340 mOsm/l while others were close to the lethal limit.

Osmotic concentration mOsm/l.
440
420
400
380
360
340
320
300
0
20
40
60
80
100
120
Hours after transfer.

samples. The values for these four samples were 405, 420, 420 and 432 mOsm/l, respectively. Giving a mean of 419 ± 11 mOsm/L.

The experiment was terminated after 120h, there being only 21 fish remaining of the 110 at the start, these were returned to fresh water where no further deaths were recorded.

After transfer the standard deviations were all large and at the 120h sample the standard deviation was 43.5 mOsm/l with a range of 352 - 434 mOsm/l, indicating that some fish were at the lethal limit while others were approaching a sea water equilibrium of 345 mOsm/l.

The recorded mortalities were almost equal in the two tanks. Very little feeding was noticed in either tank following transfer but the fish seemed to be displaying very aggressive behaviour and there was a great deal of activity with fish regularly breaking the surface.

Experiment 4. Effect of size on plasma concentration and water content during direct transfer.

Three identical trials were run using about 50 fish of three different sizes, 1) 10.8 ± 2.4g, 2) 22.8 ± 4.3g, 3) 39.2 ± 8.7g. The fish were brought to the Laboratory and placed in a 225 l tank with a fresh water input of 5°C. They remained there for at least 10 days until fully recovered and actively feeding. Sea water from the constant-head device was then run into the tanks, raising the salinity to 30 ± 5°/oo (temperature 7.5°C) over 1h, where it remained for the rest of the trial. Fish were sacrificed at intervals during the trials and blood samples taken and water contents determined. There was normally six fish in each sample. In the first trial where the fish weighed only about 10g, the plasma collected from two individuals sometimes had to be pooled to give the minimum volume of 0.2ml required for the osmometer. Each trial was

continued for at least a week.

The full table of results for this experiment is given in Appendix II. Graphs of the changes in plasma concentration are given in Fig. 5. The shapes of the curves are very similar to those in Fig. 2. The rate of rise and the extent of the rise both seem to be size-dependent. The gradients over 12h were as follows:

Fish weight g.	Gradient mOsm/l/h.
11	5.6
23	4.0
39	2.9

The time taken to return to the equilibrium point also appears to be size-dependent and the 11g fish had still not reached it by 168h.

The mortalities during the experiment varied according to size:

Hours after transfer	Recorded mortalities 11g	23g	39g
0	-	-	-
12	2	-	-
24	5	-	-
30	4	-	-
48	1	-	-
52	2	1	-
72	1	-	1
94	-	1	-
Total	15	2	1
Approx. Mort. %	42	7	4
Mean wt. of dead fish g.	9.6	14.6	30.2

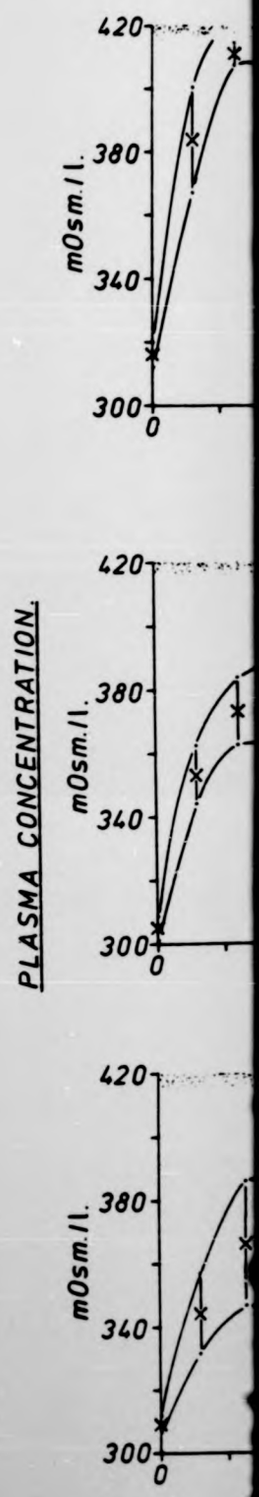


Figure 5. Change in the osmotic concentration of the plasma in fish of three different size ranges following transfer to sea water (30°/oo).

Each sample consisted of six fish; the bars show the standard deviation within each sample. The shaded line around 420 mOsm/l represents the lethal limit.

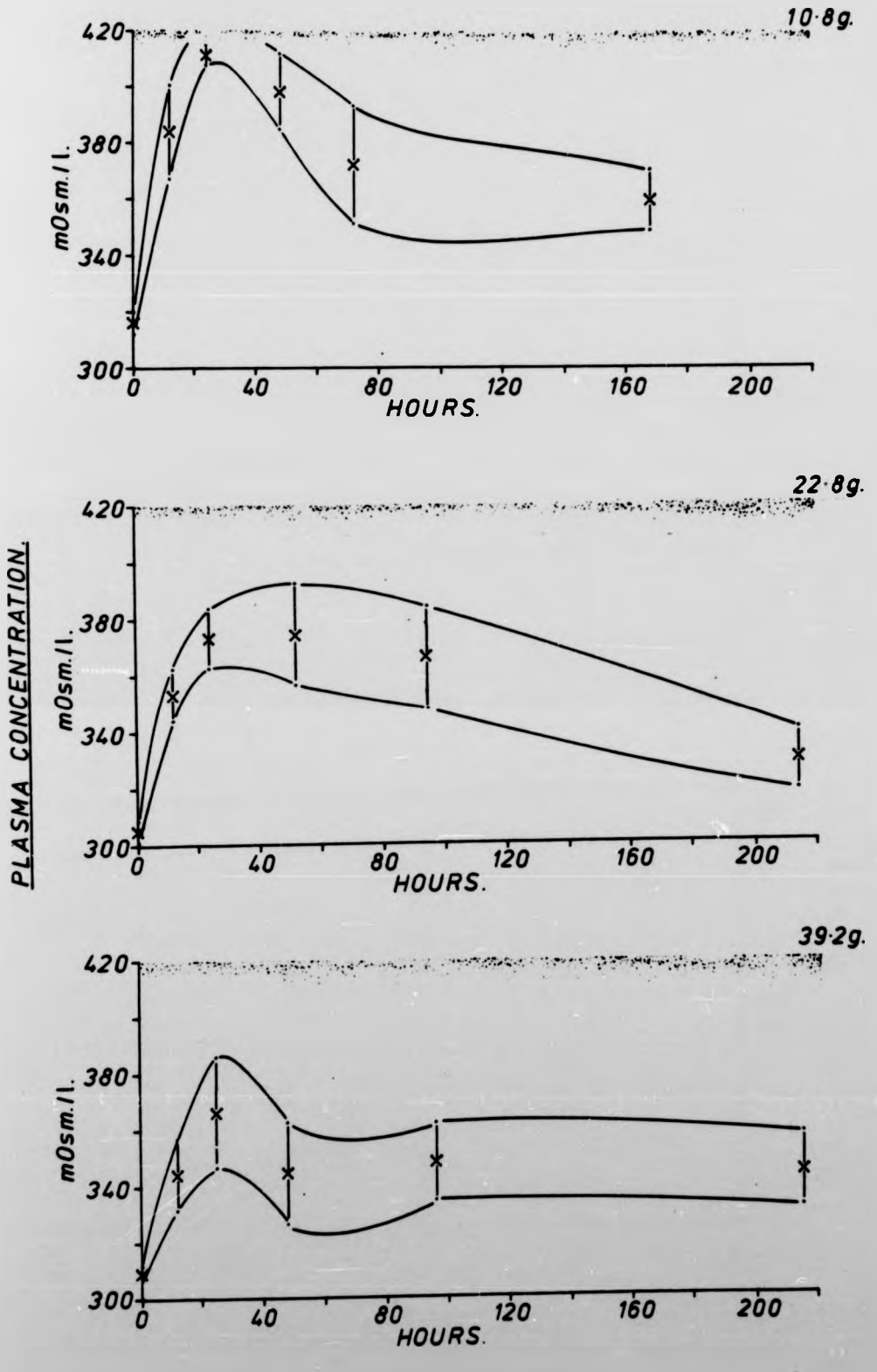

10·8g.
22·8g.
39·2g.
420
380
340
300
mOsm./l.
0
40
80
120
160
200
HOURS.
PLASMA CONCENTRATION.
TIME AFTER TRANSFER

Figure 6. The % water content of fish of three different size ranges following transfer to sea water ($30^{o}/oo$).

Mean weights: □ - 39.2 g

◇ - 22.8 g

△ - 10.8 g

Note the decreasing water content before transfer with increasing size. Standard deviations for each sample are to be found in Appendix II.

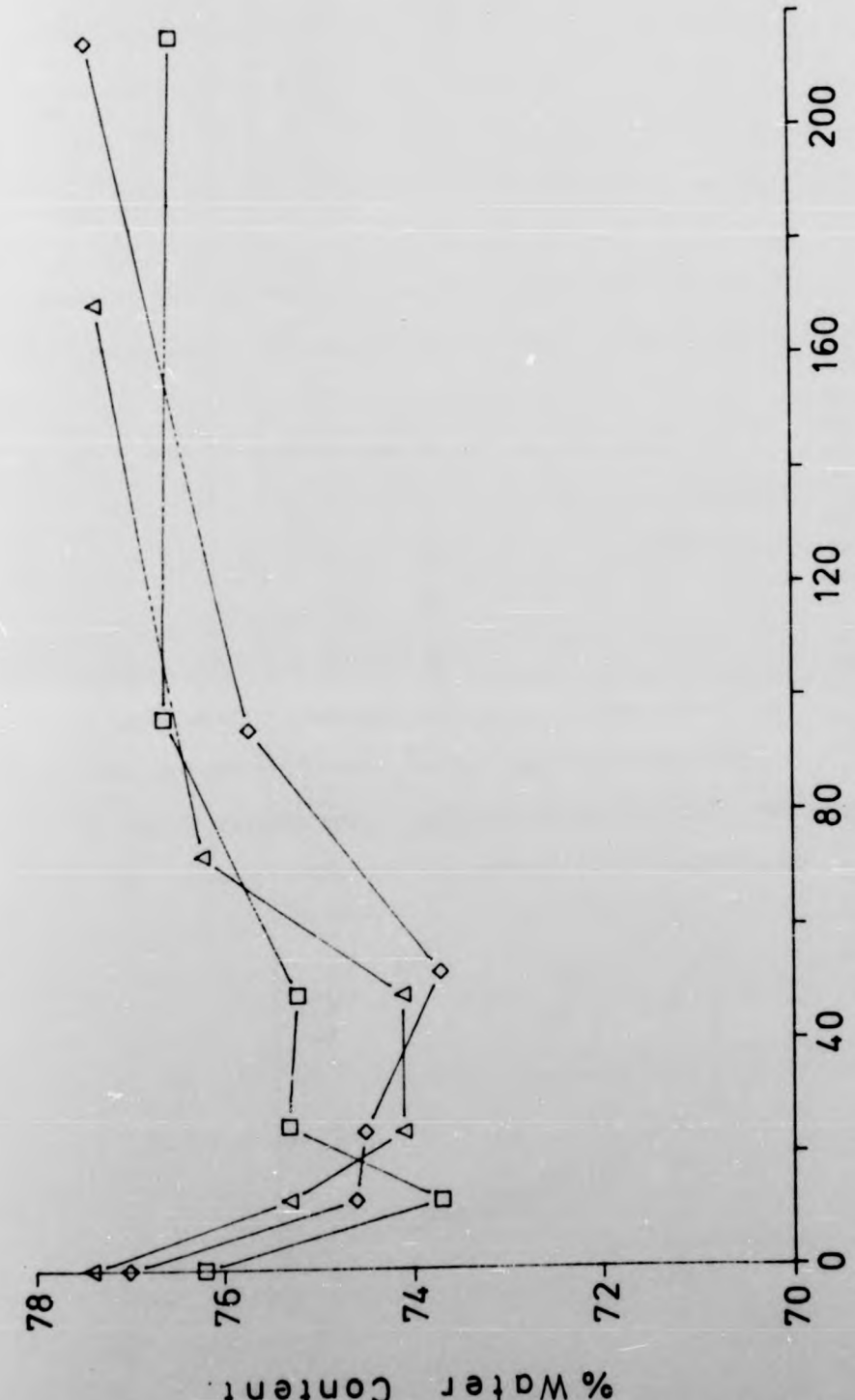

% Water Content.
78
76
74
72
70
0
40
80
120
160
200
Hours after transfer.

Figure 7. The change in % water content of fish of three different size ranges following transfer to sea water (30°/oo).

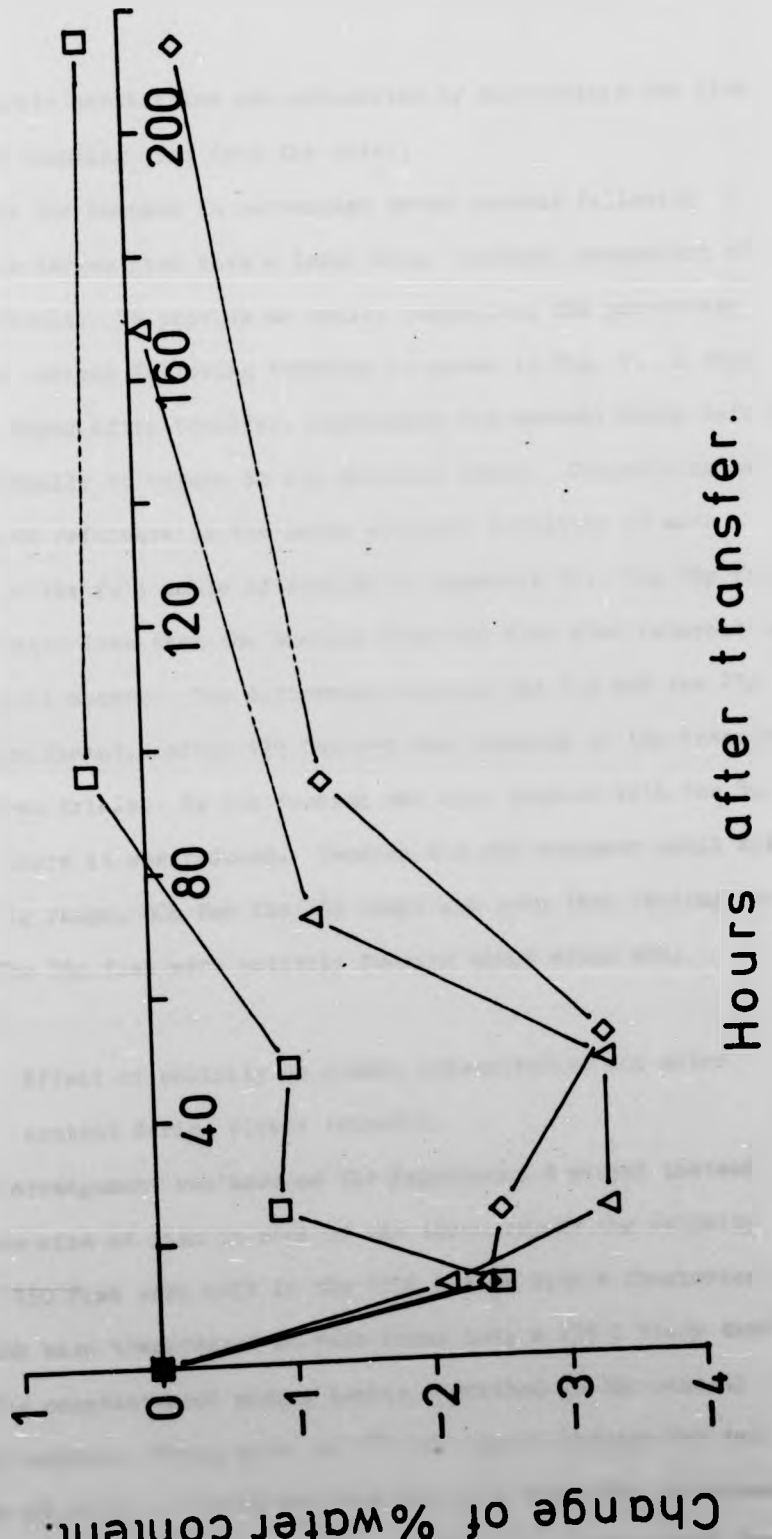
The same data as Fig. 6 but arranged to give a common origin.

Mean weights: □ - 39.2 g

◇ - 22.8 g

△ - 10.8 g

The large standard deviations results in there being no significant difference between the 10.8 g and the 22.8 g fish. The return of the 39.2 g fish to the origin is however, significantly faster than for the other two.


Change of %water content.
1
0
-1
-2
-3
-4
40
80
120
160
200
Hours after transfer.

The approximate mortalities are calculated by subtracting the fish removed at each sampling time from the total.

Fig. 6 shows the changes in percentage water content following transfer. Since larger fish have a lower water content, comparison of changes is difficult. To provide an easier comparison the percentage change in water content following transfer is given in Fig. 7. A rapid water loss was found after transfer, continuing for several hours before it started, gradually to return to its previous level. Comparisons can only be made with reference to the large standard deviation of each point as seen in the full table of results in Appendix II. The 39g fish suffered less water loss than the smaller fish and they also returned to the previous level sooner. The difference between the 11g and the 23g fish is not significant. After 12h feeding was observed in the transferred fish in all three trials. By 24h feeding was only present with the 39g fish and even there it was reduced. Feeding did not reappear until after 120h for the 11g range, 80h for the 23g range and even then feeding was only light. The 39g fish were actively feeding again after 48h.

Experiment 5. Effect of salinity on plasma concentration and water content during direct transfer.

A similar arrangement was used as for Experiment 4 except instead of altering the size of fish in each of the three trials the salinity was altered. 150 Fish were held in the 1100 l tank with a freshwater input. 50 Fish were transferred at each trial into a 225 l black tank supplied by the constant-head mixing device described in the general materials and methods. Fresh water of 7°C was passed through for two days to allow the fish to settle and then the salt water was introduced into the system. The salinity was controlled by the constant-head device,

being checked several times a day and minor adjustments made as necessary.

Trial No.	Size g.	Temp. °C.	Salinity °/oo
1	30.7 ± 5.4	7.5	15 ± 1
2	30.1 ± 5.7	7.8	28 ± 1
3	31.7 ± 5.0	8.0	32 ± 0.5

Fish were sacrificed at intervals during the trials and blood samples taken and water contents determined. Each trial lasted at least a week, by which time the fish had returned to normal feeding activity.

The full table of results is given in Appendix II. Figure 8 shows the change in plasma concentration of fish of similar size being transferred into different salinities. The rate of the rise in concentration and the extent of the rise, both seem to be salinity-dependent. The gradients over the first 12h were calculated and plotted against salinity in Fig. 9. The sharp rise in the gradient between 28 and 32°/oo suggests that osmotic control is much more difficult above 28°/oo.

The rate of return to 345 mOsm/l also appears to be salinity-dependent. At 15°/oo it is 50h, at 28°/oo 100h and at 32°/oo 214h. Feeding activity follows a similar trend. After 12h feeding continued but at a reduced level in the 32°/oo trial. By 24h feeding activity was only present in the 15°/oo trial. Feeding activity returned in the 28°/oo trial after 80h, but at a reduced level until about 120h. In the 32°/oo trial feeding was totally absent from 12h - 96h, after which very light feeding was observed. It was still at a low level after 214h.

The changes in percentage water content are shown in Fig. 10. Large

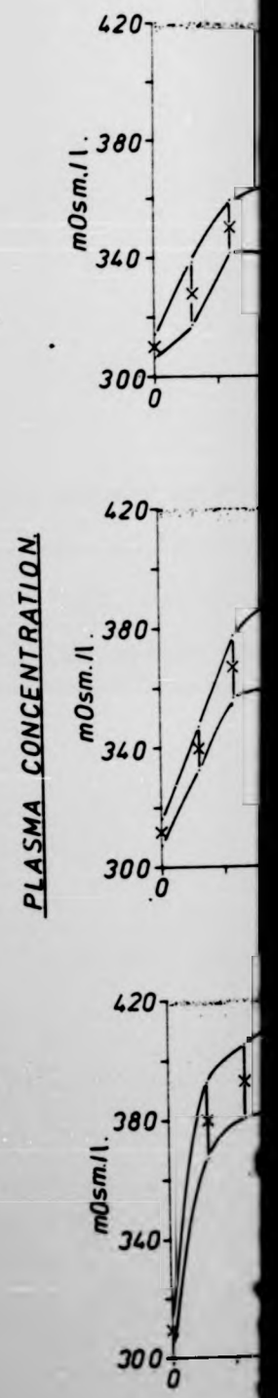


Figure 8. Change in the osmotic concentration of the plasma of fish of mean weight 30.8 g following transfer to water of three different salinities.

Each sample consisted of at least 6 fish except 15°/oo at 190 h and 28°/oo at 214 h which only contained 4 fish (see Appendix II). The standard deviation for each sample is given by the bar. The shaded line at 420 mOsm/l represents the lethal limit.

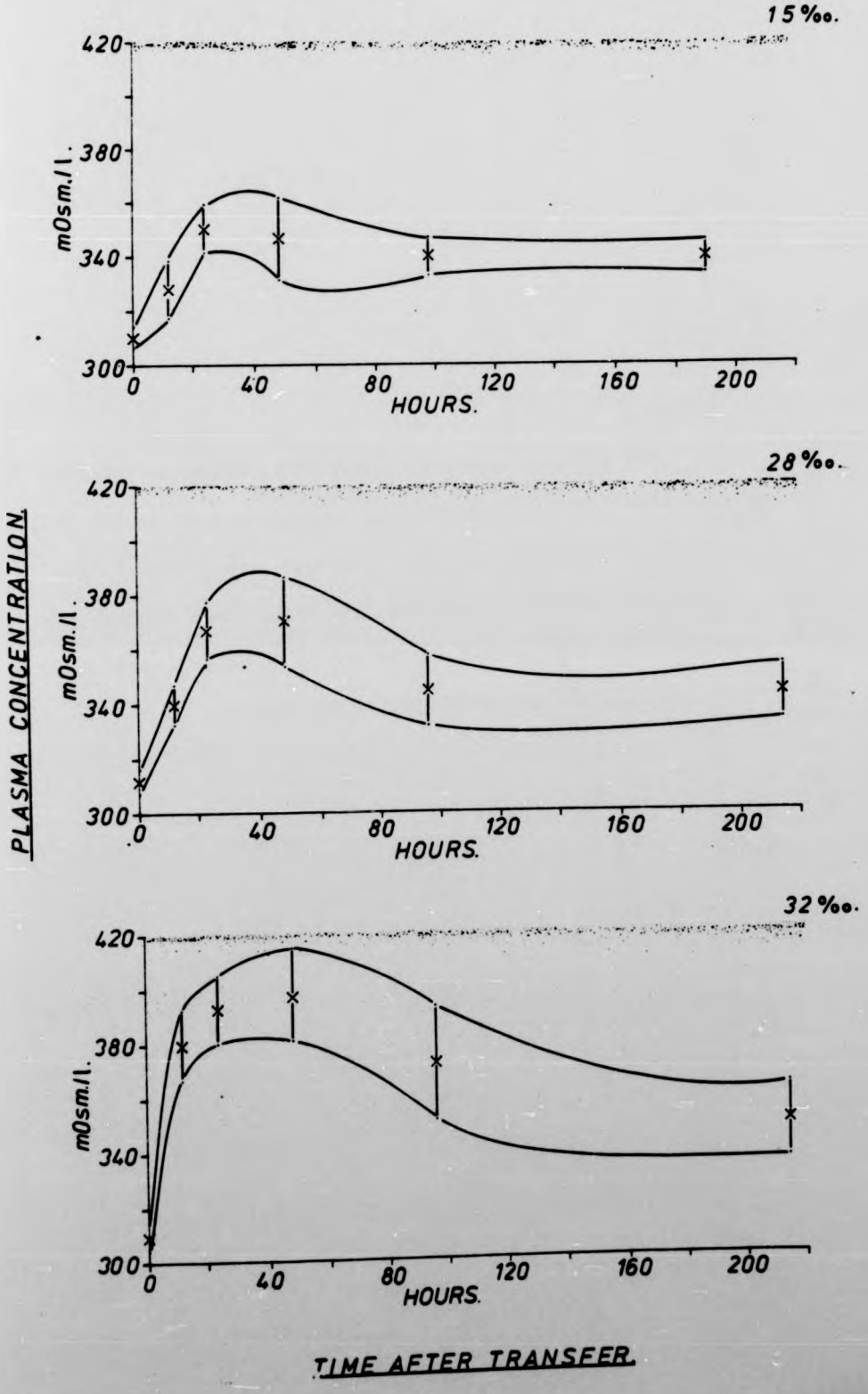

15 ‰.
420
380
340
300
mOsm./l.
0
40
80
120
160
200
HOURS.
28 ‰.
420
380
340
300
mOsm./l.
0
40
80
120
160
200
HOURS.
32 ‰.
420
380
340
300
mOsm./l.
0
40
80
120
160
200
HOURS.
PLASMA CONCENTRATION.
TIME AFTER TRANSFER.

Figure 9

The gradient over the first 12 h following transfer for the three trials shown in Fig. 8 plotted against the salinity of the water into which the fish were transferred. The assumption was made that the line would pass through the origin since no change in the salinity should cause no rise in the osmotic concentration of the plasma.

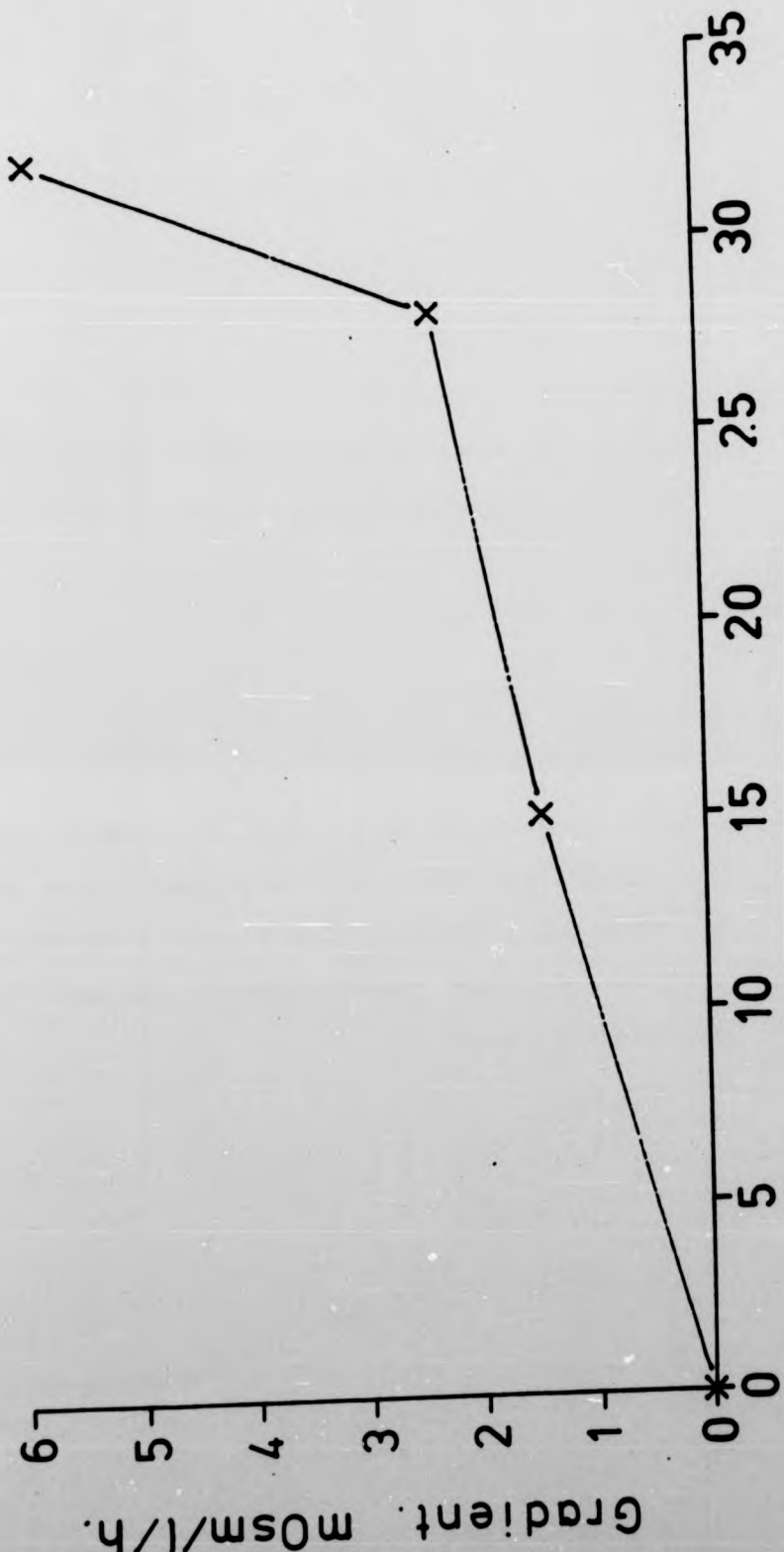

Gradient. mOsm/l/h.
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
SALINITY. ‰.

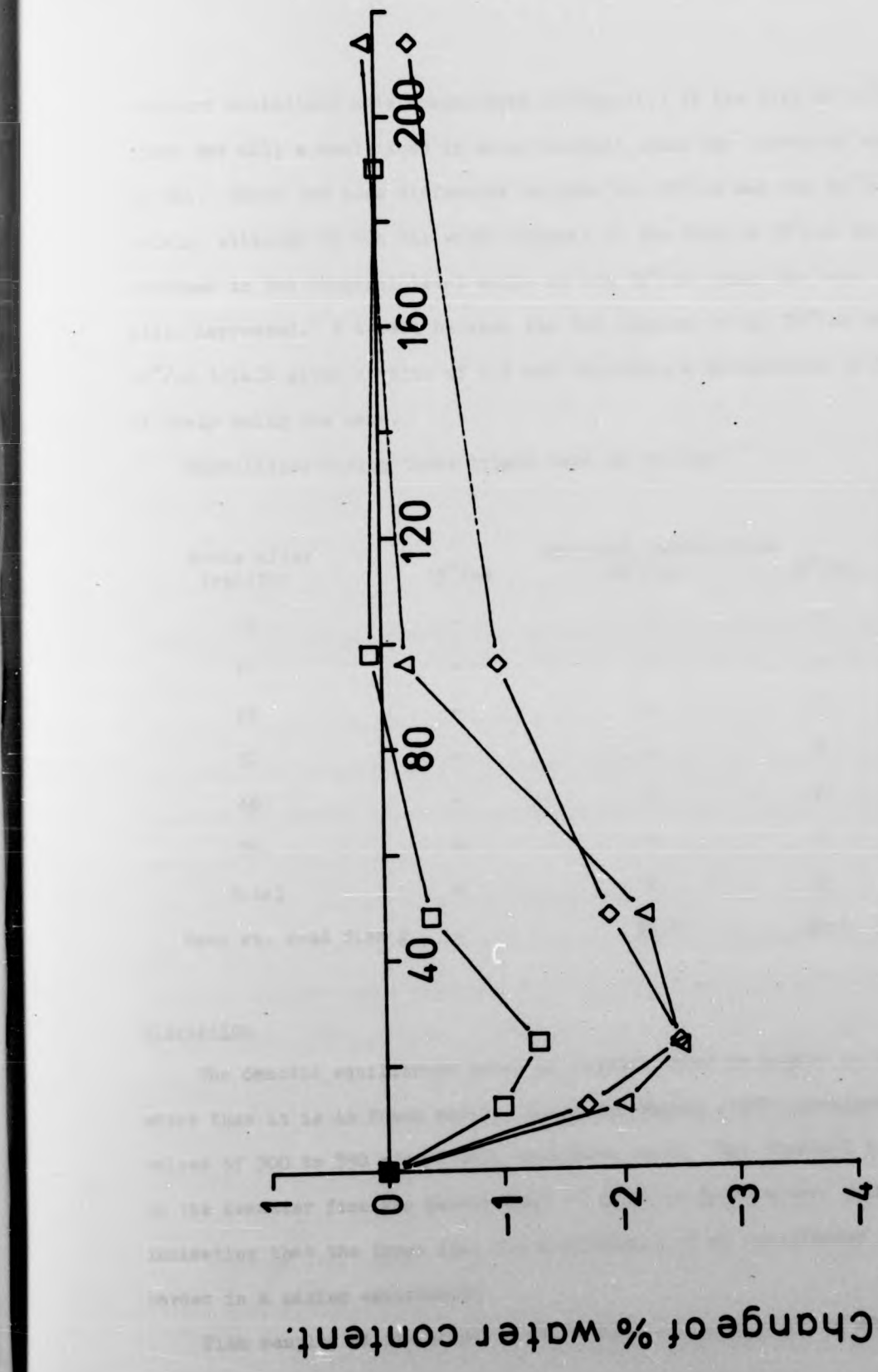
Figure 10. The change in % water content of fish of mean weight 30.8 g following transfer to water of three different salinities.

△ - 32°/oo

◇ - 28°/oo

□ - 15°/oo

Each sample consisted of at least 6 fish except 15°/oo at 190 h and 28°/oo at 214 h which only contained 4 fish (see Appendix II). There is no significant difference between the 32°/oo and the 28°/oo trials but the fish transferred to 15°/oo lost significantly less water than in the other two trials.


Change of % water content.
1
0
-1
-2
-3
-4
40
80
120
160
200
Hours after transfer.

standard deviations made comparisons difficult. In the fish at 15°/oo there was only a small loss in water content which was corrected again by 80h. There was less difference between the 28°/oo and the 32°/oo trials, although by 96h the water content of the fish in 28°/oo had returned to the original level while in the 32°/oo trial the level was still depressed. A t-test between the 96h samples of the 28°/oo and 32°/oo trials gives a value of 2.2 and therefore a probability of 0.05 of their being the same.

Mortalities during these trials were as follows.

Hours after transfer	Recorded Mortalities 15°/oo	28°/oo	32°/oo
0	-	-	-
12	-	-	-
24	-	-	3
30	-	-	3
48	-	1	2
76	-	1	1
Total	-	2	9
Mean wt. dead fish g.	-	21.3	20.5

Discussion

The osmotic equilibrium level of rainbow trout is higher in sea water than it is in fresh water. Conte and Wagner (1965) obtained similar values of 300 to 350 mOsm/l with steelhead trout. The standard deviation in the seawater fish was double that of those in fresh water, perhaps indicating that the trout find the maintenance of an equilibrium level harder in a marine environment.

Fish sampled at the point of death gave a lethal limit of 420 mOsm/l,

which is lower than the figure of 450 mOsm/l given by Conte and Wagner for steelhead. This difference could be due to the fact that the steelhead being a natural migratory fish has a greater tolerance of high osmotic levels than the fresh water rainbow. Weisbart (1968) showed that different Pacific salmon species had different lethal serum chloride concentrations depending on their life cycles. Young chinook salmon which migrate to the sea in their first summer could tolerate serum chloride concentrations as high as 230 meq/l. Young coho salmon, however, which do not migrate to the sea until at least a year old, died when the concentration rose above 170 meq/l.

The haematocrit value of 41% (S.D. = 4) agrees well with Snieszko's (1960) figure of 45.3% (S.D. = 6) for rainbow trout. The low haematocrit values found in fish showing high plasma concentration could well be the result of water movements. If the number of blood cells remains the same but the amount of blood plasma increases, the haematocrit value will decrease. A reduction of P.C.V. from 41% to 30% would require an increase in plasma volume of approximately 30%. How this increase might occur is discussed later.

The water content of about 75% in rainbow trout is a typical value for a teleost fish (Holmes and Donaldson, 1969) and compares well with values given for other salmonids; 70-76% for Atlantic salmon (Farmer et al, 1978), 77% for Brown trout (Phillips et al, 1957) and 78% for Chum salmon (Black, 1951). The decrease in water content with an increase in size was also found by Farmer et al and Black. The finding that an increase in plasma concentration is accompanied by a decrease in water content was also reported by Houston (1959) for steelhead trout.

Houston also described the ion changes that occurred in steelhead trout during acclimatisation to sea water. Two distinct phases could be

observed, the adjustive phase which was characterised by increased plasma ion concentrations and the regulative phase during which control was established over the concentration of electrolytes.

Both these phases are clearly seen in Fig. 3. The adjustive phase lasts 100 - 160h which is comparable to the 80 - 170h given by Houston (1959), and the 60 to 100h given by Conte and Wagner (1965), both working with steelhead trout. The extent of the rise is also similar although Conte and Wagner found that smolts in mid-April had a much smaller rise reaching about 360 mOsm/l.

Following transfer into high salinity water there is obviously a concentration gradient formed between the inside of the fish at 320mOsm/l and the external environment at 1000 mOsm/l. This concentration gradient will tend to cause a dehydration of the fish mostly across the gills. There could also be a net influx of ions across the gills tending to concentrate the plasma. Fish in the sea tend to drink large volumes of water to make-up this loss (Smith, 1930). Selective uptake of monovalent ions produces an osmotic gradient across the intestinal membrane resulting in passive water movement into the blood through the gut wall (Shehadeh and Gordon, 1969). The monovalent ions which are taken up remain in the blood until they are actively secreted.

The three factors, dehydration, branchial ion diffusion and intestinal ion absorption, could all influence the rapid rise in osmotic concentration seen in Fig. 3. The contribution of each factor is not at present known for Salmo gairdneri, but Kirsch and Mayer-Gostan (1972) working with the European eel and Potts et al (1970) working with the Atlantic salmon, both estimate that in sea-water-adapted fish, about one third of the ion influx is through the gut and two thirds through the body wall.

The problem of excess monovalent ions, mostly Na^+ and CL^- is overcome by active secretion through the gills (see review Maetz, 1971). This active secretory mechanism in rainbow trout does not appear to become functional immediately at transfer. During the lag between transfer and the time when ion efflux balances ion influx there is an increase in the osmotic concentration. By about 20h the active secretion of ions starts and by 40h there is a balance between influx and efflux. Between 40 and 100h the active secretion via the gills returns the plasma concentration to the sea water equilibrium level.

Fig. 4 shows a rather different picture. An adjustive phase is just visible but no regulative phase. The slower initial rise might be a result of the fish being a larger size (52g as opposed to 32g). The continued rise instead of levelling-off may be due to the conditions under which the fish were held. Rainbow trout are naturally territorial fish and when held in small tanks they set up hierarchies with often the larger fish being dominant, (Landless, Ph.D. thesis). This tendency is not so marked with fry, but larger fish can expend considerable amounts of energy in this aggressive behaviour (Li and Brocksen, 1977), which may cause the death of the smaller fish. If all the fish are of a similar size the stress can be kept to a minimum, but in this trial the weight ranged from 34.5g to 77g. Aggression may then explain the poor ability of these fish to acclimatise. Many of the fish showed loss of scales, particularly in the caudal region. This would lead to an increased area through which osmotic loss could occur.

Farmer et al (1978) demonstrated the relationship between the size of Atlantic salmon parr and their ability to osmoregulate following sea water transfer. A similar relationship for rainbow trout is shown in Fig. 5. The initial rise of the plasma concentration in the three size

groups continues in an almost linear fashion for the first 20h, after which a reduction in plasma concentration is most likely caused by the commencement of active ion secretion. As one would expect, if there is a fixed maximum rate of secretion, the higher the rise the longer the adjustive phase, until the plasma concentration returns to an equilibrium level of about 345 mOsm/l. The time taken to reach this level was >160h, 150h and 50h for the three trials in increasing size.

The lag of 20h before active secretion starts to balance the influx, agrees with measurements made by several workers on ion effluxes following transfer to sea water, (Potts et al 1970; Conte and Lin, 1967). Kirsch and Mayer-Gostan (1973), measured chloride effluxes following sea water transfer of freshwater-adapted eels Anguilla anguilla. They showed that the level was only 8.2μ equiv/h/100g during the first 6h, was still only 30μ equiv/h/100g by 15h, after which it rose rapidly to 120μ equiv/h/100g by 25h and reached a peak on the fourth day. By the 14th day a normal seawater-adapted level of 230μ equiv/h/100g was reached. Measurements of the plasma chloride concentration during this time were very similar to the osmotic concentrations shown for rainbow trout during this study. There was a rapid rise over the first 24h reaching a peak about 48h and returning slowly to equilibrium by 160h.

Potts et al (1970) showed that much of the salt entering at this time comes through the body wall, almost certainly the gills. The area of gills with respect to the body volume is therefore very important in determining the rate of rise in the osmotic concentration. Muir (1969) showed that the ratio of gill area to weight in grams decreases with growth in most species of fish. This means that larger fish will have a smaller surface area/volume ratio and therefore the rate of rise of the internal concentration will be slower. Since the rise between the fresh

water level of about 320 mOsm/l and the upper lethal limit of 420 mOsm/l is 100 mOsm/l and obvious salt reduction does not occur until 20h after transfer, a gradient of greater than 5 mOsm/kg/h will usually result in death.

From Fig. 5, 11g fish in 30°/oo gave a gradient of 5.6 mOsm/l/h and mortalities were very high. Whereas with the 23g fish the gradient was 4 mOsm/l/h and only two deaths were recorded.

It is an over-simplification to state that the only factor which causes a levelling-off is the commencement of active salt secretion. There are other physiological changes that occur that could assist this process. For example there could be a decrease in the osmotic permeability in the gill as reported in the flounder (Motais et al, 1969), the eel (Motais and Isaia, 1972a) and for the rainbow trout (Gordon, 1963). There could also be a dilution of the incoming extracellular electrolytes by a movement of water from the cellular phase as suggested by Houston (1964). These factors could, however, slow down the rate of rise, but only active ion secretion could result in a return to the regulative phase.

It is possible that older fish are more able to decrease the permeability of their gills particularly as this is thought to be under hormonal control, but Conte and Wagner (1965), showed that size at transfer was far more important than age, thus emphasising the importance of the surface area/volume ratio.

An increased osmotic gradient would obviously lead to a steeper rise in internal concentration and this is clearly seen in Fig. 8. The shapes of the curves for the three different salinities are very similar to those seen for the three different size ranges. Again the rises were almost linear over the first 20h. Fig. 9 shows that there is a very

rapid increase in the gradient between 28°/oo and 32°/oc. This is evidence that the rise is not simply a physical process which would give a linear increase with salinity, but that there are also biological factors such as altered membrane permeability and passive water movements effecting it.

Since a gradient of about 5 mOsm/l/h is likely to lead to high levels of mortality it can also be seen from Fig. 9 that a relatively small increase in salinity from 28 to 32°/oo is likely to produce a large increase in mortalities with fish of 30g. Further evidence that rainbow trout have considerably more difficulty adjusting to high salinities (30°/oo and above), is given by Landless (1976). Using fish of 55g he showed that raising the salinity to 25°/oo had very little effect on the fish, but that the rise from 25 to 33°/oo resulted in osmotic stress and several deaths. MacLeod (1977) concluded that there was little difference in food absorption or conversion efficiency in rainbow trout in salinities between fresh water and 28°/oo, but that there were detrimental effects above that range. MacLeod also reported that food intake was adversely effected by a sudden increase in salinity. This agrees with the finding reported here that when the osmotic concentration rises above about 360 mOsm/l feeding activity ceases and does not return until the plasma concentration falls below this figure again.

Black (1951) found that the percentage water content of coho salmon fry decreased from 81.6% to 76.2% during the first 24h following sea water transfer, by which time most of the fish were moribund. She also found that with chum salmon fry which had little trouble acclimating, the water content remained close to the controls at 82.6%. These results are similar to those shown in Figs 7 and 10 in that those fish which are adapting better become less dehydrated.

The loss of water matches the rise in plasma osmotic concentration with a very rapid loss over the first day, a minimum being reached between 24 and 48h before a slow return to about the previous level after 90 to 250h, depending on the extent of dehydration. Work by Kirsch and Mayer-Gostan (1973), would suggest that drinking occurs immediately after transfer but that the gut requires at least 20h before the active uptake of ions commences allowing the passive movement of water into the blood. The drinking rate then rose rapidly reaching a peak on the fourth day, before reaching an equilibrium level after three weeks.

A water loss from 77.4% to 74.1% over the first 24h - as seen in the 11g fish in Fig. 6, is a reduction of about 0.38g of water. Assuming an extracellular space of 150g/kg (Houston, 1964) this is a loss of about 22% of the extracellular phase. Evidence from the haematocrits, however, suggests that during a rise in plasma concentration the plasma volume increases by about 30%; Houston (1964) also states that the extracellular phase increases by 45%, following transfer. This discrepancy between a loss of 22% and an increase of about 45% in the extracellular phase can be explained by the fact that the observed increase in the plasma osmotic concentration causes the extracellular phase to become hypertonic relative to the cellular phase resulting in the exosmosis of the cellular water. This movement of water results in an increase in the extracellular phase and a dehydration of the cells . It is this tissue dehydration which leads to the observed reduction in percentage water content.

At death, fish had lost about 5% of their weight through dehydration, but it is impossible to say whether death was caused by dehydration or from a very high plasma osmotic concentration. Probably several factors contribute to a general physiological failure. Conte and Wagner (1965), suggested an impairment of the circulatory system leads to other

effects on vital organs such as a failure of the excretory systems with a subsequent effect on the acid-base balance. In this study it was certainly true that extracting blood from the caudal artery of moribund fish proved very difficult due to a decreased flow rate.

In conclusion therefore, the two techniques for measuring the ability of trout to adjust to a rapid change in salinity proved successful. Both techniques gave results which could be explained by the knowledge obtained from other workers and in certain cases similar experiments on other salmonids agreed with the findings presented here. The measurement of the osmotic concentration was thought to be the more reliable and useful measure. Take for example, the transfer of 11g fish into 30°/oo in experiment 4.

<u>% Water content</u>. <u>%</u>

F.W. value = 77.4 ± 1.4

Value after 48h in S.W. = 74.1 ± 0.6

Difference = 3.3

The difference as a % of the F.W. value = 4.3%

The standard deviation of the F.W. value as a % of the mean = 1.8%.

<u>Osmotic concentration</u> <u>mOsm/l</u>

F.W. value = 316 ± 3.9

Value after 48 h in S.W. = 398 ± 13.5

Difference = 82

The difference as a % of the F.W. value = 29.5%

The standard deviation of the F.W. value as a % of the mean = 1.2%.

From this it can be seen that the osmotic concentration shows a

smaller variability within the sample and a greater percentage change during the transfer. This means that the measurements of the osmotic concentration are more likely to give accurate and statistically significant results than would be the case for the percentage water content. For this reason the changes in plasma concentration were used in later experiments as the best method for assessing the ability of stocks of fish to adjust to changes in salinity.

SECTION 2. ACCLIMATISATION - CAGE TRIALS

Introduction

As discussed in the first section, both size of the fish and salinity effect the ability of rainbow trout to adjust following seawater transfer. If a group of trout are to be transferred it is important to know how these two factors will influence the mortality.

On a fish farm it is important to determine the earliest possible transfer time which will give acceptable levels of mortality. Hildingstam (1976) states that rainbow trout of 80 - 100g will acclimatise well following direct transfer to full-strength sea water. This agrees with the value of 100g to 34°/oo given by Sedgwick (1973). Purdom (1977), however, maintains that trout of 30 to 90g can withstand transfer. (Unfortunately, none of the workers reports the methods used for obtaining these figures, for example no mention being made of whether cages or tanks were used or whether there was transportation before transfer).

Landless (1976) presented a table of 11 trials with fish of different sizes transferred to floating cages, where the salinity ranged from 21 to 30°/oo. These trials gave mortalities from 0 to 44% and using this table it is possible to obtain an estimate of expected mortalities in certain cases. It was felt that if further similar trials were completed it might prove possible to construct a graph relating size, salinity and mortality for the use of fish farmers. Since Gordon (1959) has emphasised the importance of the time of year when transferring brown trout to sea water and Conte and Wagner (1965) showed that season had a great effect on the mortality when transferring steelhead trout, experiments were performed through the year.

Materials and Methods

Prior to transfer the fish were held at varying sites. Some were transported direct from commercial hatcheries and placed in the rafts. Eight of the trials involved transport from the tanks at Kilmore, and at the three trials at Kames the fish were held at their own freshwater site. This did result in there being rather more variation between trials than one would desire. But, all the trials did have the following features in common; the fish were all from freshwater-adapted rainbow trout stock and they were transported prior to transfer in crowded conditions with bottled oxygen to keep them alive. On arrival at the sea, they were placed in bins containing fresh water in which they were transported by boat to the rafts where they were tipped straight into the nets, or in the case of Kames, tipped straight from the transporting tank into the cage.

The size of the nets used on the floating cages in Dunstaffnage Bay depended on the number being transferred. For fewer than 1000 fish 2 x 2 x 2m nets were used and for larger number the 4 x 4 x 4m nets were employed. Salinity measurements were taken regularly, at different stages of the tidal cycle. The measurements during each trial were combined to give a mean value.

Once in the sea the fish were carefully checked for mortalities over at least 10 days. Dead fish were removed at regular intervals and counted. Food in the form of dried pellets was offered daily.

Results

The full set of the results for the transfers is given in Table 1 and as expected, the heavier the fish and the lower the salinity, the smaller was the percentage mortality.

TABLE 1.

Transfer No.	Month	No. of fish	Mean wt.of fish g.	Salinity °/oo	Mortality %
1	Mar.	200	44	30	7
2	Mar.	1000	48	27	2
3	Apr.	250	61	25	2
4*	Apr.	10000	59	33	9
5*	May	3350	67	33	7
6*	May	5000	69	33	4
7	June	500	13	30	47
8	June	500	14	30	55
9	June	500	15	30	47
10	June	500	17	30	49
11	July	500	18	30	33
12	Aug.	500	24	30	59
13	Aug.	500	30	30	37
14	Sept.	350	45	30	9
15	Oct.	120	9	32	100
16	Nov.	1500	14	22	6
17	Dec.	2000	11	25	90
18	Dec.	5000	14	26	87

* - Trials at Kames Fish Farm.

The seasonal effect is not as marked as reported for steelhead trout (Conte and Wagner, 1965), but a slight effect can perhaps be seen in trials 7 to 13 during the summer of 1977. These trials can be compared closely as they were all from the same stock of fish which were held in the tanks at Kilmore. 500 Fish were transferred in an identical manner at intervals from June to August, during which time the salinity in the cages remained unusually constant between 29-31°/oo, due to an extended period of low rainfall.

The fish in early June were 13g (trial 7) and when transferred suffered a mortality of 47%; by early August when the fish had nearly doubled their weight to 24g, the mortality was 59% (trial 12). The other trial in August also gave a high mortality, but by September the fish were 45g and the loss was only 9%. This could be the result of seasonal variation or increased thermal shock during August.

Since the table published by Landless (1976), (Table 2), was produced using very similar techniques and identical materials, the two can be combined to produce a more comprehensive range of results. Combining the two tables, it is possible to produce a nomogram as given in Fig. 11. The mortality scale is marked in ranges indicating the large variability for similar trials, e.g., trial 1 table 1 and trial 3 table 2. The nomogram is inaccurate in estimating high mortalities since the scales become very condensed and a small change in either size or salinity produces a very large change on the mortality scale. This is also exactly what the results dictate. Trials 10 and 11 in table 2 illustrate this. 6g in 22°/oo gives 5% mortality, while 5g in 23°/oo gives 44%. The same trials read on the nomogram would give a predicted mortality range of 5-10% in trial 10 and 60-100% in trial 11.

A similar observation can be made for trials 16 and 18 of Table 1.

TABLE 2.

From Landless (1976).

Transfer No.	Month	No. of fish	Mean wt of fish g.	Salinity o/oo	Mortality %
1	/	200	142	26	0
2	May	100	81	32	6
3	Dec.	100	36	30	0
4	Nov.	300	31	25	7
5	Nov.	3000	22	22	1
6	Nov.	2000	18	22	1
7	Nov.	6500	15	23	8
8	Nov.	6000	15	21	6
9	Sept.	100	8	23	9
10	Sept.	800	6	22	5
11	Sept.	100	5	23	44

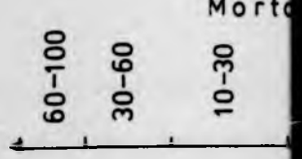
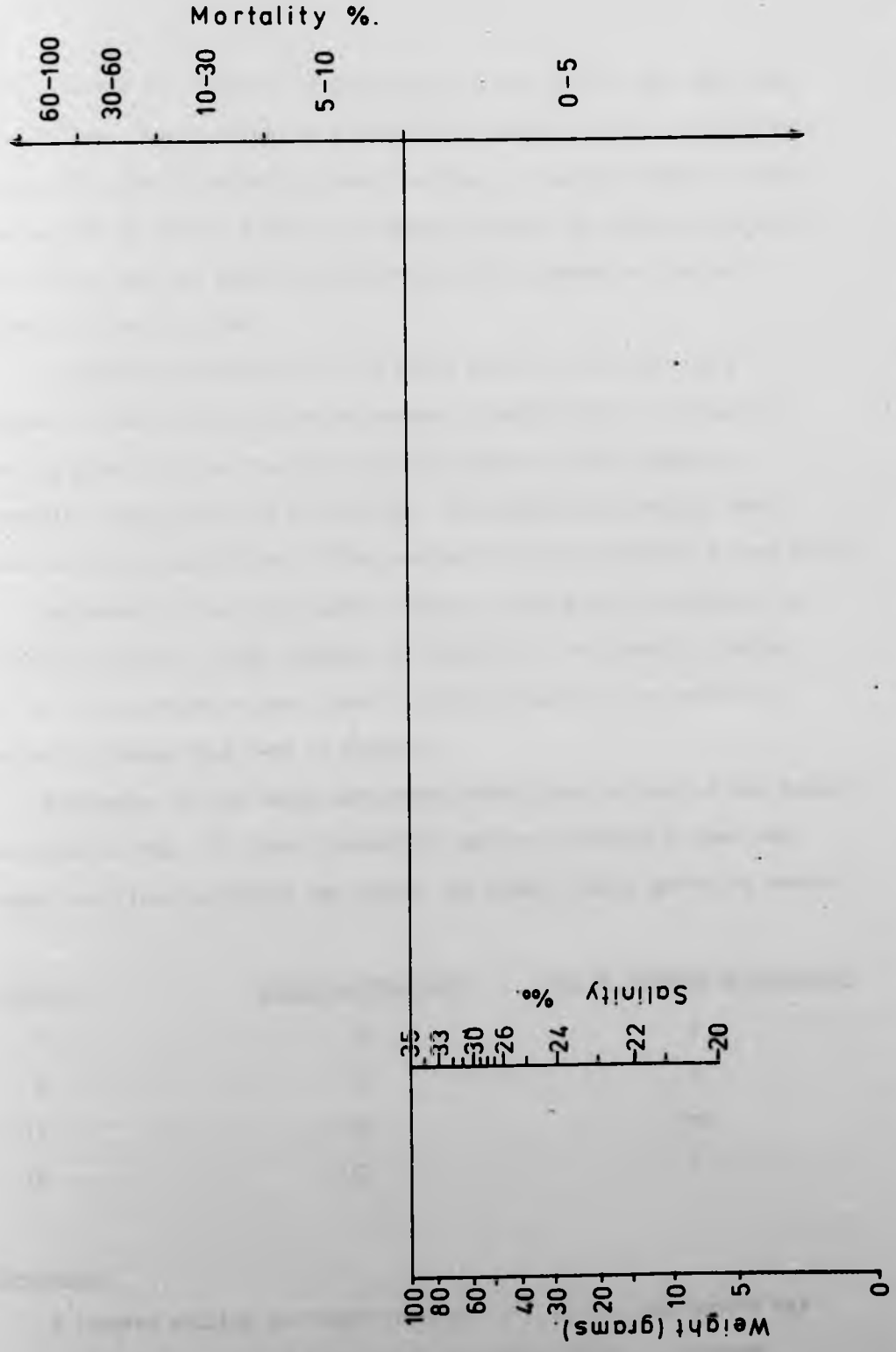


Figure 11.

A nomogram relating the weight of the fish (g) to be transferred and the salinity (°/oo) of the water to the expected percentage mortality. A straight-edge joining the mean weight of the fish with the salinity of the water will cross the mortality scale at the predicted level. The mortality scale is marked in ranges indicating that very accurate predictions cannot be made particularly for high levels of mortality. It should be noted that fish below 5 g were never used and that area should be used with particular caution.


Mortality %.
60-100
30-60
10-30
5-10
0-5
Salinity ‰
35
33
30
26
24
22
20
Weight (grams)
100
80
60
40
30
20
10
5
0

Both involved the transfer of 14g fish, one into 22°/oo and the other into 26°/oo. Despite what is a relatively small increase in salinity there is a rise in mortality from 6 to 87%. A similar effect for size can be seen in trials 9 and 11 of Table 2, where the salinity for both was 23°/oo, and the drop in size from 8g to 5g, caused a rise in mortality from 9 to 44%.

It therefore seems that for a given size of fish there is a discrete rise in salinity, which causes a drastic rise in mortality. For 10g fish the rise from 22 to 25°/oo results in the predicted mortality rising from 0-5 to 60-100%. With 30g fish a similar sharp rise occurs between 26 and 31°/oo, and with 50g fish between 30 and 34°/oo.

The same is true for a given salinity where a small reduction in size can lead to a large increase in mortality. For example, taking 26°/oo a reduction in size from 30 to 20g results in the predicted mortality rising from 0-5% to 60-100%.

Histograms of the daily percentage mortalities in four of the trials are given in Fig. 12. The interesting feature to notice is that the larger the final mortality the sooner the highest daily mortality occurs.

Trial No.	Final mortality %	Day of highest % mortality
2	2	4
5	7	3
11	33	1-2
18	88	1

Discussion

A lowered ability in summer to adapt to a marine environment was reported for non-smolting brown trout by Gordon (1959). Although

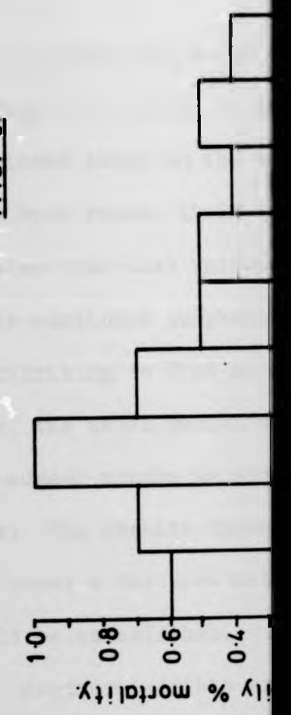
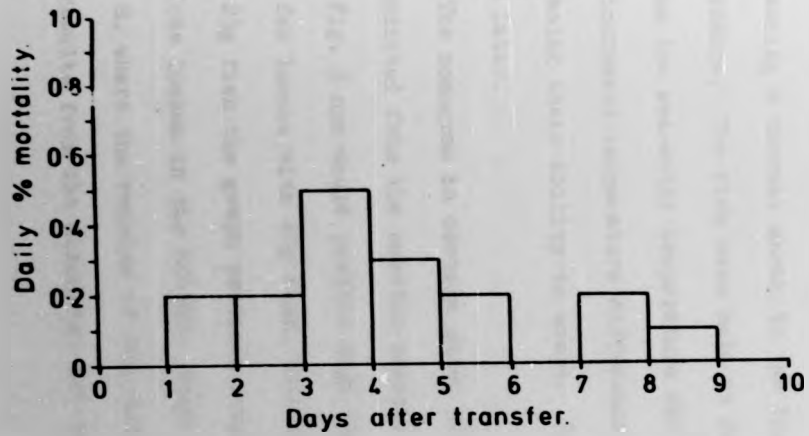
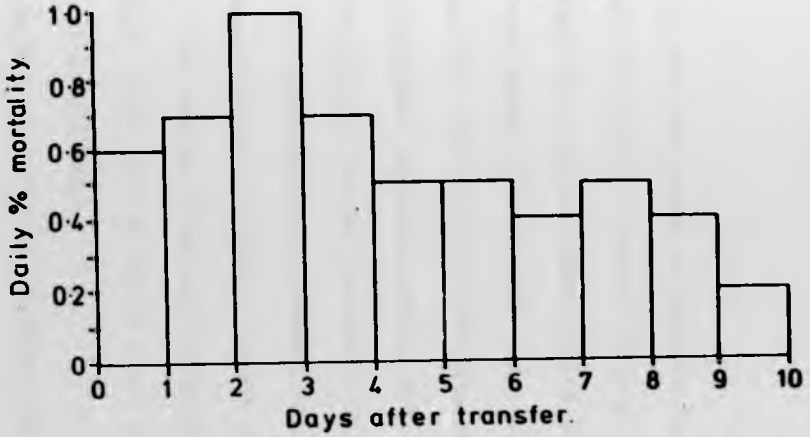
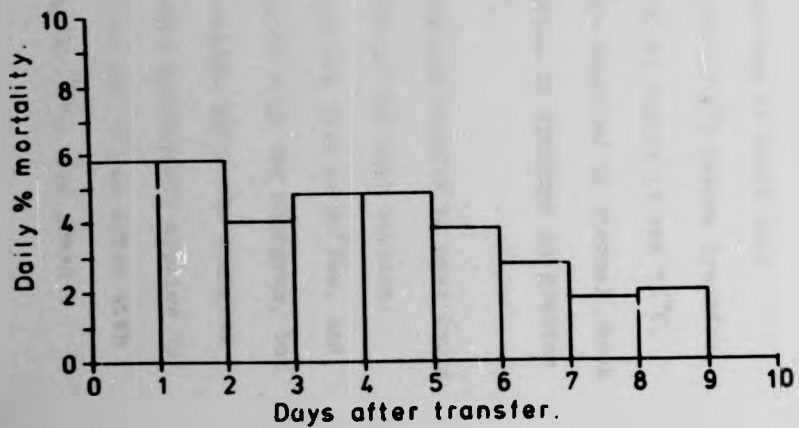
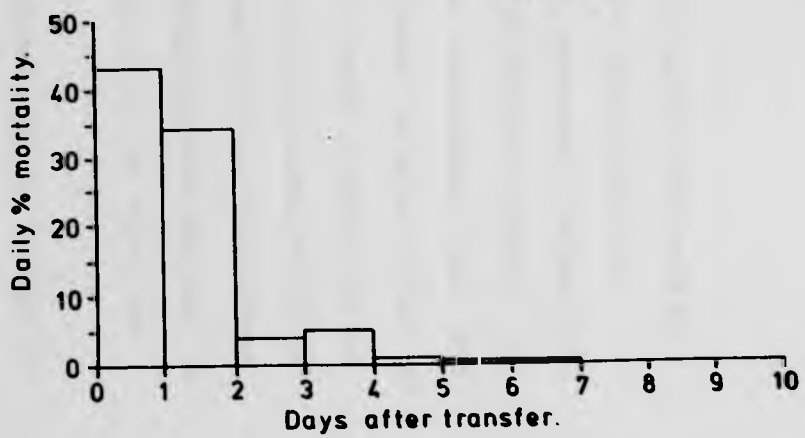


Figure 12. Histograms of the daily percentage mortalities of four trials taken from Table 1. Note that the mortality scale is different for trials 11 and 18.


Trial 2.
Daily % mortality.
1·0
0·8
0·6
0·4
0·2
0
0 1 2 3 4 5 6 7 8 9 10
Days after transfer.


Trial 5.
Daily % mortality
1·0
0·8
0·6
0·4
0·2
0
0 1 2 3 4 5 6 7 8 9 10
Days after transfer.


Trial 11.
Daily % mortality.
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10
Days after transfer.


Trial 18.
Daily % mortality.
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Days after transfer.

smolting does not occur in rainbow trout it is possible that there are seasonal variations in their ability to adapt. Rainbow trout and steelhead trout are the same species and since hatcheries in Britain have held both races, it is likely that some interbreeding has occurred. It is also true that rainbow trout are descended from migratory trout, hence their continued euryhaline abilities. Given these two facts it would not be suprising to find an increased ability to transfer in April, May and June, the usual period of seaward migration, and a decreased ability in the summer months as shown by Conte and Wagner (1965) for the steelhead race. The results shown here only give an indication that this might be the case; a far more detailed study would have to be made before this could be established.

Another possible explanation for the decreased ability to transfer in August is that the temperature difference of the two water bodies was causing a thermal shock to the fish, resulting in their poor performance. The fish were held in fresh water of 9°C before transfer. In June the sea-water temperature was 11°C and by August it was 15°C. This increased temperature difference may have resulted in thermal shock decreasing their ability to adapt. This problem is examined in greater depth later.

The nomogram in certain cases gives different results to what would be predicted from the osmotic changes reported in the last section. From Fig. 5 one would predict high losses with 11g fish in 30°/oo, and very few losses with 40g fish, both as predicted from the nomogram, but with 23g fish the graph predicts only a few losses while the nomogram predicts losses in the 60-100. range. The same discrepancy applies to Fig. 8, where the results of 30g fish in 15°/oo and 32°/oo agree with the results from the nomogram, but that with 28 °/oo the graph

indicates few deaths while the nomogram shows mortalities in the range of 10-30%.

The most obvious reason for the discrepancy is that the osmotic concentration graphs were from tank transfers involving no prior transportation, while the nomogram is based on cage transfers with prior transportation. This transportation often involved harsh conditions including initial netting and holding in very crowded conditions, sometimes for several hours. Wedemeyer (1972) showed that even moving fish 25m from one tank to another could temporarily upset the osmotic control of steelhead trout. It therefore seems likely that with the cage transfers the fish were under some osmotic stress even before entering the sea water. This could explain why fish close to the region of drastic rise in mortality tend to do considerably worse when transported prior to transfer.

Fig. 13 shows two graphs one deduced from the nomogram, the other estimated from the osmotic concentrations. This indicates how much prior transportation might effect percentage mortalities at transfer to 30°/oo. This graph does not show the wide variation between similar trials and should not be used in place of the nomogram, it merely indicates where the two methods predict the drastic rise in mortalities will occur with reducing size. The level of mortality rises above 10% at 20g for the tank trials and at 40g for the cage trials.

The nomogram predicts that a drastic rise in mortalities will occur in restricted salinity ranges for different sizes of fish. For fish of 30g the nomogram predicts that the drastic rise will occur between 26 and 31°/oo. Examination of Fig. 9 shows that the gradient over the first 12h increases rapidly between 28 and 32°/oo. With the knowledge that a gradient of around 5 mOsm/l/h will result in high mortalities it can be

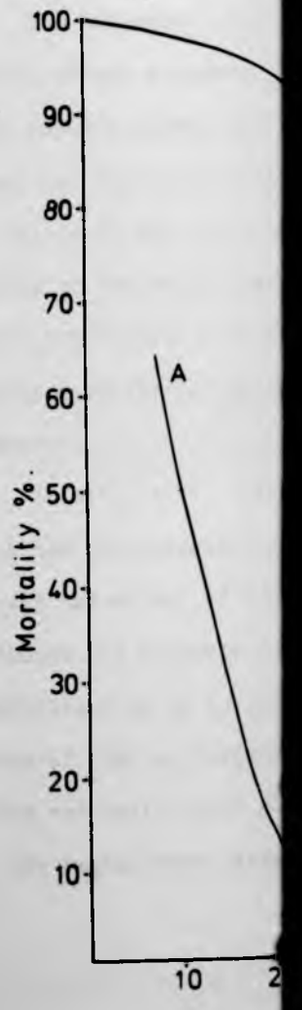
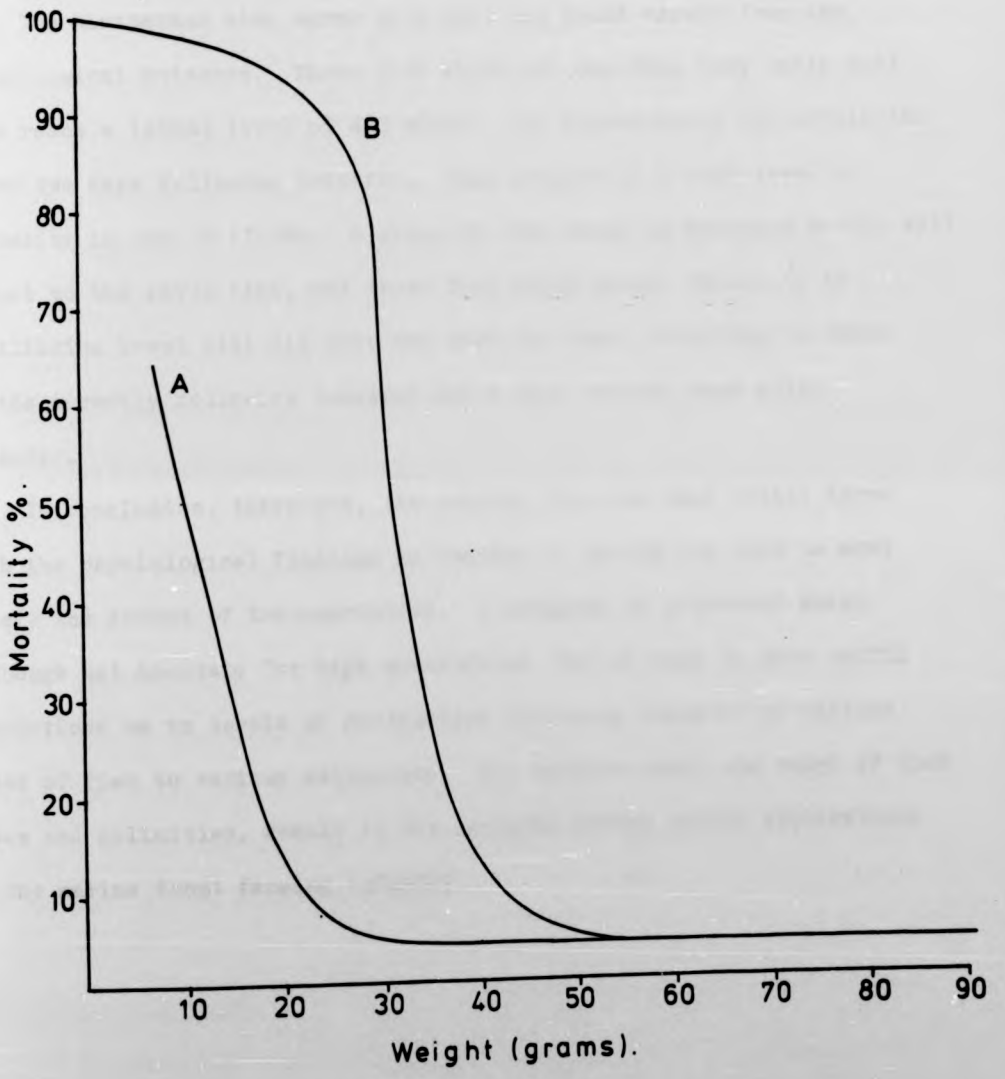


Figure 13. A graph relating predicted % mortalities with weight (g) for fish transferred into sea water of 32°/oo.

A. - Tank transfers involving no prior transportation, results from Section 1.

B. - Cage transfers involving prior transportation taken from the nomogram (Fig. 11).

It is important to note that curve A is based on only three points while curve B does not indicate the wide ranges as shown by the nomogram. This graph should therefore not be used to give accurate predictions of mortality, its purpose is only to indicate the weight at which the rapid rise in mortality appears to occur for the two different techniques.


100
90
80
70
60
50
40
30
20
10
Mortality %.
A
B
10
20
30
40
50
60
70
80
90
Weight (grams).

seen that one would predict a rapid rise in the number of mortalities with an increase from 28 to 32°/oo. These two observations coincide well, except the cage trials suggest a slightly lower salinity range, again perhaps showing the effect of prior transportation.

The histograms also agree with what one would expect from the physiological evidence. Those fish which are adapting very badly will soon reach a lethal level of 420 mOsm/l and consequently die within the first two days following transfer. This results in a high level of mortality in the first 48h. A group of fish which is adapting better will adjust to the early rise, and those fish which cannot return to an equilibrium level will die over the next few days, resulting in fewer deaths directly following transfer and a peak several days after transfer.

In conclusion, therefore, the results from the cage trials agree with the physiological findings in Section 1, except for what is most likely the effect of transportation. A nomogram is presented which although not accurate for high mortalities, can be used to give useful predictions as to levels of mortalities following transfer of various sizes of fish to various salinities. The methods used, the range of fish sizes and salinities, result in the nomogram having useful applications to the marine trout farming industry.

SECTION 3. THE INFLUENCE OF THERMAL SHOCK ON TRANSFER

Introduction

The previous section indicated that August was a poor month for transferring rainbow trout to sea water. This could be the result of a number of factors, for example a seasonal variation in osmoregulatory ability as reported in brown trout by Gordon (1959) or perhaps the effect from the increased thermal shock on the fish. The trout in the transfers under discussion were held in spring water of a constant 9°C. The sea water was 11°C in early June when 13g fish suffered a mortality of 47%; by August the water temperature was 15°C and 24g fish had a 59% mortality. Reaves et al., (1968) showed that a moderate heat shock from 11-16°C in 15 mins caused a temporary reduction in plasma electrolytes of rainbow trout in fresh water, the plasma levels of sodium and chloride decreasing by about 15% following heat stress and remaining low for up to 10 days.

An experiment was, therefore, devised which would measure the osmotic stress experienced by fish following transfer to sea water 2 deg. C and 6 deg. C warmer than the fresh water. The trials were performed at the same time and involved the same salinity so that the only variable was the heat stress caused at transfer.

Materials and Methods

In February 100 trout, weighing 18.6 g (S.D. 3.9) were brought to the laboratory and 50 fish were placed in each of the two 225 l tanks. The tanks were supplied with tap water of 6°C; the fish were left to adjust for three weeks, feeding on a normal pelleted diet. Sea water was then added and the salinity raised to 30 °/oo ± 0.5 over 30 min.

The sea water to one control tank was merely passed through a header-tank to remove any supersaturation and was held at 8°C.

Three 500 W heaters were placed in the other header tank which raised the temperature to 12°C. Thus temperature shocks of 2 deg. C and 6 deg. C were applied. To ensure a stable temperature the water entering the header-tank first passed through a constant level device which delivered a fixed flow rate. Once the system attained equilibrium there was only a small temperature fluctuation ($\pm$ 0.2°C). A plasma sample of 6 fish was taken from each tank before transfer and again 12, 24, 48 and 96 h after transfer.

To test whether a thermal shock alone has any effect on osmoregulation 24 fish of mean weight 32.4 g (S.D. 5.6), which were fully adapted to a marine environment were placed in a 225 l tank with a salinity of 30°/oo and a temperature of 8°C. After 10 days adjustment, heated sea water of 12°C and 30°/oo was run into the tank. Four samples of 6 fish were taken for blood samples, one as a control at 0 h and the others 24, 48 and 72 h after the thermal shock.

Results

The results from the blood samples are shown in Fig. 14. The graphs are very similar to the ones already reported in Section 1, showing a clear adjustive phase. The two curves are very similar, but on three occasions the mean in the 6 deg. C stress was greater than that of the 2 deg. C stress but the differences were very small when compared to the standard deviations and are not significant when using a t-test. 2 Deaths were recorded after 48 h in the 2 deg. C stress tank and 2 deaths in the 6 deg. stress tank one after 48 h and the other after 72 h. Feeding ceased after 12 h and was not re-established in either tank till about 96 h. From the results it can be concluded that no significant difference

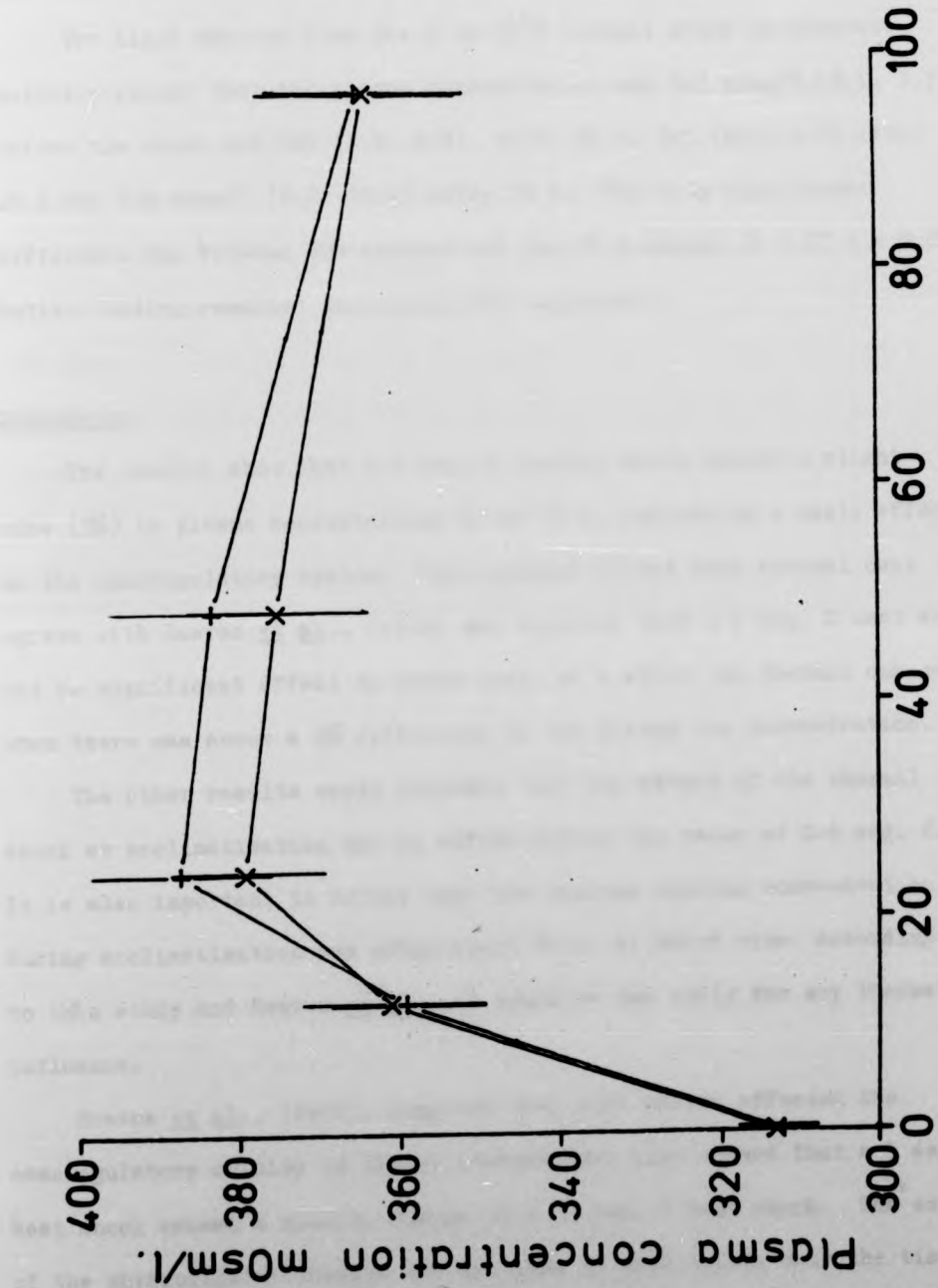
Figure 14. Effect of thermal shock on plasma concentration following transfer to sea water.

The freshwater temperature was 6°C, the salinity of the sea water was 30°/oo and the temperatures were:

X - 8°C

\+ - 12°C

The difference between the two graphs is not significant.


Plasma concentration mOsm/l.
400
380
360
340
320
300
0
20
40
60
80
100
Hours after transfer.

could be observed in the regulatory ability of fish given a 2 deg. C heat shock at transfer and those given a 6 deg. C heat shock.

The blood samples from the 8 to 12°C thermal shock in constant salinity showed that the plasma concentration was 346 mOsm/l (S.D. 7.3), before the shock and 349 (S.D. 8.8), after 24 h, 345 (S.D. 9.2) after 48 h and 358 mOsm/l (S.D. 10.4) after 72 h. The only significant difference was between the control and the 72 h sample ($t = 23$ $p = 0.05$). Active feeding remained throughout the experiment.

Discussion

The results show that a 4 deg. C thermal shock caused a slight rise (3%) in plasma concentration after 72 h, indicating a small effect on the osmoregulatory system. This gradual effect over several days agrees with Reaves et al., (1968) who reported that a 5 deg. C heat shock had no significant effect on trout until 96 h after the thermal change when there was about a 9% difference in the plasma ion concentration.

The other results would indicate that the extent of the thermal shock at acclimatisation had no effect within the range of 2-6 deg. C. It is also important to notice that the maximum osmotic concentration during acclimatisation was after about 30 h, at which time, according to this study and Reaves et al., it would be too early for any thermal influence.

Reaves et al., (1968), reported that heat stress effected the osmoregulatory ability of trout; however they also showed that a 5 deg. C heat shock caused a similar change to a 10 deg. C heat shock. The extent of the physiological changes was the same in both trials only the time course differed, being slower in the 5 deg. C shock.

It is rare on a fish farm to have a temperature difference larger

than 6 deg. C between the fresh water and the sea water, particularly at the usual times of transfer, autumn and spring. The results are also evidence that the poor August transfers in the previous section were more likely to be due to seasonal effects than increased thermal shock. Supporting this is the fact that the transfer in September (Table 1) involved 45 g fish and a heat shock of 7 deg. C, but the mortality was only 9%. The increased size as compared to the August trials is of course an important factor in this reduction in mortality, but increasing size did not reduce the mortality from June to August. This leaves seasonal variation as the most probable factor, with summer being a poor time to transfer but with an improvement by September.

SECTION 4. THE VALUE OF A WET DIET

Introduction

The results from Sections 1 and 2 show that the first 48h in sea water are the most important with respect to the final level of mortality. If during this time some means could be found to alleviate the osmotic stress, the level of mortality should be decreased. Section 1 illustrates that a rapid loss of water during this time leads to tissue dehydration. The ingestion of water to balance this loss requires the uptake of ions from the intestine, which adds to the osmotic problem. If the fish could obtain fresh water during the first 48h without having to take-up additional salt, its chances of survival ought to be increased.

Landless (1976) suggested that a wet diet of either minced squid or pre-soaked pellets could provide valuable fresh water during the early adjustive phase. Hildingstam (1976) also mentioned that wet feeds should assist the maintenance of an osmotic equilibrium in sea water. It therefore seemed worthwhile to perform some experiments to see if a wet diet could assist rainbow trout in adapting to a marine environment.

Wedemeyer (1972) demonstrated that handling coho salmon and steelhead trout in fresh water caused a temporary breakdown in the homeostatic mechanisms. The chloride plasma concentration remained depressed for up to 24h, following the handling stress. A regular practice on any fish farm is the grading of fish into different size ranges, which results in more even growth rates and less antagonistic behaviour. The grading process can involve quite harsh treatment and deaths are often recorded following gradings. Experiments were therefore also devised to measure the effect of grading in sea water on the osmotic

equilibrium and to see if the feeding of a wet diet had any beneficial results.

The Wet Diet

Freshwater soaked pellets were used as a wet diet and contained about 50% water. If soaked for only 30 min the pellets did not disintegrate on feeding. Since trout are active feeders the pellets were normally taken within a few seconds of them entering the sea water, too short a time for much of the fresh water to be displaced by sea water.

Experiment 1 - Cage transfers.

10,000 Fish of 14g were transported to the Laboratory and taken to the cages where four 2 x 2 x 2m nets were prepared, 2500 fish were placed in each net. The mean salinity for the trial was 26°/oo and the temperature 7°C. Two of the nets were fed on dry pellets (Edward Baker No. 4), and two on pellets soaked in fresh water for 30 min. Any dead fish were netted and counted.

The results of this experiments are given in Table 3. It was intended to stress the fish and give high levels of mortality so that any difference between the two diets would be more marked, but the salinity, in fact, remained higher than expected and the final levels of 85 to 90% were too high to provide satisfactory results.

The levels of mortality were very similar in all four cages and there was no significant difference between the two diets. One very likely reason for this was that about 85% of the deaths occurred during the first two days. Feeding activity during this time was either absent or very light. This means, therefore, that the fresh water intake by the

Table 3.

Table of mortalities and feeding activity over 10 days following the transfer of 10,000 fish into 4 cages, 2,500 fish in each. Two cages were fed a dry diet, the other two a wet diet.

The legend for feeding activity is:

N - None

VL - Very light

L - Light

A - Active

VA - Very active.

The exact definition of each category is given in the general materials and methods.

There was no significant difference between the mortalities and feeding activity for the two different diets.

TABLE 3.

Days after transfer		1	2	3	4	5	6	7	8	9	10	Total	% Mort.	Mean %
Salinity °/oo		26	27	26.5	28	27	25	24	24	23	24			
No. of dead fish in:														
Dry Diet.	A	1025	906	78	204	35	13	12	3	1	3	2280	91	88
	B	1140	812	102	38	17	12	5	4	2	5	2137	85	
Feeding activity		N	VL	VL	L	A	A	A	A	A	A			
Wet Diet.	C	910	918	225	25	16	9	11	5	12	7	2138	85	86
	D	850	930	300	42	12	7	10	4	9	8	2172	87	
Feeding activity		N	VL	VL	L	A	A	A	A	A	A			

Period of heavy rain shortly before the trial, but did not result in lowered salinity until towards the end of the trial.

wet diet fed fish was negligible. Feeding did not return to active until the fifth day and after this time only 2% of the deaths occurred so that any freshwater intake would have very little influence on the final result.

The reduction in feeding activity can be attributed to two factors. Transportation of fish always causes stress which results in a loss of feeding activity normally for about a day and it remains reduced for two or three days. Also as discussed in Section 1, a rise in osmotic concentration of the plasma causes feeding activity to cease. In this experiment these two factors combine resulting in poor feeding activity for the first four days after transfer.

Experiment 2 - Tank transfers.

80 Fish of 44g (S.D. = 8.4) were brought from Kilmore and divided equally into two 225l black laboratory tanks. The tanks had a fresh water input of 10°C and the fish were held in that for six days to settle into the new conditions. Sea water at (11°C) was then added and the salinity raised to 30°/oo over 1h. Food was offered at regular intervals during the experiment, the fish in one tank being given a dry diet of Edward Baker No. 5, the other the same, but soaked in fresh water. Samples of 6 fish from each tank were taken for plasma concentration and water content determinations at various times.

To ensure that some feeding occurred during the critical first 48h the two factors of transport stress and osmotic stress were reduced in this experiment. Firstly, the fish were allowed several days to recover from transport. Also at about 40g and with a salinity of 30°/oo, the fish would be osmotically stressed in a similar manner to trial 3, experiment 4, Section 1, where the plasma concentration rose to 366 mOsm/l

which is only just above the level at which feeding appears to cease.

The results of the blood samples and water determinations are shown in Figure 15. Feeding during this time was as follows (for legend, see Table 3):

Hours after Transfer	Feeding Activity	
	Dry Diet	Wet Diet
1	A	A
5	A	A
12	L	L
23	N	N
42	N	N
48	N	N
72	VL	VL
96	L	L
124	L	L

It can be seen from the graph of the plasma concentrations that four out of the five means were lower with the wet diet than with the dry. The points shown with the results of a t-test between the two trials were as follows:

Hours after Transfer	Mean Plasma Concentration mOsm/l ± S.D.		t
	Dry Diet	Wet Diet	
2	335 ± 16.7	331 ± 8.0	0.66
6	356 ± 13.9	347 ± 13.6	1.07
13	356 ± 17.0	354 ± 20.3	0.13
24	362 ± 15.0	367 ± 12.3	0.61
127	356 ± 20.0	352 ± 18.2	0.34

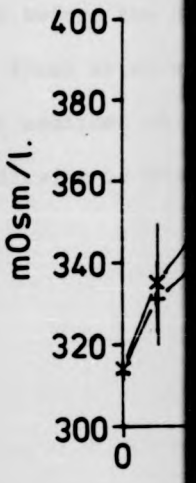
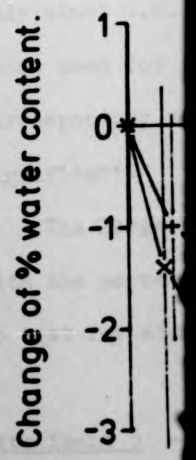


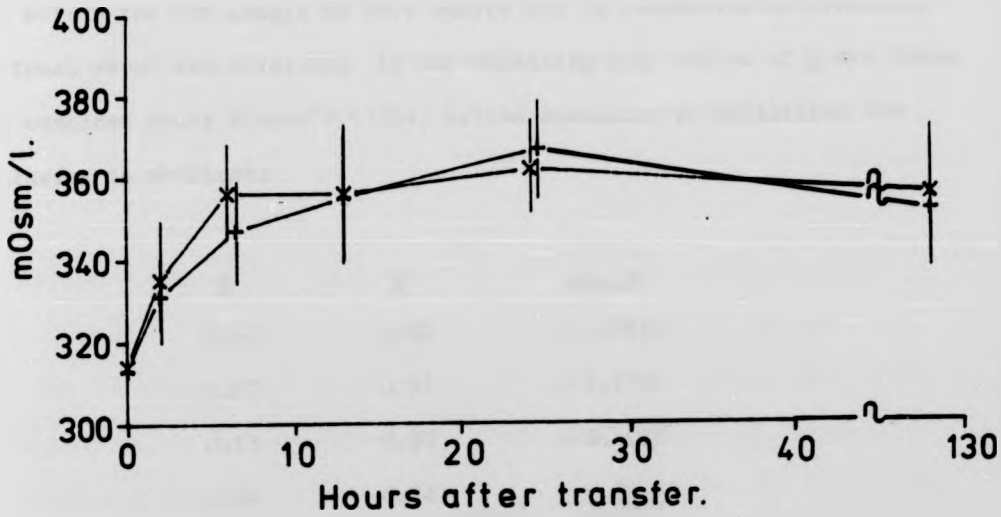
Figure 15. Change in plasma concentration and percentage water content of two groups of fish, one fed of a dry diet and the other on a wet diet.

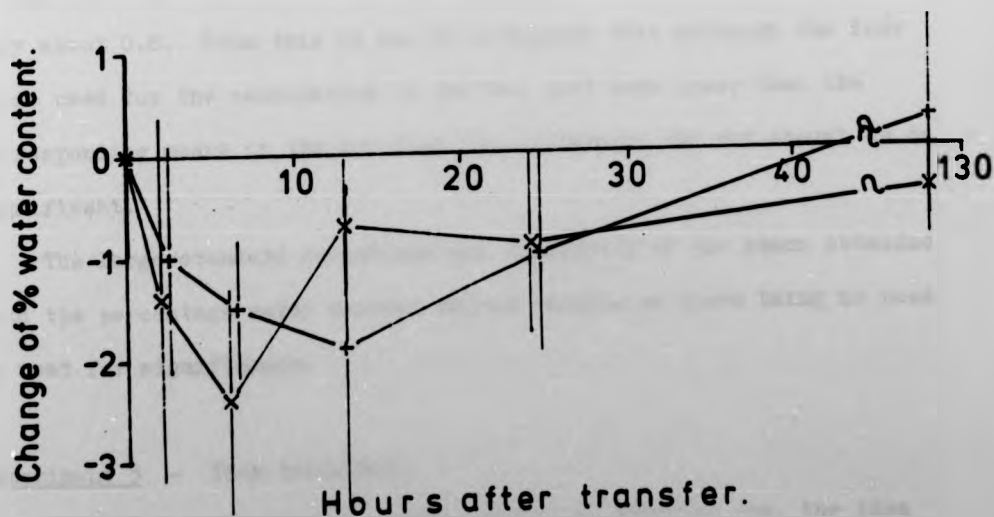
X - Dry diet

✚ - Wet diet.

For test of significance see text.




400
380
360
340
320
300
mOsm/l.
0
10
20
30
40
130
Hours after transfer.


1
0
-1
-2
-3
Change of % water content.
10
20
30
40
130
Hours after transfer.

From the feeding table it can be seen that no feeding occurred for 12h before the 24h sample so this sample can be discounted as obviously no fresh water was obtained. If the remaining four values of $\underline{t}$ are taken and combined using Fisher's (1954) method combining probabilities the following is obtained:

$\underline{t}$	$\underline{p}$	$\underline{\ln. p.}$
0.66	0.52	- 0.6539
1.07	0.31	- 1.1712
0.13	0.90	- 0.1053
0.34	0.74	- 0.3011
	Total	- 2.2315

$$-2 \Sigma \ln p \text{ or } \chi^2 = -2 (-2.2315)$$

$$\chi^2 = 4.463$$

This can be looked up under 8 degrees of freedom, there being 2 degrees for each probability value used. The resulting probability is only about 0.8. From this it can be concluded that although the four means used for the calculation in the wet diet were lower than the corresponding means in the dry diet the difference was not enough to be significant.

The large standard deviations and similarity of the means obtained with the percentage water content values results in there being no need to test for significance.

Experiment 3 - Tank transfers.

This was a very similar experiment to the previous one, the idea being to use larger sample numbers to obtain greater accuracy and also to use a slightly higher salinity - 32°/oo as opposed to 31°/oo - in the

hope that any difference caused by the different diets would be enhanced.

100 Fish of 46.5g (S.D. 12.4) were transported from Kilmore and held in the 1100 l tank for a week before being divided equally between two 225 l tanks. There they were held a further five days in fresh water at 14°C before the salinity was raised by sea water to 32°/oo at a temperature of 14.5°C. Food was given at regular intervals during the experiment, dry pellets to one tank, soaked pellets to the other. Following sea water transfer three samples of about 9 fish were taken from each tank.

The three sampling times were 16, 40 and 64h after transfer. These times were chosen as they followed periods when feeding had occurred. The feeding during the trials was as follows, (for legend see Table 3):

Hours after Transfer	Feeding Activity	
	Dry Diet	Wet Diet
0	A	A
10	A	A
12	A	A
14	L	L
18	VL	VL
34	VL	VL
36	L	L
38	L	L
40	L	L
58	L	L
60	L	L

The results obtained from the blood samples are shown in Table 4. This shows that again although the means for the wet diet fish were

TABLE 4.

Hours after transfer	DRY DIET		WET DIET		t.	p.	ln.p.
	No. in sample	mOsm/l ∓ S.D.	No. in sample	mOsm/l ∓ S.D.			
0	5	308.0 ∓ 8.8	5	315.4 ∓ 5.1	-	-	-
16	9	368.8 ∓ 13.3	9	362.3 ∓ 13.6	1.025	0.32	-1.139
40	9	396.5 ∓ 18.2	9	394.7 ∓ 13.7	0.237	0.82	-0.198
64	10	383.9 ∓ 20.7	9	370.2 ∓ 15.6	1.63	0.12	-2.120
						TOTAL	-3.457

$-2 \leqslant \ln.p$ or $\chi^2 = 6.9.$ d.f. = 6. p. = 0.32.

lower, the individual differences between the means are not significant and neither is the combined probability.

Experiment 4 - Stress experiments.

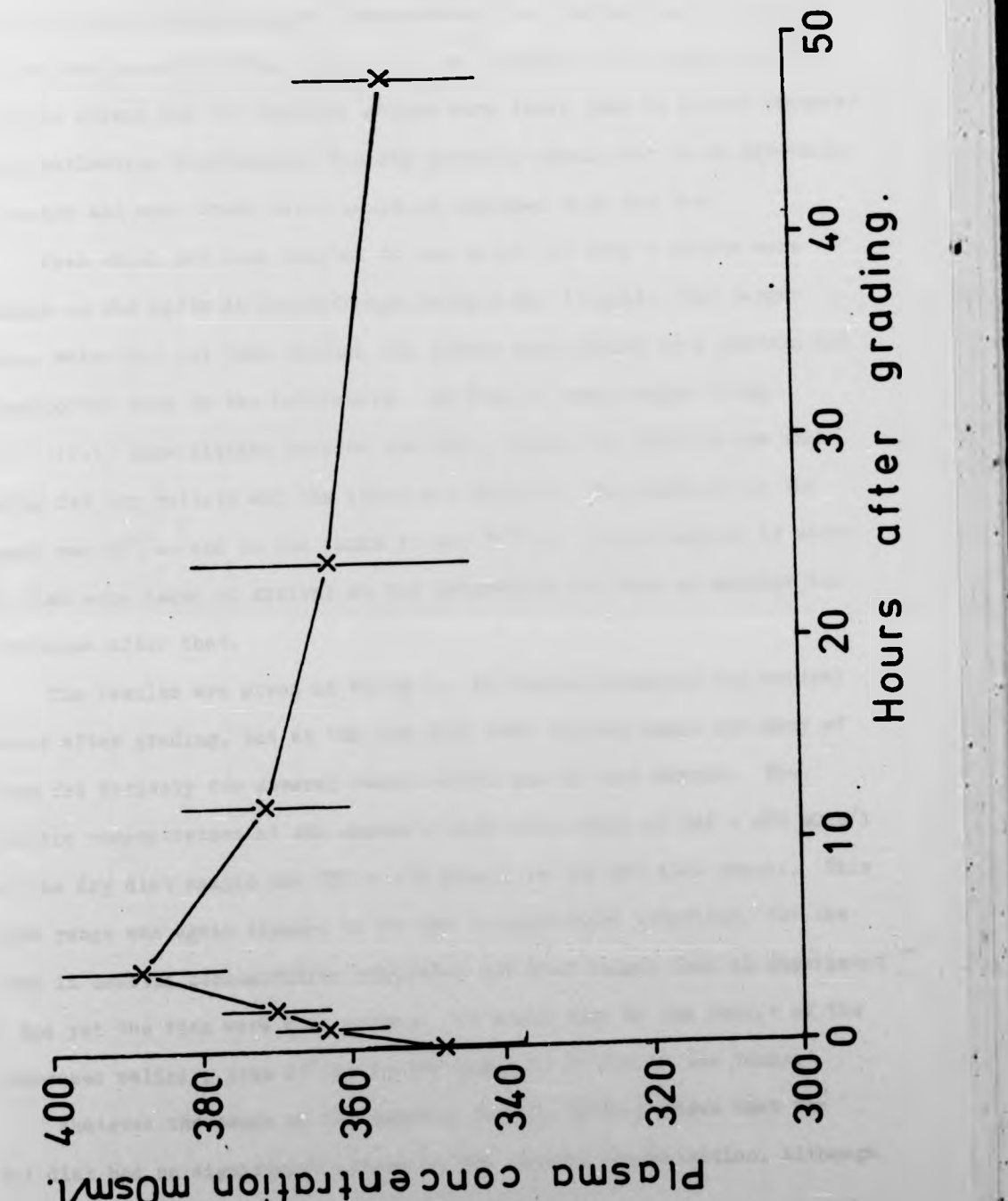
50 Fish, with a mean weight of 39.4g (S.D. 9.2), which had been living in the cages for several months, were netted and transported in dustbins to the Laboratory, where they were placed in a commercial fish grader fitted with a No. 14 grid. The fish were graded in a normal manner, the smaller fish passing through the grids into a 225 l tank, the larger fish remained in the grader and then being tipped into the same tank. The salinity and temperature in the cages was 30°/oo and 15°C, while in the tanks it was 31°/oo and 15°C. 6 Fish were sampled immediately on arrival at the Laboratory, 10 mins after leaving the cage and these were used as a control. 5 Further samples were taken, each of 5 fish, during the first 24h. Small quantities of dry pellets were offered regularly to obtain measure of appetite. The results of this experiment are shown in Fig. 16. The stress of the transport obviously causes a temporary breakdown of the osmotic equilibrium and there is a sharp rise in plasma concentration similar to that seen for the acclimation trials. The difference is, however, that the rise only lasts for the first few hours and by 12h the level is falling again. The level is still raised after 24h, but by 48h most of the fish have reached an equilibrium point, although some of the smaller fish still have raised plasma concentrations. After 6h one of the trout was showing signs of stress and by 8h it was dead.

Experiment 5 - Feeding of a wet diet after grading.

The purpose of this experiment was to determine whether the feeding

Figure 16. The change in plasma concentration of fully seawater-adapted fish (mean wt. 39 g) following grading.

The general shape of the graph is similar to Fig. 3 there being a rapid rise followed by a slower return to an osmotic equilibrium. The time course is however shorter, the peak occurring after six hours and the equilibrium being re-established by about 50 h.


Plasma concentration mOsm/l.
400
380
360
340
320
300
0
10
20
30
40
50
Hours after grading.

of a wet diet following short transportation and grading could assist in maintaining an equilibrium level. It was thought that, since both the osmotic stress and the handling stress were lower than in direct transfer acclimatisation experiments, feeding activity should not be so adversely effected and more fresh water would be consumed with the food.

Fish which had been adapted to sea water for over 6 months were graded on the rafts at Dunstaffnage using a No. 17 grid. The larger fish, which did not pass through the grader were placed in a dustbin and transported back to the Laboratory. 40 Fish of mean weight 61.9g (S.D. 16.1) were divided between two 225 l tanks, the fish in one tank being fed dry pellets and the other wet pellets. The salinity in the cages was 27°/oo and in the tanks it was 31°/oo. Large samples of about 10 fish were taken on arrival at the Laboratory and then on another two occasions after that.

The results are given in Table 5.. No feeding occurred for several hours after grading, but at 18h the fish were feeding again and many of them fed actively for several hours before the 48 hour sample. The osmotic concentration at 48h showed a very wide range of 342 - 409 mOsm/l in the dry diet sample and 335 - 410 mOsm/l in the wet diet sample. This wide range was again thought to be due to aggressive behaviour, for the rise in osmotic concentration continued for much longer than in experiment 4 and yet the fish were much larger. It could also be the result of the increased salinity from 27°/oo in the cages to 31°/oo in the tanks.

Whatever the cause of the osmotic stress, Table 5 shows that the wet diet had no significant effect on the osmotic concentration, although the mean after 48h was lower than in the control.

TABLE 5.

Hours after transfer	DRY DIET		WET DIET		t.	p.	ln.p.
	No. in sample	mOsm/l ∓ S.D.	No. in sample	mOsm/l ∓ S.D.			
0	8	341.1 ∓.9.8	7	341.7 ∓ 13.7			
19	13	354.6 ∓ 19.0	12	354.8 ∓ 19.9	0.02	0.95	-0.05
48	9	377.6 ∓ 23.7	9	368.0 ∓ 27.3	0.80	0.42	-0.87
						TOTAL	-0.92

$-2 \leq \ln.p$ or $\chi^2 = 1.84$. d.f. = 4. p. = 0.6.

Discussion

Shehadeh and Gordon (1969) showed that rainbow trout in sea water drink about 130ml/kg body wt/day. That means that 50g fish will drink about 6.5ml of water every 24h. The pellets hold almost exactly their own weight in fresh water and at 10°C 50g fish will be fed about 2% of body weight per day. (Appendix III). From this one can say that the feeding of a wet diet should provide about 13% of the fish's water requirement (Landless, 1976). This should lead to a reduction in the required efflux of chloride and sodium ions and therefore one would imagine a lowered plasma concentration during times of osmotic stress.

The results presented here, however, show no significant advantage of feeding a wet diet during times of osmotic stress. The reasons for this are not altogether clear. One obvious factor is the reduced feeding activity at times of osmotic stress. Those fish most requiring the freshwater intake, i.e., plasma levels of over 390 mOsm/l will not be feeding, while any fish with a level above 360 mOsm/l will have a very reduced feeding activity. Fish which are feeding lightly will only take about one quarter of the recommended level of food. Obviously a reduction of food intake to 0.5% body wt/day will reduce the contribution provided by the wet diet to about 3% of the freshwater requirement.

Another important reason why the wet diet may not be having a beneficial effect is due to the adaptive state of the intestinal wall. Smith (1964) and House and Green (1965) concluded from their observations on isolated intestines that the rate of water and salt absorption is much higher in seawater-adapted fish. Utida (1967) showed that the level of enzyme activity of alkaline phosphatase in the intestine of rainbow trout was much higher in seawater-adapted fish. Since the role of the intestine is very different in the two media, one would expect there to be

physiological differences. There is at present no information as to how long after transfer it takes for these changes to occur.

In the situation discussed here less active up-take of salt has to occur, the fresh water in the pellet should move osmotically across the intestine wall. However, Skadhauge (1969) demonstrated that in the European eel the osmotic permeability of the gut wall is higher in seawater-adapted eels than in freshwater animals. It therefore seems likely that at transfer the gut wall of the trout is very impermeable to water and thus acts as a barrier against the up-take of the fresh water. Shehadeh and Gordon estimated that in trout fully adapted to sea water, 80% of the water drunk was absorbed. It is therefore possible that a smaller percentage of the water from the pellets actually passes into the blood. If only 50% is absorbed as opposed to 80% then the amount of drinking saved will fall to about 6%, which, spread over several hours, would produce too small a difference to be measured.

Another important point to remember is that drinking accounts for only one fifth of the sodium influx and one sixth of the chloride influx in sea water, the rest entering via the gills (Potts et al, 1970). So, although feeding a wet diet may be reducing the sodium and chloride influx through the gut, the overall change in the influx is small.

The grading experiments indicate that the treatment causes a temporary breakdown in the equilibrium condition which in the case of experiment 4 lasts for over 24h with a peak after about 6h. This agrees with the results obtained by Wedemeyer (1972) in fresh water, except that in his experiment the ion concentration decreased - as one would expect in fresh water - and reached a minimum around 4h and was still slightly depressed after 24h. The feeding of a wet diet did not seem to effect the plasma concentration in this case either.

SECTION 5. THE VALUE OF A HIGH SALT DIET

Introduction

Section 1 showed that the first 48 hours following transfer was the critical period; there is therefore a very short period during which physiological changes might be induced to alleviate the osmotic stress. The previous section indicated that trying to reduce the osmotic stress via the intestine after transfer had no significant effect.

It is well known that fish which undergo smoltification change physiologically while still in fresh water, so that they are pre-adapted to marine conditions (see review by Hoar, 1976). Although rainbow trout do not smolt it was thought that the possibility of inducing preadaption might prove a useful line of investigation.

A possible method of producing physiological changes prior to transfer would be the use of a hormone treatment. Both prolactin and cortisol are thought to play an important role in osmoregulation of teleosts (see review by Mayer, 1970). Cortisol particularly is thought to be important in controlling salt secretion in sea water. The exact effect is still, however, under dispute and the problem and expense of administering a cortisol treatment to thousands of fish on a commercial scale would prove prohibitive.

Zaugg and McLain (1969), working with Pacific salmon fry, found that an increased level of salt in the diet (4 and 8% NaCl) resulted in longer TM 50 values (time in hours for 50% mortality), following seawater transfer. They explained this result by stating that: 'the ingestion of elevated quantities of salt would activate the excretory processes involved in the elimination of these salts when swallowed by drinking sea water in a marine environment'.

Basulto (1976) carried out similar experiments with Atlantic salmon and found that again the chances of survival after transfer from fresh water to sea water were increased following the feeding of a diet containing an elevated level of salt (12% NaCl).

Both these groups of workers were, however, using smolting species and very small numbers in their transfer trials. Zaugg and McLain used 20 fish to obtain each of their TM 50 values, while Basulto used between 25 and 38 fish to obtain his percentage survival values.

The approach did however seem to be a useful one and very easily applied commercially. It therefore seemed worthwhile to investigate whether the feeding of a diet containing a high level of salt had any effect on the percentage mortality of rainbow trout following transfer to sea water. If the H.S.D. (High Salt Diet), was to have any beneficial effect one would also expect to find lower levels of plasma concentration during the adaptive phase; this parameter was therefore also measured.

Both Zaugg and McLain and Basulto reported that high levels of dietary salt had a detrimental effect on the growth of salmon. But a more detailed study by Shaw et al (1975) concluded that levels of up to 12% NaCl had no significant effect on the growth of Atlantic salmon. This agrees with a recent paper by MacLeod (1978), where he reported that the addition of up to 8.5% NaCl to the diet of rainbow trout had no significant effect on either food intake or food conversion ratio. It therefore seemed important to monitor growth during the experiments to see if the H.S.D. effected it.

The High Salt Diet

Zaugg and McLain used three test diets containing additional salt, one with 'Instant Ocean' salts, one with a NaCl-KCl mix and one with

additional NaCl only. They found that all three seemed equally effective. It was decided to use the NaCl and after consultation with the manufacturers 10% was added by weight. The first batch of the H.S.D. showed that at a 10% level in a No. 5 sized compact pellet the texture was suitable and crumbling minimal.

The fish found the H.S.D. palatable and took all the food provided. On changing from the control diet to the H.S.D. there was occasionally a period of up to 2 days during which feeding was reduced, but once they had adapted to the change of diet, feeding returned to normal. This reduction in activity was also often observed when changing from one pellet size to another or from one brand of food to another and therefore not thought to be due to the unpalatable nature of the H.S.D.

Experiment 1 - The uptake of the salt.

This experiment was designed to see if the salt in the diet was taken-up across the gut wall into the blood stream. If this was the case the plasma osmotic concentration should rise above the normal freshwater equilibrium level.

40 Fish of mean weight 55.2g (S.D. 13.6) were placed in the large 1100 l tank at the Laboratory. They were held in tap water (9°C) for 2 weeks to settle, during this time they were fed a control diet of Edward Baker's No. 5 pellet daily. They were then starved for 3 days and 7 fish sampled for plasma osmotic concentration. The intestines were also dissected out for examination. The remaining fish were then fed a control diet to satiation and sampled again 7 hours after feeding. The fish were then fed normally again for 2 days before being starved once more for 3 days to allow the stomachs to empty. They were then fed on the H.S.D. to satiation and 2 samples taken, 7 and 24h after feeding. The state of the intestines was again examined.

TABLE 6.

Fish Type	Wt. in g.	Osmolarity mOsm/l	Mean ∓ S.D.	Stomach Contents
Unfed for 3 days	48.9	295	306.3 ∓ 7.2	All empty
	54.3	305		
	47.6	319		
	68.2	306		
	82.4	303		
	54.2	310		
	42.2	306		
7 Hours after control diet	43.2	311	313.8 ∓ 3.9	All full
	71.2	319		
	40.7	315		
	37.3	314		
	58.5	316		
	54.1	308		
7 Hours after H.S.D.	58.5	356	363.6 ∓ 13.6	Full
	57.8	349		
	47.7	357		
	44.9	375		
	57.3	381		
	50.4	320		Empty
	46.2	307		
24 Hours after H.S.D.	87.1	348	364.0 ∓ 18.8	All ¼ full
	86.2	389		
	45.3	382		
	36.2	363		
	57.8	340		
	53.2	362		

The effect on the plasma osmotic concentration of feeding the H.S.D. is shown in Table 6. As the fish had been starved for 3 days most of them had fed very actively and about 2% of body weight was consumed. As can be seen from the table, two of the fish which were sampled had not eaten and had obviously resisted the change in diet despite 3 days starvation. There is a significant difference between the unfed fish and fish 7h after being fed the control diet ($t = 2.38$ $p < 0.05$).

More importantly, there is a significant difference between 7h after the control diet and 7h after the H.S.D. ($t = 7.9$ $p. < 0.001$). This therefore means that the salt from the diet is taken up into the blood where it is obviously retained and not immediately eliminated. The two fish which had refused the H.S.D. had much lower plasma salt concentrations and they were ignored from the calculations of the mean. The level was still significantly higher 24h after feeding the H.S.D. ($t = 6.4$ $p < 0.001$), again showing that the salt was not immediately eliminated via the kidney or through the gills.

Experiment 2 - Long term effect of the H.S.D.

This experiment was to determine the longer term effect of the H.S.D. on the plasma concentration. Another 40 fish of mean weight 86.9g (S.D. 23) were placed in the 1100 l tank and allowed to adjust. Tap water of 15°C was run through the tank. A plasma sample was taken while the fish were still on the control diet and the remaining fish were then fed the H.S.D. The fish were fed twice daily on the H.S.D., 1% of body weight being offered at each feed. Three samples were taken during the next 2 weeks to see if there was any effect on the osmotic concentration..

Table 7 shows the result of continued feeding of the H.S.D. All

TABLE 7.

Fish Type	Wt. in g.	Osmolarity mOsm/l	Mean ∓ S.D.
Control 4 hours after last feed.	54.8	304	312.7 ∓ 9.7
	38.9	311	
	63.8	323	
	52.0	301	
	62.9	312	
	52.8	325	
36 Hours after 1st feed on H.S.D.	97.4	327	341.0 ∓ 16.0
	83.0	340	
	64.9	344	
	95.5	355	
	92.2	361	
	112.4	319	
8 Days after 1st feed on H.S.D.	113.9	341	350.6 ∓ 14.3
	109.8	367	
	118.7	343	
	74.1	331	
	110.2	362	
	124.0	360	
14 Days after 1st feed on H.S.D.	80.1	336	350.8 ∓ 11.2
	96.2	362	
	109.8	367	
	191.8	359	
	116.2	361	
	91.6	342	

3 samples following the change to the H.S.D. were significantly higher than the control sample ($p = < 0.05$). The rise is not as large as seen in Experiment 1, but this is perhaps due to each feed being only 1% of body weight as opposed to the 2% at the single feed in the previous experiment. A sample was also taken from fish which had been on the H.S.D. for 8 days at Kilmore. There the level was 348 mOsm/l (S.D. 12.7) as compared to a control value of 313 mOsm/l (S.D. 3.6).

From this it can be seen that trout on the H.S.D. have a significantly higher equilibrium level than do fish on a control diet.

Experiment 3 - Cage transfers.

7,000 Fish were transported from Cloan hatchery and placed in the 4 tanks at Kilmore. They were held for a week to recover during which time they were fed a control diet. Each tank had its own feeder wired to a central clock to ensure equal feeding at a rate of 2% of body weight per day. The fish in two of the tanks were then fed the H.S.D. while the other two remained on the control.

Every week 250 fish were taken from each tank and transported to the cages in Dunstaffnage Bay where the mean salinity was 30°/oo. The H.S.D. fed fish were placed in one net (2 x 2 x 2m) and the control fish were placed in another. The dead fish were removed regularly and food was offered daily to make an assessment of feeding activity. These weekly transfers continued for 5 weeks; there was then a gap of 5 weeks and another transfer was made 10 weeks after the start. There was a final transfer after 12 weeks. The final transfer consisted of only 400 fish which had been on the H.S.D. and 295 as a control.. The control fish for the last 2 trials were from a tank which was being hand fed 1% daily so their growth rate was slower and the sizes were unfortunately not identical.

Fish weighings were made during the course of the experiment to determine growth rates. The method of weighing was to net out 50 fish and to place them in a bucket containing water on a tared balance. Two such weighings were taken from each tank giving 4 control and 4 H.S.D. measurements and from these a mean weight for each diet was obtained.

The growth of the fish during the experiment is shown in Fig. 17. This shows that after 38 days on the diet the control fish are nearly 1g heavier than those on the H.S.D. However, it must be remembered that 10% of the H.S.D. is salt which cannot be utilised for tissue growth, so effectively instead of being 2% of body wt/day they are receiving 1.8% body wt/day. If the only effect the salt is having on the growth is caused by "diluting" the diet, they should grow at the same rate as fish fed on 1.8% body wt/day of a control diet. Conversely, if the salt is having a detrimental effect on growth the weight gain of the fish should be slower than when fed 1.8%. It is possible to determine the effect using the method of growth prediction devised by Landless (pers. comm.).

The equations used for the growth prediction are:

$$\text{Specific Growth Rate, S.G.R.} = \frac{\text{Feeding Rate (\% per day)}}{\text{Food Conversion Ratio F.C.R.}} \qquad \text{Equation 1.}$$

$$\text{No. of days to increase weight by factor of t} = \frac{\log. t}{\log \left(\frac{1+\text{S.G.R.}}{100}\right)} \qquad \text{Equation 2.}$$

With these equations it is possible to construct a table of predicted growth rate for fish fed 1.8% body wt/day. First, however, an estimation of the conversion ratio has to be calculated. This is possible using the growth of the control fish with the equation:

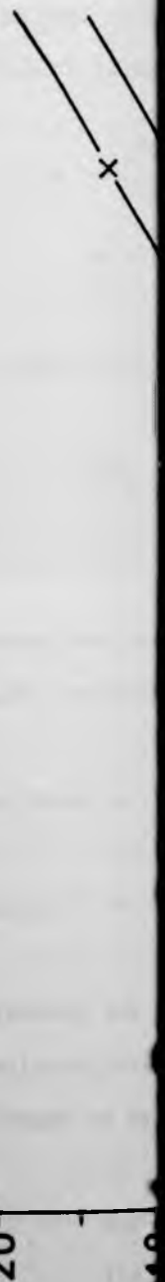
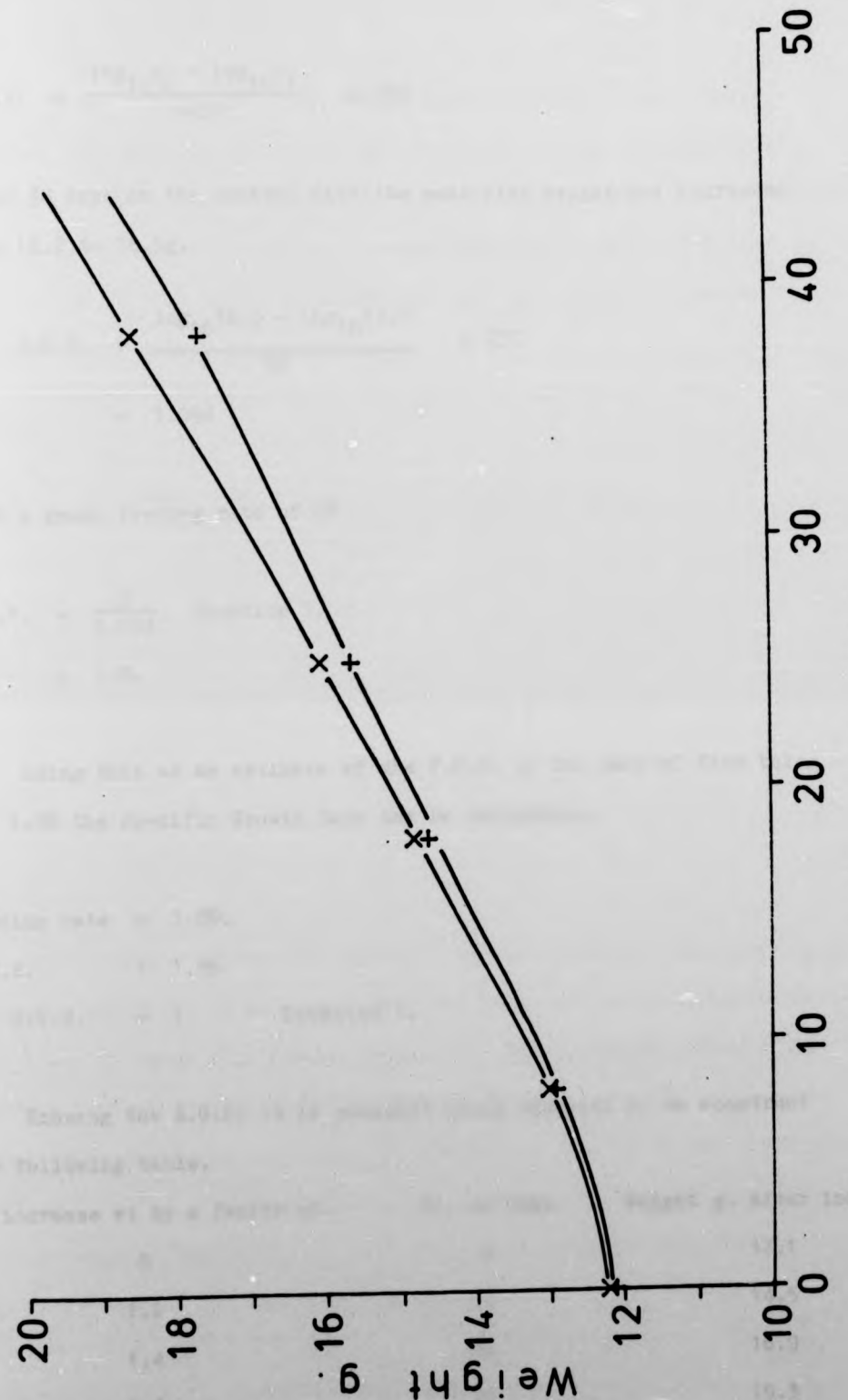


Figure 17. Growth of fish being fed 2% body weight/day in water of 9°C.

X - Control Diet

+ - 10% High Salt Diet.


Weight g.
20
18
16
14
12
10
0
10
20
30
40
50
Days on Diet.

$$S.G.R. = \frac{\log_{10} w_2 - \log_{10} w_1}{\text{days}} \times 230$$

After 38 days on the control diet the mean fish weight had increased from 12.2 to 18.5g.

$$S.G.R. = \frac{\log_{10} 18.5 - \log_{10} 12.2}{38} \times 230$$

$$= 1.094$$

With a known feeding rate of 2%

$$F.C.R. = \frac{2}{1.094} \quad \text{Equation 1.}$$

$$= 1.8.$$

Using this as an estimate of the F.C.R. in the case of fish being fed 1.8% the Specific Growth Rate can be calculated.

Feeding rate = 1.8%.

F.C.R. = 1.8%.

S.G.R. = 1 Equation 1.

Knowing the S.G.R. it is possible using equation 2, to construct the following table.

To increase wt by a factor of:	No. of days	Weight g. after increase
0	0	12.1
1.2	18	14.5
1.4	34	16.9
1.6	47	19.3

If these points are plotted on a graph with the actual results obtained from feeding the H.S.D. at 2% per day the curves shown on Fig. 18 are the result. The growth rates of the predicted and the actual results are almost identical and it can therefore be concluded that the salt in the diet is merely diluting the food by 10% rather than having any inhibitory effect on growth.

The weights of the trout at the seven transfer trials were:

Weeks from start	Weight of fish g. Control	H.S.D.
1	13.0	12.8
2	14.1	13.8
3	15.2	14.8
4	16.5	15.9
5	17.9	17.1
10	24.5	26.0
12	30.0	32.0

The mortalities recorded during the first nine days following each transfer are given in Fig. 19. In every trial the level of mortality was lower in those fish fed on the H.S.D. The divergence between the two was obvious in most cases even after 25h and continued to increase until 100h, the number of deaths recorded then diminished and the graphs flatten.

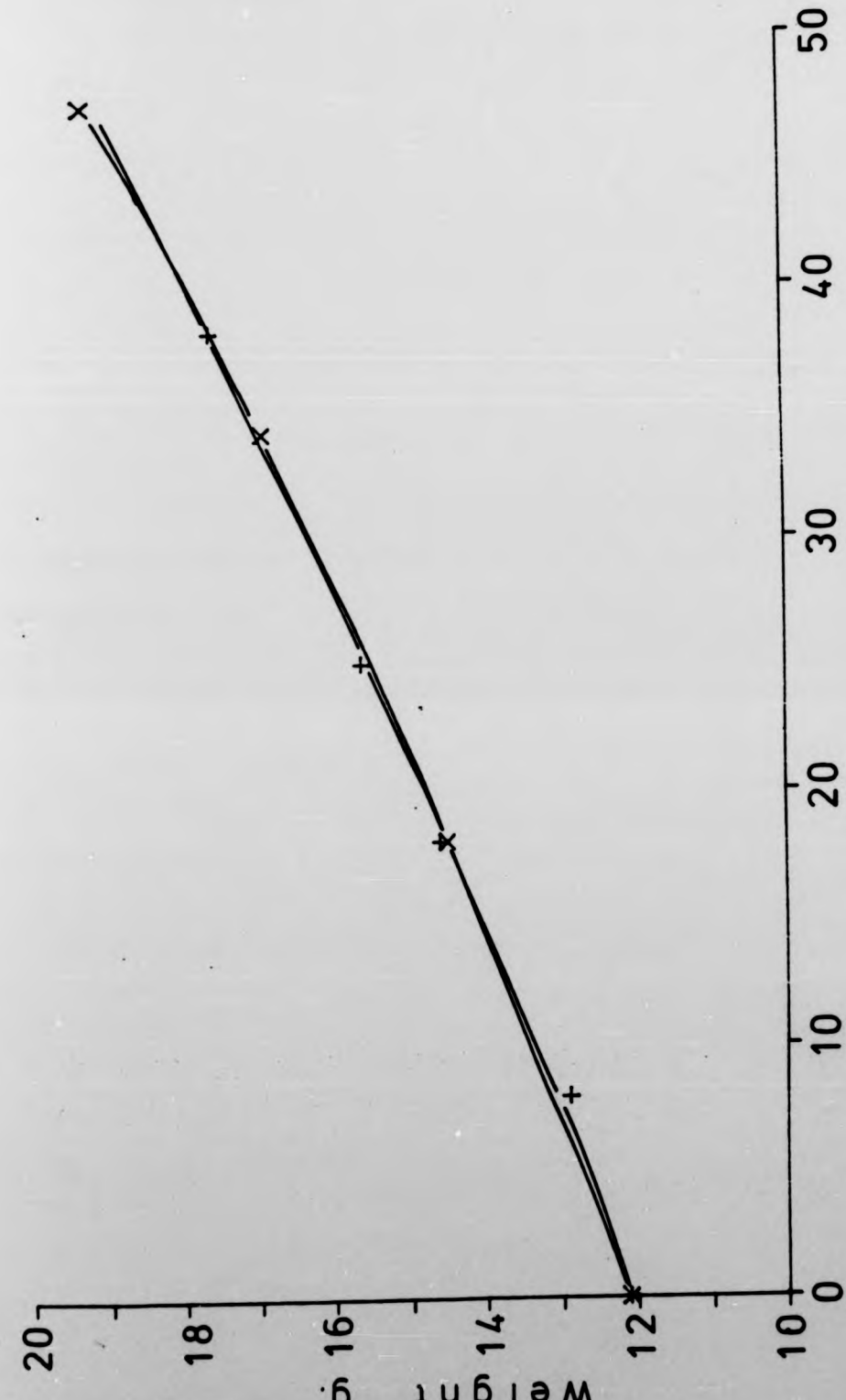
To compare the overall mortalities it is possible to calculate the percentage saving in fish using the equation:

$$\% \text{ saving in fish} = \frac{\% \text{ Mortality Control} - \% \text{ Mortality H.S.D.}}{\% \text{ Mortality Control}} \times 100$$

Figure 18. Comparison of growth of fish being fed 2% body wt/day of the H.S.D. with the predicted growth of fish being fed 1.8% body wt/day of control diet.

\+ - 2% body wt/day H.S.D.

X - Predicted growth on 1.8% body wt/day control diet.



% body

sh being

.

1.8%
diet.

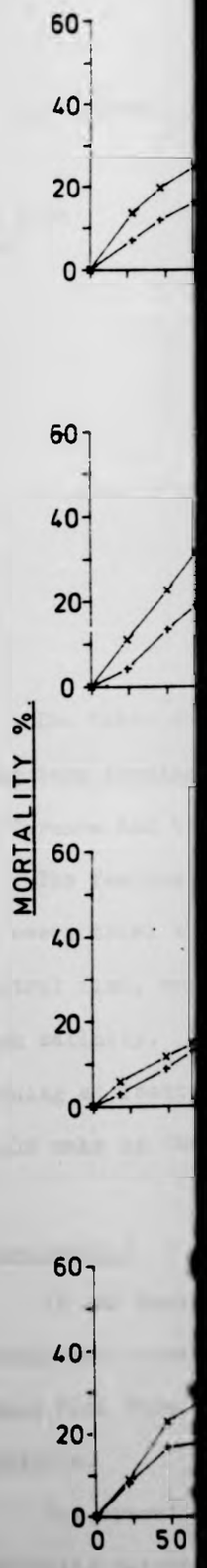
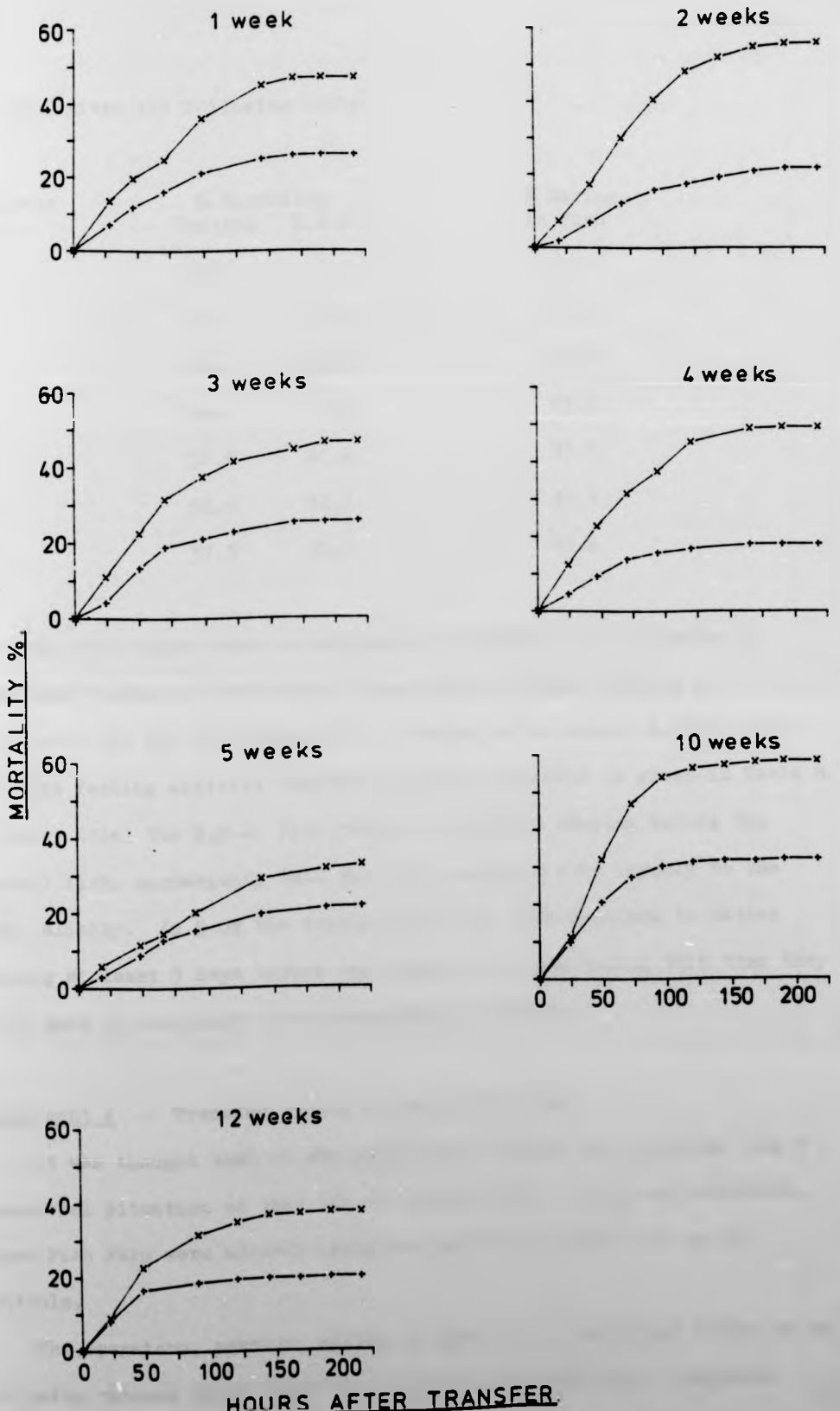


Figure 19. Observed mortalities following seawater transer of fish fed on a control diet and on the H.S.D. for an increasing number of weeks.

X - Control Diet

+ - 10% H.S.D.



transer of

increasing

That gives the following table:

Week from start	% Mortality Control	% Mortality H.S.D.	% Saving in fish
1	47.4	26.2	44.7
2	55.6	21.8	60.8
3	46.8	25.8	44.9
4	49.4	18.0	63.5
5	32.6	21.6	33.7
10	58.6	32.2	41.1
12	37.5	20.0	46.6

The table shows that no additional advantage is to be gained by long term feeding of the H.S.D. Even after one week there is a difference and the 61% saving after 2 weeks is the second highest value.

The feeding activity observed following transfer is given in Table 8. In every trial the H.S.D. fish returned to active feeding before the control fish, emphasising that they were adapting more rapidly to the high salinity. In 5 of the trials the H.S.D. fish returned to active feeding at least 3 days before the control fish and during this time they would make up the weight lost during H.S.D. feeding.

Experiment 4 - Transfer trials at Kames Fish Farm

It was thought that it was important to obtain some results from a commercial situation so that the technique could be properly evaluated. Kames Fish Farm were already using the diet but without the use of controls.

The operators, however, agreed to allow some controlled trials to be run using rainbow trout which were ready for transfer and 2 separate

TABLE 8.

Weeks after start	Type of diet	Days after transfer: 1	2	3	4	5	6	7	8	9
1	Cont.	N	VL	VL	L	L	L	L	A	A
	H.S.D.	N	VL	L	L	A	A	A	A	A
2	Cont.	N	N	N	L	L	L	A	A	A
	H.S.D.	N	VL	VL	L	A	A	A	A	A
3	Cont.	N	VL	L	L	L	L	L	A	A
	H.S.D.	N	L	A	A	A	A	A	A	A
4	Cont.	N	N	VL	VL	L	L	L	A	A
	H.S.D.	N	N	L	A	A	A	A	A	A
5	Cont.	VL	L	A	A	A	A	A	A	A
	H.S.D.	VL	A	A	A	A	A	A	A	A
10	Cont.	N	N	VL	VL	VL	L	L	L	A
	H.S.D.	N	VL	L	L	A	A	A	A	A
12	Cont.	N	N	N	N	L	L	A	A	A
	H.S.D.	N	N	VL	L	A	A	A	A	A

N - None; VL - Very light; L - Light; A - Active.

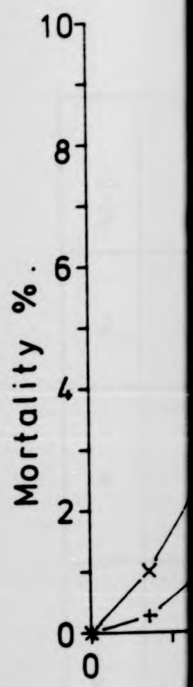
trials were performed. Before each trial the fish were starved for 2 days and then graded, the larger fish from the grading (mean weight 52g) being placed in three tanks. In the first trial 10,000 in two of these tanks were fed on a control diet and 5540 fish in the third tank on the H.S.D. The fish were fed a level of 1.5% body weight daily for 2 weeks, by which time the mean weight was 59g. The fish were then starved for 2 days and transferred from the fresh water ($8^{o}C$) to the sea cages ($10^{o}C$) with a salinity of $33^{o}/oo$. The 10,000 control fish were placed in a large cage and the 5,440 H.S.D. fish in a small cage.

A similar procedure was followed for the second trial, except the number of control fish was 3,350 (1 tank) and the number on the H.S.D. was 11,000 (2 tanks). The mean weight at grading was 57g and after 2 weeks at transfer the mean weight was 67g. The fresh water was $10^{o}C$ and the sea water was also $10^{o}C$ with a salinity of $33\ ^{o}/oo$.

Once in the sea the fish were monitored for feeding activity and mortalities. Also at various times blood samples were taken for osmotic concentration determinations. The period of surveillance lasted at least 2 weeks for each trial.

The recorded mortalities are shown in Fig. 19, again the H.S.D. fish had lower levels of mortality. The saving in trial 1 was 26.3% and 42.4% in trial 2. Although the difference between the levels of mortality were small the large number of fish used meant a high degree of accuracy. One fish was between 0.03 and 0.009% of the total, so although as in trial 1 the difference was only 2.5% it still represented a large number of fish.

As in Experiment 3, active feeding returned to the H.S.D. fish before the control fish, again indicating less osmotic stress. This is confirmed by the blood samples taken during the trials (Tables 9 and 10). Table 9 shows that there is no significant differenta between the H.S.D.-fed



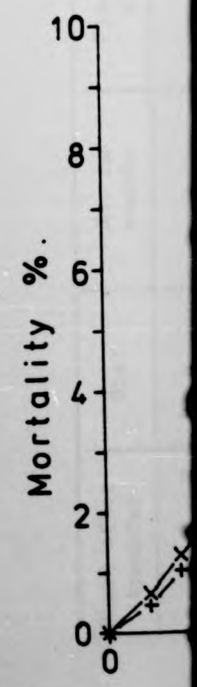


Figure 20. Observed mortalities following the seawater transfer of control fed fish and fish fed on the H.S.D. for two weeks prior to transfer.

X - Control Diet

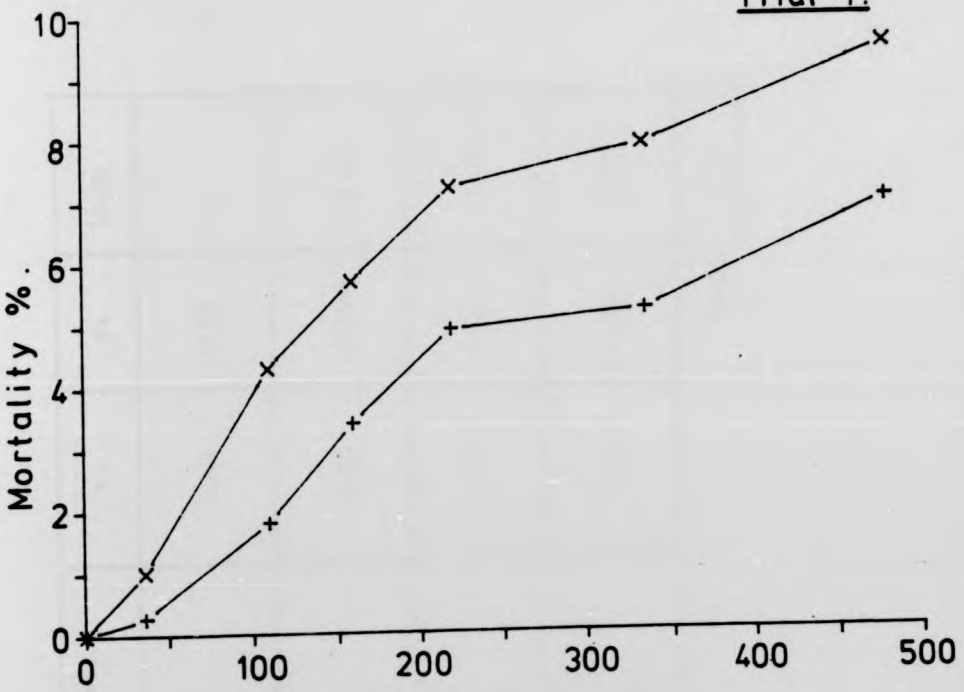
+ - 10% H.S.D.

Both trials were completed at Kames Fish Farm.

ter transfer

wo weeks

Trial 1.



Trial 2.

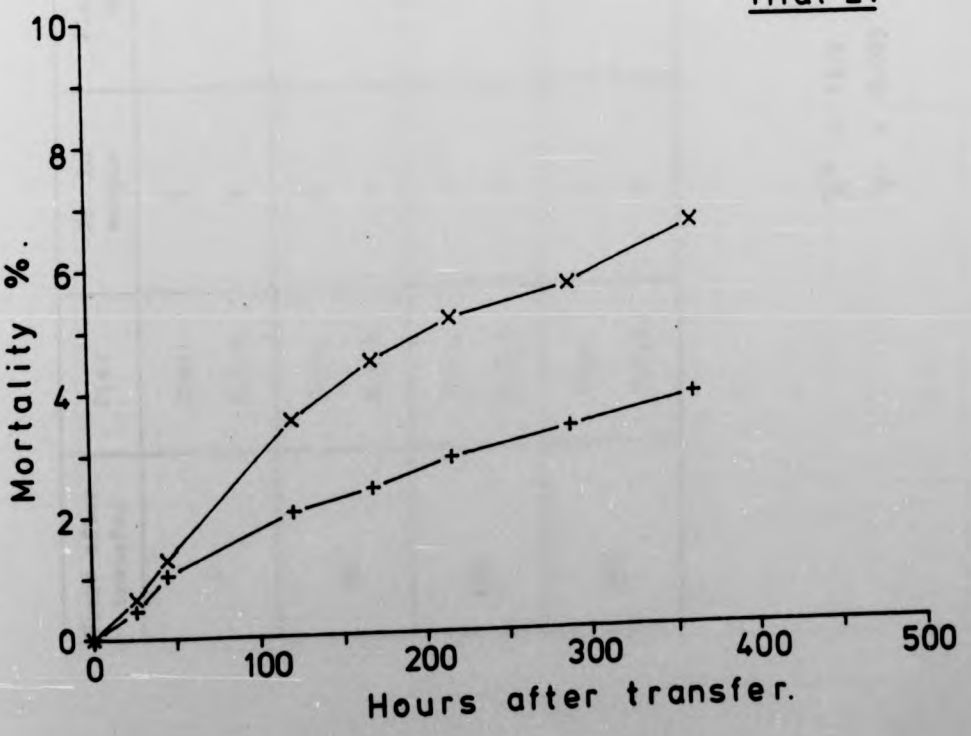


TABLE 9. Trial 1.

Hours after transfer	Diet	No. in sample	Plasma concentration mOsm/l ∓ S.D.	t.	p.	ln.p.
0	Cont.	3	313 ∓ 2.6	0.61	0.55	
	H.S.D.	5	311 ∓ 6.5			
18	Cont.	5	384 ∓ 6.2	3.75	0.008	-4.83
	H.S.D.	4	358 ∓ 12.7			
110	Cont.	4	363 ∓ 3.4	1.24	0.24	-1.43
	H.S.D.	5	356 ∓ 12.7			
192	Cont.	4	354 ∓ 6.7	0.97	0.35	-1.05
	H.S.D.	4	346 ∓ 17.3			
					TOTAL	-7.31

χ^2 = 14.6

p. = 0.025

TABLE 10. Trial 2

Hours after transfer	Diet	No. in sample	Plasma concentration mOsm/l ∓ S.D.	t.	p.	ln.p.
0	Cont.	6	320 ∓ 5.1	3.26	<0.01	
	H.S.D.	6	334 ∓ 9.2			
7	Cont.	5	381 ∓ 6.2	1.75	0.12	-2.12
	H.S.D.	4	371 ∓ 10.0			
21	Cont.	6	365 ∓ 13.6	1.43	0.18	-1.71
	H.S.D.	6	354 ∓ 13.0			
47	Cont.	6	379 ∓ 14.8	4.25	0.002	-6.21
	H.S.D.	6	350 ∓ 7.8			
119	Cont.	4	374 ∓ 7.5	3.12	0.015	-4.2
	H.S.D.	6	357 ∓ 9.7			
218	Cont.	4	349 ∓ 8.7	1.81	0.12	-2.12
	H.S.D.	4	338 ∓ 8.5			
					TOTAL	-16.36

$\chi^2 = 32.7$. p. = <0.001

fish and the control fish just prior to transfer. This may be the result of the 2 day starvation period before the sample was taken.

Once in the sea, however, the means of the H.S.D. fish were lower than those of the control. At the 18h sample there was a significant difference but not in the other two. When the probabilities are combined using the method described in Section 4 the probability of the osmotic concentration of the control fish during the adjustive phase being the same as the H.S.D. fed fish is only 2.5%.

Table 10 shows that on this occasion the plasma concentration of the H.S.D-fed fish was significantly higher at transfer despite the 2 days starvation. Following transfer two of the samples were significantly lower in the H.S.D.-fed fish and in the other three samples the difference was just outside the 5% significance level. When combined, however, the overall probability of there being the same was less than 0.001.

Experiment 5 - Tank transfers

50 Control-fed fish and 50 H.S.D.-fed fish were brought from Kilmore and placed in two 225 l tanks in the Laboratory with a freshwater input at 12°C. They were then left for a week to recover, by which time the H.S.D. fish had been on the diet for 3 weeks. The mean weight of the control fish was 51g (S.D. 10.3) and the mean of the H.S.D. was 49g (S.D. 8.7). After a recovery period of one week the sea water was added (14°C and 33°/oo). Blood samples were taken from each tank while they were still in fresh water and then at intervals following transfer.

The results of the blood samples are given in Table 11. After 24h there was no difference between the 2 trials but by 45h there was a difference of 35 mOsm/l between the means. The probability that they

TABLE 11.

Hours after transfer	Diet	No. in sample	Plasma concentration mOsm/l ∓ S.D.	t.	p.
0	Cont.	6	321 ∓ 12.3	1.57	0.15
	H.S.D.	6	334 ∓ 16.1		
24	Cont.	6	366 ∓ 11.6	0	1.0
	H.S.D.	6	366 ∓ 18.0		
45	Cont.	6	381 ∓ 21.0	1.94	0.08
	H.S.D.	7	358 ∓ 21.7		
72	Cont.	6	380 ∓ 19.0	2.2	0.05
	H.S.D.	6	361 ∓ 9.3		
96	Cont.	7	363 ∓ 12.8	0.43	0.75
	H.S.D.	5	366 ∓ 11.0		
120	Cont.	7	362 ∓ 12.2	0.29	0.85
	H.S.D.	7	364 ∓ 13.2		

were the same is still above the 5% level. By 72h the difference was similar but the smaller standard deviations results in the probability decreasing to the 5% level. There was no significant difference between the 2 diets in the remaining 2 samples.

Although not conclusive the results indicate that the H.S.D. reduces the extent of the rise in the plasma concentration during the adjustive phase. No deaths were recorded and feeding was reduced in both tanks on the second day and was only returning to normal by the end of the experiment.

Discussion

A proportion of the salt in the diet is absorbed into the blood causing a rise in the osmotic concentration. The extent of the rise varied from 12 to 18% which is lower than the figure of 19 to 34% reported by Basulto (1976) and higher than the 7% reported by Shaw et al (1975), both following a feeding of 12% salt diet to Atlantic salmon.

The trout fed on the H.S.D. have slower growth rates. If one considers, however, that the food has been diluted by the salt, then the high level of salt does not seem to have any inhibitory effect on growth. This agrees with Shaw et al (1975) and MacLeod (1978), but disagrees with the findings of Zaugg and McLain, and Basulto. They reported that a high level of salt in the diet has an inhibitory effect on food utilisation. The disparity with Zaugg and McLain may be due to the fact that their growth trials were run with salmon fry initially weighing less than 1g.

It seems reasonable to assume that if the salt has to be actively secreted from the blood this would be reflected in a decreased growth rate. The fact that the salt does not cause a reduction in growth rate is

evidence that it is not actively secreted. The salt is likely to be eliminated either via the renal system which in fresh water produces large amounts of hypoosmotic urine or by passive diffusion through the gills.

The acclimation trials show that the H.S.D. reduces the number of mortalities following transfer. This is in agreement with the increased TM_{50} values reported by Zaugg and McLain and the increased percentage survival reported by Basulto following the feeding of a H.S.D. The actual saving in fish varied but on every occasion was above 25% and the mean saving was 45% (S.D. = 12). Two weeks feeding is long enough to produce the desired physiological changes. Zaugg and McLain also reported beneficial effects after two weeks on a salt-enriched diet.

The plasma concentration measurements give a strong indication as to the effect of the H.S.D. Following transfer the plasma osmotic concentrations of the fish prior fed on the H.S.D. were consistently lower during the adjustive phase than those of the control. This results in fewer fish reaching the lethal level of 420 mOsm/l and therefore reduced levels of mortality. These findings are reinforced by the feeding activity reported in Table 8. With the knowledge that the raised plasma concentrations result in reduced feeding activity it is clear that the H.S.D. fish returned to active feeding sooner than the control fish indicating lower plasma concentrations.

How the H.S.D. produces these lower plasma osmotic concentrations is of some interest. The work of Zaugg and McLain shows that high levels of dietary salt caused a rise in the level of Na^+/K^+-stimulated ATPase

activity in the gills of salmon. Work by several authors has shown that seawater-adapted fish have much higher levels of Na^+/K^+ ATPase activity than freshwater-adapted fish (Epstein et al, 1967; Kamiya and Utida, 1968; Utida et al, 1971; Zaugg and McLain, 1970; Zaugg and Wagner, 1973; Sargent et al, 1975; Thomson and Sargent, 1977). The rise in activity following the feeding of a diet containing 12% NaCl was from 11 to 25 μ moles/mg/h; this compares with a normal sea-water level of 61 μ moles/mg/h. So it is possible that the H.S.D. is stimulating the salt secretory mechanism situated in the gills, while the fish are still in fresh water.

Mayer and Nibelle (1970), working with Anguilla anguilla showed that if freshwater-adapted fish were given a salt (NaCL) loading injection, the efflux of sodium increased while the influx decreased to bring about a return to equilibrium conditions. They concluded that any modification of the concentration of the plasma was a sufficient stimulus to evoke changes in intensity of the sodium exchanges at the level of the gill.

An increased level of salt in the blood (due to injection or inteatinal absorbtion) could either act directly on the branchial target or through an endocrine loop. Ma yer (1970) emphasised the importance of endocrine control of osmoregulation. A likely site for monitoring the osmotic, or more likely the ionic, concentration of the blood is the hypophysis. The hypophysis controls the production of prolactin and ACTH which itself stimulates interrenal cortisol production. Both prolactin and cortisol are known to play important roles in osmoregulation (Mayer 1970).

The artificially raised plasma concentration could also be reducing the water permeability of the gill or increasing the permeability of the intestine, both of which are known to occur following transfer to sea

water, again possibly via endocrine loops. The most likely explanation is that since the effects of the H.S.D. on the plasma is analog ous to what occurs following transfer to brackish water, some of the control systems, including various endocrine loops and enzyme systems, required for a sea-water existence become partially stimulated. Thus, when transfer is completed the sea-water systems are already present and their activity has only to increase and this is reflected in lower plasma concentrations and fewer deaths.

Clarke and Blackburn (1977) used the plasma ion concentration 24h after sea-water transfer as a measure of the extent of smolting in salmon. If the fish had high plasma levels they were considered not to be fully smolted; low levels and smolting was considered complete and the fish ready for entry to the sea. Smolting includes the development of the sea-water osmoregulatory systems while the fish are still resident in fresh water and is also thought to be controlled by the endocrine system (see review Hoar , 1976).

SECTION 6. HISTOLOGY OF THE CHLORIDE CELL

Introduction

As discussed in the previous section the High Salt Diet would appear to have beneficial effects on subsequent transfer to sea water by holding down the osmotic concentration during the adjustive phase. Zaugg and McLain (1969) showed that the feeding of a H.S.D. resulted in a doubling of the Na^+/K^+ ATPase activity which is associated with active secretion of ions in sea water. More recent work has shown that most of the Na^+/K^+ ATPase activity is localised in the chloride cells of the gills (Mizuhira et al., 1970; Kamiya, 1972; Shirai, 1972; Sargent et al., 1975).

The facts would, therefore, point to the chloride cells being the most likely site of salt secretion in marine fish. Utida et al. (1971) demonstrated that following transfer of eels to sea water, there was an increase in both the number of chloride cells and the level of Na^+/K^+ ATPase activity. Shirai and Utida (1970) also reported a change in the shape and size of the chloride cells as well as an increase in the number following transfer to sea water. Vickers (1961) working with the guppy Lebistes reticulatus reported that the salt load to which a fish was exposed was closely associable with both the structure of the chloride cells and with their number and distribution.

As Zaugg and McLain had demonstrated, a significant increase in Na^+/K^+ ATPase activity in salmon gills followed the feeding of a H.S.D., it was thought that an examination of the chloride cells of rainbow trout would be of interest. A light microscope study of trout gills was therefore undertaken examining freshwater and sea-water-adapted fish as well as fish which had been fed a H.S.D. while still in fresh water. As early as 1948 Copeland had shown that injecting sea water with added

salt into the digestive tract of Fundulus heteroclitus produced an observable change in the chloride cells. He even stated that on occasions, changes could be seen in the chloride cells following feeding, he called this food-enteric stimulation.

Materials and Methods

The fish which were to be sampled were first anaesthetised in a 1:10,000 solution of MS222 (Sandoz) and the second fill arch on the right side of the fish removed and washed to remove as much of the blood as possible before fixation.

The method chosen to show the chloride cells was the Altmann aniline fuchsin method for the staining of mitochondria. A similar method was used by Copeland (1948) and Shirai and Utida (1970). The exact technique used was a modified Altmann method given by Drury and Wallington (1967). This stained mitochondria, nucleoli and erythrocytes red and nuclei, cytoplasm and connective tissues pale yellow. The 3 μm sections were then counterstained with methyl green to show the nuclei (Getman, 1950).

Freshwater and seawater (for at least one month)-adapted fish of varying sizes were sampled as well as fish which had been fed on the H.S.D. for several weeks.

Results

Seawater-adapted fish

The gill filaments show a typical arrangement for teleost fish (Fig. 21), as described by many workers (see review Conte, 1969). Each filament has an eccentrically placed cartilaginous rod which lies nearer the afferent artery and is attached to the gill arch thereby providing

Figure 21. Transverse section of a primary filament from a seawater - adapted trout (x 320).

C - Cartilage

AA - Afferent artery

EA - Efferent artery

SF - Secondary filaments

Cell types:

SE - Surface epithelial

ND - Non-differentiated

MC - Mucous goblet

CC - Chloride.

Figure 22. Oblique section of a primary filament from a seawater-adapted trout (x 320).

CC - Chloride cells

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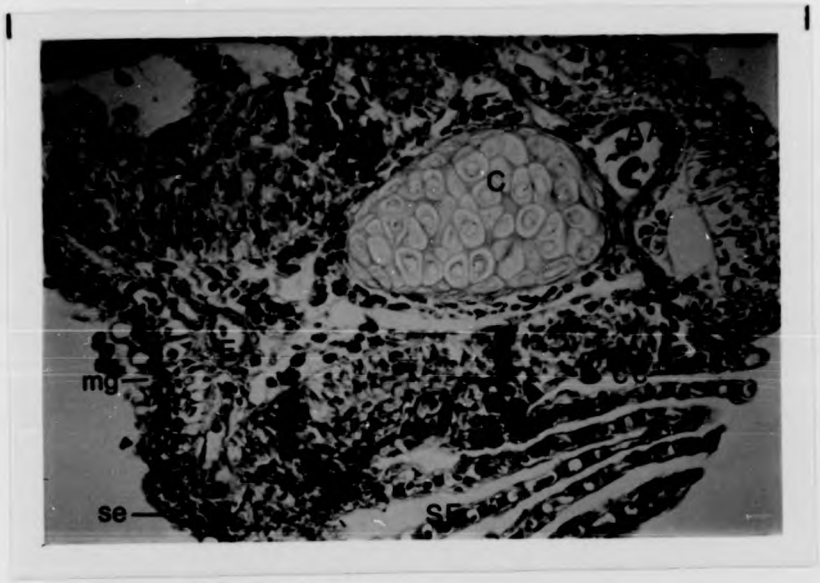
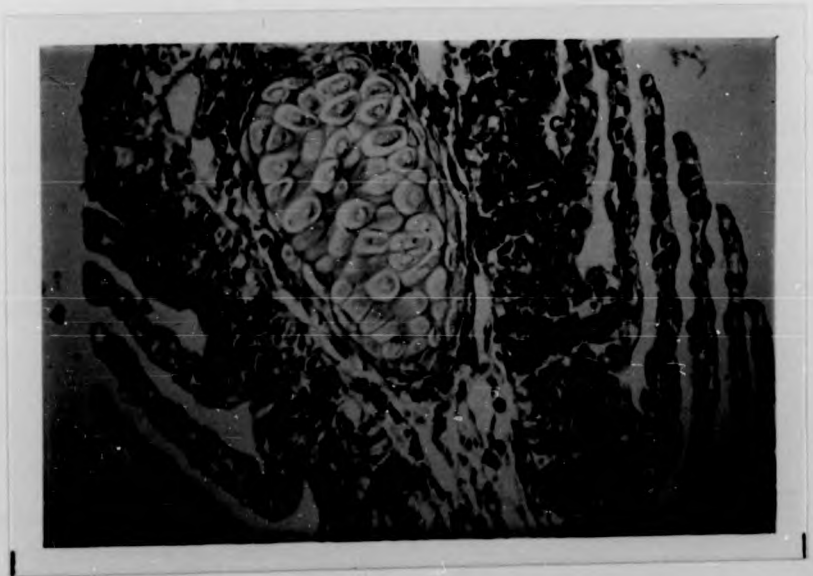
from a





support for the primary filament. There are two blood vessels in each primary filament, the afferent artery close to the cartilage and the efferent artery on the opposite side. The two arteries are connected by the respiratory or secondary filaments which were flattened leaflike structures projecting at regular intervals on either side of the primary filaments, thereby providing a large surface area for gaseous exchange. Each secondary filament consists of a network of interconnecting blood spaces delimited by pillar cells.

The epithelial cells of the primary filaments are of four types:

1) The surface epithelial of platelet cells which comprise the main surface area.
2) Small non-differentiated cells normally lying between the basement membrane and the surface epithelial cells.
3) Mucous goblet cells dispersed randomally between the surface epithelial cells, but particularly abundant at the filament tip.
4) Large cells which stained red using the Altmann method indicating a high density of mitochondria, these cells were taken to be mitochondria-rich cells, otherwise known as chloride cells.

It is the fourth group of cells which were thought to be of interest to this study. The chloride cells were large, often columnar cells up to 20 µm long, with a granular appearance (Fig. 22). They could be found anywhere on the primary filaments, but were absent from the secondary filaments, except at the bases. It was also very noticable that the chloride cells were more abundant where the afferent artery joins the respiratory filaments than the same site on the efferent side, this is in agreement with other workers (Copeland, 1948; Getman, 1950; Vickers, 1961).

support for the primary filament. There are two blood vessels in each primary filament, the afferent artery close to the cartilage and the efferent artery on the opposite side. The two arteries are connected by the respiratory or secondary filaments which were flattened leaflike structures projecting at regular intervals on either side of the primary filaments, thereby providing a large surface area for gaseous exchange. Each secondary filament consists of a network of interconnecting blood spaces delimited by pillar cells.

The epithelial cells of the primary filaments are of four types:

1) The surface epithelial of platelet cells which comprise the main surface area.
2) Small non-differentiated cells normally lying between the basement membrane and the surface epithelial cells.
3) Mucous goblet cells dispersed randomally between the surface epithelial cells, but particularly abundant at the filament tip.
4) Large cells which stained red using the Altmann method indicating a high density of mitochondria, these cells were taken to be mitochondria-rich cells, otherwise known as chloride cells.

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The fully-developed chloride cells which were often grouped together were usually in contact basally with the branchial blood supply while the apical membrane was in contact with the external medium (Fig. 23). No apical pit was observed as described in Fundulus heteroclitus by Copeland (1948), in Anguilla anguilla by Shirai and Utida (1970) and in Lebistes reticulatus by Vickers (1961), but this agrees with Threadgold and Houston (1964) working on Salmo salar. The nature, position and number of these chloride cells would therefore, indicate their active secretory role in seawater-adapted trout.

Freshwater-adapted fish

The same gill filament arrangement was observed in the freshwater-adapted fish as for the seawater-adapted ones. There were also the same four different types of epithelial cell (Fig. 24). The chloride cells on the primary filament appeared identical to those described for seawater-adapted fish; they were columnar in shape and were most abundant in vascular regions (Figs 25 & 26). The degree of staining also appeared to be identical, indicating a similar density of mitochondria within each cell.

Shirai and Utida (1970), described an eight-fold increase in the number of fully-developed chloride cells following seawater transfer of the Japanese eel. No similar increase was, however, observed with rainbow trout; as can be seen in Fig. 24 the freshwater-adapted fish had a number of fully-developed chloride cells. Freshwater trout of various sizes were sampled, the smallest being 7g, and similar densities of chloride cells found in all cases.

The only observable difference between the chloride cells of freshwater and seawater-adapted fish was that in the former, chloride cells were common on the secondary filaments (Figs 24 & 25), but were

Figure 23. Base of a secondary filament from a seawater-adapted trout (x 1250).

C - Cartilage

CC - Chloride cell

E - Erythrocyte.

Figure 24. Oblique section of a primary gill filament of a freshwater-adapted trout (x 320).

C - Cartilage

AA - Afferent artery

CC - Chloride cells.

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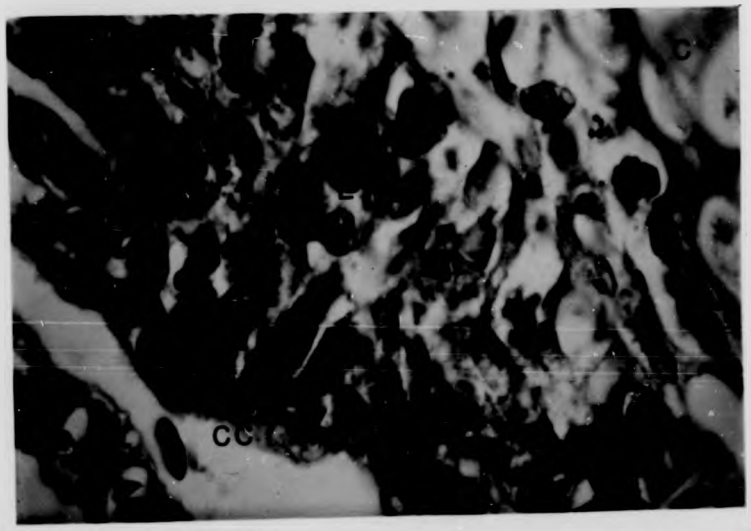


Figure 25. Oblique section through a primary filament of a freshwater-adapted trout (x 500).

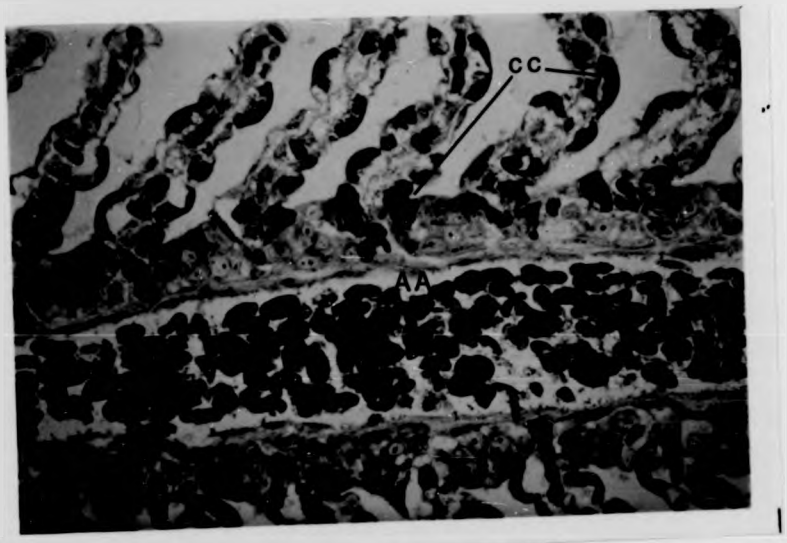
AA - Afferent artery

CC - Chloride cells.

Figure 26. Base of a secondary filament of a freshwater-adapted trout (x 1250).

AA - Afferent artery

CC - Chloride cells.


CC
AA


CC
AA

absent there in seawater-adapted trout. These chloride cells were more ovoid than those on the primary filaments and they usually had a convex apical membrane (Fig. 25 & 26).

Fish fed on the H.S.D. for up to six weeks had identical chloride cells on their primary filaments as control fed fish. They also had ovoid chloride cells on their secondary filaments. It was difficult to estimate the relative abundance of the chloride cells on the secondary filaments in the control and H.S.D. fish, but it appeared equal in both cases.

Discussion

The presence of well developed chloride cells in the gills of freshwater fish has been described by Datta-Munshi (1964) in Catla catla. by Coleman et al., (1977) in Tilapia aurea and by Morgan and Tovell (1973) in Salmo gairdneri. Sargent et al., (1978) working with Anguilla anguilla also described chloride cells in freshwater-adapted eels.

Pettengill and Copeland (1948) first proposed that chloride cells in the gills of Fundulus heteroclitus were responsible for the active uptake of ions in fresh water. Recent reviews by Maetz (1971 and 1976), present the evidence for NH_4^+/Na^+ (or H^+/Na^+) and HCO_3^-/CL^- exchange pumps most likely situated in the chloride cells. If such exchanges were occuring at the apical membrane, and Shirai (1972) demonstrated a concentration of Na^+ in the mucous layer of freshwater chloride cells, a large surface area for active absorption would be desirable. The need for a large surface area could explain the convex nature of the apical membrane on the chloride cells of the freshwater-adapted fish and the microvilli described by Morgan and Tovell (1973) also working with rainbow trout. The large ovoid chloride cells on the secondary filaments

could, therefore, provide a large surface area for the collection of Na^+ and Cl^- ions from the surrounding medium. The ingestion of Na^+ and Cl^- from the H.S.D. should have led to a decrease in the number of ions required to be taken-up by the gill and therefore possibly a decrease in the number of chloride cells on the secondary filaments. This, however, did not seem to occur, although an ultrastructural examination would be of interest, for Threadgold and Houston (1964), described ultrastructural changes that occurred within the chloride cells following smolting in salmon.

The large increase in the number of chloride cells following transfer to sea water, as described in the eel species Anguilla anguilla by Sargent et al., (1978) and Anguilla japonica by Shirai and Utida (1970), does not seem to occur in rainbow trout. Coleman et al., (1977) reported that the number of chloride cells also remains constant following seawater transfer of Tilapia aurea. The absence of chloride cells on the secondary filaments after transfer would, however, indicate a change in their role. Most of the chloride cells in the seawater-adapted fish are columnar cells collected in groups at the bases of the secondary filaments adjacent to the afferent branchial artery. The exact biochemical means of transport across the chloride cells in sea water is still under investigation, but a recent model proposed by Sargent et al., (1978) suggests that the fluid in the spaces between adjacent chloride cells is concentrated by active pumps basally and laterally situated on the cells until the fluid becomes more concentrated than the external sea water. Diffusion of NaCl ions then occurs outwards through the ion permeable junction between chloride cells. The model, therefore, requires groups of columnar chloride cells basally in contact with the blood and apically with the sea water, exactly as seen in this study.

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DISCUSSION - Practical applications

Some of the findings presented in this thesis have practical applications for the marine trout farmer. Fig. 11 is a nomogram from which, knowing the salinity and the size of the fish, it is possible to estimate the expected level of mortality following transfer to the sea. Although the nomogram is less accurate at predicting high levels of mortality, fish farmers are more concerned with lower levels of mortality below 10%, and within this range the nomogram is more accurate. It is of most use at sites where reduced salinities occur.

The nomogram also shows, as does the physiological evidence, that there is a range over which a drastic rise in mortality occurs for a small change in either the salinity or size. Taking for example the case of a farmer transferring his fish into full-strength sea water (33°/oo), 80g fish should suffer very little mortality, 60g fish should still be below the 10% level, but a reduction to 50g would result in a mortality around 30%. It is therefore unwise for the farmer to transfer his fish under 60g and conversely there is little advantage in holding back 80g fish until they reach 100g.

The physiological evidence and the nomogram were combined to produce Fig. 13, which illustrates the effect of transportation. Prior transportation means that fish must be double the size of non-transported fish to experience the same survival. In Fig. 13, 25g fish with no prior transportation suffered a mortality of about 8% when transferred to 30°/oo sea water. In contrast, the same mortality was suffered by 50g fish if they had undergone prior transportation. It is therefore important that the farmer ensures that transportation stresses are kept to a minimum.

Evidence is also presented which suggests that if possible summer should be avoided when making transfers. Some farmers at present wait till they are forced to make a transfer by rising temperatures and falling flow rates with the intention of transferring the fish at the maximum size. This can result in transfers being made in July and August with often unexpectedly high mortalities (Cannon, pers. comm.). The results in Table 1 would indicate that fish transferred in June have similar mortalities to those transferred in August despite their considerably smaller size.

Section 4, on the value of a wet diet, demonstrates that there is little advantage in feeding a wet diet directly after transfer and that it will have no beneficial effect on the mortality. There might be a long term advantage in feeding a wet diet but this was not investigated.

The High Salt Diet would, however, appear to reduce mortalities following transfer and it is therefore worth examining whether it has a practical application. The cost of the H.S.D. is the same as a normal diet, for although there is a reduction in the cost of the ingredients the manufacturers at present charge an additional manufacturing cost. Growth on the H.S.D. is slower as discussed in Section 5. However, 2 weeks on the diet is adequate to produce the reduction in mortality. Thus, a farmer wishing to transfer his trout at a weight of 60g should change to a H.S.D. when they weighed 52g. The growth lost during the 2 weeks of feeding the H.S.D. can be calculated, assuming a food conversion ratio of 1:1.8 and a feeding rate of 2% body weight per day. For the equations used see Section 5.

On a control diet

$$\text{Specific Growth Rate} = \frac{2}{1.8}\%/\text{day}$$

$$\therefore \text{S.G.R} = 1.1\%/\text{day}$$

The factor by which weight increases in 14 days = 1.165

$\therefore$ Weight at transfer = 60.6g.

On a H.S.D. (10%)

$$\text{Specific Growth Rate} = \frac{1.8}{1.8}$$

$$\therefore \text{S.G.R.} = 1.0\%/\text{day}$$

The factor by which weight increases in 14 days = 1.15

$\therefore$ Weight at transfer = 59.8g.

$\therefore$ Weight lost by feeding H.S.D. for 14 days = 0.8g.

The fish fed on the H.S.D. return to active feeding after transfer several days before the control fish. Two days of normal feeding will more than make-up the loss of 0.8g, so there is no overall reduction in weight caused by the H.S.D.

Continuing the example, if a farm produces about 50,000kg of 250g trout per annum then 200,000 60g trout will need to be transferred into the sea. If the level of mortality is normally 10% then 20,000 fish will be lost. By feeding the H.S.D. fewer fish will die; taking the saving to be 30% only 14,000 fish will be lost. The 6,000 extra fish represent an immediate monetary saving of about £ 500 (1978 prices) and at harvest will yield extra fish weighing 1500kg and worth about £ 2000.

The H.S.D. can, therefore, be used to increase the annual production of a farm. Also for the same mortality fish which have been on a H.S.D. can be transferred into full-strength sea water at 5 to 10g lighter, which at a feeding rate of 2% is 10 to 20 days sooner. As discussed in

On a control diet

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the introduction, there is considerable pressure to transfer trout into the sea at the smallest possible size with acceptable mortalities. Although not obviating the problem entirely, a H.S.D. could be of assistance in reducing mortality rates of small fish.

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APPENDIX I. Dechlorination Filter

A 100 l plastic bin was converted into an activated charcoal dechlorinator. The movement of the water through the charcoal was by gravity and the required delivery was 2-3 l/min. If the flow rate fell below this figure the filter was backwashed for $\frac{1}{2}$ h. Every 2 months the filter was dug-out and the activated charcoal replaced. The chlorine levels of the water entering the filter varied from about 0.1 ppm to below the level of detection using the DPD method (APHA et al., 1975) about 0.02 ppm. The water leaving the filter had no detectable chlorine and the fish fed actively and showed no signs of depressed activity as described by Dandy (1972). It was therefore assumed that the level in the tanks must have been below 0.005 ppm. Seegert and Brooks (1978) using a charcoal depth of 0.2 m and a flow rate of 4.5 l/min reduced the chlorine level from 0.13 ppm to 0.025 ppm even after 3 months in service.

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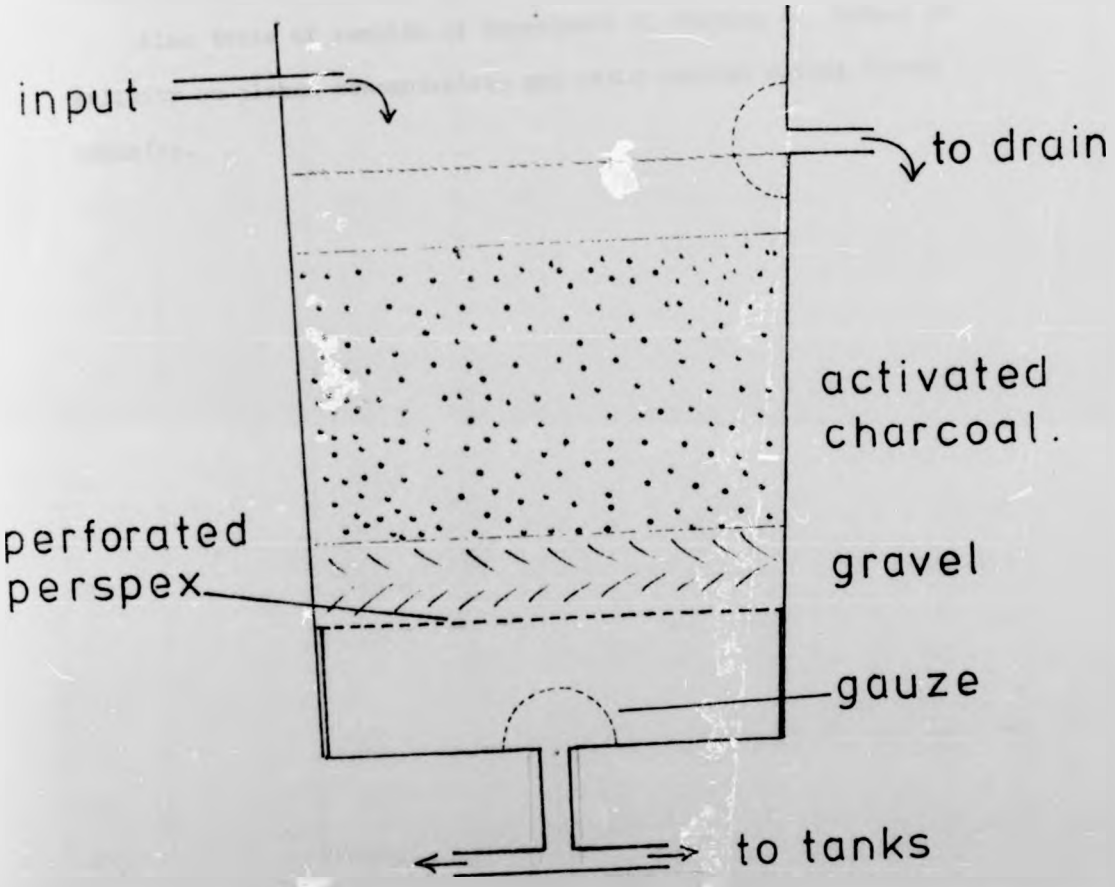

APPENDIX II

Table of results of Experiment 4, Section 1. Effect of size on plasma concentration and water content during direct transfer. Three different size ranges were used.

Also table of results of Experiment 5, Section 1. Effect of salinity on plasma concentration and water content during direct transfer.

ACCLIMATION OF SMALL FISH

Time hours	No. in sample	Mean wt. gms.	Mean lng. cms.	% Water ± S.D.	Osmolarity ± S.D. mOsm/l.
0	7	12.7	10.5	77.4 ± 1.4	316 ± 3.9
12	8	10.8	9.0	75.3 ± 0.8	383 ± 16.7
24	6	10.9	10.7	74.1 ± 0.8	411 ± 3.6
48	6	11.7	10.7	74.1 ± 0.6	398 ± 13.5
72	6	10.6	10.1	76.2 ± 0.6	372 ± 21
168	6	10.7	10.2	77.3 ± 1.7	359 ± 10.8
0	5	24.8	13.4	77.0 ± 1.5	305 ± 3.9
12	6	25.6	13.6	74.6 ± 1.2	353 ± 9.2
24	6	21.6	13.1	74.5 ± 1.5	373 ± 10.6
52	6	22.1	13.3	73.7 ± 0.9	374 ± 18.0
94	6	23.8	13.3	75.7 ± 0.7	366 ± 17.2
214	6	22.2	12.8	77.4 ± 0.9	329 ± 10.8
0	6	39.1	15.0	76.2 ± 1.0	309 ± 2.6
12	6	35.7	15.3	73.7 ± 0.7	344 ± 12.7
25	6	37.2	15.5	75.3 ± 0.8	366 ± 19.7
48	6	40.1	15.8	75.2 ± 2.0	344 ± 18.1
96	6	39.5	15.6	76.6 ± 1.0	348 ± 13.8
215	6	44.7	16.1	76.5 ± 1.5	345 ± 13.2

EFFECT OF SALINITY

Time hours	No. in sample	Mean wt. g.	Mean lng. cms.	% Water ± S.D.	Osmolarity ± S.D. mOsm/l.
15 p.p.t.					
0	6	33.1	14.4	76.9 ± 0.5	310 ± 3.9
12	7	32.9	14.4	75.9 ± 0.6	327 ± 10.6
24	6	26.9	13.8	75.6 ± 0.7	350 ± 9.0
48	6	29.1	13.9	76.5 ± 0.5	346 ± 15.2
98	6	31.6	14.0	77.0 ± 0.6	340 ± 7.0
190	4	30.2	14.1	76.9 ± 0.4	339 ± 6.0
28 p.p.t.					
0	6	29.3	13.8	77.1 ± 0.7	312 ± 4.5
12	6	28.7	14.3	75.1 ± 0.8	340 ± 7.3
23	7	30.1	14.1	74.6 ± 1.7	367 ± 10.5
48	6	31.5	14.2	74.9 ± 1.2	370 ± 16.5
96	6	34.0	14.6	76.9 ± 0.6	344 ± 13.0
214	4	31.8	14.4	77.2 ± 0.9	344 ± 10.1
32 p.p.t.					
0	6	33.2	14.4	77.0 ± 0.6	309 ± 5.3
12	6	29.7	14.2	75.3 ± 1.1	379 ± 12.9
24	6	31.2	14.4	74.5 ± 0.8	392 ± 12.5
48	6	28.8	13.9	75.1 ± 1.3	397 ± 18.0
96	6	34.5	14.6	76.0 ± 0.8	372 ± 20.9
214	6	32.9	14.4	76.7 ± 0.7	350 ± 13.8

Recommended Feeding Rates for Rainbow Trout in % of Total Fish Weight per Day

Water temp. °C.	Fish Weight. Grams: 1.5 - 5	5 - 12	12 - 25	25 - 40	40 - 60	60 - 90	90 - 135	135 - 200	200 - 250
5	2.3	1.8	1.6	1.4	1.2	1.0	0.9	0.8	0.7
7	2.7	2.3	1.8	1.6	1.4	1.2	1.1	1.0	0.9
9	3.2	2.6	2.1	1.9	1.6	1.4	1.3	1.2	1.1
11	3.7	3.0	2.4	2.1	1.9	1.6	1.5	1.4	1.3
13	4.3	3.5	2.7	2.5	2.2	1.9	1.7	1.6	1.5
15	5.1	4.0	3.2	2.8	2.5	2.2	2.0	1.9	1.8
17	5.3	4.3	3.5	3.0	2.6	2.3	2.1	2.0	1.9
19	2.8	2.5	1.9	1.7	1.5	1.2	1.1	1.0	0.9
21	2.2	1.9	1.5	1.3	1.1	0.9	0.8	0.7	0.6

APPENDIX IV.

Graph of results from cage acclimatisation trials presented in Tables 1 and 2. The figures next to each point are the percentage mortalities from each trial. From this graph the nomogram (Figure 11) can be constructed. Due to the discrepancy on either side of each mortality line the mortality scale on the nomogram is marked in ranges indicating that very accurate predictions cannot be made particularly for high levels of mortality.