Photoperiodic manipulation and its use in the all year round production of Atlantic salmon, *Salmo salar*.

A thesis submitted to the University of Stirling

for the degree of Doctor of Philosophy

by

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Declaration.

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged the work described in this thesis has been conducted independently and has not been submitted for any other degree.

Signature of candidate: ..... Neil J. Duncan .....

Date: ..... 6th June 1997 .....

N. Bernaye (Supervisor)
7th June 1997

Abstract.

The Atlantic salmon (*Salmo salar*) has two developmental processes which are clearly seasonal; smoltification is restricted to the spring and maturation to late autumn. In the farming industry the seasonality of juvenile production is primarily responsible for the seasonal production in market-sized fish. Both smoltification and maturation appear to be controlled by mechanisms timed by photoperiod. This thesis investigates the effects of photoperiod on smoltification and maturation. The performance of both out-of-season eggs and particularly smolts are closely examined and the possible application of photoperiod techniques in the industry discussed.

Both potential S1 and S2 parr were subjected to natural photoperiods or a range of artificial photoperiods under constant and ambient temperature conditions. S1 and S2 smolts reared under natural photoperiods were transferred to sea water during April and May. A 2-3 month period of short days followed by a period of long days was observed to advance smoltification; 0+ and 1+ smolts were transferred to sea during December (4 month advance) and October (6 month advance), respectively. A 12 month seasonal photocycle delayed by 3 months delayed smoltification by 2 months.

Out-of-season smolts were produced in January, March, April, May, June, July, October, November and December. All groups of out-of-season smolts exhibited the same growth potential as natural smolts. The different transfer date of out-of-season smolts therefore resulted in different harvest periods compared to natural smolts. The age at maturity of out-of-season smolts was positively related to the length of the seawater growing period prior to the completion of maturation. Out-of-season smolts exhibited increased maturation in association with an increased size (compared to natural smolts) for a particular time of year. These large maturing fish were generally harvested as superior salmon prior to flesh deterioration. Mortality during the first month in sea water was significantly higher in a number of out-of-season smolt groups compared to

natural smolts. This was considered to be related to site-specfic environmental conditions rather than the smoltification process.

Smoltification was also examined under constant photoperiods and a 4 week period of short daylengths. Dissociation and suppression of certain aspects of smoltification were observed. The different aspects of smoltification: the development of bimodality, hypoosmoregulatory ability, decrease in condition and smolt coloration appeared to be controlled by different independent mechanisms. The development of bimodality, hypoosmoregulatory ability and possibly coloration appeared to be controlled by independent endogenous rhythms. The decrease in condition factor, associated with the parr-smolt transformation required a period of short daylength greater than 2 months followed by a period of long daylength.

Smoltification and maturation were examined in salmon retained in constant 10°C borehole water. Smolts held in fresh water grew steadily and matured producing eggs and sperm both in- and out-of-season. There was, however, a period of increased mortality after smoltification and egg quality was reduced especially in fish subjected to photoperiod manipulation. The salmon responded to photoperiodic manipulation. Abrupt changes in photoperiod advanced spawning by 12 weeks and a reciprocal seasonal photoperiod (6 month out of phase with a natural photoperiod) advanced spawning by 22 weeks. The maturation process in the Atlantic salmon would appear to be controlled by similar mechanisms to those described for the rainbow trout. Freshwater holding conditions appeared to decrease the age at maturity and reduce egg quality. The progeny of freshwater broodstock (F1 generation) successfully completed smoltification and seawater transfer.

Photoperiodic manipulation can be used to produce out-of-season smolts and eggs. Through the use of photoperiod, farms could increase and target production. This could remove the seasonality from the production cycle and help stabilise the pricing structure for market salmon.

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Much of the project was carried out in conjunction with farms. Thank you to all the staff at these farms who helped and gave technical assistance. In particular I would like to thank the following: At Terregles Salmon, Jake Elliott, Eric (Eck) Baxter and especially Vincent (Big Vinny) Muir who made me welcome in his home where we regularly sorted out the salmon farming industries problems, unfortunately we rarely remembered the solutions in the morning; Alan Pendred, Iain and Duncan McLoad of Summer Isles Salmon, who gave excellent highland hospitality; Rob Gardiner of Shetland Sea Farms; Iain Webster of West Coast salmon; Niall Auchinachie and Paul Finnagin at Buckieburn; Rob Murray at Howietoun and lastly at Machrihanish Marine Laboratory, I thank Dr. Bill Roy, Simon (the wizard) Barnett and Fraser Griffiths for their assistance. I found writing the thesis particularly difficult and I sincerely thank my supervisor Prof. Niall Bromage, who's help was invaluable (and annoyingly correct). I must also thank Janet (Mum) Duncan and Ana Roque for their help. Lastly I should thank all my friends who have offered support and encouragement; I will mention some but thank you to you all:- Michael Bruce, Briony Campbell, Ann Gilmore, Andy Shinn, Serawut Klingbunga, Mark Porter, Clive Randall, Penpun Serisakultiew, James Bron, Brian Howie, Graeme Taylor, David Nickell, Charlie Harrower, Luis Carrasco, Bill Gaze, Jane Lewis, Steve Powell, etc.....

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I feel I should dedicate this thesis to myself
as I worked so hard on it, but that would be too much.

**I dedicate this thesis to the wild Atlantic salmon,
who wishes I was never born.**

Chapter 1. General Introduction.

1.1 Atlantic Salmon Life History.

The Atlantic salmon is native to the North Atlantic and once inhabited rivers from latitudes 41 to 60 degrees north, on the east coast of North America and 40 to 70 degrees north on the west coast of Europe. However, as a result of pollution and over fishing the salmon has all but disappeared from the highly populated areas in the lower latitudes. Today the only large salmon runs which remain are in Canada, Russia, Norway, Sweden, Scotland and Ireland.

The Atlantic salmon is an anadromous fish with a complex and variable life history. The life cycle begins in the autumn when the adult salmon spawn into redds, a gravel hole dug by the female. The eggs are fertilised and buried. The time from fertilisation to hatching is dependent on water temperature and takes approximately 500 degree days. In Scotland this period lasts approximately 80 to 90 days, whilst in the north of Norway incubation can take 180 days.

During the spring as the water temperature begins to rise the alevins hatch from the eggs. The alevins remain in the gravel redd utilising their large yolk sacs. In Scotland, it is early April when the young fish, fry emerge from the gravel and begin to actively feed. The fry grow rapidly and in the summer become parr. Development of the parr depends on the opportunities for growth, principally the availability of food in the environment and the length of the growing season (latitude dependant). Aspects of the parr's growth rate and acquisition of stored energy are believed to influence whether parr mature in fresh water and the age at which parr undergo smoltification (Thorpe 1986, 1989, 1994).

Smoltification refers to the changes in morphology, physiology and behaviour which take place when the parr changes from a freshwater fish to a seawater fish. Whether the

parr undergoes smoltification is thought to be dependant on attaining a certain critical size and/or physiological-biochemical threshold during late summer / early autumn (Thorpe 1986, 1989). Parr which attain the size and/or physiological-biochemical threshold continue to feed during the winter months, whilst parr which do not attain the threshold reduce foraging and avoid predation. The parr-smolt transformation takes place in the spring, the smolts migrate down the river systems and enter the sea in large numbers when food availability in the sea is high. The period of residence in the river is therefore determined by the number of years required to reach the size/physiological-biochemical threshold. This residence time varies from 1-4 or more years. A productive river in the lower latitudes will produce a high percentage of 1 year old smolts (S1's) whilst a river with poor productivity in the high latitudes will produce 2 (S2's) or 3 (S3's) year old smolts and a low percentage of 1 year old smolts; seven year old smolts have been recorded in northern Norway.

Precocious maturation (the term commonly used to describe maturation during the parr stage) is a common strategy adopted by male parr. Male parr which have missed the threshold for smoltification will again actively forage for food in the spring. Whether these parr undergo precocious maturation is thought to be dependant on attaining a certain size and / or physiological-biochemical threshold during the spring / early summer (Rowe & Thorpe 1990). Parr which attain this threshold in the spring, mature in the autumn. The percentage of parr maturing is again thought to depend on the productivity of the river system and the attainment of a genetically pre-determined threshold (Thorpe 1986, 1994). Precocious parr use 'sneaking' tactics to fertilise the adults eggs which are only released in the presence of a mature adult male. Precocious parr which survive the autumn spawning have been observed to transform into smolts during the following spring (Saunders *et al.* 1994).

On leaving the river environment the smolts begin a long migration to rich feeding grounds near Greenland, Iceland and northern Norway. Here they feed on shrimps and young fish before returning to their natal river to spawn. The number of winters spent at

sea can vary from 1 to 6 or 7 in some cases. Fish returning after one sea winter are called grilse or one sea winter fish and range in size from 1.5-3 kg, maiden two sea winter fish range from 3-7 kg, and maiden three sea winter fish from 7-12 kg. In late autumn / early winter after the return migration to the headwaters of the river, the female adult excavates a redd. Accompanied by a mature male the two fish spawn into the redd, which the female then covers with gravel. Clearly the varying age at maturity and different periods of freshwater and seawater residence highlight the extremly flexible life history of the Atlantic salmon.

There are advantages and disadvantages to the many possible life strategies. Combinations of long or short residences in the river or seawater environments are possible. The two environments offering different advantages and disadvantages. The river environment often has low predation and poor food availability, while the sea environment is rich in food, with high predation. The river and sea environments interact to select the genetic make up of a rivers stock and hence the life strategies which the stock adopts. Ultimately the selection pressures on the stock determine the life strategies which will produce the highest survival through to the next generation.

1.2 Life Cycle of Farmed Atlantic Salmon.

When the present study was initiated in 1990, few farms were attempting to manipulate the natural life cycle. Although the selection of low maturing stocks and the high availability of food to the farmed fish had resulted in differences in the life cycle, the seasonal timing of maturation and smoltification in the farmed salmon's life cycle were generally the same as in the natural environment.

On the farm the maturation of broodstock fish is still generally carried out under natural environmental conditions. The broodstock, therefore, mature in the autumn and are either moved to fresh water or retained in sea water during final maturation. Under farm conditions the broodstock will not release ova or milt, these must be stripped from the fish by applying pressure to the abdomen. The ova are fertilised and placed in incubation

trays, where a slow flow of water up-wells through the eggs. The incubation period for the ova is temperature dependant, the holding water is commonly heated to reduce the incubation time to approximately 50 days.

This practice of incubating eggs in heated water is quite recent and heated water is often also utilised through the alevin and fry stage until late spring when ambient water temperatures rise. Under these conditions the fry quickly develop into parr. During the autumn the parr are size graded into potential S1 and S2 parr. The high growth rates achieved under farm conditions result in high proportion of potential S1 parr, usually > 90%. Many farms consider it to be uneconomical to grow S2 smolts and the potential S2 parr are culled. The culling of potential S2 parr and the increasing proportion of potential S1 fish, due to better farm practices and the use of heated water during the early stages, has resulted in a gradual decline in S2 production (figure 1.1). The methods researched in the present work and other studies and the improved production practices have resulted in the increasing production of 0+ smolts (smolts less than 1 year old) (figure 1.1). However, the majority of a farms produce S1's under natural photoperiod conditions.

The majority of the smolts produced are transferred directly to sea water during the spring. The fish grow rapidly in the sea and after 1 winter in the sea a proportion of the fish begin to mature as grilse. The proportion which mature is dependant on the growth of the fish (Thorpe 1986, Thorpe *et al.* 1990) and the genetic stock (Herbinger & Newkirk 1990). Maturing grilse can be identified by coloration during late May - early June. During late July the flesh quality of the maturing grilse begins to deteriorate as body reserves are directed into gonadal growth (Aksnes 1986), maturing fish harvested after July do not command a high price from the fish processor. The farmer, therefore, selects and harvests the grilse (grilse grade and harvest) from late May to early July. This can result in fish being harvested at a size which is considered uneconomical. In the past research has focused on avoiding maturation by genetic means, selecting low maturing stock or producing female triploids. However, improved production facilities

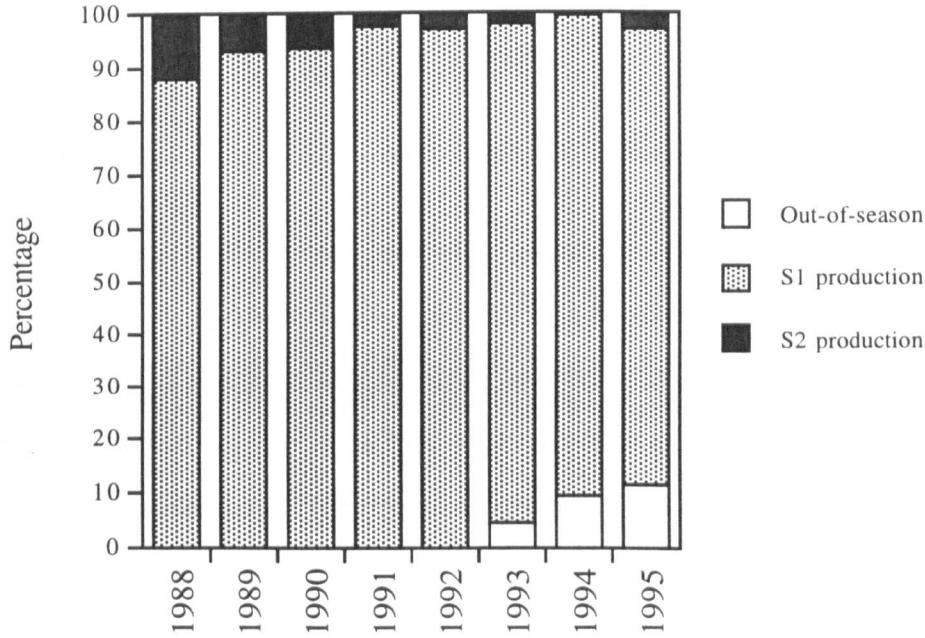


Figure 1.1 The percentage of the total smolt production for each year produced as out-of-season smolts (open bar), 1 year old smolts (S1, speckled bar) and two year old smolts (S2, filled bar). Data from The Scottish Office, Agriculture, Environment and Fisheries Department (1988-1995).

and particularly improved high oil feeds have enabled farmers to produce 3-4 kg grilse and the early harvest of grilse is no longer considered to be a problem.

When this study was initiated the majority of market-sized fish (2-4 kg) were produced in 18-24 months. With the use of high oil diets the majority of market-sized fish (2-4 kg) are now produced in 12-18 months. This is demonstrated by the increasing percentage of a year class harvested during the year following transfer (figure 1.2) and the increasing average harvest size of these fish (figure 1.2). This difference in production time for fish grown in sea water should be considered if the early results from this study are compared with present or future growth profiles.

1.3 Production Problems and Aims of the Study.

The salmon farming industry has grown rapidly both in Scotland and the other salmon farming nations particularly Norway and Chile. In Scotland production has increased from 6,921 tonnes in 1985 to 70,060 tonnes in 1995. However, this rapid expansion has resulted in periods of overproduction when the price of salmon has collapsed. The salmon price first crashed in 1989 and has subsequently fell during 1991, 1993 and 1994 (Sutherland 1992, Jaffa 1994, Ritson 1994, Chamberland 1994) (figure 1.3). The problems of overproduction and the associated price instability are not uncommon in the agriculture industry, particularly where livestock have a long period of growth before attaining market size.

The farmed salmon production cycle of 1.5-2.5 years requires that production targets must be established 1-2 years prior to the production being realised, possibly when the market price for salmon is good. Ritson (1994) pointed out that when smolts were transferred into the sea in 1992 salmon prices were 50% higher than those actually realised when the fish were harvested towards the end of 1993. Co-ordination of the industry's production to meet a correctly predicted market demand is difficult and the imbalance of these two factors have resulted in periods of both over- and underproduction and major price instability.

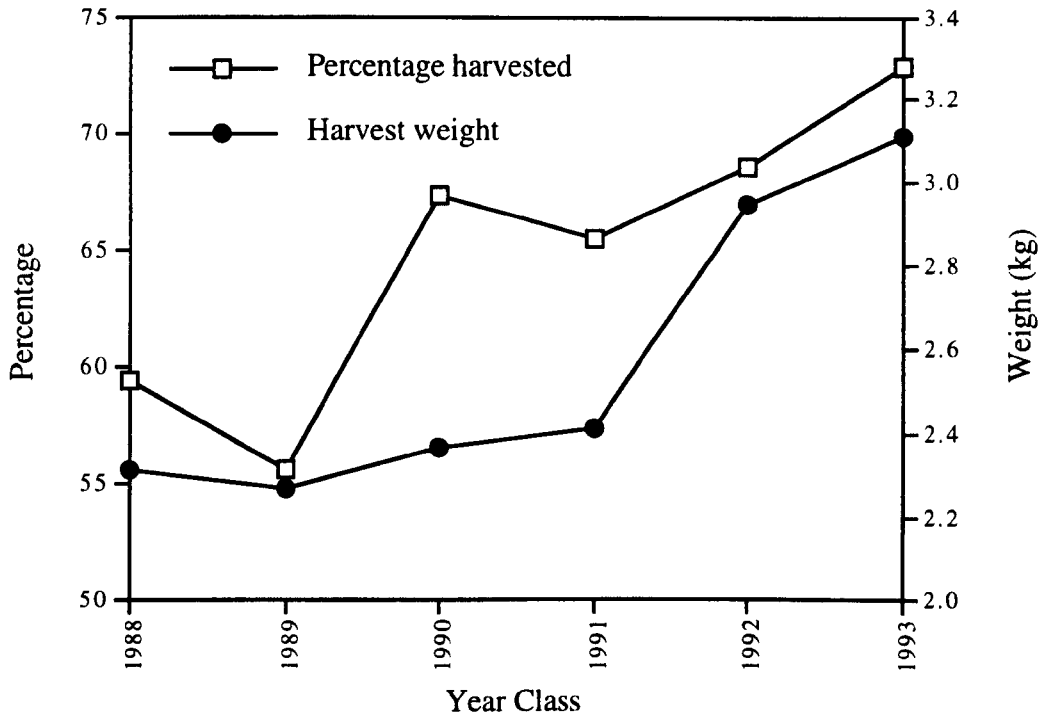


Figure 1.2 The percentage of a year class (year of entry to sea) of fish harvested during the year following transfer to sea water and the mean harvest weight of the fish harvested during the year following transfer to sea water. Data from The Scottish Office, Agriculture, Environment and Fisheries Department (1995).

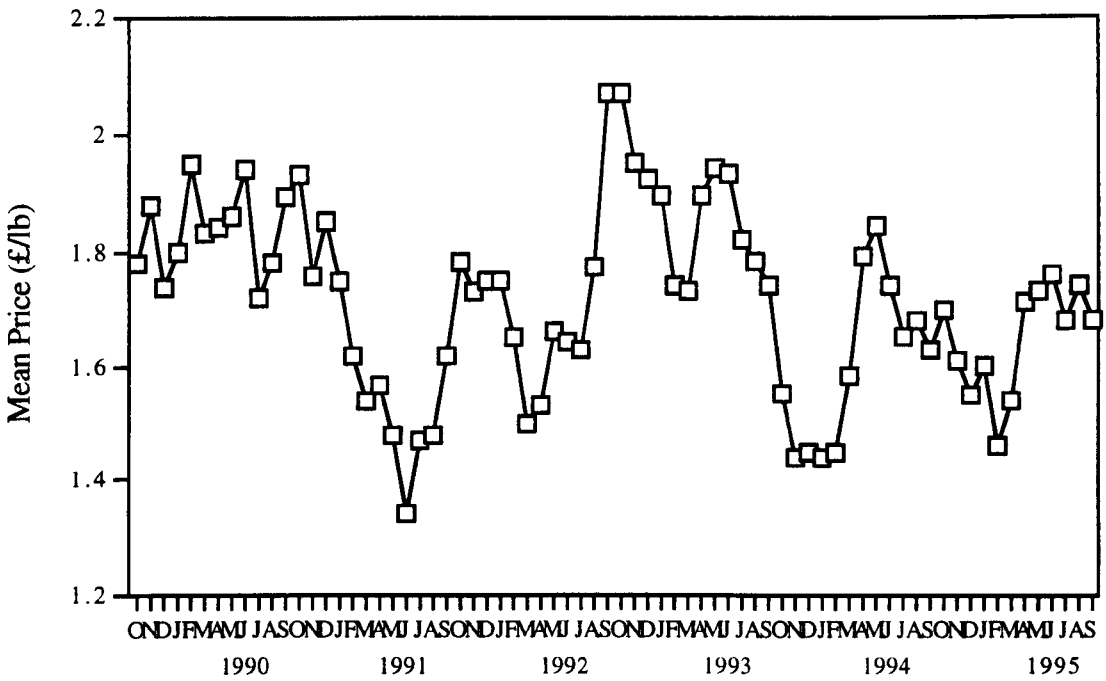


Figure 1.3 Change in monthly mean price (£/lb) of Scottish farmed salmon sold through Billingsgate fish wholesale market. Figures supplied by the Fishmongers Society (1990-1995).

Marketing strategies to manipulate the market have been suggested as a solution (Sedgwick 1990, Jaffa 1994). Although marketing can increase demand it would be difficult to manipulate the market to meet the fluctuations in supplies. Chamberland (1994) indicated that although marketing was important it could not provide a solution to the problem of price instability.

The price instability is supply induced and Chamberland (1994) suggested that a solution must smooth out the fluctuations in supply. However, this is difficult to achieve when the product is perishable, as in the case of fresh salmon. Non-perishable foods can be stored during periods of overproduction and released during periods of underproduction. This approach would work for frozen salmon. However, the frozen salmon market is a different market compared with fresh salmon and commands lower prices. Perishable foods can not be stored for release when there is a shortage in supply. It has been suggested that producer organisations should be set up to co-ordinate production (Chamberland 1994, Ritson 1994), enabling the industry to collectively meet predicted market demand. A producer organisation could influence the annual production by controlling smolt input. However, this overlooks the seasonal fluctuation in availability of product (Duncan 1995, Ritson 1994) which is a major contributory factor to the price instability. The seasonal fluctuations can be attributed principally to the seasonal supplies of eggs and smolts.

The trout industry was afflicted by similar problems of fluctuation in supply. However, the use of out-of-season eggs, produced both in the UK and abroad, has allowed the industry to stabilise, in part, its seasonal production. This, in conjunction with producer organisations and centralised processing facilities, has lead to a more stable pricing structure for the trout industry.

After the initial collapse of salmon prices in 1989, Jake Elliott and Willie Baxter of Terregles Salmon Ltd approached Niall Bromage and Mark Thrush, who were examining aspects of the photoperiodic control of smoltification. It was decided to set up a project

to investigate the use of photoperiodic manipulation to increase the window of smolt supply. The aim of the project was to smooth out the seasonal fluctuations in supply of market sized 2-4 kg salmon. Late in 1990 the project was initiated as a PhD study, which resulted in the production of this thesis. The aims of the study were as follows:-

1) To investigate the mechanisms involved in the photoperiodic control of smoltification, establishing photoperiod techniques for the production of out-of-season smolts.

2) To examine the potential seawater performance of out-of-season smolts.

3) To investigate the use of photoperiod in the manipulation of reproduction and the production of out-of-season eggs.

Chapter 2. General Materials and Methods

2.1 Fish

All experiments were carried out on Atlantic salmon *Salmo salar*. Different stocks were used in certain experiments, however all stocks used were commonly farmed strains of Atlantic salmon of mixed Scottish and Norwegian origin. These farmed stocks are considered medium grilsing strains with grilsing rates of 15-20% under sea cage culture conditions. Both diploid and triploid stocks were used in experiment 4. A triploid stock was used in experiment 3. All the other experiments were conducted on diploid stock.

2.2 Holding Facilities.

2.2.1 Freshwater Holding Facilities.

2.2.1.1 Site FW1.

The site was located 56° north, with a gravity fed water supply from a reservoir. The water supply was ambient with temperatures ranging from 1-15°C. The hatchery had heating facilities which had the capability of raising the ambient temperature by 8-9°C

Three sizes of tank were used 1m ($0.25m^3$) tank, 1.5m ($0.7m^3$) tank, and 5m ($25m^3$) tank. The water was supplied through an angled elbow joint to give a circular flow. Out flow in the 1 and 1.5m tanks was through a central stand pipe with a sleeve which induced a self cleaning flow. The 5m tank had a central drain which was connected to an external stand pipe. Stocking densities varied but never exceeded $30kg.m^{-3}$. Flow rates were maintained which gave oxygen readings in excess of 6.5-7 $mg.l^{-1}$ at the outflow. Oxygenation and aeration was used during the warm summer months.

Each tank was covered with a black light-proof cover. The light source the fish experienced in the 1m and 1.5m tanks was from a 60 watt tungsten light suspended 0.5m above the water's surface giving a light intensity of 60 lux at the water's surface. The 5m

tanks were illuminated with two banks of two 'daylight' neon tubes (4 tubes) giving a light intensity at the water's surface of 250 lux. The lights were controlled by individual electronic timers or a light sensitive switch which mirrored the natural daylength. The lights were switched on at full intensity with no twilight period. The on/off times of the two banks of lights in the 5m tanks were staggered by 15 minutes, to provide an initial period of illumination with a light intensity at the water's surface of 125lux.

The fish were fed by automatic feeders and by hand feeds. Trouw and BOCM Pauls feeds were used at rates according to the manufacturers feed tables.

2.2.1.2 Site FW2.

Site FW2 was situated 55° north, the water supply was pumped borehole water which had a constant temperature of 10±1°C. The water was supersaturated with nitrogen. Degassing towers were used which reduced the super saturation to 101-103%.

The fish were held in concrete raceways or earth ponds. Three sizes of raceway were used 1.5m^3 (4.5x0.8x0.6m), 7m^3 (10.6x1.4x0.8m) and 18m^3 (25x1.4x0.8); each raceway was gravity fed, the outlet pipe was adjustable to set the water level in the raceway. The raceways were stocked at 30-60kg/m^3 to facilitate feeding and self-cleaning. Flows were adjusted to maintain oxygen levels in excess of 50% saturation at the outlet.

The ponds had a volume of 38m^3 and were gravity fed with second use water which varied from 10°C by ± 3°C. Stocking densities were similar to the raceways. The second use water had oxygen levels of 70-80%. The resulting flows were higher to maintain an oxygen level at the outlet of 50% saturation.

The raceways were housed in light-proof sheds which had dual light sources, 100 watt tungsten lighting which gave 20 lux at the water's surface and 400 watt metal halide floodlights which gave 600 lux at the water's surface. Either light source could be used, each being controlled by a pre-set electronic timer or a light sensitive switch which

mirrored the natural daylength. The fish housed in the light proof sheds were not subjected to a twilight period. The ponds and a number of raceways were uncovered, the only illumination being natural daylight.

The fish were hand fed at 1-2 hourly intervals. Both Ewos and Biomar feeds were used in accordance to the manufacturers guides and tables.

The site appeared to have a water quality problem, possibly related to gas supersaturation or heavy metals. The problem possibly affected the health of the fish; gill damage was identified in health reports.

2.2.2 Seawater holding facilities.

2.2.2.1 Site SW1.

The fish were transported to the site by road in a 0.7m^3 transport tank. The fish were stocked to a maximum of 70 $kg.m^{-3}$, the transport tank was continuously aerated. The journey from site FW1 was approximately 3-4 hours. The journey from site FW2 was approximately 5-6 hours.

Site SW1 was a land-based site situated 56° north on the west coast of Scotland. The sea water was pumped into a central holding tank and gravity fed to the fish holding tanks. The salinity averaged 33‰ and the temperature ranged from 4-15°C.

The fish were held in 3m (7m^3) tanks which had a central drain connected to an external standpipe. Stocking densities were kept below 20$kg.m^{-3}$. Flows varied in relation to the head of water which was available; flows in excess of 0.5 $l.min^{-1}.kg^{-1}$ of fish were always maintained. Each tank was, on a daily basis, checked for mortality and cleaned. All tanks were continuously aerated. The tanks had no photoperiod covers, the only light source was natural daylight.

The fish were fed BOCM Pauls commercial salmon diets to demand. This was in the range 0.1-1.5% body weight.day^{-1}, a reduced rate in comparison with the manufacturers tables.

2.2.2.2 Site SW2.

The fish were transported to the site by road in 6 x 2m^3 transport tanks. The fish were stocked to a maximum of 150-170 kg.m^{-3}; the transport tanks were aerated and oxygenated for the initial 0.5 hour of transport, after which the aeration was stopped. The journey from site FW2 was approximately 8-10 hours. On arrival at site SW2 the fish were transported to the cages in a bucket suspended below a helicopter. The helicopter journey was approximately 5 minutes.

Site SW2 was a cage site 57° north on the west coast of Scotland. A mixture of 12m and 15m cages were employed. The site had a good tidal exchange of full strength 32‰ sea water which had a temperature range of 7-14°C.

Each cage was initially stocked with 15000-20000 smolts. The biomass of the cages were always maintained below 15000kg by transferring fish to new cages. Cages were checked for mortalities by divers 2-3 days after stocking and then once a week for the first month. The fish were fed Biomar commercial salmon diets at a reduced rate (0-1%) to that recommended in the manufacturers tables.

The site had a high disease risk (furunculosis and pancreas disease); consequently fed rates were reduced and crowding stress was kept to a minimum. The fish could not be crowded for sample weighing especially during periods of warm water temperatures.

2.2.2.3 Site SW3.

The fish were transported to the site by road and well boat. During the road transport to the well boat the fish were stocked to a maximum of 150-170 kg.m^{-3} in 6 x 2m^3 transport tanks. The transport tanks were aerated and oxygenated for the initial 0.5 hour of the journey, after which the aeration was stopped. The journey to the well boat was

approximately 2 hours, on arrival the fish were piped by gravity into the wells of the well boat. The well boat had two wells which were stocked with approximately 57000 fish. Each well contained oxygenated sea water. Once the boat was making passage a flow of sea water was maintained through the well and the oxygenation was stopped. The well boat trip was approximately 36 hours; on arrival the fish were pumped into the cages.

Site SW3 consisted of 3 cage sites 62° north on the west coast of the Shetland Isles. These sites employed 12m, 15m and 20m cages. The sites all had a tidal exchange of oceanic water which had an average salinity of 33‰ and a temperature range of 6-13°C.

The 12m and 15m cages were stocked with 15000-20000 smolts and the biomass was maintained below 20000kg. Larger fish were transferred to the 20m cages where the biomass was maintained below 45000kg. Cages were checked for mortalities by divers 2-3 days after stocking and then once a week for the first month.

The fish were fed Biomar commercial salmon diets in accordance to the manufacturers tables.

2.2.2.4 Site SW4.

The fish were transported to the site by road in 6 x $2m^3$ transport tanks. The fish were stocked to a maximum of 150-170 $kg.m^{-3}$. The transport tanks were aerated and oxygenated for the initial 0.5 hour of transport, after which the aeration was stopped. The journey from site FW1 was approximately 3-4 hours.

Site SW4 was a land-based site situated 56° north on the west coast of Scotland. The sea water was pumped into a central header tank and then to the fish holding tanks. The salinity averaged 34‰ and the temperature ranged from 4.5-14°C.

The fish holding tanks were 18.5m ($1000m^3$) and stocking densities were maintained below $52kg.m^{-3}$. The water was oxygenated both in the header tank and the fish holding tanks. Oxygenation of the fish holding tank was computer monitored; the out-flow was kept in the range 8-8.5ppm. A daily check was made for mortalities.

The fish were fed Ewos diets at 90% satiation (demand). Automatic feeders were used in combination with lighting to allow feeding over an 18 hour period. The lighting was a combination of 500 watt halogen and 70 watt Son (Sodium) lights which enabled the fish to feed during darkness. The lighting resulted in a dual photoperiod, the natural photoperiod and a night illumination giving an effective photoperiod of LD18:6. It is unclear whether the fish responded to the natural or LD18:6 photoperiod.

2.3 Fish Sampling.

During the course of all the experiments fish were regularly sampled for weight, length and/or blood sampled. Fish were starved for 24 hours before any sampling procedure was carried out. Random samples of fish were netted from the populations, which were crowded into a confined area. The netted samples were then placed in a holding tank. and measured and/or blood sampled.

In circular tanks and raceways the stand pipe was removed or lowered to reduce the water level and crowd the fish. In raceways and the ponds a seine net or mesh screen was also used to crowd the fish to one end of the raceway/pond. The fish held in sea cages were crowded using a seine net and a sample taken from the population in the enclosed area.

2.3.1 Anaesthesia.

To enable the fish to be accurately measured for weight and length, or blood sampled, the fish were anaesthetised. A solution of 1ppt of 2-phenoxyethanol (Sigma Chemicals Company Ltd, Poole, Dorset, UK) was used to anaesthetise the fish. A suitable volume was made up depending on the size of the fish. The fish were rendered unconscious after approximately two minutes in the anaesthetic. Recovery usually occurred in approximately 5 minutes in a well aerated recovery tank. Anaesthetic solutions were made up to the same concentration with fresh water, sea water and artificial sea water.

2.3.2 Growth.

At the sites FW1, FW2 and SW1 the fish were anaesthetised and weighed individually to the nearest 0.1 g (Sartorius L12000S balance; Sartorious Instruments Ltd, Epsom, Surrey, UK.). Individual weighing of fish was not possible on cage sites (unless conditions were flat calm) due to the movement of the cages. At site SW3 batch weights were obtained for batches of 10-20 fish. At site SW2, crowding the fish was not possible as the site was considered to have a high disease risk and therefore any practice which may have stressed the fish was avoided. The sea site managers in charge of the cage sites recorded a monthly sample weight for each cage of fish. These farm sample weights were obtained from either a batch weight taken from fish caught whilst feeding and/or an estimated weight taken from the size of the fish and their feed requirement. These farm sample weights were close to weights obtained from a random sample and were used when it was not possible to obtain a random sample. Farm sample weights were used for site SW4.

2.3.2.1 Specific Growth Rate.

In a number of experiments the specific growth rate (SGR) for the period between sampling dates was calculated and recorded. The SGR or percent increase in body weight per day was calculated with the equation below.

$$SGR = (\ln W2 - \ln W1) \cdot 100 \cdot T^{-1}$$

SGR = specific growth rate; W2 = final weight expressed in g; W1 = initial weight expressed in g; T = the time interval between the sampling for W1 and W2 expressed in days.

2.3.3 Smoltification.

2.3.3.1 Condition factor.

The parr to smolt transformation is marked by a fall in condition factor (Hoar 1939). A fall in condition factor, in association with growth, can be used as an indication of the

parr to smolt transformation. The condition factor was monitored in all experiments examining the change from parr to smolts.

The weight and fork length of the fish were accurately determined and used to calculate the condition factor. Fish smaller than 100g were weighed to the nearest 0.1g while fish greater than 100g were weighed to the nearest 1g. Fork length from the nose of the fish to the fork of the tail was measured to the nearest mm. The condition factor was then calculated using the equation below.

$$k = w \cdot 100 \cdot l^{-3} \qquad \text{(Bolger \& Connolly 1989)}$$

k = Condition factor, w = weight (g), l = length (cm)

In order to obtain a profile of changes in condition factor the fish were sampled every 2-3 weeks during the experiments.

During the measurement of fork length and weight, the visibility of parr marks and the presence of darkened fin edges were noted; these observations were not quantitative. A score was attributed to the fish as follows:- 1- parr, no silvering; 2- parr with some silvering; 3- per-smolt, silver, parr marks just visible; 4- smolt, parr marks not visible. The health status of the fish was also observed, evidence of fin damage was noted.

2.3.3.2 Seawater Challenge Test.

During the parr to smolt transformation, the ability of the fish to osmoregulate increases (Folmar and Dickhoff 1980). This change was monitored using a standardised seawater challenge as outlined by Clarke and Blackburn (1977) and Blackburn and Clark (1987). Fish with a good osmoregulatory ability will actively drink water whilst excreting salt ions to maintain a low blood Na ion and Cl ion level or osmolality (320-330 mOsmo.kg^{-1} a level similar to an unchallenged fish). A fish with poor osmoregulatory ability will not prevent fluid being lost whilst excessive salt ions enter the fish to give the blood a high Na ion and Cl ion level or osmolality.

As a single test provides no indication of changes in the ability of the fish to osmoregulate, fish were tested at 2-3 weekly intervals during experiments which examined the change from parr to smolt.

Procedure

1. Prior to the test the fish were starved for 24 hours.

2. A tank of 50 litres of well aerated 28‰ sea water was made up using artificial sea salt (Instant Ocean, Animal House, Batley, UK). Salinity is measured in ppt; therefore 28g of artificial sea salt was dissolved in 1 litre of pure water to give a salinity of 28‰. The tank was maintained at 10°C with a water bath or heaters/coolers. The artificial sea water was left for at least 1 hour to fully dissolve. It was often convenient to leave the artificial sea water overnight to fully dissolve.

3. The bottom of the tank was checked to ensure the salt had fully dissolved. If undissolved salt was found the tank was stirred and left a further hour. When fully dissolved the salinity was checked with a salinity refractometer.

4. The tank was then stocked with 10-15 fish (approx. $10g.l^{-1}$) and left for 24 hours.

5. After the 24 hours each fish was anaesthetised and blotted dry using tissue. Plastic gloves were worn. A blood sample in excess of 0.2ml was removed from the caudal vein using a 1 ml disposable syringe (Terumo, Fisher Science, Loughborough, UK) and a 21 gauge needle (Terumo, Fisher Science, Loughborough, UK). The blood sample was then placed in a 1ml eppendorf (Sarstedt, Life Sciences Int., Basingstoke, UK). During this procedure care was taken not to contaminate the sample with sea water.

6. Each blood sample was immediately refrigerated or put on ice.

7. Within 12 hours each sample was centrifuged at 14000 rpm for 5 minutes. The clear serum was removed using a pasteur pipette and placed in a 1ml eppendorf (Sarstedt, Life Sciences Int., Basingstoke, UK). The red blood cells were discarded.

8. If the sample was not analysed immediately it was stored at -20°C.

9. The osmolality of the sample was then determined using a freezing point depression osmometer (micro-osmometer, model 3MO, Advanced Instruments Inc., Norwood, MA, USA). Each sample was measured twice. A line standard was tested each time samples were analysed.

2.3.3.3 Seawater Survival Test.

The sea water survival test as first described by Komourdjian (1976) is another useful test to determine the osmoregulatory ability of a fish. The survival of the fish in high 37.5-40‰ sea water is determined over a 96 hour period. Fish with poor osmoregulatory ability die (0% survival) while fish with a good osmoregulatory ability survive (100% survival). This test was useful as a final check of the osmoregulatory ability of the fish before transfer and as an assessment of the changing ability of the fish to osmoregulate during the parr to smolt transformation.

Procedure

1. Prior to the test the fish were starved for 24 hours.

2. A tank of 100 litres of well aerated high salinity (37.5‰) sea water was made up by adding artificial sea salt (Instant Ocean, Animal House, Batley, UK) to fresh water. The artificial sea salt was left for at least 1 hour to fully dissolve. In order to maintain the tank at the same temperature as the holding water of the fish, the tank was placed in a second larger tank or raceway which was used as a flow through water bath.

3. The bottom of the tank was checked to ensure the salt had fully dissolved. If undissolved salt was found the tank was stirred and left a further hour. When fully dissolved the salinity was checked with a salinity refractometer.

4. The tank was then stocked with 10-15 fish (approx. $5g.l^{-1}$) and left for 96 hours. The fish were checked every 12 hours and any mortalities were removed and recorded.

2.3.3.4 Assessment of Smoltification.

During the parr-smolt transformation of Atlantic salmon, the fish change morphologically and physiologically (see reviews Wedermeyer *et al* 1980, Hoar 1988). Morphologically, the condition factor decreases significantly and the fish develop the appearance of a smolt. Physiologically, the fish develop the ability to hypo-osmoregulate. To determine when smoltification was complete the condition factor, coloration and serum osmolality, after a 24 hour seawater challenge (28‰), were monitored. A significant decrease in condition factor to a value close to 0.9 (Hoar 1939) indicated the fish had the morphology of a smolt. A smolt coloration, silver with darkened fin edges, is not quantifiable. However, an experienced fish farmer or scientist can easily distinguish a smolt from a parr. A fully developed smolt appearance was recognised and recorded. A challenged serum osmolality within the range of 330-350 $mOsm.kg^{-1}$, the expected value of a smolt or unchallenged parr (Duston & Saunders 1990), showed the fish had the hypo-osmoregulatory ability of a smolt. These 3 parameters were used to identify the completion of the parr-smolt transformation. The fish were transferred to sea water when smoltification was considered complete.

In all experiments condition factor measurements and seawater challenge tests or seawater survival tests were carried out at intervals of 2-3 weeks. Sampling in each experimental group was initiated before the start of the 'spring' increase in the photoperiod and continued until the fish were transferred to a seawater holding facility or the experiment was terminated. The appearance of the fish and the maturity (presence of milt when pressure was applied to the abdomen) were also assessed during sampling.

2.3.4 Maturation.

2.3.4.1 GSI determination.

In a number of experiments in conjunction with the above assessment of maturity, gonadosomatic index (GSI) was calculated and recorded. For the calculation of the GSI fish were anaesthetised and killed with a blow to the head. The fish were then dried and

weighed to the nearest 0.1g. The gonads of the fish were removed and weighed to the nearest 0.1g. The GSI was calculated with the equation below and expressed as a percentage.

$$GSI = W2 \times 100 / W1$$

GSI = gonadosomatic index, W1 = total fish weight, W2 = gonad weight.

2.3.4.2 Assessment of Maturation.

During all sampling procedures the fish were assessed for maturation. Male parr were recorded as mature if milt ran from the sperm duct when pressure was applied to the abdomen. Fish which had been transferred to sea water were assessed to be mature by appearance. During the months from June-November mature fish are recognisable from their colouring, a purple/brown colour with a large round belly and protruding oviduct in the females and a red/brown colour with a kype in the males. At site SW2 the assessment of maturation rate was carried out in June/July when the fish were grilse graded (the removal of grilse, fish which mature during the second maturation episode, from the cage population). The grilse were harvested, while the salmon were kept for on growing. At sites SW1 and SW3 the number of mature fish present in the random weight sample was recorded.

The time of completion of maturation, ovulation in females and milt production in males, was assessed by checking the fish at two weekly intervals. Under farm conditions Atlantic salmon will not oviposition or release milt. The correct time for stripping must be assessed and the eggs/milt stripped from the fish. The date milt ran freely from the males sperm duct when pressure was applied to the abdomen was recorded. At two week intervals the females were checked for tightness and shape of the belly to determine if the eggs had been ovulated, released into the abdomen. Females which had released eggs into the abdomen were stripped into a separate dry bowl. Each female was weighed before and after the eggs were stripped. The eggs were fertilised with the pooled milt from two males. Milt was checked for motility under x100 magnification; 1ml of milt

was used to fertilise 1 litre of eggs. Water was added to the fertilised eggs which were left 30 minutes to water harden. During hardening, the temperature was maintained at the same temperature as the holding water of the fish. The dry volume of water hardened eggs from each fish was recorded and the number of eggs on a horizontal 25cm egg measure were counted. From the above measurements the below parameters were calculated and recorded.

egg diameter	$ED = 250 \cdot E25^{-1}$
Eggs / litre	$EL = 10^{(-0.283 \cdot ED+5.41)}$ (Von Bayer 1950)
Total fecundity	$Tf = Tv \cdot EL$
Relative fecundity	$Rf = Tf \cdot W2$

Were; E25 = number of eggs on a 25 cm egg measure; ED = egg diameter in mm; EL = eggs per litre; Tf = total fecundity, number of eggs per fish; Tv = the total volume of hardened eggs obtained from a fish; Rf = relative fecundity, number of eggs per kilogram of somatic weight; W2 = post stripped weight of fish in kg.

The eggs were laid out in up-welling hatchery trays supported in a hatching trough. The development of the eggs and alevins were monitored to the end of the first month of first feeding. Survival during the following stages was recorded, fertilisation to hatching, hatching to first feeding and the first month of first feeding.

2.4 Statistics.

All means have been expressed ± 1 standard mean error.

2.4.1 Comparison of two samples.

The two sample distributions were tested for normality by calculating the correlation of the sample with normal scores (Minitab release 8.1). This is equivalent to the Shapiro-Wilk test (Shapiro and Wilk, 1965). The samples variance was tested and compared

using the F-test (Snedecor and Cochran, 1980). If the sample distributions satisfied the normality and variance tests, the sample means were compared using a Student's t-test (Minitab release 8.1). If the sample distributions did not satisfy the tests for normality (at ∂=0.01), or similar variance ($p<0.05$), the sample distributions were compared using the non-parametric Mann-Witney test (Minitab release 8.1).

2.4.2 Multiple comparison.

The sample distributions were tested for normality (Shapiro and Wilk, 1965) and for homogeneity of variance using the Bartletts test (Snedecor and Cochran, 1980). If the sample distributions satisfied the normality and homogeneity tests, the sample means were compared using a one way analysis of variance (ANOVA). A parametric multiple comparisons t-test using the residual mean square from the ANOVA was used to identify specific differences between sample means. If the sample distributions did not satisfy the tests for normality (at ∂=0.01), or homogeneity ($p<0.05$), the sample distributions were compared using Kruskal-Wallis test followed by DUNN'S multiple range procedure (Zar 1984) to identify specific differences between sample distributions.

2.4.3 Comparison of proportions.

Proportions or percentages were compared by calculating a 95% confidence limits for each proportion (Fowler and Cohen 1987). If the confidence limits did not overlap, the proportions were considered significantly different ($P<0.05$).

2.4.4 Correlation.

Correlation's were made between data sets using the Pearson's paired correlation (Minitab release 8.1). The critical value r was compared (at ∂=0.05) with a table of critical values for the linear correlation coefficient.

Chapter 3. Photoperiodic Manipulation of Smoltification.

3.1 Introduction.

Smoltification is a complicated process involving an array of morphological, physiological and behavioural changes which result in the juvenile Atlantic salmon changing from a parr adapted for residence in a freshwater (hypo-osmotic) environment to a smolt adapted for an oceanic (hyper-osmotic) environment. Smoltification has been extensively reviewed, see reviews Wedemeyer *et al.* 1980, Folmar & Dickhoff 1980, Langdon 1988 and Hoar 1988.

The smoltification process is thought to begin in the summer / autumn approximately 9-12 months prior to the completion of smoltification. During the autumn, populations of Atlantic salmon parr in Scottish (Thorpe 1977, Thorpe *et al.* 1980, 1982), Canadian (Bailey *et al.* 1980, Saunders *et al.* 1982) and Norwegian (Knutsson & Grav 1976) hatcheries and in the wild (Bagliniere & Maisse 1985, Nicieza *et al.* 1991) have been observed to form a bimodal size distribution. The parr which are destined to smoltify during the following spring form the upper mode and those which defer smoltification for another year form the lower mode. Observations of the fish in the two modes have shown that upper mode fish exhibit a higher growth rate compared to the lower mode fish (Thorpe 1977, Thorpe *et al.* 1982, Kristinsson *et al.* 1985). Kristinsson *et al.* (1985) demonstrated that fish in the upper mode exhibited the highest growth rates, both before and after the bimodal distribution was established, and suggested that the bimodal distribution was formed as a result of diverging growth rates within the population.

The divergence of growth rates has been attributed to a 95% drop in appetite in the lower mode fish between September and December (Metcalfe & Thorpe 1992). In the wild the lower mode fish spend the winter hiding under rocks. Higgins & Talbot (1985) showed that the low appetite persisted in the lower mode fish even when food was supplied in

excess. In contrast the upper mode fish maintained and possibly increased (Kristinsson *et al.* 1985) appetite and growth over the same period. It would appear that there is a 'decision' period during the summer, when parr destined to smoltify the following spring make a developmental decision to continue to feed and grow through the winter, whilst parr destined to defer smoltification to another year make a developmental decision to lose appetite and hide from predators. This 'decision' period results in the observed divergence of growth rates which in turn results in the formation of a bimodal size distribution during the autumn.

The 'decision' to maintain feeding and growth has been related to a critical size (Elson 1957, Thorpe *et al.* 1980, Evans *et al.* 1984). However, Thorpe (1986) suggested that fish are not aware of their size. Thorpe (1986, 1989) and Saunders (1986) proposed that a physiological / biochemical state related to growth rate or energy storage, must exceed a genetically pre-determined threshold before growth is maintained or increased.

In support of the suggested genetic component, variation in the percentage of parr recruited into the upper mode has been observed between families maintained under identical growth conditions (Thorpe *et al.* 1980). The hypothesis also suggests that the proportion which form each mode is related to the opportunity available to the fish to attain the physiological / biochemical threshold. Studies have demonstrated that both increased daylength (Villarreal *et al.* 1988, Thorpe *et al.* 1989, Saunders *et al.* 1989) and increased temperature (Thorpe *et al.* 1989) increase the percentage of parr recruited to the upper mode. Thorpe *et al.* (1989) related opportunity for growth (thermal sum including hours of daylight and °C) to the percentage of parr contained in the upper mode and concluded that recruitment to the upper mode was dependant on the past opportunity for growth. This has also been demonstrated in wild stocks (Metcalfe & Thorpe 1990) where 82% of the variation in smolt age between rivers can be explained by an index of growth opportunity which included temperature and the change in daylength with latitude.

In addition to affecting the percentage of parr included in the upper mode, photoperiod has also been shown to affect the timing of the development of the bimodal distribution (Villarreal *et al.* 1988, Duston & Saunders 1992). Parr subjected to an accelerated photoperiod developed bimodality prior to a control group (Duston & Saunders 1992), whilst parr subjected to a decelerated photoperiod developed bimodality after the control group (Villarreal *et al.* 1988, Duston & Saunders 1992). Both Villarreal *et al.* (1988) and Duston and Saunders (1992) concluded that bimodality developed under a decreasing photoperiod and was controlled by an endogenous rhythm which was entrained by photoperiod.

After a bimodal size distribution has been established, recruitment into the upper mode group has been observed to continue (Kristinsson *et al.* 1985, Duston & Saunders 1992). Kristinsson *et al.* (1985) suggested that recruitment into the upper mode continued until the water temperature dropped below 10°C. Duston and Saunders (1992) observed that if growing conditions were favourable, recruitment into the upper mode continued until the photoperiod began the spring increase.

It would, therefore, appear that as the autumn photoperiod is decreasing a 'decision', based on a genetically pre-determined threshold, is made to maintain feeding and growth or to suppress appetite and hide during the winter. The combination of attaining the physiological threshold and continued feeding during the winter should ensure the parr are large enough and have sufficient energy stored to complete, the energetically costly, parr-smolt transformation.

The juvenile salmon in the upper mode change from a parr to a smolt in a series of morphological, physiological and behavioural changes. This parr-smolt transformation is observed during the spring following the development of bimodality.

In the wild, the differences in behaviour between parr and smolts are related to the change from a territorial parr maintaining a feeding position in a fast flowing environment to a smolt which must migrate down the river system to the estuary. Parr maintain a

position in a fast flowing environment by actively swimming against the current and sheltering behind boulders, whilst smolts maintain a higher position in the water column and passively and/or actively migrate downstream with the water flow. Smolts have been observed to exert 4x less effort in swimming compared to parr (Thorpe & Morgan 1978, Wankowksi & Thorpe 1979). This was confirmed by the finding that parr swam to exhaustion in a flow chamber (Kutty & Saunders 1973). The higher position in the water column, maintained by smolts, has been attributed to an increased swim bladder volume in smolts compared to parr (Saunders 1965).

Morphologically, parr and smolts are very different. The parr is an olive green fish with distinct parr marks (bars of dark green) on the lateral surface. The smolt has a silvery appearance with a silver grey dorsal surface, a white ventral surface and darkened fin edges. During the parr-smolt transformation the olive green coloration visible in the parr is gradually obscured by the deposition of silvery purines (quanine and hypoxabthine) in the scales and the upper dermis (Johnston & Eales 1967, 1968). Another visible change is that of the ratio of length to weight. The smolt is a more streamlined slimmer fish. The change in length to weight ratio is manifest as a decrease in the condition factor (section 2.3.3.1) as the parr changes to a smolt (Hoar 1939). In coho the decrease in condition factor was attributed to an increase in the length of the post-anal region (Winans & Nishioka 1987).

The behavioural and morphological changes described indicate the extreme physiological changes the juvenile salmon goes through during the parr-smolt transformation. Other physiological changes include: changes in metabolism and growth rate (Higgins 1985) and changes in the ionic regulation of the fish (Folmar & Dickoff 1980, Wedemeyer *et al.* 1980, McCormick *et al.* 1987).

Smolts exhibit higher metabolic rates compared to parr (Higgins 1985). The increased metabolic rate has been associated with increased oxygen consumption (Higgins 1985), increased growth rates (Higgins 1985) and the catabolism of stored lipid (Sheridan 1989). The catabolism of stored lipids is in turn associated with changes in the body

composition: reduced total body lipid (Komourdjian *et al.* 1976, Sheridan 1989), decreased liver and muscle glycogen (Sweeting & McKeown 1989, Mayer *et al.* 1994) and increased body moisture content (Komourdjian *et al.* 1976); and in plasma composition: changes in glucose, amino acid nitrogen and free fatty acids (Sweeting *et al.* 1985, Sweeting & McKeown 1989). It has been suggested that the energy from the catabolism of stored lipids is channelled into the hypoosmoregulatory adjustment (Sheridan 1989), as similar catabolism of lipids has been observed in association with seawater exposure (Woo *et al.* 1978).

The increased hypoosmoregulatory ability observed in the smolt is perhaps the most important physiological change and pre-adapts the smolt for a hyper-osmotic seawater environment (Folmar & Dickoff 1980). The change from a hypo-osmotic environment to a hyper-osmotic environment requires that the juvenile salmon change from a parr with the ability to excrete excess water whilst retaining salts, to a smolt with the ability to excrete salts whilst retaining water (hypoosmoregulatory ability).

During the parr-smolt transformation an increase in the number and size of chloride cells has been observed in the gills of the juvenile salmon (Langdon 1983, Langdon & Thorpe 1985, Lubin *et al.* 1991). The chloride cell has been implicated as the site of Na^+ K^+ ATPase activity (Foskett & Scheffey 1982) which is involved in the active transport of ions from the blood into the external medium (Hoar & Randall 1984). The increase in Na^+ K^+ ATPase activity during the parr-smolt transformation has been recorded in a number of studies (Folmar & Dickhoff 1980, Wedemeyer *et al.* 1980, Langdon 1988, Hoar 1988).

Few studies have examined the hypoosmoregulatory changes associated with the intestines and kidney; these aspects are poorly understood. During the parr-smolt transformation there was no increase in the Na^+ K^+ ATPase activity associated with the kidney either in fresh water (McCartney 1976, McCormick *et al.* 1989) or when transferred to sea water (McCormick *et al.* 1989). The kidney probably has a minor role in the hypoosmoregulation of the smolt. However, Eddy and Talbot (1985) showed that

urine production doubled during the parr-smolt transformation (urine production was not measured in sea water). It was suggested that the increased urine production was a response to the drinking of water. Veillette *et al.* (1993) observed that fluid transport across the posterior gut wall increased during the parr-smolt transformation. Fish in a hyper-osmotic environment drink water to replace water lost through body membranes. Smolts undergo pre-adaptive changes for a hyper-osmotic environment whilst still in the hypo-osmotic fresh water. Reduced sodium and chloride ion concentrations (Houston & Threadgold 1963) and increased body moisture content (Komourdjian *et al.* 1976) have been observed in smolts prior to transfer into sea water.

Many studies have examined the increase in hypoosmoregulatory ability by examining the osmoregulatory capability of the fish using a 24 hour seawater challenge test (section 2.3.3.2) or survival in high salinity sea water (section 2.3.3.3). Increased hypoosmoregulatory ability has been recorded in many studies (Komourdjian *et al.* 1976, Saunders & Henderson 1978, Saunders *et al.* 1985).

These multiple changes which represent the parr-smolt transformation are associated with a surge of endocrine activity. Changes in plasma thyroid (Dickoff *et al.* 1982, Grau *et al.* 1982, Boeuf *et al.* 1985, Virtanen & Soivio 1985, Boeuf *et al.* 1989, Specter *et al.* 1989), plasma prolactin (Prunet & Boeuf 1985, Prunet & Boeuf 1989), growth hormone (Clarke *et al.* 1989, Bjornsson *et al.* 1989, Boeuf *et al.* 1989, Schmitz *et al.* 1994), cortisol (Veillette *et al.* 1993, Cornell *et al.* 1994) and insulin (Mayer *et al.* 1994) have been recorded during the parr-smolt transformation. This endocrine activity is considered part of the mechanism which controls the timing and instigates the changes associated with smoltification.

Smoltification, in common with many seasonal biological processes at temperate latitudes, is closely associated with the seasonally-changing daylength. Photoperiod is strongly implicated as the environmental control of smoltification, probably acting through the endocrine system. Photoperiod has been shown to be a cue for the parr-smolt transformation, in steelhead trout (Wagner 1974, Zaugg & Wagner 1973), Atlantic

salmon (Saunders & Henderson 1970, 1978, Komourdjian 1976), coho salmon (Clarke *et al.* 1981) and masu salmon (Okumoto *et al.* 1989).

Saunders and Henderson (1970), in an early study, showed that Atlantic salmon parr subjected to a reciprocal photoperiod beginning in March did not complete the parr-smolt transformation, a decrease in condition factor was not observed. Two later studies (Komourdjian 1976, Saunders & Henderson 1978) advanced the completion of smoltification by altering the photoperiod to which the parr were exposed. During December parr were subjected to a direct step-up from the natural photoperiod to LD 16:8 followed by a reciprocal photoperiod. The fish completed smoltification approximately two months in advance of fish reared under a natural photoperiod. These early studies showed that the timing of smoltification could be modified by photoperiod.

The use of artificial photoperiods, to alter the timing of the parr-smolt transformation in Atlantic salmon, is now well established (see reviews Hoar 1988, Duston & Saunders 1990b, Clark 1989). Two important aspects of the natural photoperiod are the period of increasing daylength, the 'spring', and the period of decreasing daylength, the 'autumn'. Many studies have manipulated the timing of smoltification by altering the timing of the 'spring' or 'autumn' photoperiod in relation to the natural photoperiod. The 'spring' period has been advanced, i.e. the increase in daylength was given earlier in the experimental photoperiod than under the natural photoperiod. This advance in the 'spring' period, resulted in an advance in the parr-smolt transformation (Sigholt *et al.* 1989, Duston & Saunders 1990, Thrush *et al.* 1994, Solbakken *et al.* 1994). Similarly, if the decrease in daylength was delayed compared to a natural photoperiod, the parr-smolt transformation was delayed, and if the decrease was delayed into December, smolt quality was reduced (McCormick *et al.* 1987, Saunders *et al.* 1989, Saunders & Harmon 1990). Maximum changes in the timing of the completion of smoltification have been obtained by altering both the decrease and the increase in the photoperiod. The completion of smoltification was advanced 7 months by advancing both the 'autumn' and the 'spring' periods (Thrush *et al.* 1994, Berge *et al.* 1995) and smoltification was

delayed 5 months by delaying the 'autumn' and 'spring' periods (Duston & Saunders 1992).

Atlantic salmon parr have responded to various types of photoperiods. A one month delayed photoperiod delayed the timing of smoltification (Soivio 1989, Solbakken *et al.* 1994). A decrease to 6 and 5 month advanced 12 month photocycles advanced smoltification (Berge *et al.* 1995). Accelerated photoperiods, involving the compression of the natural photoperiodic cycle into 6 months (Duston & Saunders 1992, Thrush *et al.* 1994), 7 months (Clarke *et al.* 1985) or 10 months (Thrush *et al.* 1994), advanced the timing of the parr-smolt transformation. By contrast a decelerated photoperiod, comprising of a natural photoperiodic cycle expanded to 18 months (Duston & Saunders 1992) delayed smoltification.

'Square wave' photoperiods (direct changes from different constant daylengths) have both delayed and advanced smoltification, depending on when, during the natural photoperiod, the direct change in daylength was applied. A squarewave of LD 16:8 (Saunders *et al.* 1989) or LD 18:6 (Saunders & Harmon 1990) applied during the Autumn effectively extended the summer solstice delaying the decrease in the photoperiod and in turn the completion of smoltification was also delayed. Similarly, fish reared under continuous light (LL) followed by a reduction to a natural photoperiod in October (McCormick *et al.* 1987) exhibited a delay in smoltification. A direct increase or step up in December or January from the natural photoperiod to LD 16:8 (Komourdjian 1976, Saunders & Henderson 1978, Duston & Saunders 1990a), LD 20:4 (Thrush *et al.* 1994) or LL (Solbakken *et al.* 1994) effectively advanced the spring increase in the photoperiod and in turn advanced the completion of smoltification. Bjornsson *et al.* (1989) demonstrated that direct changes between short and long daylengths resulted in the development of aspects of smoltification. Groups of parr held on LL were exposed to a square wave photoperiod of LD14:10, LD8:16 or LD2:22 for a duration of 5 months. All the groups appeared to successfully complete smoltification (Bjornsson *et al.* 1989).

These manipulations in the timing of the parr-smolt transformation, demonstrate that photoperiod is a controlling factor in the smoltification process. The smoltification process appears to be timed by a decrease followed by an increase in the photoperiod. The parr-smolt transformation coincides with the increasing photoperiod. However, if a decreasing photoperiod is preceded by a rapid increase in daylength, the parr-smolt transformation can be completed under a decreasing photoperiod (Komourdjian 1976, Saunders and Henderson 1978, Duston & Saunders 1992, Thrush *et al.* 1994). It has been suggested that the development of smolt characteristics under a decreasing photoperiod is a delayed response to the preceding rapid increase in the photoperiod; this is referred to as a phase delay (Duston & Saunders 1992, Thrush *et al.* 1994). A typical phase delay of smoltification has been observed following an accelerated photoperiod (Duston & Saunders 1992, Thrush *et al.* 1994). A phase delay in relation to a driving zeitgeber (in this case photoperiod) is typical of a biological process controlled by an endogenous rhythm (Bromage & Duston 1986, Duston & Saunders 1992).

Potential S2 parr held for 14 months under LD12:12 and constant 11±0.5°C showed a cycle in morphological characteristics, body silvering and condition factor (Eriksson and Lundqvist 1982). The cycling approximated to 10 months. Fish held on a constant LD 8.25:15.75 from the winter solstice, developed a characteristic smolt osmoregulatory ability at the same time as control fish held under a natural photoperiod (Duston and Saunders 1990a). The development of biological processes under an unchanging photoperiod and particularly the cycling of the process with a periodicity close to but not precisely a year, indicates an underlying endogenous rhythm (Bromage & Duston 1986, Duston & Saunders 1990b, 1992).

Gwinner (1986) proposed that circannual rhythms allow an organism to time a biological process (e.g. smoltification), which can not react immediately to ultimate factors (e.g. food availability), by using proximate cues such as photoperiod. The organism can not react immediately to ultimate factors as the organism requires a considerable time period for the biological process to develop. Gwinner (1986) also stated that an endogenous

circannual rhythm should free run under constant conditions with a periodicity close to but significantly different from a year and entrain to yearly cycles of varying periodicity; it should also exhibit temperature compensation. It is arguable if these exacting criteria have been demonstrated in any organism. The mechanism controlling maturation in the rainbow trout has been closely examined and meets most of the criteria for an endogenous circannual rhythm (Duston & Bromage 1986, 1991, Randall 1992), except under a constant long daylength, when maturation has been observed to cycle with a 6 month periodicity (Duston & Bromage 1986, 1991).

The ultimate factor for smoltification is that the smolts enter the marine environment when the marine conditions ensure a high survival. Smoltification is a process which requires time to develop. The fish can not react instantly to the ultimate factor and it would appear that the fish uses photoperiod as a proximate cue to time aspects of smoltification. The phase delay of smoltification and the cycling of the morphological aspects of smoltification provides strong evidence to support the hypothesis that smoltification is controlled endogenously. However, these observations do not meet the criteria indicated above and some observations, particularly the suppression and dissociation of aspects of smoltification, contradict the hypothesis or are difficult to explain using the hypothesis that smoltification is controlled by an endogenous rhythm.

Suppression of the whole smoltification process has been observed (Saunders *et al.* 1985, McCormick *et al.* 1987). Fish, exposed to LL and ambient temperature from the 22nd Nov. (Saunders *et al.* 1985) and from hatch (McCormick *et al.* 1987), did not develop smolt characteristics at a time similar to controls reared under a natural photoperiod. A biological process controlled by an endogenous rhythm should cycle with a period close to but not precisely a year when held under a constant photoperiod (Bromage & Duston 1986, Duston & Saunders 1990b, 1992). It is possible that smoltification was delayed beyond the end of the experiment. A delay in the photoperiodic decrease has been observed to cause a delay in the completion of

smoltification (McCormick *et al.* 1987, Saunders *et al.* 1989, Saunders & Harmon 1990).

Suppression of the decrease in condition factor alone has also been observed (Saunders & Henderson 1970, Bjornsson *et al.* 1989, Duston & Saunders 1990a, Solbakken *et al.* 1994). Duston and Saunders (1990a) demonstrated that fish, held on LD 8.25:15.75 from the winter solstice, developed the osmoregulatory ability of a smolt at the same time as controls reared under a natural photoperiod. However, unlike the control fish, the fish reared under LD 8.25:15.75 did not exhibit a decrease in condition factor from a parr to a smolt condition. Bjornsson *et al.* (1989) obtained a similar result from fish held on LL and exposed to a square wave short day period of one month. Duston & Saunders (1990a) suggested that condition factor is not controlled endogenously but requires a minimum period of short days followed by an increase in daylength. However, this is in contradiction with Eriksson and Lundqvist (1982) who observed a cycling of condition factor under constant LD 12:12 and constant 11±0.5°C. Solbakken *et al.* (1994) recorded no decrease in condition factor in fish subjected to ambient conditions and suggested that a combination of photoperiod, temperature and feeding regime had obscured the reduction in condition factor.

Photoperiod manipulation has resulted in the dissociation as well as the suppression of smolt parameters. Dissociations of osmoregulatory ability and condition factor (Bjornsson *et al.* 1989, Thrush *et al.* 1994), osmoregulatory ability and Na^+,K^+ -ATPase (Solbakken *et al.* 1994, Berge *et al.* 1995), and coloration and condition factor (Eriksson & Lundqvist 1982) have all been described. Bjornsson *et al.* (1989) recorded the development of osmoregulatory ability in advance of the decrease in condition factor, after a direct increase in daylength from a short day to a long day. Solbakken *et al.* (1994) found that osmoregulatory ability developed soon after an increase in temperature in advance of a photoperiodic associated increase in Na^+,K^+ -ATPase activity. Berge *et al.* (1995) observed increased salinity tollerance in the absence of increased Na^+,K^+ -ATPase activity. Na^+,K^+ -ATPase activity appeared to be related to growth rate (Berge

et al. 1995). Eriksson and Lundqvist (1982) observed the dissociation of silvering and the decrease in condition factor in individual fish held under constant LD 12:12.

These observations indicate that different parameters of the parr-smolt transformation are controlled by different endogenous rhythms or mechanisms (Bjornsson *et al.* 1989 Duston & Saunders 1990a). These separate endogenous rhythms or mechanisms respond differently to constant (Eriksson and Lundqvist 1982, Duston and Saunders 1990a) or accelerated photoperiods (Bjornsson *et al.* 1989, Duston & Saunders 1992 Solbakken *et al.* 1994, Thrush *et al.* 1994) and the smolt parameters become dissociated or suppressed. The evidence suggests that an endogenous circannual rhythm entrained by photoperiod controls the development of osmoregulatory ability and possibly a separate endogenous rhythm controls the changes in condition factor and growth related smoltification parameters. The parr-smolt transformation is clearly a complicated process, with a whole range of physiological, morphological and behavioural changes timed to take place during the same time period.

Despite the complexity of the mechanisms controlling the smoltification process, it is clear that photoperiod can be used to control the development of smoltification and alter the time when smolts are available for transfer to sea water. Further investigation is required to establish artificial photoperiod regimes which can be used to produce smolts. Ideally the smolts should be produced out-of-season: ie at a time when smolts are not produced naturally (see section 1.2). Experiments 1 and 2 examined the production of smolts during the winter using artificial photoperiod regimes.

Experiment 1 examined both freshwater and seawater performance of large autumn parr and autumn photoperiod smolts, transferred directly to sea water. The transfer of large parr directly from fresh water to sea water is a method already practised by the industry to increase the sea water transfer 'window'. The industry has had mixed results with this practice, reporting both high and low mortalities. Bergheim *et al.* (1990) and Bjerknes *et al.* (1992) showed that parr which were acclimatised to sea water prior to transfer exhibit survivals of 80-90%. However, acclimation in association with seawater

temperatures of 1-2°C resulted in survivals of 40% (Duston and Knox 1992). The size of the parr acclimatised is important. Small parr (<9.5cm) exhibited survivals of 18% (Bjerknes *et al.* 1992). Bjerknes *et al.* (1992) also reported that direct transfer to sea water of parr can result in low survival rates. Cold seawater temperatures and the production of large autumn parr are not problems in Scotland. However, few farms have the facility to acclimatise fish prior to transfer. The production of autumn photoperiod smolts could be an alternative to the transfer of large parr and the two methods should be compared.

Experiment 2 further examined the use of artificial photoperiods in the production of out-of-season smolts, investigating the use of square wave photoperiods. The square wave photoperiods used, highlighted the potential of photoperiod manipulation to produce smolts and aspects of the mechanisms controlling smoltification. The mechanisms controlling smoltification were further examined in experiment 3 which examined the effect of a constant photoperiod and temperature regime on smoltification. Experiment 3 was carried out on triploid fish to try and exclude the effects of the rhythm controlling maturation.

The aims of this chapter are:-

1. To compare the performance of smolts produced by photoperiod manipulation with large parr reared under a natural photoperiod.

2. To manipulate smoltification using squarewave photoperiods and determine the period of short days which is required for the completion of smoltification.

3. To investigate the mechanism(s) which controls the development of the individual smolt parameters and how the mechanism(s) operates when the individual smolt parameters are dissociated from each other and/or the photoperiod.

3.2 Experiment 1 : Comparison of growth, seawater tolerance and mortality for Atlantic salmon parr and photoperiod smolts transferred to sea water in December.

3.2.1 Materials and Methods.

The experiment was carried out at site FW1 (section 2.2.1.1). The eggs, alevins and fry were reared in heated water (8.4±0.2°C), until the 23rd April. The eggs and alevins were held in darkness, until first feeding. The fry were subjected to LD 23:1 from first feeding to the 23rd April. The hatchery's main stock was transferred to a natural photoperiod and water at ambient temperature, on the 23rd April. The largest fish (batch weights from 2.5-2.9g) which represented the top 50% of the hatchery's stock were divided into 2 groups. One of these groups consisted of 50000 parr. This group of parr was started on an artificial photoperiod (group PL) on the 4th June. The artificial photoperiod began with a decrease from natural daylength to LD 14:10; this was continued at 4 hours/week to LD 6:18. The LD 6:18 was held for 2 months before beginning an increase of 1 hour / week to LD 18:6 (figure 3.1). The second group from the same 50% of the hatchery's stock was maintained under a natural photoperiod (group NL). The fish were stocked from 4-20 $kg.m^{-3}$ while in fresh water. The PL group was transferred from 2m (2-4m^3) to 5m (25m^3) tanks on the 5th Nov. The NL group remained in the 2m (2-4m^3) tanks until transfer to sea water. During September both groups were size graded, the potential S1 parr being retained. The PL group was graded on the 8th Sept. and the NL group was graded on the 15th Sept. Fish greater in size than 8g were retained as the S1 fraction; this size grade was based on past experience and observations from the pre-grade populations (figure 3.2).

On the 7th Dec., 1000 fish from each group were transferred to seawater site SW1 (2.2.2.1), where the fish were held for 5 months at stocking densities from 5-10kg/m^3. The tanks were checked daily and mortalities were recorded.

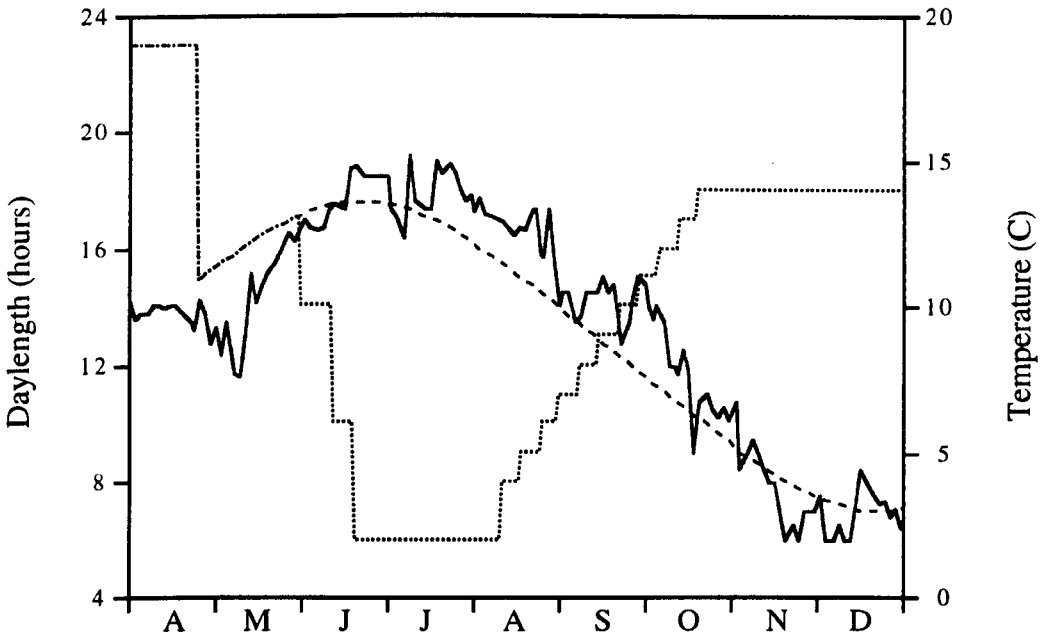


Figure 3.1 The photoperiod regimes and the temperature profile experienced by the two groups. The dotted line represents the photoperiod exposed to the photoperiod group PL and the dashed line represents the photoperiod exposed to the control group NL. The solid line represents the temperature profile experienced by both groups.

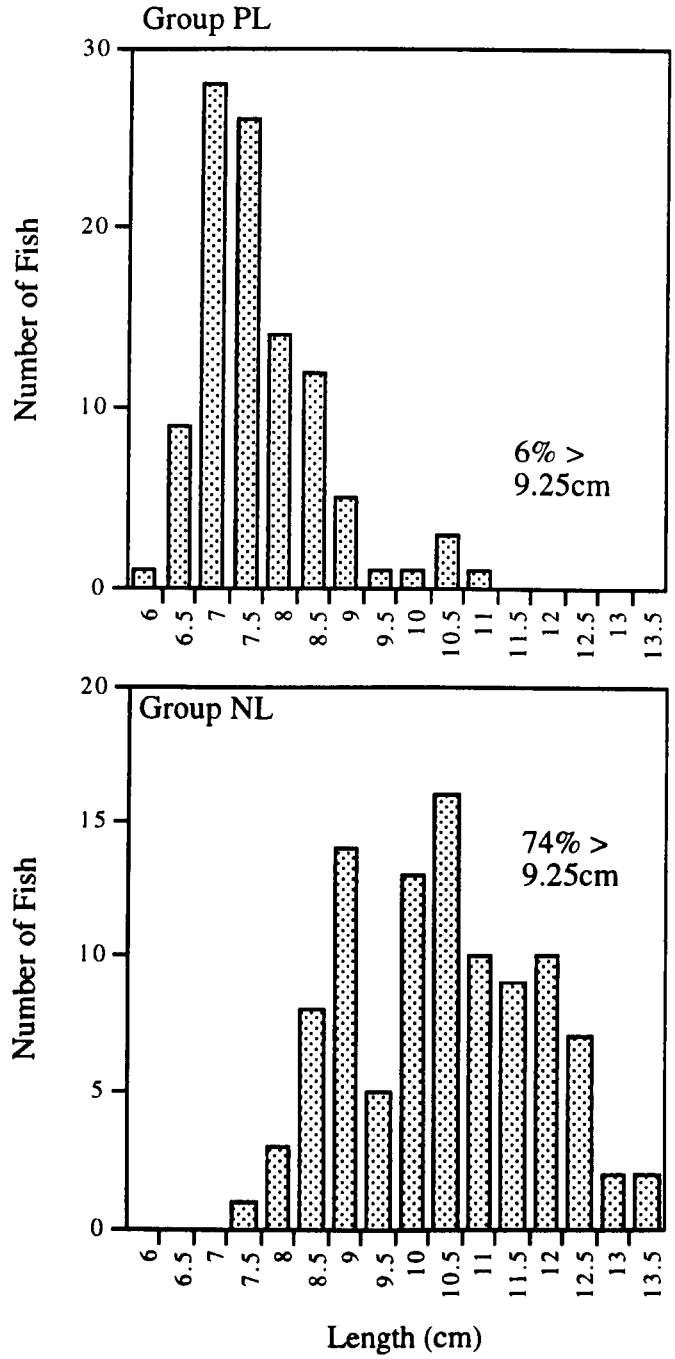


Figure 3.2 The number of fish observed in the length catagories (mid-point displayed) for the two groups PL and NL. Group PL was sampled (n=101) on the 8th Sept. and group NL was sampled (n=100) on 21st Aug.

Throughout the experiment, weights and lengths were recorded at intervals of 2-4 weeks for a random sample (n = 50-100) of fish from each group. The osmoregulatory ability of a sample of fish (n = 10-11), from both groups, were tested in a seawater survival test (section 2.3.3.3) on the 25th Sept. 15th Oct., 6th Nov., 20th Nov. and 2nd Dec. The survival tests were carried out at ambient temperature.

Specific growth rate (SGR) was calculated (section 2.3.2.1). Weight and condition factor data were compared using DUNN'S multiple comparison test (section 2.4.2). Confidence limits for the proportions of potential S1 smolts, tolerance test data and seawater mortality were calculated and compared (section 2.4.3).

3.2.2 Results.

Significantly different ($p<0.01$) proportions of potential S1's were obtained from the PL and NL groups during the grading. The percentages yielded from the PL and NL groups were 14.5% and 88.5% (table 3.1) respectively. These percentages obtained from the grades of the entire populations were slightly higher than the percentages observed in the samples (n=100) (figure 3.2), 6 % and 74 % from group PL and NL respectively.

The seawater tolerance tests on 25th Sept. and 15th Oct. showed no significant difference in seawater survival (figure 3.3) between the two groups. The seawater tolerance tests on 6th Nov., 20th Nov. and 2nd Dec. showed a significantly ($p<0.01$) higher survival in the PL fish. The PL group exhibited 80-100% survival indicating that the fish had developed osmoregulatory ability.

The condition factor in both groups decreased significantly ($P<0.01$) (figure 3.4). The PL group exhibited a significant ($P<0.01$) decrease in condition factor from the 14th Oct. to the 5th Nov., other changes in the condition factor were insignificant. The NL group exhibited a significant ($p<0.01$) decrease from the 29th Sept to the 14th Oct before the condition factor levelled at 1.14-1.17. The condition factor of the PL group was significantly ($p<0.05$) lower than group NL on the 2nd Dec, the sample date prior to

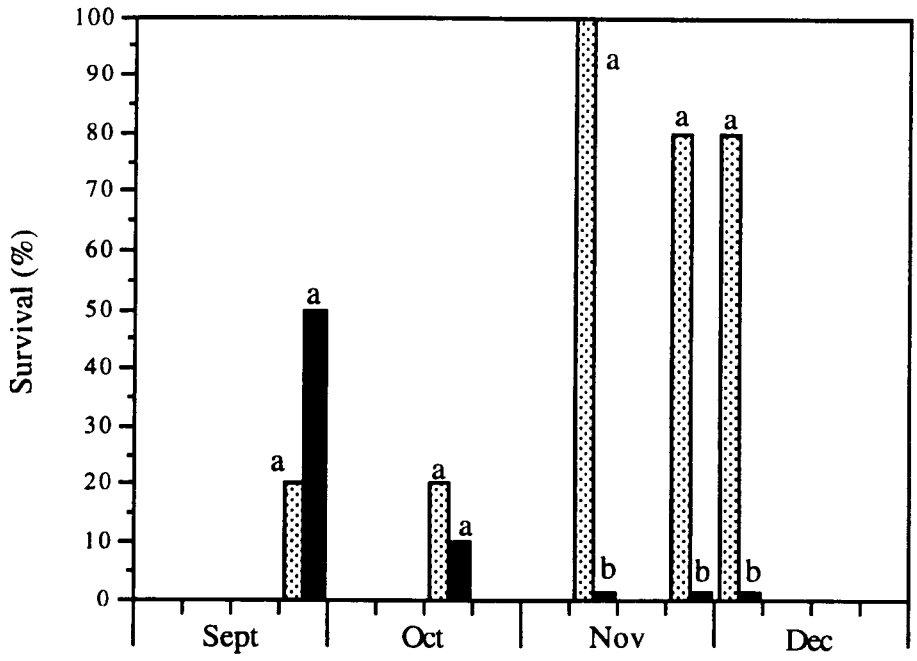


Figure 3.3 Percentage survival of fish (n = 10-11) seawater tolerance tested for 96 hours at 37.5‰. The dotted histogram represents fish subjected to an artificial photoperiod (group PL) and the filled histogram represents fish subjected to a natural photoperiod (group NL). The tests were carried out on 5 occasions, 25th Sept., 15th Oct., 6th Nov., 20th Nov. and 2nd Dec. Different letters on a the same sample date indicate a significant difference ($p<0.05$).

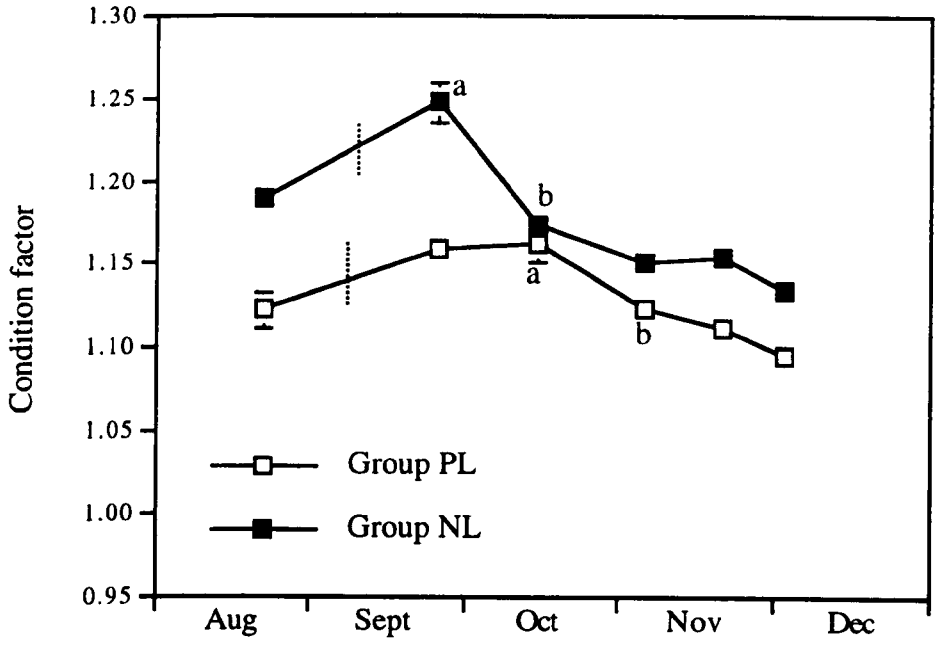


Figure 3.4 Change in condition factor (mean ± 1 SEM) with time. The open symbol represents fish subjected to an artificial photoperiod (group PL) and the filled symbol represents fish subjected to a natural photoperiod (group NL). If no error bar is visible the plot symbol has obscured the error bar. Dotted line indicates when the groups were graded. Different letters indicate significant ($p<0.05$) changes over time for each group.

seawater transfer.

Table 3.1 The SGR values, weights, mortality and proportion of potential S1 fish observed in a group of fish reared under a natural photoperiod (group NL) and a group of fish reared under an artificial photoperiod (group PL). Different letters in the same row indicate a significant difference ($p<0.05$).

	NL group	PL group
Initial weight (g).	2.01±0.05	2.01±0.05
SGR (%.day^{-1}) range 9th June-21st Aug	1.97-2.97	1.18-1.24
Proportion of potential S1's (%)	88.5^{a}	14.5^{b}
SGR (%.day^{-1}) range 29th Sept.- 2nd Dec	-0.01-0.5	-0.34-2.28
Mortality month prior to transfer (%).	0	0
Transfer weight (g).	39.5±0.9^{a}	25.0±0.6^{b}
Mortality month post transfer (%).	19.4	6
SGR (%.day^{-1}) range 2nd Dec.-3rd May	0.04-0.57	0.31-1.17
Weight (g) after 5 months seawater growth.	59.9±2.0^{a}	60.2±2.9^{a}

During the short day (LD 6:18) part of the photoperiod, the PL fish exhibited a lower SGR (9th June-31st July, SGR=1.24) than the NL fish (9th June-31st July, SGR=2.97). As a result of this lower growth rate the PL fish were significantly ($p<0.01$) smaller on the 31st July (figure 3.5). The situation was reversed in October, the PL group exhibited a higher SGR (14th Oct-5th Nov, SGR=2.28) than the NL fish (14th Oct-5th Nov, SGR=0.19). Despite the increased growth the PL fish were still significantly ($p<0.01$) smaller when the groups were transferred (table 3.1) to sea water.

The PL group had the osmoregulatory ability of smolts, a silver smolt appearance and a reduced condition factor in late November. The NL group had the osmoregulatory ability of parr and parr marks were still clearly visible. The NL group fish had a mean weight of 39.5±0.9g and were significantly ($p<0.01$) larger than the PL fish which had a mean weight of 25.0±0.6g (table 3.1). However, growth had declined, particularly in the NL

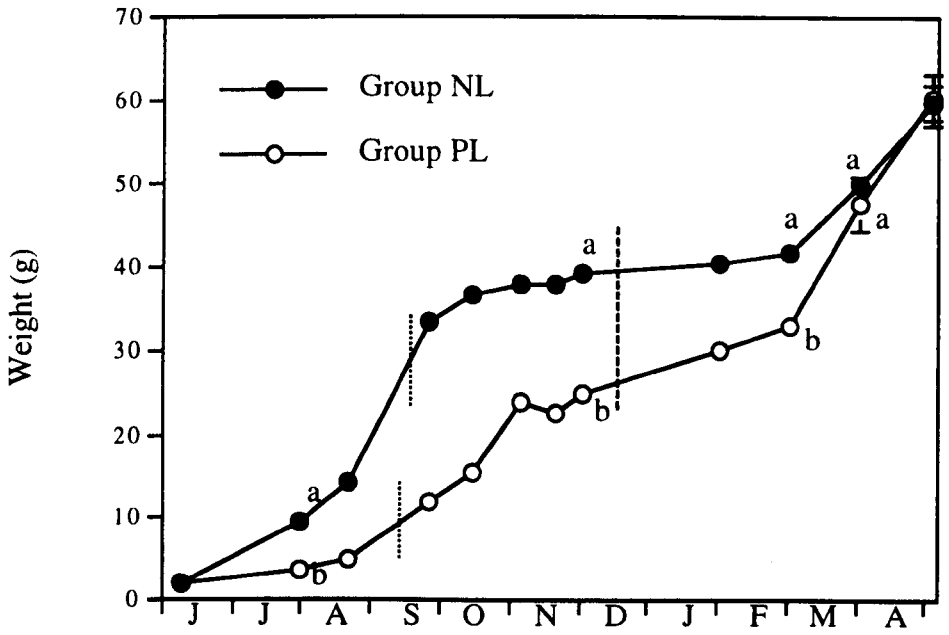


Figure 3.5 Changes in weight (mean±1 SEM) with time. The open symbol represents fish subjected to an artificial photoperiod (group PL) and the filled symbol represents fish subjected to a natural photoperiod (group NL). If no error bar is visible the plot symbol has obscured the error bars. Dotted line indicates when the groups were graded and the dashed line indicates when the groups transferred to sea water.

group, due to decreasing winter temperatures. The 2 groups were transferred directly to sea water on the 7th Dec.

The seawater growth of the PL group was consistently higher (SGR=0.31-1.17) than the NL group (SGR=0.04-0.58). After 4 months in sea water there was no significant difference between the mean weight of the two groups (figure 3.5). This situation remained until the end of the experiment on the 3rd May (table 3.1).

The NL group gained the silver appearance of smolts during the first month after transfer to sea water.

Seawater mortality (figure 3.6) during the first two months was significantly ($p<0.01$) higher in the NL group. This was comparable with the survival test results (figure 3.3) which showed the NL fish had poor survival in sea water. From February to April mortality was significantly ($p<0.01$) higher in the PL group (figure 3.6), which suffered from a *Vibrio* sp. infection. Vibriosis was identified on the 10th March. and treated with a course of antibiotic.

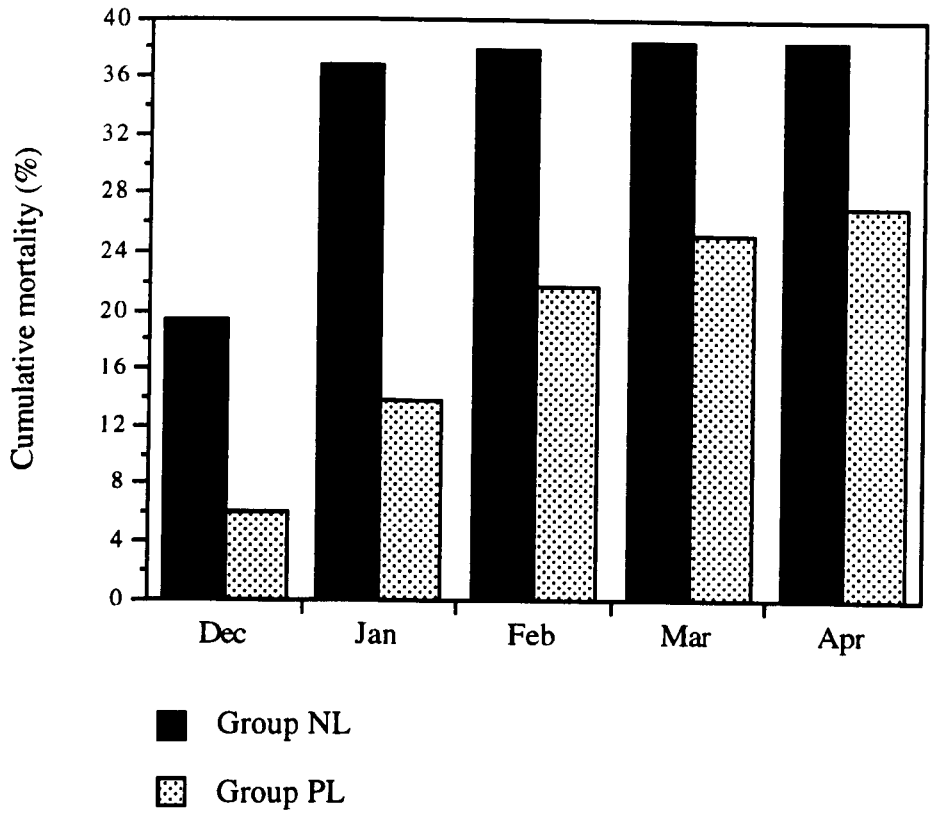


Figure 3.6 Cumulative percentage mortality for each month for two groups of fish transferred directly to sea water on 7th Dec. The dotted histogram represents fish subjected to an artificial photoperiod (group PL) and the filled histogram represents fish subjected to a natural photoperiod (group NL).

Summary of Results from Experiment 1.

1) The artificial photoperiod experienced by group PL resulted in a recruitment of 14.5% into the upper mode compared to 88.5% in group NL.

2) The condition factor decreased in both groups.

3) The hypoosmoregulatory ability of the group PL fish increased in response to the artificial photoperiod whilst the hypoosmoregulatory ability of the group NL fish decreased in response to the natural photoperiod. This resulted in significantly higher mortalities in group NL during the first 2 months in sea water. Due to a *Vibrio* infection in group PL there was no difference in cumulative seawater mortality at the end of the experiment.

4) At transfer the group PL fish were significantly smaller than the group NL fish. After 4 months in sea water there was no significant weight difference between the 2 groups.

5) The group PL fish gained a silver smolt appearance prior to seawater transfer. The group NL fish gained a silver smolt appearance after seawater transfer.

3.3 Experiment 2 : The effects of 'winter' length on smoltification and the production of 0+ Atlantic salmon (*Salmo salar*) smolts.

3.3.1 Materials and Methods.

Experiment 2 was carried out at site FW1 (section 2.2.1.1). The eggs, alevins and fry were reared in heated water averaging 10.6±0.3°C (eggs 5-8.2, alevins 9.7-10.8, fry 5.5-14.6), until the 20th May. The eggs and alevins were held in darkness until first feeding and the fry were subjected to LL from first feeding to the 20th May. On the 20th May a sample of 63000 of the largest fish, which represented the top 50% (mean weight 3g) of the hatchery's stock, were transferred to 5m ($25m^3$) tanks which were supplied with water at ambient temperature (figure 3.7) and illuminated by natural daylight. On the 6th July 2375 fish were randomly selected and divided between 5 tanks (1m, $0.25m^3$) to form 5 groups. On the 1st Oct. each group was transferred to a separate 1.5m ($0.75m^3$) tank. The 5 groups were subjected to the following photoperiods.

Group SNP - From the 6th July until transferred to a sea site SW1 (section 2.2.2.1), the fish were held on a simulated natural photoperiod (SNP) (figure 3.8a).

Group L - From the 6th July until transferred to a sea site SW1, the fish were held on LD 19.5:4.5 (figure 3.8b).

Group 1 - From the 6th July to the 7th Sept. the fish were held on LD 19.5:4.5. For 1 month from the 7th Sept. until the 5th Oct. the fish were held on LD 8:16. The fish were held on LD 19.5:4.5 from the 5th Oct. until transferred to a sea site SW1 (figure 3.8c).

Group 2 - From the 6th July to the 8th Aug. the fish were held on LD 19.5:4.5. For 2 months from the 8th Aug. until the 5th Oct. the fish were held on LD 8:16. The fish were held on LD 19.5:4.5 from the 5th Oct. until transferred to a sea site SW1 (figure 3.8d).

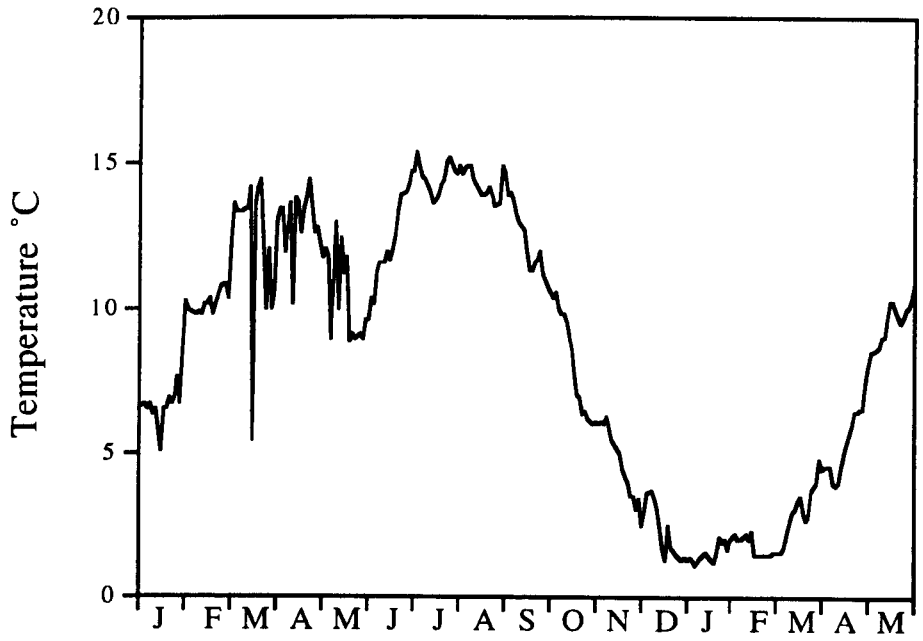
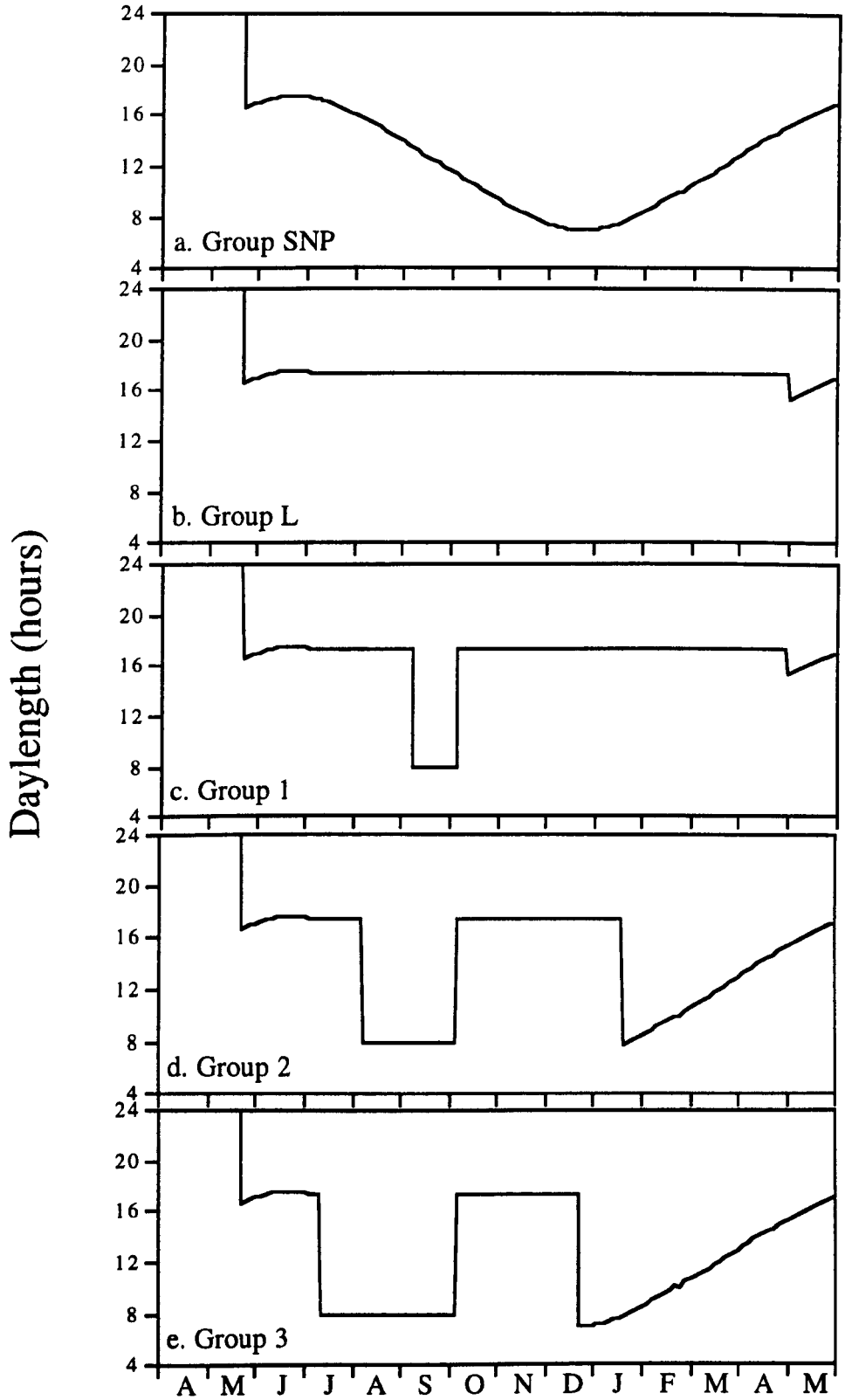


Figure 3.7 Temperature regime experienced by the fish in Experiment 2.

Figure 3.8 a-e. Photoperiod regimes to which the 5 groups of fish were subjected; a simulated natural photoperiod - group SNP (figure 3.8a.); a constant long daylength LD 19.5:4.5 - group L (figure 3.8b.), a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 - group 1 (figure 3.8c.); a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 - group 2 (figure 3.8d.) and a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 - group 3 (figure 3.8e.).


Daylength (hours)
24
20
16
12
8
4
a. Group SNP
b. Group L
c. Group 1
d. Group 2
e. Group 3
A M J J A S O N D J F M A M

Group 3 - From the 6th July to the 9th July the fish were held on LD 19.5:4.5. For 3 months from the 9th July until the 5th Oct. the fish were held on LD 8:16. The fish were held on LD 19.5:4.5 from the 5th Oct. until transferred to a sea site SW1 (figure 3.8e).

The weight, condition factor, smolt index (n = 50-100) and serum osmolality of challenged fish (n = 9-11) (section 2.3.3.2) were determined for the 5 groups at 2-3 week intervals. All groups developed a bimodal length and weight distribution. Fish from the upper mode were selected for the seawater challenge test. During data analysis the length distribution was used to separate the data for upper mode and lower mode fish. The distributions of condition factor and weight (SGR) for each mode were analysed separately. The population of each mode (UM and LM) changed during the course of the experiment, as fish were recruitment from the LM to the UM. This should be remembered, particularly when changes in condition and weight are examined.

Upper mode fish were selected from all groups and transferred to site SW1. The length of the fish was used to identify upper and lower mode fish. Group 3 was transferred on the 21st Dec., group 2 on the 18th Jan. and group SNP, group L and group 1 were transferred on the 18th May. All groups were transferred into the same 3m tank under natural illumination. Prior to transfer the fish in each group were marked with separate freeze brands. Daily mortality checks were made and at monthly intervals a random sample of fish (n =30-50) was weighed from each group.

Specific growth rate (SGR) was calculated (section 2.3.2.1). Weight, condition factor data and serum osmolality were compared using DUNN'S multiple comparison test and the t-test (section 2.4.1). Confidence limits for the proportions of sea water mortality were calculated and compared (section 2.4.3).

3.3.2 Freshwater Results.

Bimodality.

All groups exhibited a bimodal distribution of length (figure 3.9) and weight. The length distribution of group SNP first exhibited bimodality on the 31st Aug. (figure 3.10a), 41 % of the population forming an upper mode. Recruitment appeared to have continued during November and December. From December to May the percentage of the population in the upper mode varied from 66% to 83 %. A bimodal length distribution in group L was not observed until 6th Oct. (figure 3.10b), when 52 % of the population were in the upper mode. The upper mode gradually increased and during April and May the percentage of the population in the upper mode varied between 74-89 %. Group 1 exhibited a bimodal length distribution on the 20th Oct. (figure 3.10c), when 47 % of the population formed the upper mode. The upper mode gradually increased and from December to May the percentage of the population in the upper mode varied between 61-72 %. Group 2 first exhibited a bimodal length distribution on the 22nd Sept. (figure 3.10d). During the freshwater period the percentage of the population in the upper mode ranged between 36-47 %. Group 3 exhibited a bimodal length distribution on 31st Aug. (figure 3.10e), 28% of the population forming the upper mode. During the freshwater period the percentage of the population in the upper mode ranged between 28-45 %.

Growth.

The specific growth rates (SGR) observed in the groups were between -1.4 and 2 (figure 3.11). The freshwater period was during the winter and the water temperatures were low (figure 3.7, below 3°C from December to March); this resulted in slow growth in all groups. Generally the SGR observed in the upper mode was slightly higher than the SGR observed in the lower mode (figure 3.11).

In the SNP group the SGR decreased during the Autumn as the water temperature decreased (figure 3.11a.). Throughout the winter period the SGR for both the upper and

Figure 3.9. Length (mid-point of category) distributions (n=100) showing the development of bimodality in each of the 5 groups:- group SNP which experienced a simulated natural photoperiod; group L which experienced a constant long daylength LD 19.5:4.5; group 1 which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. The two distributions shown for each group were classed as unimodal (left distribution) and bimodal (right distribution). See figure 3.10 for percentage in each mode.

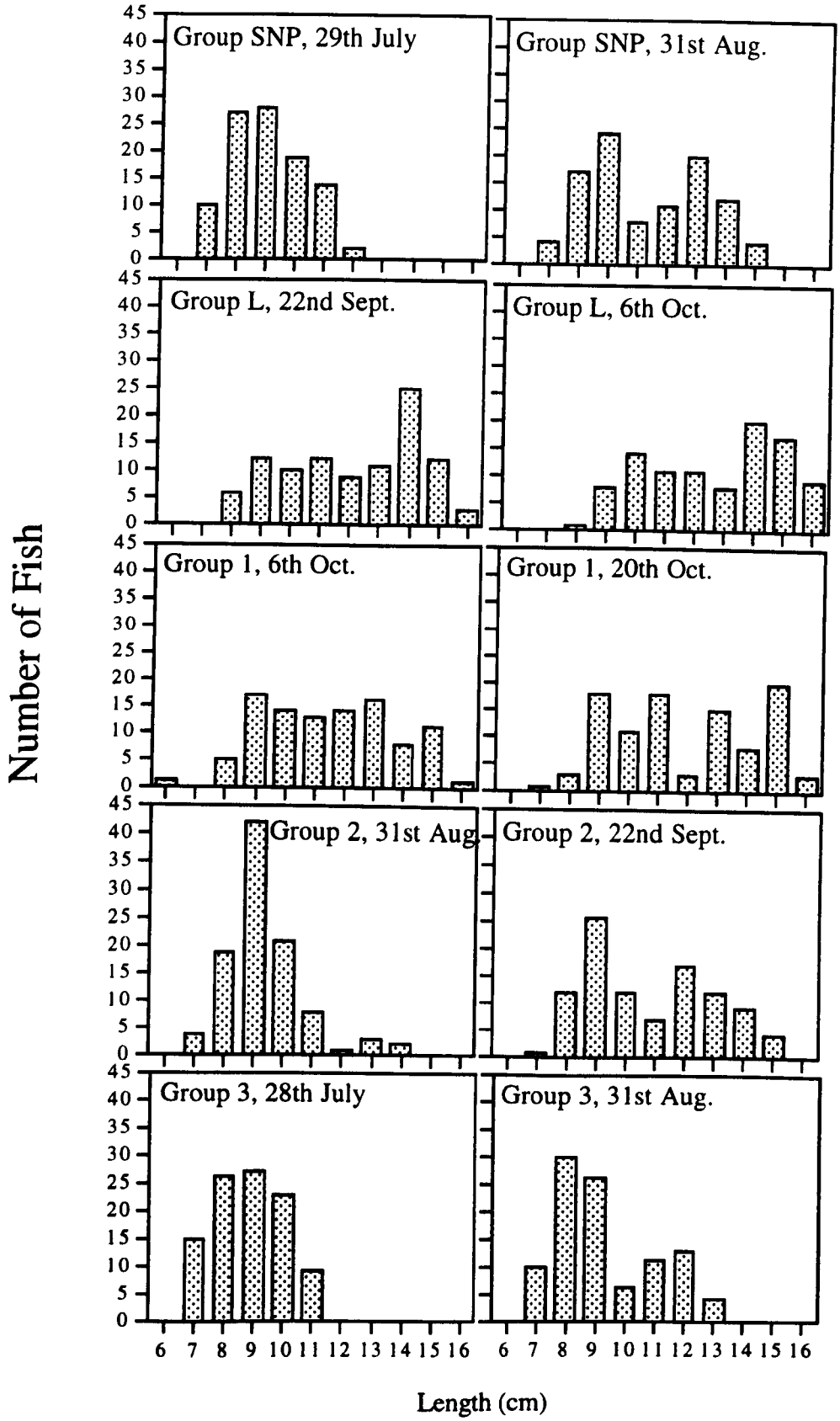

Group SNP, 29th July
Group SNP, 31st Aug.
Group L, 22nd Sept.
Group L, 6th Oct.
Group 1, 6th Oct.
Group 1, 20th Oct.
Group 2, 31st Aug.
Group 2, 22nd Sept.
Group 3, 28th July
Group 3, 31st Aug.
Number of Fish
Length (cm)
0 5 10 15 20 25 30 35 40 45
6 7 8 9 10 11 12 13 14 15 16

Figure 3.10 a-e. Percentage of fish in the upper mode for the 5 groups:- group SNP (a.) which experienced a simulated natural photoperiod; group L (b.) which experienced a constant long daylength LD 19.5:4.5; group 1 (c.) which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 (d.) which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 (e.) which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. The filled bar in figure 3.10 c, d and e indicates the period of short days. The percentage of fish contained in the upper mode was assessed from length distribution data. Groups showing no bimodal distribution were recorded as containing 0 % of fish in the upper mode. Error bars are 95% confidence limits (section 2.4.3), error bars which are not visible are obscured by the plot symbol.

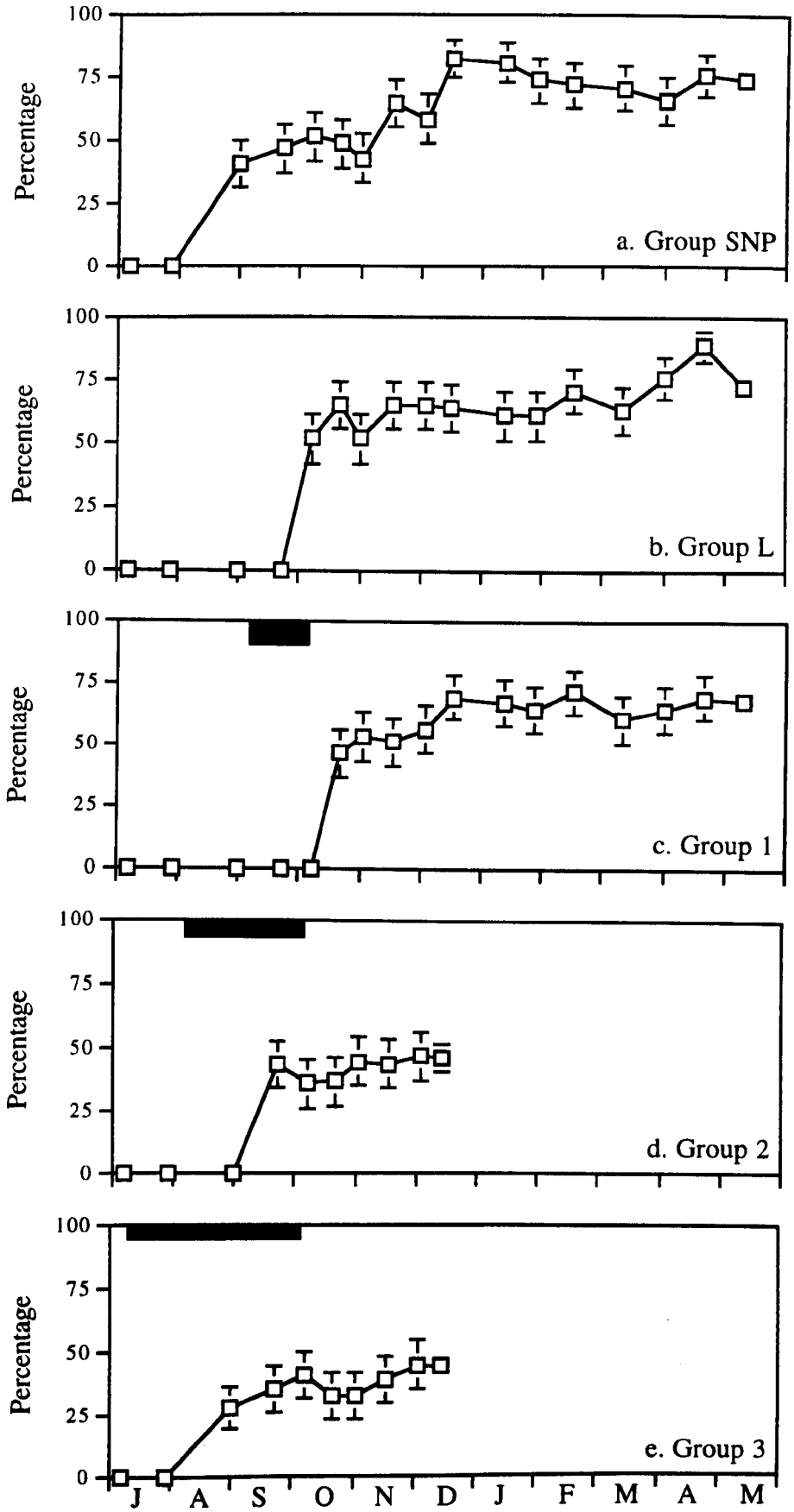
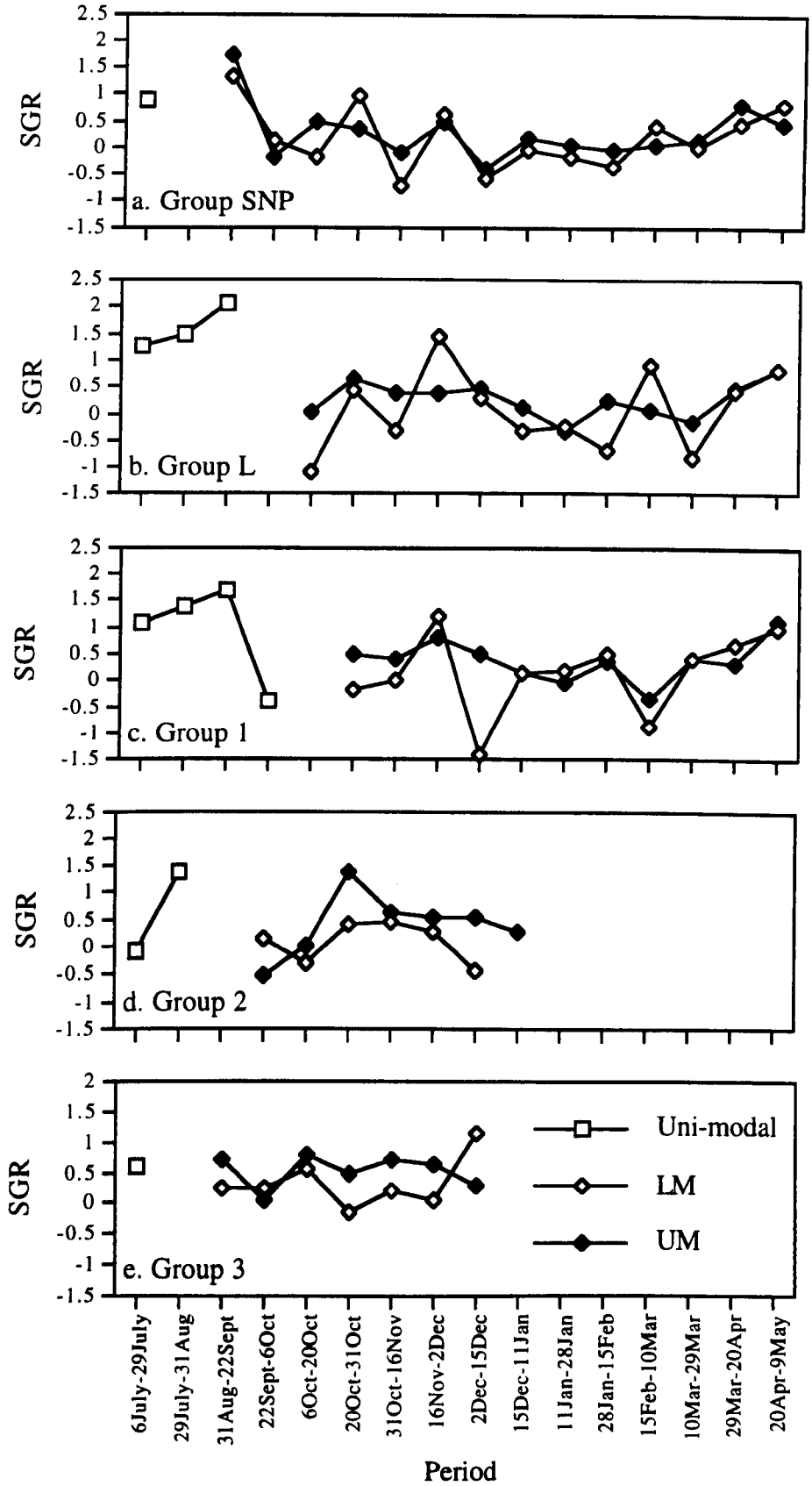

Percentage
100
75
50
25
0
a. Group SNP
b. Group L
c. Group 1
d. Group 2
e. Group 3
J
A
S
O
N
D
J
F
M
A
M

Figure 3.11 a-e. The specific growth rate (SGR) during the periods between sampling dates for the five groups:- group SNP (a.) which experienced a simulated natural photoperiod; group L (b.) which experienced a constant long daylength LD 19.5:4.5; group 1 (c.) which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 (d.) which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 (e.) which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. Open squares represent the entire population prior to the bimodal split; open diamonds refer to lower mode (LM) fish and filled diamonds refer to upper mode (UM) fish.


SGR
a. Group SNP
b. Group L
c. Group 1
d. Group 2
e. Group 3
Uni-modal
LM
UM
6July-29July
29July-31Aug
31Aug-22Sept
22Sept-6Oct
6Oct-20Oct
20Oct-31Oct
31Oct-16Nov
16Nov-2Dec
2Dec-15Dec
15Dec-11Jan
11Jan-28Jan
28Jan-15Feb
15Feb-10Mar
10Mar-29Mar
29Mar-20Apr
20Apr-9May
Period

lower modes was close to 0. Generally the SGR for the upper mode was positive but below 0.5 and the SGR of the lower mode oscillated around 0. During the spring (March - April) the SGR for the upper and lower modes increased (figure 3.11a.).

The pattern of SGR under the constant LD 19.5:4.5 in group L was different from the SNP group (figure 3.11 a. and b.). Prior to the development of bimodality, which was later (figure 3.10b. and 3.11b.) than in group SNP, the SGR increased to 2.05. After the formation of the bimodal population the SGR values observed in the lower mode again oscillated around 0 but over a greater range (figure 3.11b.). In the upper mode the pattern was similar to the SNP upper mode with values generally between 0 and 0.5. During the spring (March - April) the SGR for the upper and lower modes increased (figure 3.11b.).

In group 1 an increase in SGR, similar to that observed in group L, was observed before the development of bimodality. However, a decrease was observed after this increase, prior to the development of bimodality (figure 3.11c.). The pattern observed after the development of bimodality was similar to the pattern observed in group L. During the spring (March - April) the SGR for the upper and lower modes increased (figure 3.11c.).

In group 2 (figure 3.11d.) and group 3 (figure 3.11e.), after the formation of the bimodal distribution, the SGR observed in the upper modes was low (close to 0 or below). The SGR then increased to between 0.5 and 1 until a slight decrease prior to transfer to sea water. The average SGR for the period 20th Oct. to the 15th Dec. was 0.77 in group 2 and 0.53 in group 3. This compares to 0.56 in group 1, 0.48 in group L and 0.07 in group SNP. The lower mode SGR values in groups 2 and 3 were close to 0 over most of the sampling period (figure 3.11d. and 3.11e.).

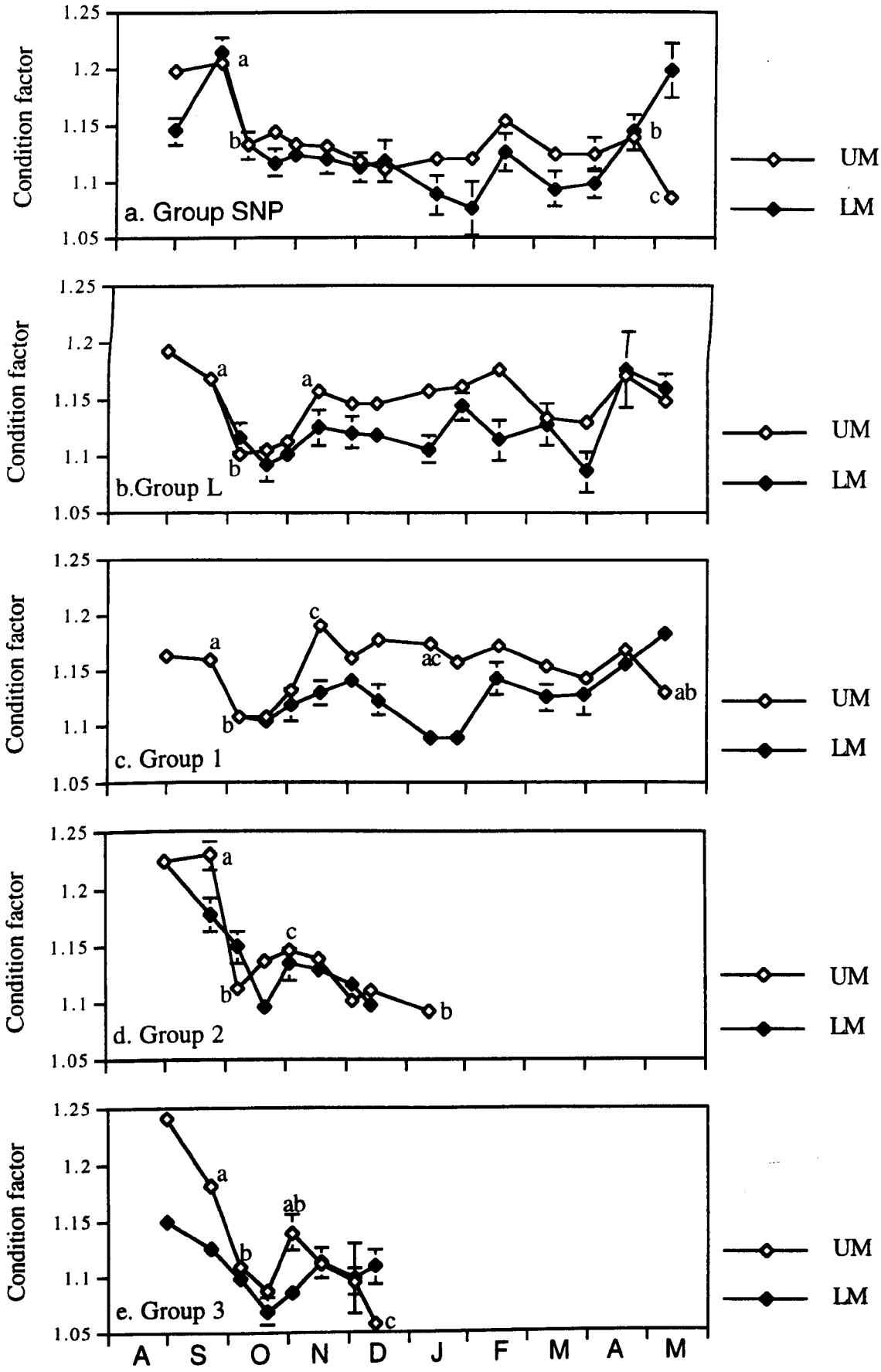
Condition Factor.

All groups exhibited a significant decrease ($p<0.01$) in condition factor during September (figure 3.12a-e). After the September decrease the upper mode from group SNP (figure 3.12a) remained between 1.15±0.006 and 1.11±0.007 until the 20th April when the condition factor decreased significantly ($p<0.01$) from 1.14±0.005 to 1.09±0.006 on the 9th May. The lower mode fish from group SNP (figure 3.12a) exhibited a similar pattern remaining between 1.13±0.01 and 1.07±0.02 during early spring. However, in contrast to the upper mode fish, the lower mode fish exhibited no significant change.

The condition factor of the upper mode fish from group L (figure 3.12b) and group 1 (figure 3.12c) increased significantly ($p<0.01$) during November. After this, there were no further significant changes in condition factor prior to transfer. At transfer the upper mode condition factor was 1.13±0.008 and 1.15±0.007 in group L and group 1 respectively. The lower mode fish in group L (figure 3.12b) exhibited no significant changes after the September decrease. The lower mode fish in group 1 (figure 3.12c) exhibited a significant increase during April and May. In comparison no significant changes were observed in group L.

After the significant decrease ($p<0.01$) in September the upper modes in both group 2 (figure 3.12d) and group 3 (figure 3.12e) appeared to increase during October. The condition factor in both groups again decreased significantly ($p<0.01$), in group 2 from 1.15±0.007 on the 2nd Nov. to 1.09±0.01 on the 12th Jan. and in group 3 from 1.095±0.01 on the 3rd Dec. to 1.058±0.006 on the 12th Dec. The lower modes of group 2 (figure 3.12d) and group 3 (figure 3.12e) exhibited a similar pattern as the upper modes. However, the decrease in December was less pronounced. There was no change in either of the lower mode groups after September.

Figure 3.12 a-e. Changes in condition factor over the freshwater experimental period for the five groups:- group SNP (a.) which experienced a simulated natural photoperiod; group L (b.) which experienced a constant long daylength LD 19.5:4.5; group 1 (c.) which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 (d.) which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 (e.) which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. Open diamonds represent the upper mode (UM) condition factors and filled diamonds represent the lower mode (LM) condition factors. Over the sampling period different letters denote significant differences ($p<0.05$) in upper mode condition. Error bars are $\pm$ 1 sem; error bars which are not visible are obscured by the plot symbol.


Condition factor
1.25
1.2
1.15
1.1
1.05
a. Group SNP
b.Group L
c. Group 1
d. Group 2
e. Group 3
UM
LM
A
S
O
N
D
J
F
M
A
M

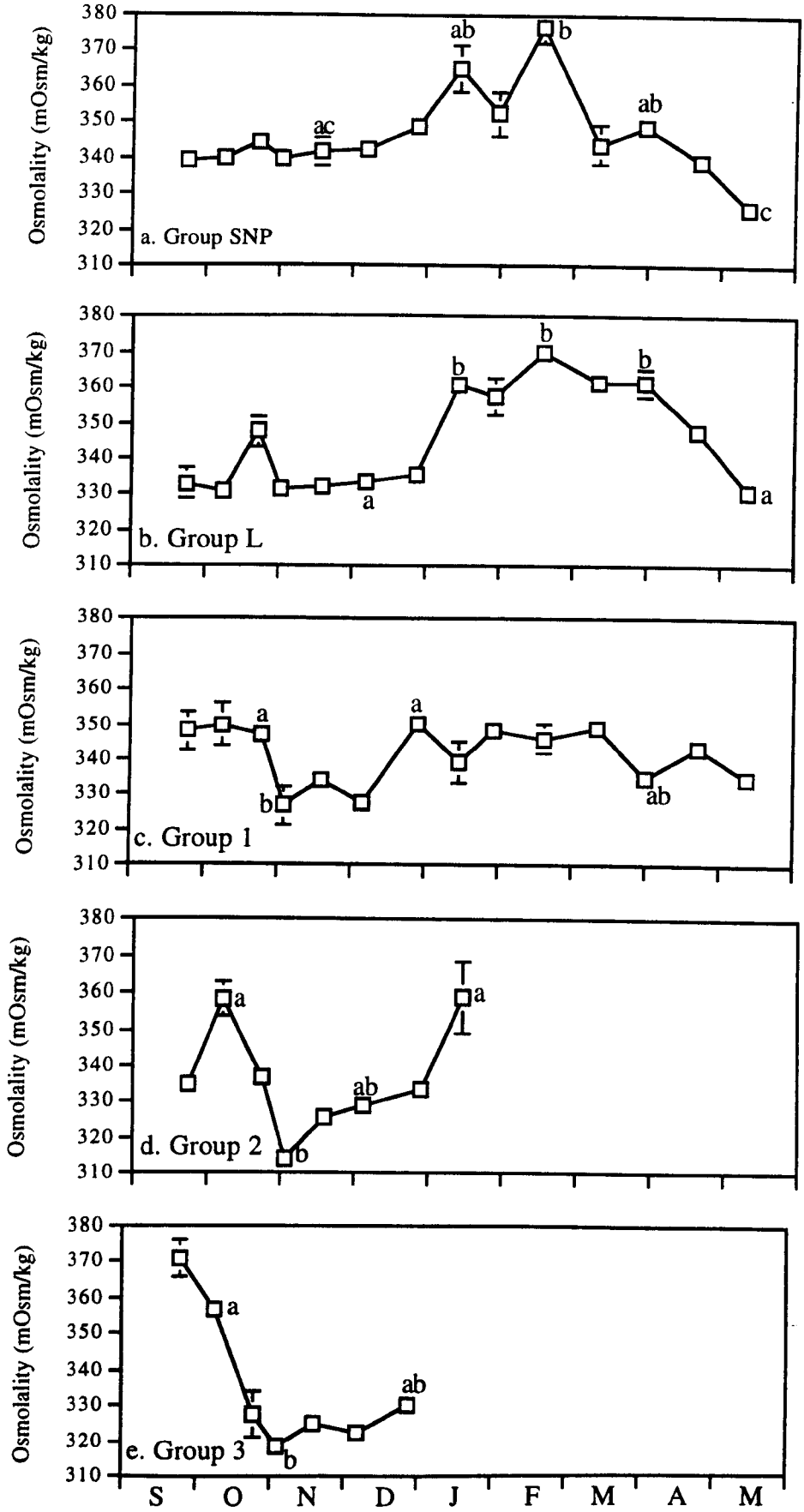
Serum Osmolality of Challenged Fish.

The serum osmolality of challenged fish from group SNP (figure 3.13a.) exhibited little change during September, October, November and December ranging from 339.45±3.02 to 348.85±2.96 mOsm.kg^{-1}. The serum osmolality increased to between 352.48±5.97 and 376.6±4.35 mOsm.kg^{-1} during January and February before decreasing from March through to April. The highest osmolality reading on the 17th Feb. (376.6±4.35 mOsm.kg^{-1}) was significantly greater ($p<0.01$) than the readings in September and the pre-transfer readings on the 21st April (339.4±1.52mOsm.kg^{-1}) and on the 11th May (325.95±3.56 mOsm.kg^{-1}). The serum osmolality of challenged fish from group L (figure 3.13b.) exhibited a similar pattern as observed in group SNP (figure 3.13a.). The serum osmolality was between 330.7±2.4 and 347.55±4.05 mOsm.kg^{-1} from September to December, the values were elevated during January and February before decreasing significantly ($p<0.01$) from 361.1±3.94 mOsm.kg^{-1} on the 29th March to 331.17±1.05 mOsm.kg^{-1} on the 11th May.

The serum osmolality of challenged fish from group 1 (figure 3.13c.) was between 346.95±3.05 and 349.9±6.35 mOsm.kg^{-1} during September and October. The serum osmolality appeared depressed during November. From 327.95±1.95 mOsm.kg^{-1} on the 4th Dec. the serum osmolality increased significantly ($p<0.01$) to 350.05±3.72 mOsm.kg^{-1} on the 17th Dec. During January-May the serum osmolality remained between 348.65±2.36 and 334.56±3.2 mOsm.kg^{-1} with no significant variation.

Changes in the serum osmolality of challenged fish from group 2 (figure 3.13d.) and group 3 (figure 3.13e.) exhibited a similar pattern. Group 2 increased significantly ($p<0.05$) before decreasing significantly ($p<0.01$) from 358.33±4.43 on the 23rd Oct. to 313.95±1.58 mOsm.kg^{-1} on the 2nd Nov. Group 3 also decreased significantly during October from 356.65±3.3 on the 21st Oct. to 318.3±2.02 mOsm.kg^{-1} on the 1st Nov. During November and December both groups displayed an increasing trend in serum osmolality. In group 3 the change was slight while the increase observed in group 2 was

Figure 3.13 a-e. Changes in serum osmolality of challenged fish over the freshwater experimental period for the five groups:- group SNP (a.) which experienced a simulated natural photoperiod; group L (b.) which experienced a constant long daylength LD 19.5:4.5; group 1 (c.) which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 (d.) which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 (e.) which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. Over the sampling period different letters denote significant differences ($p<0.05$). Error bars are $\pm$ 1 sem; error bars which are not visible are obscured by the plot symbol.


Osmolality (mOsm/kg)
380
370
360
350
340
330
320
310
a. Group SNP
ab
b
ac
ab
c
b. Group L
b
b
b
a
a
c. Group 1
a
a
b
ab
d. Group 2
a
a
ab
b
e. Group 3
a
ab
b
S
O
N
D
J
F
M
A
M

significant ($p<0.01$), increasing from 318.3±2.02 on the 1st Nov. to 359.05±9.74 $mOsm.kg^{-1}$ on the 14th Jan.

Smolt Index.

On the 11th-12th Jan. there were differences in appearance of the fish in the different groups (figure 3.14). During sampling 86% of the upper mode fish in Group 2 and 98 % of the upper mode fish in group 3 (sampled on 12th Dec. prior to transfer) scored smolt indices 3 and 4. The percentage of upper mode fish scoring smolt indices 3 and 4 in groups SNP, group L and group 1 was 3.7 %, 3.3 % and 2.9 % respectively.

On the 9th - 10th May prior to the transfer of groups SNP, L and 1 the three groups exhibited a similar percentage of fish scoring smolt index 3 and 4 (figure 3.15). Group SNP and group 1 contained almost entirely smolt indices 4 fish; 94.6% in group SNP and 94.3 % in group 1. Group L, however contained 59.5 % smolt index 4 fish, 31.1 % smolt index 3 fish, and 9.5 % smolt index 2 fish.

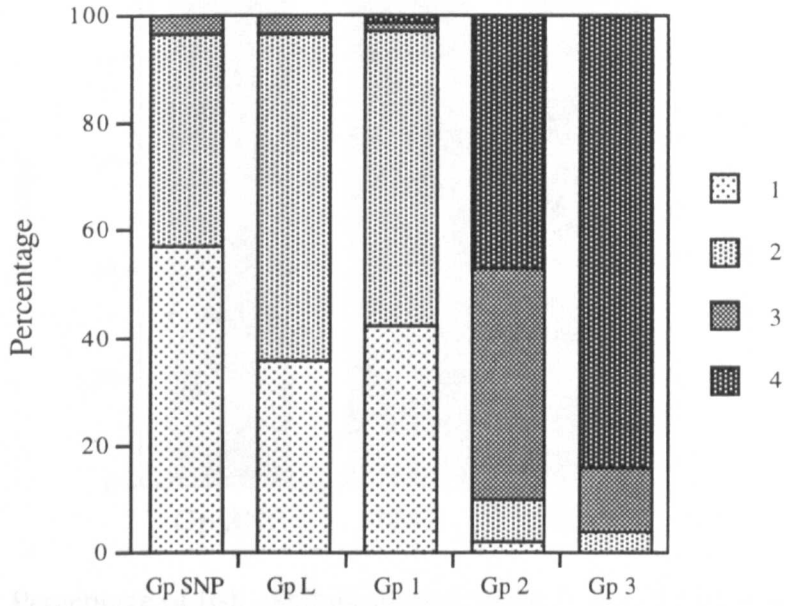


Figure 3.14 Percentage of fish exhibiting smolt index 1 (parr no silvering), 2 (parr with some silvering), 3 (pre-smolt, silver parr marks just visible) and 4 (smolt) in the upper mode of each of the 5 groups:- group SNP which experienced a simulated natural photoperiod; group L which experienced a constant long daylength LD 19.5:4.5; group 1 which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. The groups were assessed on the 11th and 12th Jan. (group 3 was assessed on the 12th Dec.).

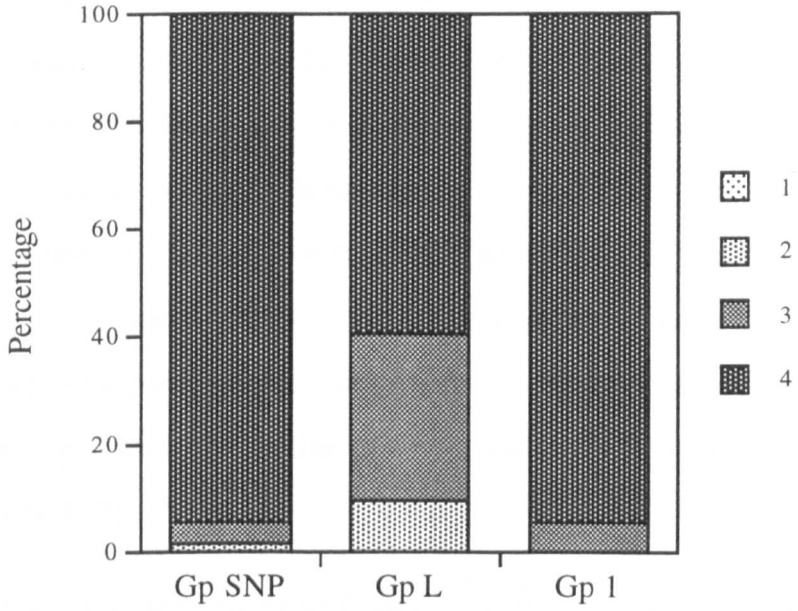


Figure 3.15 Percentage of fish exhibiting smolt index 1 (parr no silvering), 2 (parr with some silvering), 3 (pre-smolt, silver parr marks just visible) and 4 (smolt) in the upper mode of each of the 3 groups:- group SNP which experienced a simulated natural photoperiod; group L which experienced a constant long daylength LD 19.5:4.5 and group 1 which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. The groups were assessed on the 9th and 10th May.

3.3.3 Seawater Results.

Growth.

The 5 groups displayed different growth patterns when transferred to sea water. Group 3 and group 2 were transferred on the 21st Dec. and 18th Jan. respectively. Prior to the transfer of group 3 on the 13-14th Dec., there was no significant difference between the 2 groups population or upper mode mean weights. On the 2nd Feb group 3 displayed a significantly ($p<0.01$) greater mean weight than group 2 (figure 3.16). Group 3 maintained this weight advantage, displaying a significantly ($p<0.01$) greater mean weight on all subsequent sample dates except the 9th June. The maintained weight difference is explained by the similar pattern of specific growth rates exhibited by the 2 groups (figure 3.17).

Group SNP, group L and group 1 were all transferred to sea water on the 18th May. On the 9-10th May there was no significant difference between the population means. However, the upper mode means were 49.3±1.4 g for group SNP, 57.4±2.0g for group L and 66.1±3.1 g for group 1. Group 1 were significantly ($p<0.01$) heavier than group SNP. There was no significant difference between group L and either group SNP or group 1. During the first 2 months in sea water the situation remained the same. Group 1 were significantly ($p<0.01$) heavier than group SNP whilst there was no significant difference between group L and groups SNP or 1 (figure 3.16). On the 2nd Aug. there was no significant difference between the 3 groups. On the 1st Sept. there was no significant difference between group L and group 1 whilst group SNP was significantly ($p<0.01$) larger than both group L and group 1. The changes in weight were reflected in the specific growth rates (figure 3.17). All groups lost weight during the first month and grew (SGR = 0.33-0.92) during the second month. During the third month the specific growth rate of group L and group 1 dipped while the specific growth rate of group SNP increased. During August and September group SNP maintained a higher specific growth rate than group L and group 1.

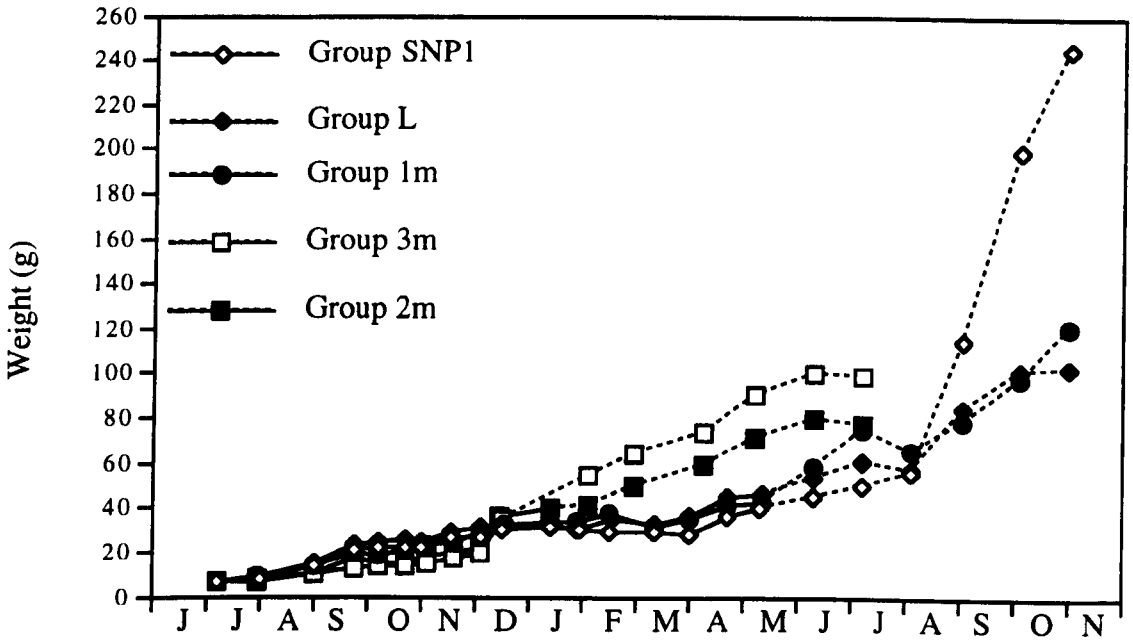


Figure 3.16 Growth in weight for the 5 groups:- group SNP which experienced a simulated natural photoperiod; group L which experienced a constant long daylength LD 19.5:4.5; group 1 which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5. The solid line represents freshwater growth and the dashed line represents seawater growth. The error bars (± 1 sem) are obscured by the plot symbols.

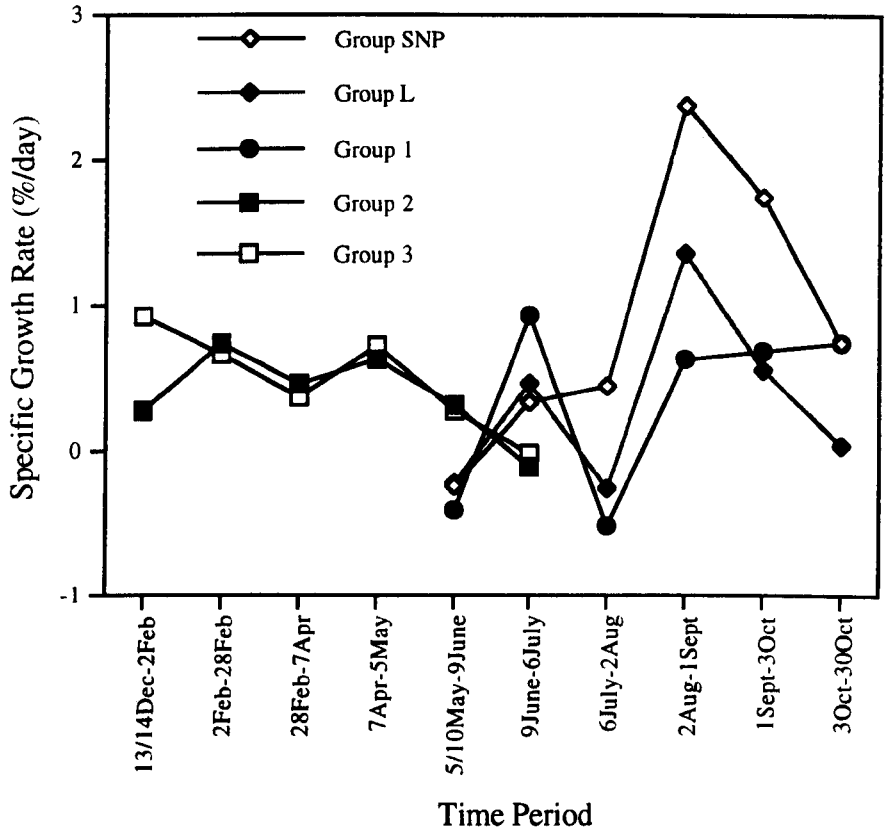


Figure 3.17 Specific growth rate during consecutive time periods for the five groups:- group SNP (open diamond symbol) which experienced a simulated natural photoperiod; group L (filled diamond symbol) which experienced a constant long daylength LD 19.5:4.5; group 1 (filled circle symbol) which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 (filled square symbol) which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 (open square symbol) which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5.

Mortality.

Mortality over the 6 month seawater period varied between groups. The mortality for groups 2 and 3 was steady during the 6 month growing period (figure 3.18). The 3 groups transferred on the 18th May displayed similar patterns of mortality. However, there were significant ($p<0.01$) differences in the total cumulative mortalities. The mortalities stopped in September for group SNP and in October for group 1.

Groups 2 and 3 were transferred earliest and displayed the lowest cumulative mortalities over the entire seawater period, 20.6% and 10.1% respectively (figure 3.18). There was no significant difference between the total cumulative mortalities for group 2 and group 3. The cumulative mortality for group 3 was significantly ($p<0.01$) lower than the corresponding mortality for all the groups transferred on the 18th May and group 2 was significantly ($p<0.01$) lower than group L and group 1. Group SNP exhibited the lowest cumulative mortality from the groups transferred on 18th May, group 1 exhibited a slightly higher mortality and group L exhibited the highest cumulative mortality. The cumulative mortalities for group SNP and group 1 were significantly ($p<0.01$) lower than the cumulative mortalities for group L.

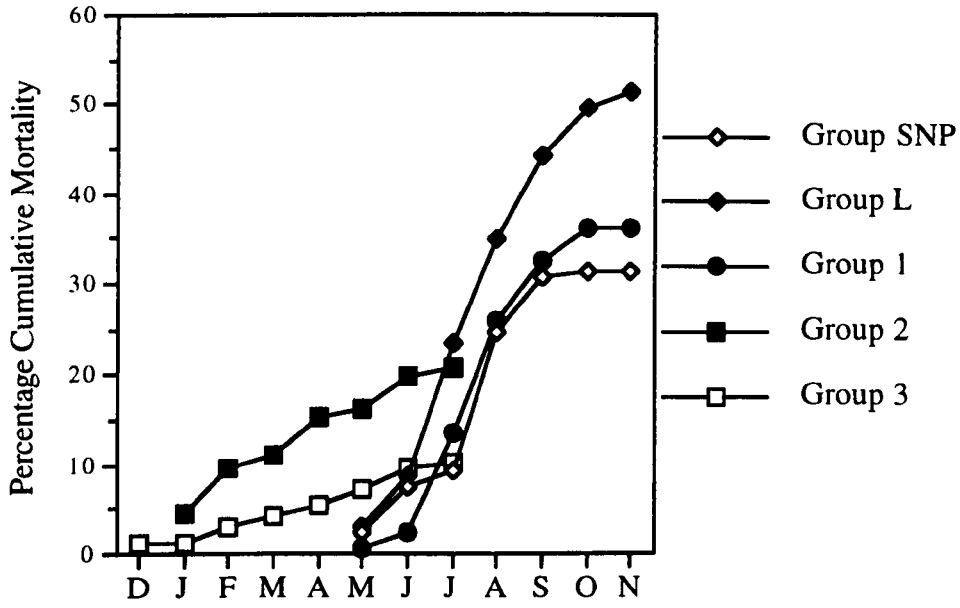


Figure 3.18 Cumulative percentage mortality for each month in sea water for the 5 groups:- group SNP (open diamond symbol) which experienced a simulated natural photoperiod; group L (filled diamond symbol) which experienced a constant long daylength LD 19.5:4.5; group 1 (filled circle symbol) which experienced a 1 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5; group 2 (filled square symbol) which experienced a 2 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5 and group 3 (open square symbol) which experienced a 3 month period of short daylength LD 8:16 during a constant long daylength LD 19.5:4.5.

Summary of Results from Experiment 2.

1) All groups developed a bimodal distribution. Bimodality developed earlier in groups with earlier decreases in photoperiod. Longer periods of short days resulted in lower recruitment into the upper mode. Bimodality developed under a constant photoperiod (group L).

2) The initial decrease in condition factor occurred at the same time in all groups. A second decrease in condition was observed in groups which experienced a winter period greater than 2 months (groups 2, 3 and SNP).

3) Hypoosmoregulatory ability generally increased as the photoperiod increased and decreased as the photoperiod decreased. Similar changes in hypoosmoregulatory ability were observed under a simulated natural photoperiod (group SNP) and a constant photoperiod (group L).

4) The development of a smolt appearance was advanced by an artificial photoperiod with a winter period of 2 months (groups 2 and 3) or more. Fish held under constant conditions (group L) or a 1 month (group 1) winter period developed a smolt appearance at the same time as the fish held under a simulated natural photoperiod (group SNP).

5) Groups 2 and 3 which experienced a 2 and 3 month short day period exhibited the highest survival in sea water, whilst group SNP which experienced a simulated natural photoperiod exhibited the highest growth in sea water. Group 1 (1 month of short days) and Group L (constant LD 20:4) exhibited the poorest growth and survival in sea water.

3.4 Experiment 3. The effects of a constant LD 20:4 photoperiod and constant 10°C temperature on growth and smoltification in juvenile triploid Atlantic salmon (*Salmo salar*).

3.4.1 Materials and Methods.

Experiment 3 was carried out at site FW 2 (section 2.2.1.2). The eggs and alevins were reared in darkness at a constant temperature of 10±1°C. The fry and parr were reared under an LD 20:4 daylength and a constant 10±1°C temperature regime. The fry and parr were reared in 1.5 m^3 and 7 m^3 raceways.

On the 4th Nov. a raceway (7 m^3) of 20000 triploid parr (mean weight 9g) was selected. The parr had been held on LD 20:4 and supplied with constant 10±1°C water from first feeding. The parr were considered to be potential S1 parr. A random sample of 250 parr was taken from the raceway and transferred to a 1m (0.25 m^3) tank. These parr formed 'group 20' and were maintained on constant LD 20:4 and constant temperature (10±1°C) during the entire experiment.

The parr remaining in the raceway were monitored as a control group (group C). Group C was maintained in the 7 m^3 raceway. On the 4th Nov. a 2 hour per week decrease in photoperiod was initiated. On the 9th Dec. the photoperiod was decreased from LD 10:14 to LD 8.5:15.5, which was maintained until 20th Jan. when a 0.5 hour.$week^{-1}$ increase was initiated. On the 28th Jan. the parr were transferred to an earth pond. The only illumination was natural daylight. On the 30th March half of the fish were transferred into a second pond.

At intervals of 2 weeks a random sample of fish (n = 10-15) from both groups C and 20 were seawater challenged (section 2.3.3.2) and a second sample of fish (n = 50) assessed for weight, length and smolt index. Fish which were sampled were returned to the experimental groups after sampling. Serum osmolality, SGR (section 2.3.2.1) and

condition factor (section 2.3.3.1) were determined from the samples. Sampling of group 20 and group C was initiated on the 19th Nov. and the 9th Jan. respectively. Group C was transferred to sea site SW 3 on the 29th May. Group 20 was maintained in fresh water and sampled at intervals of 2 weeks until the 28th Sept., two years after the experiment was initiated.

Consecutive serum osmolalities and condition factors were compared using the Mann-Whitney test (section 2.4.1). Changes in condition factor and SGR were correlated using the Pearson correlation (section 2.4.4).

3.4.2 Results.

Growth and Condition factor.

Both groups grew steadily over the sampling period. Group 20 fish consistently exhibited greater sample weights than those of group C (figure 3.19). The SGR was variable for both groups (figure 3.20). Group 20 exhibited a negative SGR (lost weight) during the 6 time periods 4th April to 16th April (-0.31), 19th Sept. to 4th Nov. (-0.61), 16th Nov. to 21st Dec. (-0.02), 7th Jan. to 20th Jan. (-0.19), 15th Feb. to 3rd March (-0.02) and 12th April to 27th April (-0.42). Group C exhibited a SGR of -0.38 during the time period from 6th Feb. to 20th Feb.

The mean SGR over the entire sample period was 0.6±0.09 for group 20 and 0.54±0.19 for group C. Group C exhibited 2 extended periods from the 6th Feb. to 19th March and the 4th April to 26th April when the SGR exceeded the groups mean SGR. Group 20 exhibited 7 extended periods from the 19th Nov. to 20th Feb., the 25th May to 20th June, the 2nd Sept. to 1st Oct., the 4th Nov. to 3rd Dec., the 20th Jan. to 15th Feb., the 16th March to 12th April and the 14th June to 10th Aug. when the SGR exceeded the groups mean SGR.

The condition factor for group C generally decreased during the sampling period (figure 3.21). The condition factor was recorded at 1.15±0.04 on the 9th Jan. From the 9th Jan.

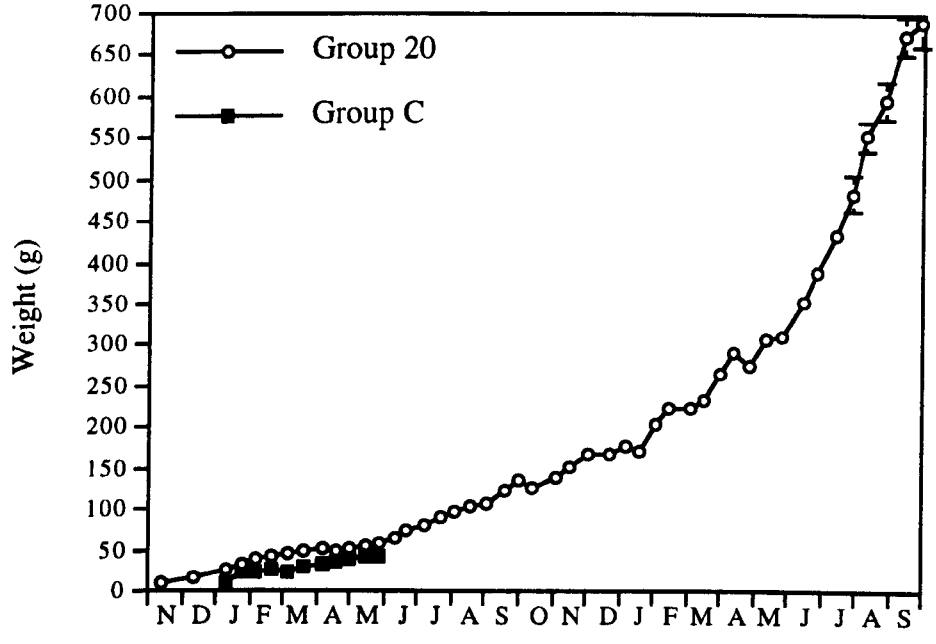


Figure 3.19 Changes in weight during the sampling period for group 20 (open circle) held under a constant LD 20:4 and group C (filled square) the control group.

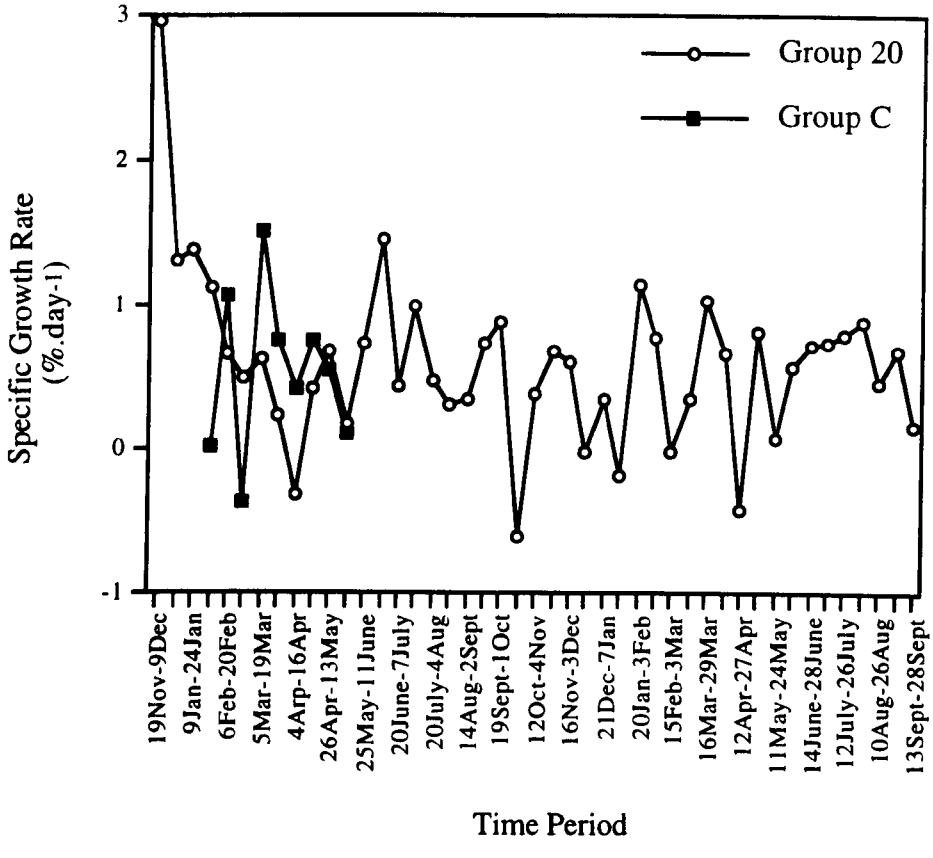


Figure 3.20 Specific growth rate (SGR) during the time periods between sampling dates. Data is presented for group 20 (open circle) held under a constant LD 20:4 and group C (filled square) a control group.

the condition decreased and increased significantly ($p<0.05$) with a low point of 1.09±.007 on the 2nd Feb. The condition factor remained above 1.09±.007 until the 16th April when the condition factor decreased significantly ($p<0.05$) from the 4th April to 1.07±0.007 on the 16th April. The condition factor again decreased significantly from the 29th April to the 13th May and from 13th May to the 25th May. The final condition factor recorded was 0.97±0.007 on the 25th May when the fish were transferred into sea water. The period of decrease from the 4th April to the 25th May was associated with positive SGR values and the significant decrease from the 29th April to the 13th May was associated with a period of above average SGR (figures 3.20 and 3.21).

The condition factor for group 20 exhibited a degree of cycling (figure 3.21). The condition factor increased during November and December then decreased during March, April and May, increased again during June and July and decreased during September through to December. Finally the condition factor increased during February, March, and June through to August. The first period of decrease from the 20th Feb. to the 25th May was associated with positive SGR values except for the period from the 4th April to 16th April. The period from the 5th March to the 19th March exhibited a significant ($p<0.05$) decrease in condition factor in association with a period of above average SGR (figure 3.20 and 3.21). The period from the 4th April to 16th April also exhibited a significant ($p<0.05$) decrease in condition factor. However, this period was associated with a negative SGR. Other significant ($p<0.05$) decreases were observed from the 20th June to 7th July, 20th July to 4th Aug., 4th Aug. to 14th Aug. and the 12th April and 27th April. All these periods were associated with a period of below average, but positive SGR except the period from 12th April to 27th April which exhibited a negative SGR. Generally for group 20 a significant decrease in condition factor was associated with a period of decreasing SGR.

The correlation of change in condition factor (condition factor at sample time 1 - condition factor at sample time 0) against SGR gave an r value of 0.54 for group 20 (figure 3.22a) and 0.37 for group C (figure 3.22b). The correlation between growth rate

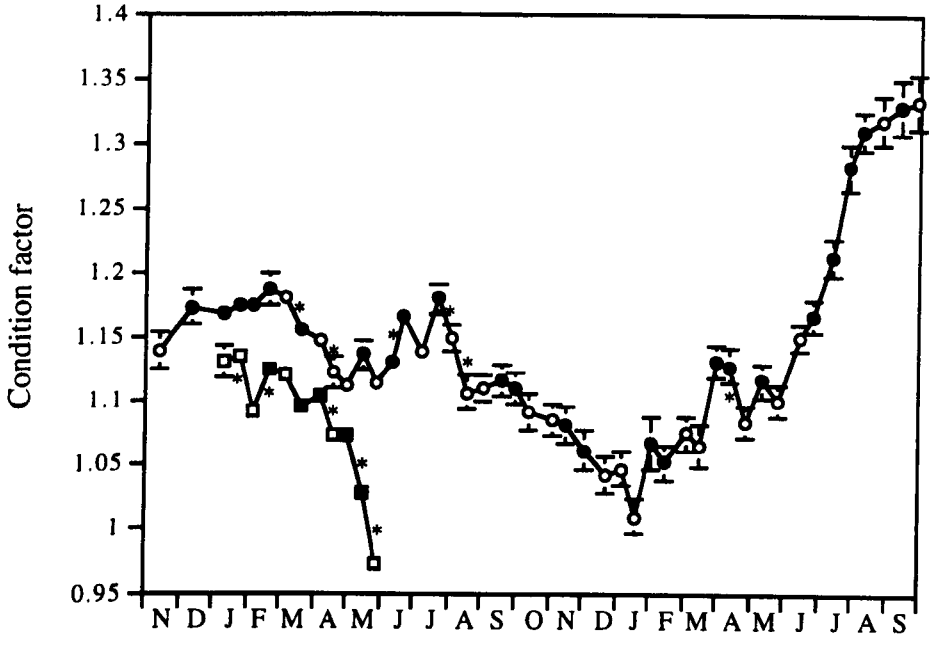


Figure 3.21 Changes in condition factor during the sampling period for group 20 (circular symbol) held under a constant LD 20:4 and group C (square symbol) the control group. Filled symbols indicate that an above average SGR was observed in the period prior to the sample point. An * between two sample points indicates that the two sample points were significantly different ($p<0.05$).

Figure 3.22a.

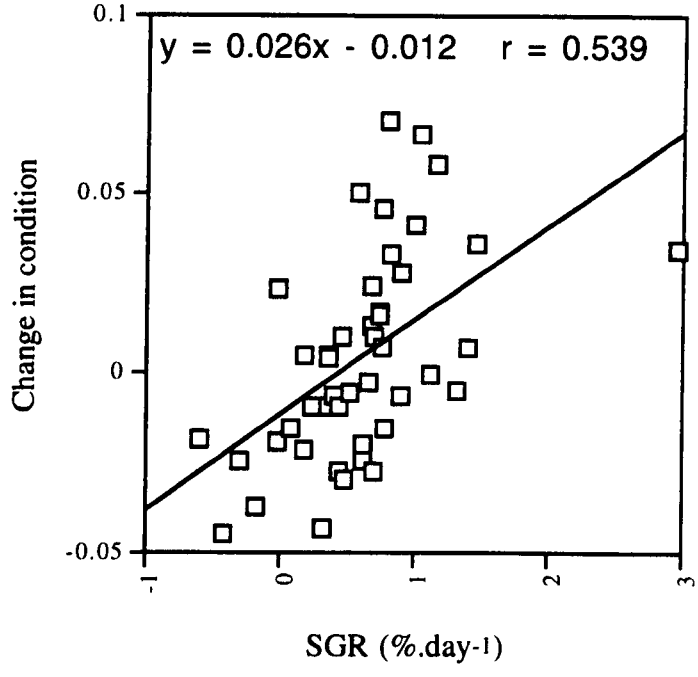


Figure 3.22b.

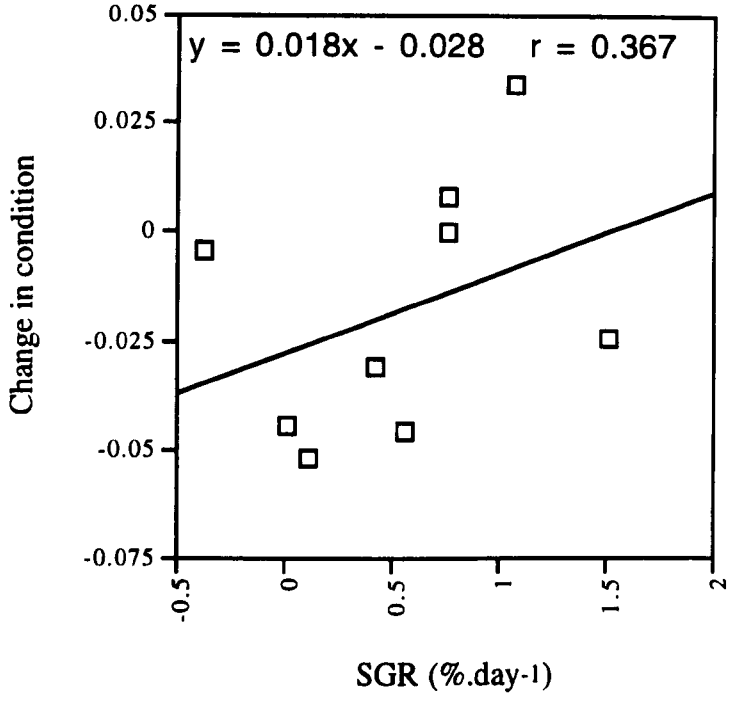


Figure 3.22 a and b. Correlations of change in condition (condition factor at sample time 1 - condition factor at sample time 0) against SGR for group 20 (a) (n=45) held under constant LD20:4 and group C (b) (n=9) a control group.

and change in condition factor was significant (∂<1%) for group 20 and was not significant (∂>1%) for group C.

Smolt Index.

The percentage of parr and smolts in the population changed over the sampling period. In group C (figure 3.23b) the population was pre-dominantly (80-100%) parr until the 4th April when the percentage of pre-smolts in the population increased. This situation continued during April and on the 26th April the percentage of smolts in the population began to increase. On the 25th May 100% of the fish in the population had the coloration of smolts.

In group 20 (figure 3.23a) the population was also initially predominately (87-100%) parr until 24th Jan. when the percentage of pre-smolts in the population increased. During February and March the population was divided 50-58% parr and 38-44% pre-smolts. During April the percentage of parr increased by 10-20%, the percentage of pre-smolts decreasing. From May through to October the percentage of parr gradually decreased and the percentage of smolts gradually increased. The percentage of pre-smolts was static over this period as the percentage recruited from parr to pre-smolts and pre-smolts to smolts was similar. The population was predominantly (76-100%) smolts from 4th Nov. until the end of the experiment.

The pattern of change in the percentages of parr, pre-smolts and smolts in the 2 groups was similar. However, the change in group C was over 5 months whilst the change in group 20 was over 12 months.

Challenge serum osmolality.

The serum osmolality of the challenged fish from group C (figure 3.24) initially increased during January and February before significantly (P<0.05) decreasing from 410.75±8.4 mOsm.kg^{-1} on the 7th Feb. to 368.72±5.7 mOsm.kg^{-1} on the 21st Feb.. After a small increase the serum osmolality again decreased significantly (P<0.05). After

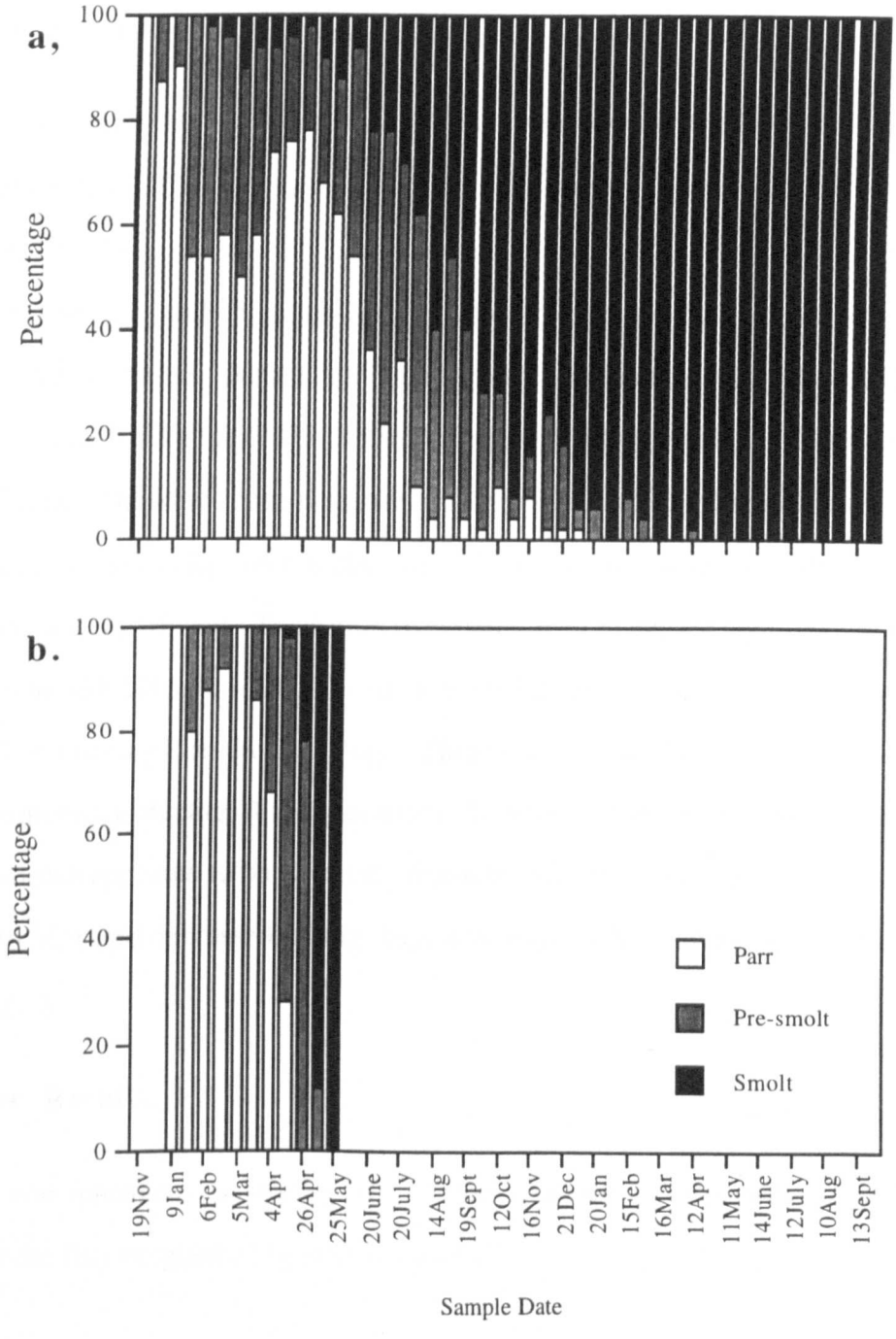


Figure 3.23 The percentage of the population recorded as parr (open bar), pre-smolts (specked bar) and smolts (filled bar) on each sampling date. a: refers to group 20 which were held under a constant LD 20:4 daylength and b: refers to group C the control group.

a small decrease a 3rd significant ($P<0.05$) decrease was observed, resulting in a serum osmolality of 324.46±1.02 $mOsm.kg^{-1}$ on the 26th May, prior to seawater transfer.

The serum osmolality of the challenged fish from group 20 (figure 3.24) changed significantly ($P<0.05$) on a number of occasions during January through to March. From the 10th Dec. to the 10th Jan. it decreased; from the 10th Jan. to 25th Jan. it increased; from the 7th Feb. to the 21st Feb. it decreased and from the 6th March to the 20th March it increased. During April and May the serum osmolality remained between 362.35±3.82 $mOsm.kg^{-1}$ and 373.21±3.6 $mOsm.kg^{-1}$ before a gradual decrease through May and June. The May / June decrease was from 373.21±3.6 $mOsm.kg^{-1}$ on 17th April to 344.62±3.67 $mOsm.kg^{-1}$ on the 20th June. However, no consecutive decrease during this period was significant. The serum osmolality then increased significantly from the 20th June to 364.24±3.74 $mOsm.kg^{-1}$ on the 7th July before decreasing significantly to 346±3.38 $mOsm.kg^{-1}$ on the 21st July. There were no further significant changes in serum osmolality during the experiment. Generally the serum osmolality of the challenged fish appeared to be elevated (approximately 360 $mOsm.kg^{-1}$) during February through to May and depressed during August through to November (approximately 340 $mOsm.kg^{-1}$).

Seawater Results.

Group C was transferred to sea site SW3 on the 29th May. The transfer mortality was 5.3% and the fish weighed 311g after 6 months.

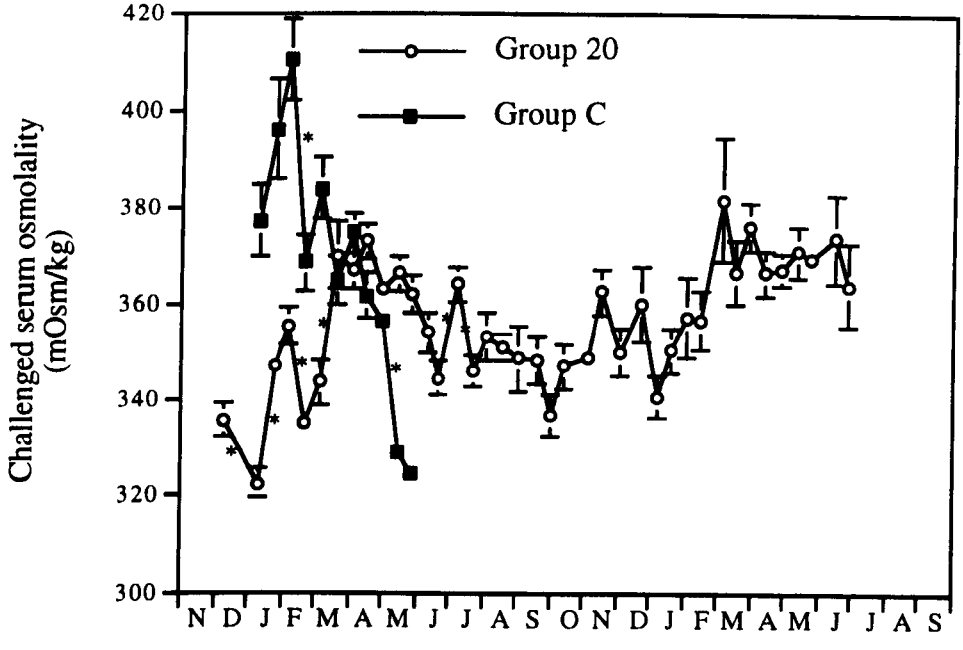


Figure 3.24 Changes in serum osmolality of challenged fish ($mOsm.kg^{-1}$) during the sampling period for group 20 (open circle) held under a constant LD 20:4 daylength and group C (filled square) the control group. An * between two sample points indicates that the two sample points were significantly different ($p<0.05$).

Summary of Results from Experiment 3.

1) Group C (simulated natural photoperiod) exhibited a decrease in condition factor in relation to the increase in photoperiod. Group 20 (constant 20 hour day) exhibited a cycle of change in condition factor, which was significantly correlated with SGR. The change in condition in group C was not significantly correlated with SGR.

2) Group C (simulated natural photoperiod) exhibited a decrease in serum osmolality of challenged fish in relation to the increase in photoperiod. Group 20 (constant 20 hour day) exhibited a series of increases and decreases in serum osmolality.

3) A smolt appearance developed in both groups. In group C development was over 5 months and was in association with the increasing photoperiod. In group 20 the development of a smolt appearance occurred over a 12 month period.

3.5 Discussion.

The three experiments included in this chapter show clearly that photoperiod influences smoltification. Photoperiod affected the timing and / or development of the smoltification parameters: bimodality, condition factor, hypoosmoregulatory ability and coloration.

The growth dynamics of the fish in experiments 1 and 2 were also affected by the artificial photoperiods. In addition to changes in growth, the timing of development of the upper mode and the proportion of fish entering this mode were altered.

In experiment 1 the decrease to LD 6:18 and the 2 month hold on LD 6:18 to which the fish were subjected reduced the growth of the fish in group PL, compared to the growth of fish which experienced a natural photoperiod (group NL). Similar suppressions of growth, following a decrease in daylength to a short day, have been reported by Saunders *et al.* (1985), McCormick *et al.* (1987) and Berge *et al.* (1995). The 1 hour . week^{-1} increase in daylength stimulated growth in the PL group, compared to the growth of the fish under the decreasing natural photoperiod in group NL. Increasing daylength has been shown to stimulate growth (Saunders *et al.* 1985, McCormick *et al.* 1987, Stefansson *et al.* 1991, Solbakken *et al.* 1994).

The significantly higher proportion of potential S1 parr in the NL group indicates that the artificial photoperiod experienced by group PL reduced recruitment into the potential S1 upper mode. The reduced recruitment was probably related to the reduced opportunity for growth, which was reflected in the significantly smaller mean weights and the lower SGR observed in group PL. Thorpe *et al.* (1989) demonstrated that decreasing the opportunity for growth during the decision period reduced recruitment into the upper mode.

A similar result was observed in experiment 2. Group 3 which experienced 3 months of LD 8:16 exhibited the lowest percentage of fish in the upper mode. As the period of LD 8:16 was decreased to 2 months and 1 month, the percentage of upper mode fish

increased. Group L which experienced a constant long daylength (LD 19.5:4.5) exhibited the highest percentage of fish in the upper mode. This result is also in agreement with Thorpe *et al.* (1989). In experiment 2 the timing of bimodal development was also affected by the photoperiod. Bimodal development was observed later in the groups exposed to artificial photoperiods, compared to the fish which experienced a simulated natural photoperiod (group SNP).

Duston and Saunders (1992) proposed a model where development of bimodality was initiated under the decreasing photoperiod and recruitment continued until the increasing photoperiod initiated the parr-smolt transformation. The 3 groups in experiment 2 which experienced different periods of short daylength all developed bimodality approximately 1 month after the decrease from LD 19.5:4.5 to LD 8:16. The decrease in the photoperiod was phased at monthly intervals and consequently the development in bimodality occurred at monthly intervals. This supports the hypothesis that recruitment and in turn the development of bimodality is initiated by the decreasing photoperiod.

In groups 2 (2 months short days) and 3 (3 months short days) recruitment into the upper mode appeared to stop in the month after bimodality developed. This suggests that the growth opportunity was insufficient to allow further recruitment. The temperature dropped considerably during September. Kristinsson *et al.* (1985) observed that recruitment stopped in parr held under a natural photoperiod when the temperature dropped below 10°C. Duston and Saunders (1992) observed continued recruitment during the winter period when photoperiod and temperature presented sufficient growth opportunity. However, the recruitment stopped after the spring increase terminated the winter period.

In contradiction to Duston and Saunders (1992) group 1 (1 month of short days) appeared to develop bimodality after the spring increase. This suggests the 1 month period of LD 8:16 did not have the effect of a short day photoperiod or the development of bimodality was phase delayed. When a biological process which is controlled by an endogenous rhythm, timed by photoperiod, is subjected to a rapidly-changing

photoperiod (i.e. a forcing zeitgeber) the process may be observed to develop later in the photoperiodic cycle than expected i.e. a phase delay of the endogenous rhythm. However, the development of bimodality in group 1 was delayed in comparison to group SNP. It would appear that the 1 month short day period was not acting as a driving zeitgeber to advance the timing of development of bimodality. This discounts the suggestion that the development of bimodality was phase delayed and suggests that the 1 month period of LD 8:16 did not have the effect of a short day photoperiod. Group L (constant long daylength) was not subjected to any period of short daylength. Early in October, bimodality was observed to develop in group L. This result is similar to the result observed in group 1 and supports the suggestion that the 1 month period of LD 8:16 did not have the effect of a period of short days. The decrease to the short day did not initiate the development of bimodality and the increase to a long day did not terminate recruitment into the upper mode.

Bimodality was observed under a simulated natural photoperiod (group SNP) during late August compared to early October under a constant long daylength (group L). The development of a seasonal biological process under a constant photoperiod, at a time close to but different from the time under a natural photoperiod, indicates that the process is controlled by an endogenous rhythm timed by photoperiod. The development of bimodality observed under a constant long daylength (group L) was delayed compared to group SNP. A number of studies have observed a delay in the parr-smolt transformation in groups which experienced a summer solstice hold (Saunders *et al.* 1989, Saunders and Harmon 1990, McCormick *et al.* 1987). It would appear that the constant long day initially delayed the development of bimodality and subsequently, in the absence of a photoperiod cue, the rhythm free ran resulting in a delay in the development of bimodality, compared to the group under a simulated natural photoperiod (group SNP).

These observations support the hypothesis that the development of bimodality is controlled by an endogenous rhythm, timed by the changing photoperiod. Entrainment

of the timing of bimodal development by photoperiod was possible when the short day period of the photoperiod was greater than 2 months.

Changes in condition factor were observed in all groups in the three experiments. In experiment 1 under both an artificial photoperiod (group PL) and a natural photoperiod (group NL) the changes in condition factor were similar. Condition factor is a recognised measure of the 'fitness or well being' of a fish (Bolger & Connolly 1989). Under natural conditions and in a year when the salmon does not smoltify or mature, condition factor increases during spring and summer and decreases in autumn and winter (Hoar 1939, Farmer *et al.* 1978). These changes in condition are related to the changing opportunity for growth. During the spring, increasing food availability, temperature and photoperiod provide the environment for the condition or 'fitness' of the fish to increase. Conversely during the autumn the decreasing food availability, temperature and photoperiod result in a decrease in condition. The decrease observed during the autumn under a natural photoperiod (NL group) was the natural autumn decrease observed in parr. The cause of the decrease observed under the artificial photoperiod (group PL) is not clear. The decreasing temperature could have possibly reduced the fitness of the fish in the group causing a decrease in condition. However, the growth observed during this period would suggest that the condition or fitness of the fish should have increased. During the natural spring period of increased growth, the condition increases in parr. However, the condition decreases in fish which transform from parr to smolts (Farmer *et al.* 1978). The decrease in condition observed in group PL was associated with an increasing 'spring' photoperiod and is therefore probably associated with the parr-smolt transformation.

In experiment 2, a similar situation was observed. The fish held under a simulated natural photoperiod (group SNP) exhibited an autumn decrease in condition, which remained depressed throughout the winter period. This has been observed in fish under natural conditions (Hoar 1939, Farmer *et al.* 1978). However, all groups exhibited a decrease in the condition factor during September and early October. This decrease in

condition coincided with the period when the temperature was falling and all groups were transferred into larger tanks. Groups 1 (1 month short day period), 2 (2 month short day period) and 3 (3 month short day period) had also experienced a decrease in photoperiod. This suggests that the decrease in condition observed in groups 1, 2 and 3 was also related to the natural autumn decrease in condition and/or the transfer to new tanks. Group L (constant long day) did not experience a decrease in photoperiod, but the condition factor did decrease again indicating that the decrease in condition factor is closely related to temperature and/or the transfer to new tanks. The decrease in condition was in association with the observed development of bimodality. The development in bimodality is growth-related and probably related to an endogenous growth rhythm. The close association between development of bimodality and decrease in condition suggests that the initial decrease in condition was possibly also related to a endogenous growth rhythm.

In groups L (constant long day), 1 (1 month short day period), 2 (2 month short day period) and 3 (3 month short day period) condition factor increased after the initial decrease. The increase in condition was under a long daylength. The cause of the rise in condition under a constant long day (group L) is unclear, because the temperature was low and an endogenous growth rhythm should promote a similar pattern in condition, as was observed under the simulated natural photoperiod (group SNP). The increase in condition may have been related to the constant long day promoting growth, a higher SGR was observed in group L compared to group SNP. The increase in condition observed in groups 1, 2 and 3 was close to the increase in photoperiod from the short day period to a long daylength. An increase in daylength has been observed to stimulate growth (Saunders *et al.* 1985, McCormick *et al.* 1987, Stefansson *et al.* 1991, Solbakken *et al.* 1994) and the SGR during the autumn period was higher in the groups 1, 2 and 3 compared to group SNP. The rise in condition was probably related to the increased growth. However, in group 2 (UM and LM) and 3 (UM) the condition factor again fell approximately 6-8 weeks after the photoperiod increased. This decrease in condition was probably the decrease associated with the parr to smolt transformation.

The suggested parr-smolt decrease in condition is supported in the 3 month short day group (group 3) as the LM condition increases in contrast to the UM decrease. In the absence of a parr-smolt transformation, condition naturally increases during the spring (Hoar 1939, Farmer *et al.* 1978). However, in the 2 month short day group (group 2) the condition in both the UM and LM decreased.

It would appear that fish which experienced a constant long day (group L) and a 1 month short day period (group 1) exhibited a complex sequence of changes in condition. The changes are difficult to explain and possibly result from the interaction of temperature, photoperiod and an underlying endogenous growth rhythm. The fish which experienced a 2 month period of short days (group 2) or a 3 month period of short days (group 3) exhibited a natural cycle of change in condition (as observed in group SNP) compressed into 4 months, from September to January.

In experiment 3, the parr-smolt transformation associated decrease in condition was again observed under a natural photoperiod. Under conditions of constant temperature and photoperiod, the changes in condition factor appeared to be related to growth and not to the parr-smolt transformation. In the control group (group C), which was exposed to a natural photoperiod the decrease in condition was in association with an above average SGR during a period when fitness of the fish increased. There was no correlation between SGR and change in condition factor in group C. The fish held under a constant 20 hour daylength (group 20) and constant temperature showed a positive correlation between SGR and change in condition factor. This suggested that the changes in condition in group 20 were related to changes in growth rate. The initial decrease in condition corresponded with both poor and good growth and may have been influenced by the parr-smolt changes.

The absence of a parr-smolt associated decrease in condition under constant conditions would suggest that the parr-smolt associated decrease in condition is controlled by photoperiod and/or temperature. The result does not appear to support the hypothesis, that the parr-smolt associated decrease in condition is controlled endogenously. A

number of studies have observed the absence of a parr-smolt decrease in condition factor when fish were: maintained under a constant photoperiod (McCormick *et al.* 1987), were not subjected to a period of short days (Saunders *et al.* 1985), subjected to a short day period but no increase in daylength (Duston & Saunders 1990a) or subjected to a short day period of one month (Bjornsson *et al.* 1989). Duston and Saunders (1990a) suggested that the parr-smolt associated decrease in condition is not controlled endogenously, but requires a period of short days followed by an increase in photoperiod.

Eriksson and Lundqvist (1982) provide the strongest evidence that the decrease in condition is controlled by an endogenous rhythm. Eriksson and Lundqvist (1982) observed a cycling of changes in condition factor under constant LD 12:12 and constant 11±0.5°C. However, the initial decrease in condition observed by Eriksson and Lundqvist (1982) may have been in response to an increase in photoperiod. The experiment was initiated in December and may have started with an increase from a short natural daylength to the LD 12:12 photoperiod, but this is speculation as the pre-experimental photoperiod is not described in the materials and methods. The second decrease in condition is in association with decreasing growth and may not be a parr-smolt change in morphology. It should be noted that Eriksson and Lundqvist (1982) is the only study which has monitored condition in individually marked fish. It is possible that the growth of individual fish quickly becomes de-synchronised and the general change of the population is not truly representative of the changes of individuals. However, Eriksson and Lundqvist (1982) did not observe a high degree of de-synchronised growth.

The changes in condition factor observed under a constant LD 20:4 and constant 10±1°C in experiment 3 suggest that the change in condition factor associated with the parr to smolt transformation are controlled by temperature and / or photoperiod. The observations support the suggestion, that the change in condition factor associated with

the parr to smolt transformation, requires a period of short days followed by an increase in photoperiod (Duston & Saunders 1990a).

However, the results from experiment 3 also suggest that growth and the associated changes in condition (excluding the change in condition factor associated with the parr to smolt transformation) are controlled by factors other than photoperiod and temperature, perhaps an endogenous rhythm. Endogenous rhythms controlling growth have been demonstrated in brown trout (Brown 1946) and rainbow trout (Wagner & McKeown 1985).

A number of studies have suggested that photoperiod can have a direct affect on growth. Periods of long daylength have been shown to elicit an immediate increase in growth (Saunders *et al.* 1985, 1989, McCormick *et al.* 1987, Stefansson *et al.* 1989, 1991, Duston and Saunders 1990a, experiment 1). This direct effect of photoperiod on growth possibly contradicts the presence of a controlling endogenous growth rhythm. However, the timing of the application of the long daylength in relation to the hypothesised rhythm is important in relation to the effect observed. The application of a long daylength close to the spring increase caused a direct increase in growth (Stefansson *et al.* 1989, 1991, Duston and Saunders 1990a, Krakenes *et al.* 1991), whilst the application of a long daylength prior to the winter solstice caused an initial decrease followed by an increase in growth (Hansen *et al.* 1992). These observations are consistent with an endogenous rhythm entrained by an zeitgeber such as photoperiod.

These experiments support the hypothesis that growth and the associated changes in condition factor (excluding the parr to smolt associated decrease in condition) are controlled by an endogenous rhythm, timed by the photoperiod. The parr to smolt associated decrease in condition appears to require a period of short days greater than 2 months, followed by an increase in daylength.

Changes in the hypoosmoregulatory ability of the fish were observed in all groups. Generally the fish appeared to exhibit hypoosmoregulatory ability under long daylengths and little or no hypoosmoregulatory ability under short daylengths.

In experiment 1, the hypoosmoregulatory ability of the fish held under a natural photoperiod (group NL) decreased as the photoperiod decreased, whilst the hypoosmoregulatory ability of the fish held under an artificial photoperiod (group PL) increased as the photoperiod increased. During November the fish in group PL had the hypoosmoregulatory ability of a smolt, whilst the fish in group NL had no hypoosmoregulatory ability. The parr to smolt increase in hypoosmoregulatory ability has been recorded in association with an increase in photoperiod (Komourdjian *et al.* 1976, Saunders & Henderson 1978, Saunders *et al.* 1985).

An autumn hypoosmoregulatory ability in parr and the loss of this hypoosmoregulatory ability in association with a decreasing photoperiod has not been well documented. An autumn increase in Na^+ K^+ ATPase activity has been observed in parr (Langdon 1983, Cunjak *et al.* 1990). However, the activity of the enzyme during the autumn was 2-3 times less than the activity observed in smolts. This autumn increase in Na^+ K^+ ATPase activity may account for the level of hypoosmoregulatory ability observed in group NL in September. Despite the hypoosmoregulatory ability observed in the parr during September in experiment 1, it is unlikely the parr would survive if transferred to sea water. High mortalities have been observed in parr transferred to sea water during the autumn (Bergheim *et al.* 1990, Bjerknes *et al.* 1992) especially in small (<9.5 cm) parr (Bjerknes *et al.* 1992).

A similar situation was observed in experiment 2. Hypoosmoregulatory ability increased under an increasing photoperiod and decreased under a decreasing photoperiod.

The hypoosmoregulatory ability observed under a natural photoperiod (group SNP) exhibited this trend, decreasing as the photoperiod decreased in the autumn and increasing as the photoperiod increased in the spring. The observed period of

hypoosmoregulatory ability during the autumn was similar to the period observed in experiment 1 (group NL) and the increase in hypoosmoregulatory ability observed in the spring was comparable to changes observed during the normal parr-smolt transformation (Komourdjian *et al.* 1976, Saunders & Henderson 1978, Saunders *et al.* 1985).

The changes in serum osmolality observed in the challenged fish which were held under a constant long daylength (group L) were similar to the changes observed in group SNP. This observation would suggest that the changes in hypoosmoregulatory ability were either related to a third factor, possibly an endogenous rhythm or a third variable which both groups experienced, such as temperature. If temperature was the controlling factor similar changes would be expected in the other groups. Temperature has been demonstrated to be a variable which affects the rate of development and more importantly the loss of hypoosmoregulatory ability, but does not control the process (Duston *et al.* 1989, Duston and Saunders 1990b, Duston *et al.* 1991). Duston & Saunders (1990a, 1990b) observed that fish, held on a constant short daylength from the winter solstice, developed osmoregulatory ability at the same time as control fish held under a natural photoperiod and suggested that the development of a hypoosmoregulatory ability was controlled by an endogenous rhythm. The loss of a natural autumn hypoosmoregulatory ability followed by the spring development of hypoosmoregulatory ability in fish subjected to a constant LD 19.5:4.5 supports the hypothesis, that the development of a hypoosmoregulatory ability is controlled by an endogenous rhythm.

The groups subjected to a period of short days (groups 1, 2 and 3), exhibited a decrease in serum osmolality of challenged fish during the month after the short day (LD 8:16) period. This increase in hypoosmoregulatory ability was in association with the increase from a short day to a long day and was probably related to the parr-smolt transformation. In other studies the development of hypoosmoregulatory ability has been advanced using an artificial photoperiod (Bjornsson *et al.* 1985, McCormick *et al.* 1987, Duston & Saunders 1990b). The changes observed, after the 1 month period of short days (group 1), were not as distinct as the changes in group 2 and 3. It has been demonstrated that an

increase in daylength results in a direct increase in Na^+ K^+ ATPase activity (Duston & Knox 1992). However, this increase was approximately 1 third of the increase observed in association with the parr-smolt transformation. It is possible that the increase to a long day experienced by group 1 had stimulated a degree of hypoosmoregulatory ability, but not the full hypoosmoregulatory ability normally associated with a smolt.

The group held under a constant temperature of 10±1°C and a constant 20 hour daylength (group 20), exhibited a degree of cycling in serum osmolality of challenged fish. The trend observed in serum osmolality in group 20 showed elevated levels from February through to May and depressed levels from August through to November. A degree of variation was observed around this trend. The amplitude of oscillation decreased and the standard error of the mean for each sample point increased during the sampling period. These observations indicate that a proportion of the fish may have been desynchronised from the general changes observed in the population. A characteristic of an endogenous rhythm, oscillating in the absence of environmental signals, is that the individuals become desynchronised from each other (Gwinner 1986, Duston & Bromage 1987). Another possibility, which would explain the variation, is that as the size of the fish increased the hypoosmoregulatory ability of the fish increased. This increase in hypoosmoregulatory ability would reduce the amplitude of the oscillation, by decreasing the degree of change in hypoosmoregulatory ability. An effect of fish size on hypoosmoregulatory ability has been observed (Duston & Saunders 1990a). The cycling was approximately 12 months and the timing of hypoosmoregulatory ability was different from the timing observed in the control group (group C). The control (group C) exhibited a parr to smolt associated decrease in hypoosmoregulatory ability in association with the naturally increasing photoperiod. This has been previously observed (Komourdjian *et al.* 1976, Saunders & Henderson 1978, Saunders *et al.* 1985).

These experiments support the hypothesis that changes in hypoosmoregulatory ability are controlled by an endogenous rhythm which is synchronised by photoperiod.

A smolt coloration developed in all groups monitored in the 3 experiments. The development of a smolt coloration was observed earlier in groups subjected to an advanced short day period followed by a long daylength.

In experiment 1, the fish which experienced a period of short days prior to an increasing photoperiod (group PL) developed a smolt coloration before the fish were transferred to sea water. A long daylength after a period of short days has been observed to advance the development of a smolt coloration (Sigholt *et al.* 1989, 1995). The fish in group NL developed the appearance of smolts after transfer into sea water. There was no change in photoperiod when the fish were transferred and this suggests that the change in coloration was caused by the change in salinity. However, this observation contradicts Duston & Knox (1992) who reported that parr transferred into sea water in the autumn retained a parr coloration until the following spring when the parr appeared to smoltify.

The fish in experiment 2, which experienced a 2 month (group 2) and 3 month period of short days followed by a long daylength, exhibited smolt coloration during January and December, respectively. A period of short days followed by a long daylength appears to advance the development of a smolt coloration (Sigholt *et al.* 1989, experiment 1). The groups held under a constant long day (group L) and a 1 month period of short days (group 1) developed a smolt coloration at a similar time as fish held under a simulated natural photoperiod (group SNP) (Sigholt *et al.* 1995). These data suggest that the development of a smolt coloration is controlled by an endogenous rhythm and that the 1 month period of short days experienced by group 1 did not alter the timing of the endogenous rhythm, controlling coloration of the fish. These observations agree with Eriksson & Lundqvist (1982) and Sigholt *et al.* (1995) who both suggested that coloration was controlled by an endogenous rhythm. Eriksson and Lundqvist (1982) observed a cycling of coloration under constant temperature and photoperiod.

However, in experiment 3 under constant temperature (10±1°C) and a constant 20 hour daylength (group 20), the development of a smolt appearance was observed, but did not cycle. The development of a smolt coloration in group 20 supports the hypothesis of an

endogenous rhythm. However, the absence of a cycle of coloration does not support the hypothesis. Eriksson and Lundqvist (1982) observed that smolts held under constant conditions changed back to a silvery parr, but not to a full parr appearance. Johnston and Eales (1970) observed that silvering was related to the size of fish. Large fish developed a smolt appearance earlier and under constant conditions never displayed a full parr appearance. It is possible that the retained smolt coloration observed in experiment 3 was size related.

The development of the smolt coloration in group 20 was similar to the control group (group C), but occurred over a period of 12 months compared to 5 months in group C. Previous studies have observed the development of a smolt coloration at the expected time (Johnston & Eales 1970) and earlier than expected (Eriksson & Lundqvist 1982). It would appear that the fish held under constant conditions in experiment 3 were delayed. The delay resulted in large fish developing a smolt appearance which was retained until the experiment was terminated.

These experiments support the hypothesis that the development of a smolt coloration is controlled by an endogenous rhythm timed by photoperiod. However, there is a suggestion that size influences the loss of a smolt coloration; large fish retain their smolt coloration.

The timing of the different smolt parameters in relation to each other is important. In fish reared under a simulated natural photoperiod (experiment 2, group SNP) the development of the smolt parameters appeared to coincide. The condition factor and development of hypoosmoregulatory ability were observed during late April and early May, when the fish had the appearance of smolts. A similar pattern was observed in fish reared under a 2 month period of short days followed by an increasing photoperiod (experiment 1, group PL). The development of hypoosmoregulatory ability and the decrease in condition coincided but at an earlier time in November.

In experiment 2, fish which experienced a 2 month (group 2) and 3 month (group 3) period of short days followed by a long daylength, developed hypoosmoregulatory ability during November, whilst the condition factor decreased during December. In group 3 a smolt coloration developed in December, whilst a large proportion of fish in group 2 were still coloured as pre-smolts in January. Bjornsson *et al.* (1989) also recorded the development of osmoregulatory ability in advance of the decrease in condition factor, after a direct increase in daylength. Eriksson and Lundqvist (1982) observed the dissociation of silvering and the decrease in condition factor in individual fish, which were held under a constant LD 12:12.

In experiment 3, fish held under a constant 20 hour day (group 20) exhibited hypoosmoregulatory ability during January, soon after the experiment was initiated. A smolt coloration was not observed until November the same year i.e. some 10 months later. It is questionable whether any smolt associated decrease in condition factor was observed. The initial period of hypoosmoregulation was not in association with any of the other smolt parameters which were monitored. These observations suggest dissociation between hypoosmoregulation and coloration or a delay in the period of hypoosmoregulatory ability, which has been observed in parr during the autumn (experiment 2, group SNP).

These observations of dissociation of the smolt parameters, development of bimodality, hypoosmoregulatory ability, decrease in condition and smolt coloration suggest that either each parameter is controlled by an independent mechanism or the parameters are controlled by a single mechanism, from which the parameters can be dissociated. The parr to smolt associated decrease in condition factor is controlled directly by photoperiod. The decrease in condition requires a period of short daylength greater than 2 months followed by a period of long daylength. The control of this parameter is different compared to the other parameters. The decrease in condition is therefore probably controlled by a different mechanism. The other smolt parameters, development of

bimodality, hypoosmoregulatory ability and possibly coloration are controlled by one or more endogenous rhythms.

The number of endogenous rhythms involved in the smoltification process can not be stated and is dependent on how the smolt parameters are linked to the endogenous rhythm(s). If the smolt parameters can be dissociated from the endogenous rhythm, then the different aspects of smoltification could be controlled by a single endogenous rhythm. The different aspects of smoltification have different rates of development and require a particular period of time to develop. In a situation when the time period to develop is limited the different rates of development of the smoltification parameters could result in different degrees of dissociation from the endogenous rhythm. These different degrees of dissociation from the endogenous rhythm could result in the dissociation of parameters observed in smoltification. A driving zeitgeber such as photoperiod could result in a limited time period for development. Gwinner (1986) observed incomplete moulting in European starlings held under a compressed 2, 1.7 and 1.5 month photoperiod cycles and suggested that this was due to insufficient time to complete the moult.

Maturation in rainbow trout has been advanced and delayed, when the fish were placed under a constant photoperiod (Duston & Bromage 1986, 1988). The constant photoperiod was then maintained for two maturation cycles. The first spawning time was altered by the change to a constant photoperiod. The second spawning under the constant conditions was after approximately 12 months. This indicates that the endogenous rhythm and the cycle of maturation were altered by the same degree when the fish experienced the change to the constant photoperiod. This suggests that the endogenous rhythm is linked directly and in time with the biological process (maturation). If the different smolt parameters are directly linked and in time with an endogenous rhythm, the dissociation of the smolt parameters, development of bimodality, hypoosmoregulatory ability and coloration indicates that each parameter is controlled by an independent endogenous rhythm.

The controlling endogenous rhythms are probably long term rhythms and the evidence discussed above would suggest that the rhythms are circannual. However, the evidence is not conclusive. Gwinner (1986) stated that under constant conditions, an endogenous circannual rhythm should free run over 2-3 cycles with a periodicity close to but significantly different from a year. A number of studies have observed the development of aspects of smoltification under constant conditions (Johnston & Eales 1968, 1970, Eriksson & Lundqvist 1982, experiment 3) and possibly the loss of smolt parameters under constant conditions (Eriksson & Lundqvist 1982, experiment 3). However, the cycling of smolt parameters is not convincing as fish retained a full or partial smolt appearance and hypoosmoregulatory ability increased as the size of the fish increased (Eriksson & Lundqvist 1982, experiment 3). Perhaps it is incorrect to try and apply theories of circannual rhythms to the rhythm controlling the smoltification process. Under natural conditions, smoltification is a once in a lifetime event; the parr transform into smolts and enter the marine environment. Fish held in natural conditions have been observed to lose smolt characteristics in response to increasing temperature (Duston *et al.* 1989, Duston and Saunders 1990b, Duston *et al.* 1991) and decreasing photoperiod (Kurokawa 1989). However, a second smoltification in the same fish, held under natural conditions, has not been documented.

The proposed hypothesis of a series of long term endogenous rhythms controlling the different aspects of smoltification highlights the complexity of the smoltification process. It is a difficult problem to manipulate all the aspects of smoltification in unison using an artificial photoperiod and to establish when the smoltification process is complete. The monitoring of smoltification is further complicated when it is realised that a change in a parameter, which is characteristic of smoltification, can be caused by other processes which do not relate directly to smoltification. The problems in determining when a fish has completed smoltification have been investigated by both farmer and scientist. Saunders (1994) suggested, that the true indication whether a fish had successfully completed smoltification was survival and growth after transfer to sea water.

The groups in experiment 1 and 2 showed a range of different survivals and growth in the 6 months after the fish were transferred to sea water. The groups which received a period of 2 months or more of short days exhibited better seawater performance as assessed by growth and survival.

In experiment 1, the fish reared under a natural photoperiod (group NL) exhibited a significantly higher mortality during the first two months in sea water compared to the fish reared under a 2 month period of short days followed by a long daylength (group PL). Mortality in parr transferred to sea water during the Autumn, particularly in small parr (<9.5cm), was attributed to an inability to maintain ion/osmotic balance (Bergheim *et al.* 1990, Duston and Knox 1992 and Bjerknes *et al.* 1992). The fish in group PL were better prepared for survival in the seawater environment and therefore exhibited lower mortality during the first two months in sea water. However, a *Vibrio* sp. infection in the PL fish contributed in part to the two groups exhibiting a similar cumulative mortality when the experiment was terminated. It has been suggested that infections such as vibriosis indicate that the fish were under stress and were not fully adapted to the marine environment (Bergheim *et al.* 1990). However, these observations were made in relation to parr transferred to sea water and there was no suggestion that the fish resembled smolts. At transfer the NL group was significantly larger than the PL group. After the first two months in sea water there was no significant difference between the mean weights of the two groups. The PL smolts exhibited adequate growth during the five months in sea water. Growth of the parr in the NL group was poor for the first two months in sea water. Stunting or poor growth has been observed in smolts not fully adapted to the marine environment (Saunders *et al.* 1985 and McCormick *et al.* 1987) or in parr (Duston and Knox 1992, Bjerknes *et al.* 1992 and Duston 1994). In agreement with Duston (1994) after the initial period of poor growth the NL parr were observed to grow at rates similar to the smolts.

In experiment 2, the fish, which experienced a 2 month (group 2) short day period, were transferred 4 months in advance of fish reared under a simulated natural photoperiod

(group SNP) and fish, which experienced a 3 month (group 3) short day period, were transferred 5 months in advance of group SNP. The significantly lower mortalities observed in groups 2 and 3 and the adequate growth over a period when photoperiod and temperature were not stimulating growth indicate that the fish had completed smoltification in response to the artificial photoperiod. Group SNP exhibited a higher growth rate than the other groups. Smolts transferred into sea water in the spring, when temperature and photoperiod are increasing, are capable of high growth rates after an initial period of acclimatisation. However, the growth observed in group SNP was suppressed during the first 3 months. The fish reared under a constant long day (group L) and a 1 month period of short days (group 1) were also transferred to sea water during May. The 3 groups (group SNP, L and 1) transferred during May were transferred into the same tank as groups 2 and 3. It would appear that groups 2 and 3 suppressed the growth of the 3 groups transferred during May. When groups 2 and 3 were removed from the tank in July, the fish in group SNP grew rapidly. Group L and 1 exhibited the highest mortalities and the growth rates were poor. The growth observed in groups 1 and L during the spring were comparable to the growth observed in groups 2 and 3 during the winter. The observations of group 1 and L agree with studies, which have recorded poor growth in fish held under constant conditions prior to being transferred into sea water (Saunders *et al.* 1985, McCormick *et al.* 1987).

The groups which received a short day period of 2 months or more, smolted as assessed by the smolt parameters monitored and despite a suggestion of dissociation between some parameters, the smolts exhibited an adequate seawater performance. This would suggest that an artificial photoperiod consisting of a short day period exceeding 2 months followed by a long daylength similar to the photoperiods experienced by group PL (experiment 1) and groups 2 and 3 (experiment 2) could be used by the fish farming industry to produce smolts during December.

The production and transfer into sea cages of smolts during December should allow both freshwater and seawater facilities to be used more efficiently. In fresh water this would

allow the facility to be fully stocked twice during the production year. This would enable the facility to produce an annual production which was greater than the facilities overall holding capacity. During December, the December smolts would be transferred making tanks available for the continued growing of the natural 'in-season' smolts. A similar situation would be possible with seawater facilities. The autumn is a period of high sales and harvesting of market-sized salmon. December smolts could be stocked in the vacant cages which result from the harvesting period. The growth of the December smolts would result in these fish being larger when the normal smolts were transferred (experiment 2) and may offer a different harvesting period. However, the sea water performance of out-of-season smolts requires further investigation (see chapter 4).

Aspects of the freshwater production also require further investigation. In experiment 2 and particularly experiment 1, the proportion of potential S1 smolts was reduced by the artificial photoperiod. This could be avoided, by the selection of fish before the artificial photoperiod is decreased. This selection could be based solely on size; a critical size which parr must attain in autumn has been described for potential S1 smolts under natural conditions (Elson 1957) and is used in the fish farming industry to select potential S1's during an autumn size grade. However, the critical size under an artificial photoperiod may be different.

The period of short days in the artificial photoperiod reduced the growth rate resulting in significantly smaller fish (experiment 1). The selection of potential S1's and the use of a 'longer' short daylength during the period of short days may reduce the problem. However, the reduced growth rate in association with the short day period may be unavoidable. The farmer will have to balance these drawbacks with the benefits of the spread of availability of smolts to decide whether out-of-season smolt production is economically viable.

Chapter 3 Conclusions.

1) A 2-3 month period of short days followed by a period of long days applied from August through to December advanced the smoltification process enabling the production of smolts during December and January.

2) The different aspects of smoltification: the development of bimodality, hypoosmoregulatory ability, decrease in condition and smolt coloration appear to be controlled by different independent mechanisms.

3) The development of bimodality, hypoosmoregulatory ability and possibly coloration are controlled by independent endogenous rhythms.

4) The decrease in condition factor, associated with the parr-smolt transformation requires a period of short daylength greater than 2 months followed by a period of long daylength.

Chapter 4. The seawater performance of out-of-season Atlantic salmon smolts.

4.1 Introduction.

Photoperiod is an important cue in the control of the smoltification process. There have been reviews of the effects of artificial photoperiods and reports of the use of photoperiod in production of out-of-season smolts (see Duston & Saunders 1990b, Clarke 1989, Berge *et al.* 1995 Chapter 3). Smoltification has been advanced 4-7 months (experiment 1 and 2, Thrush 1994, Duston & Saunders 1995) and delayed 5 months (Duston & Saunders 1992) using photoperiod techniques. However, questions relating to the performance of such fish both in fresh water and particularly after transfer to sea water require investigation.

One of the difficulties associated with both normal and out-of-season transfer of smolts is the assessment of smolt quality. Data recording the seawater performance of out-of-season smolts would indicate whether the fish had successfully completed smoltification and whether the use of such smolts is an economically viable option to the salmon farmer. A smolt should survive and grow well in the seawater environment. Naturally-produced spring smolts grow rapidly during the spring, summer and early autumn reaching mean weights of 1 kg in the Autumn. After a period of slow growth during the winter, the spring again stimulates rapid growth and potential grilse can be harvested early in the summer. Grilse harvest weights are 2-3 kg. The slower growing fish are harvested at mean weights from 2 to 5 kg from September through to March the following year. However, the majority of the fish are harvested after 18 months (personal observations). It has been reported (Scottish Office, Agriculture, Environment and Fisheries Department 1995) that, of the smolts transferred during 1993, 65.5% were harvested during 1994 (mean weight 3.11 kg) and 24.4% were harvested during 1995 (mean weight 4.27 kg). The survival over the entire growing cycle was 89.8%. The

seawater performance of out-of-season smolts should be comparable to the seawater performance of normal smolts.

Many studies on photoperiodic manipulation of the timing of smoltification do not consider the seawater performance of the out-of-season smolts. Saunders *et al.* (1985) and Stefansson *et al.* (1991) transferred and monitored the seawater performance of photoperiod manipulated out-of-season smolts. However, the photoperiods employed resulted in incomplete smoltification. Other studies (McCormick *et al.* 1987, Solbakken *et al.* 1994, Sigholt *et al.* 1995) transferred smolts reared under an artificial photoperiod at the same time as natural smolts. The development of smolt characteristics in the photoperiodically-manipulated smolts did not coincide with the development of smolt characteristics in the natural smolts. The photoperiod smolts were transferred at a time when the fish were possibly losing some smolt characteristics. The quality of the artificially produced smolts in these studies was again questionable.

Thrush (1994) observed that the transfer of photoperiodically manipulated potential S2 smolts during November, December and March exhibited similar survivals to natural smolts and the early transfer to sea allowed these fish to gain a growth advantage over control fish transferred during the spring. Duston and Saunders (1995) examined the growth of November parr and November photoperiod smolts through to harvest. The smolts exhibited better survivals but similar growth to the parr. This resulted in an alternative harvest period compared to natural spring smolts and suggested that out-of-season smolts could provide out-of-season market sized fish. This chapter aimed to further examine the growth of out-of-season smolts comparing the growth and survival of both in-season and out-of-season smolts, transferred through-out the year. It also considered the incidence of maturation in these fish.

Maturation is a major constraint for the salmon farming industry. Grilse maturation during the 2nd year in sea water in particular is a problem. The deterioration of flesh quality associated with maturation (Aksnes, 1986) dictates that maturing fish must be harvested early in the maturation process, when there is possibly no market for the fish.

The farmer requires fish which attain harvest size prior to maturation. Atlantic salmon display a flexible life history pattern with large variation in age at maturity. The age at maturity refers to the trade off between low age at maturity, characterised by high survival to maturity and low fecundity, and high age at maturity, characterised by low survival to maturity and high fecundity. The mechanisms which control the age at maturity are complicated and no single factor can be identified (Gardner 1976, Power 1986). Maturation strategies have been related to both genetic (Naevdal 1983, Gjerde 1984, Glebe & Saunders 1986, Ritter *et al.* 1986) and biological factors (Chadwick *et al.* 1986) which in turn are also affected by environmental conditions.

Different genetic strains of salmon have been reported to have different rates of maturation at different ages both in fresh water (Thorpe *et al.* 1983, Glebe & Saunders 1986) and sea water (Naevdal 1983, Ritter *et al.* 1986) suggesting that age at maturity is related to genetic strain. It has been suggested that the selection of these genetic determinants is environmental (Schieffer & Elson 1975, Myers 1986). Schieffer and Elson (1975) demonstrated that the gradient and length of a river was positively related to the mean age at maturity.

A variety of biological factors are thought to influence age at maturity including growth rate, size, availability of food and age or size at smolting (Chadwick *et al.* 1986). Growth rate is the most important of these biological factors and many studies have related high growth rate to low age at maturity (see Thorpe 1986). Growth rate is clearly related to other biological factors e.g. size is a measure of past growth (Thorpe 1986), age at smoltification is related to growth (Thorpe & Morgan 1978, Kristinsson 1985) and food availability obviously affects growth rate. Other environmental factors clearly affect growth rate i.e. temperature and length of growing season.

The complex interaction of these factors makes the prediction of the age at maturity under natural environmental conditions extremely difficult. This has resulted in much controversy in the literature (see Dempson *et al.* 1986, Chadwick *et al.* 1986). Thorpe (1986) proposed that fish were physiologically aware of their rate of acquisition of

surplus energy and provided that this rate was above a genetically determined threshold during the spring the fish would proceed with maturation. Evidence for this spring threshold was provided when maturation was suppressed by restricting feed and hence growth during the spring (Thorpe *et al.* 1990, Rowe & Thorpe 1990).

The initiation of maturation would appear to be before this spring decision period, possibly during the winter. The decision of the fish to mature results in increased androgen levels (Hunt *et al.* 1982) which increase the potential for growth (Berglund *et al.* 1992b). If food availability during the spring is sufficient to allow growth rates and/or acquisition of surplus energy, above a certain genetic threshold, maturation will proceed (Thorpe 1986). In the only study to date to consider seawater maturation in out-of-season smolt, Thrush (1994) observed 7% post-smolt maturation in smolts transferred in November and 24% post-smolt maturation in smolts transferred during March. These maturation rates were considerably higher than would be expected from smolts transferred during spring. Thrush (1994) argued that this result was in agreement with the hypothesis of Thorpe (1986) outlined above, i.e. the growth advantage gained from early transfer resulted in increased maturation. This chapter aims to examine the effects of transfer date of out-of-season smolts on age at maturity.

The seawater performance, growth, mortality and maturation must be determined in order to predict when out-of-season smolts will attain market size and to assess whether the production of such smolts is a viable production alternative for the salmon farmer.

The aims of this chapter are:-

1) To produce and transfer out-of-season smolts, recording the parameters: serum osmolality of challenged fish and condition factor in order to determine the peak of smoltification.

2) To determine the seawater growth profiles for out-of-season smolts transferred throughout the year.

3) To monitor the maturation exhibited by out-of-season smolts.

4) To compare transfer mortality (mortality during transport and the first month in sea water) of out-of-season smolts with natural smolts.

4.2 Experiments 4 and 5: The seawater growth, maturation and survival of diploid and triploid, out-of-season Atlantic salmon smolts produced using artificial photoperiod and constant temperature conditions.

Experiments 4 and 5 were both carried out at site FW2 (section 2.2.1.2) during consecutive years on different year classes of the same stock. The experiments were designed to produce smolts throughout the year using artificial photoperiods and test the seawater performance of these smolts. The methods used to produce the smolts were similar and the testing of smolt status was identical. The materials and methods, and freshwater results for the two experiments have been presented together, in one section divided into three subsections; the sampling procedures and data analysis common to both experiments are presented in subsection 4.2.1.1; the photoperiod regimes, freshwater and seawater holding conditions and freshwater results for experiments 4 and 5 are presented in subsections 4.2.1.2 and 4.2.1.3 respectively. For summaries of the range of treatments in experiments 4 and 5 refer to figures 4.1 and 4.16. The seawater results for the two experiments have been presented together in section 4.2.2 to aid comparison between the experiments and clarify the results.

4.2.1 Materials and Methods, and Freshwater Results.

4.2.1.1 Sampling Procedures and Data Analysis

Condition factor (section 2.3.3.1) was determined and a seawater challenge test (section 2.3.3.2) carried out at intervals of 2 weeks. Sampling in each group was initiated before the start of the 'spring' increase in the photoperiod and continued until the fish were transferred to a seawater holding facility. The appearance of the fish and the maturity (presence of milt when pressure was applied to the abdomen) were also assessed during sampling. The serum osmolality of challenged fish, the condition factor and the

appearance of the fish were assessed to establish when smoltification was considered complete.

Weight, condition factor data and serum osmolality was compared using DUNN'S multiple comparison test and the t-test (section 2.4.2). Pair wise comparisons of condition factor, serum osmolalities and weight data were made using the Mann-Whitney test (section 2.4.1). Confidence limits for the proportions of sea water mortality were calculated and compared (section 2.4.3).

4.2.1.2 Photoperiod regimes, holding conditions and freshwater results for experiment 4.

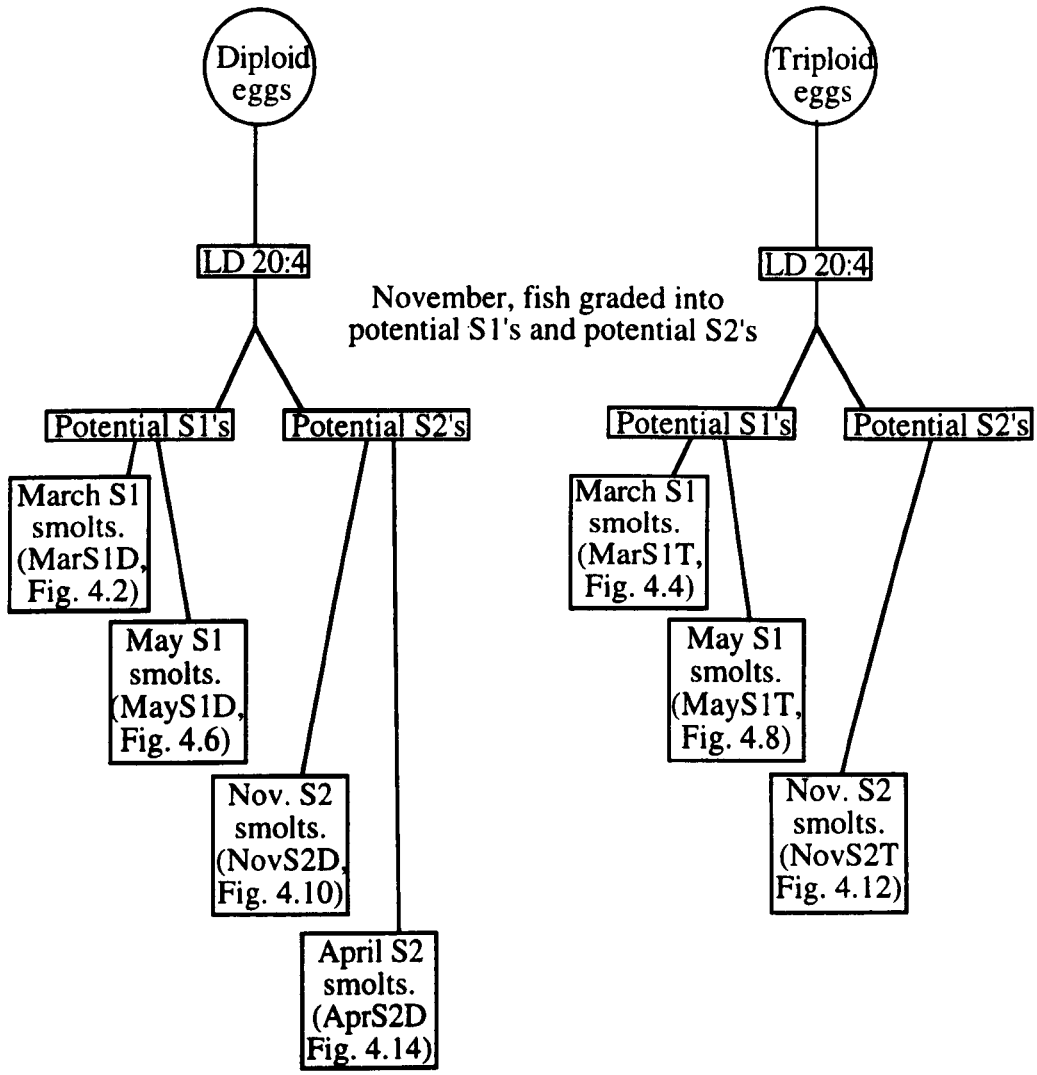
Diploid and triploid eggs were hatched in January. All fish were initially held in 1.5 m^3 concrete raceways. During Aug. and Sept. the fish were transferred to 7 m^3 raceways. During November both diploid and triploid salmon parr were graded into two groups: potential S1's and potential S2's. The different grades of diploid and triploid fish were divided into several experimental groups and subjected to different photoperiod regimes (see figure 4.1).

The photoperiod regimes were designed to produce smolts during March, May (S1 control), November and April (S2 control). The photoperiod regimes and holding conditions for each group are outlined below. The group name refers to the month of transfer (i.e. May, Mar, Nov. etc.), whether the fish were potential S1's (S1) or potential S2's (S2) and whether the fish were diploid (D) or triploid (T) stock. The initial photoperiod for all groups was LD 20:4 from first feeding until the 22nd Oct. when a reduction in photoperiod of 1 hour . $week^{-1}$ was initiated.

Group MarS1D (transferred in March, potential S1 diploid fish)

15000 of the diploid potential S1 smolts were maintained in 7 m^3 raceways. The fish were subjected to the 1 hour . $week^{-1}$ decreasing photoperiod from the 22nd Oct. to the 19th Nov. On the 19th Nov. a 2 hour . $week^{-1}$ decrease was initiated. The 2 hour .

Figure 4.1 The origin and relationship of each group in experiment 4. The month of transfer of each group is stated and the group's name is described as follows: the month of transfer (Mar, May etc.), whether the fish were potential S1's or S2's (S1 or S2) and whether the fish were diploid or triploid (D or T). The figure number quoted for each group refers to the photoperiod which was employed to produce smolts in that particular month.



week^{-1} decrease was stopped at LD 8:16 on the 10th Dec. The LD 8:16 was maintained until 14th Jan. when the fish were exposed to a direct increase in daylength from LD 8:16 to LD 18:6 (figure 4.2).

Sampling was initiated on the 5th Jan. The condition factor (figure 4.3) of the fish decreased throughout the sampling period, decreasing significantly ($p<0.01$) between the 14th Feb. and the 28th Feb. and again between the 14th March and the 28th March; the condition factor prior to transfer was 1.06±0.007. The serum osmolality (figure 4.3) of the challenged fish decreased significantly ($P<0.01$) from the 1st Feb. to the 1st March and remained between 348.1±3.25 and 336.1±1.98 mOsm.kg $^{-1}$ during March.

13700 smolts (mean weight 42.4±0.9g) were transferred to a 12m cage at site SW2 (section 2.2.2.2) on the 29th March. Mortalities and monthly farm sample weights were recorded. The fish were counted and divided into two cages on the 28th Jan. During this operation a random sample of 100 fish was obtained. The 100 fish were individually assessed for weight, length and maturity. Monthly monitoring was continued on 4900 fish which were restocked into a 12m cage. A grilse grade was carried out on the 5th June and the numbers and mean weight of salmon and grilse recorded. The fish were harvested in August and September, 17-18 months after seawater transfer.

Group MarS1T (transferred in March, potential S1 triploid fish).

16000 from the triploid potential S1 smolts, were maintained in 7 m^3 raceways under the same photoperiod as the MarS1D group (figure 4.2 and 4.4).

Sampling was initiated on the 5th Jan. The condition factor (figure 4.5) of the fish remained between 1.14±0.005 and 1.13±0.005 before decreasing significantly ($P<0.01$) from the 14th Feb. to the 28th Feb. and again from the 28th Feb. to the 14th March. The condition factor on the 14th March was 1.05±.006; this value was maintained until transfer. The serum osmolality of challenged fish (figure 4.5) after an initial increase

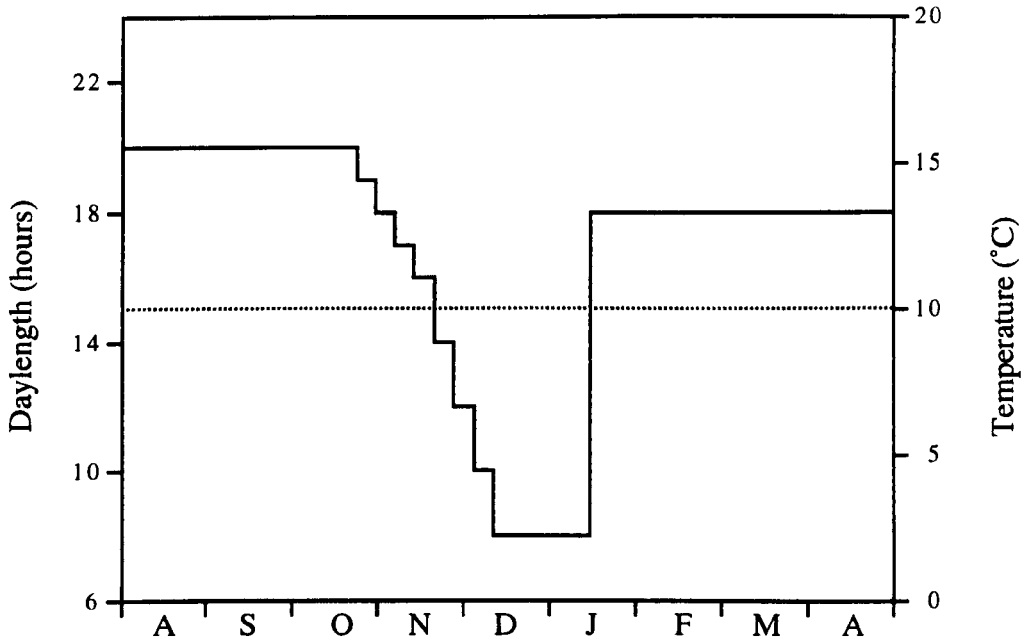


Figure 4.2 Temperature (dotted line) and photoperiod regime (constant line) experienced by the diploid fish transferred into sea water during March (group MarS1D).

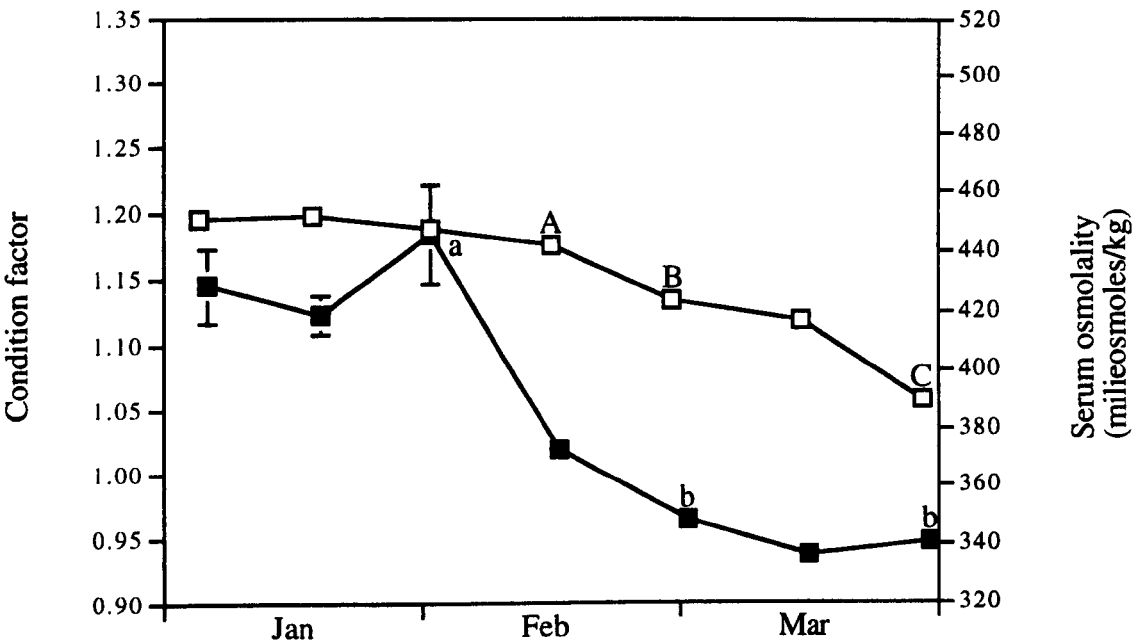


Figure 4.3 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) diploid fish transferred into sea water during March (group MarS1D). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

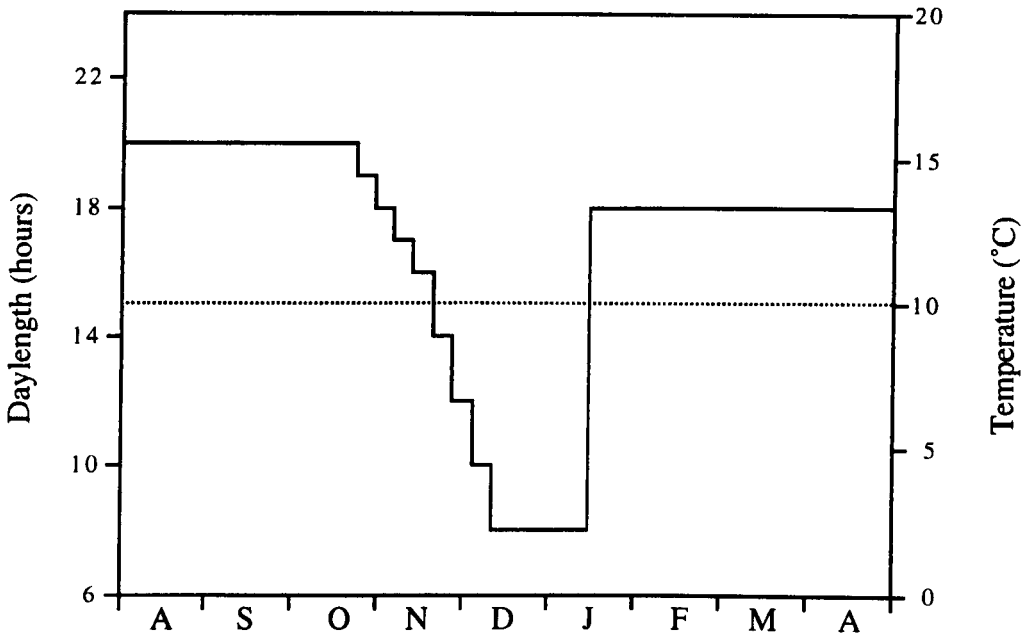


Figure 4.4 Temperature (dotted line) and photoperiod regime (constant line) experienced by the triploid fish transferred into sea water during March (group MarS1T).

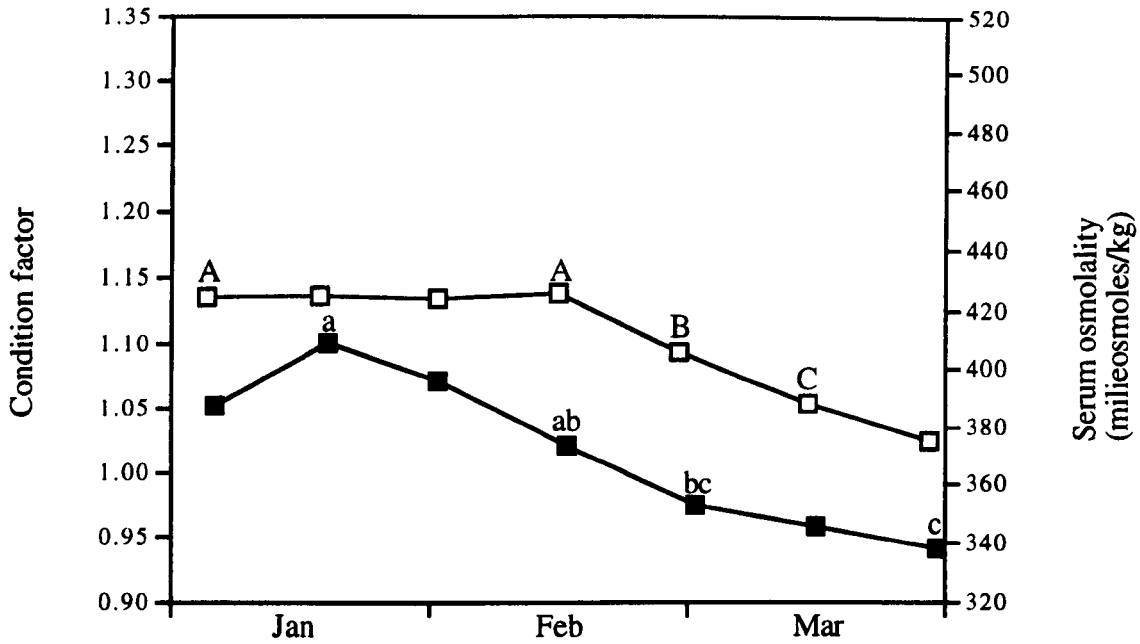


Figure 4.5 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) triploid fish transferred into sea water during March (group MarS1T). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

decreased significantly ($P<0.05$) through February. The serum osmolality prior to transfer was 338.1±2.67 mOsm.kg $^{-1}$.

15700 smolts (mean weight 48.0±0.7g) were transferred to a 12m cage at site SW2 (section 2.2.2.2) on the 29th March. Mortalities and monthly farm sample weights were recorded. The fish were counted and divided into two 12m cages on the 28th Jan. During this operation the fish were crowded and the individual weights and lengths of a random sample of 100 fish were recorded. Monthly monitoring continued on 4200 fish which were restocked into a 12m cage. The fish were randomly sampled for length and weight on 2 other occasions, the 4th June and the 16th Sept. over 1 year after transfer. In September over one year after transfer, due to constraints in the running of the site, the fish were mixed with other fish. Further data collection was not possible.

MarS1D v MarS1T

Prior to transfer on 29th March the osmoregulatory abilities of the triploid and diploid smolts were not significantly different. The diploid group had a significantly higher ($P<0.05$) condition factor than the triploid group on the 29th March (figures 4.3 and 4.5).

Group MayS1D (transferred in May, potential S1 diploid fish)

20000 of the diploid potential S1 smolts, were maintained in 7 m^3 raceways. The fish were subjected to a 1 hour . week^{-1} decreasing photoperiod from the 22nd Oct. to the 27th Nov. On the 27th Nov. the fish were transferred to an earth pond, where the only illumination was natural daylight (figure 4.6).

Sampling was initiated on the 5th Jan. The condition factor (figure 4.7) of the fish remained between 1.15±0.004-1.18±0.006 until 11th April, from which time it decreased significantly ($p<0.01$) to 1.11±0.006 on the 22nd April. The serum osmolality of the challenged fish (figure 4.7) ranged from 404.8±6.08-435.3±9.43 mOsm.kg $^{-1}$ during January and February. From the 28th Feb. to 11th April the serum

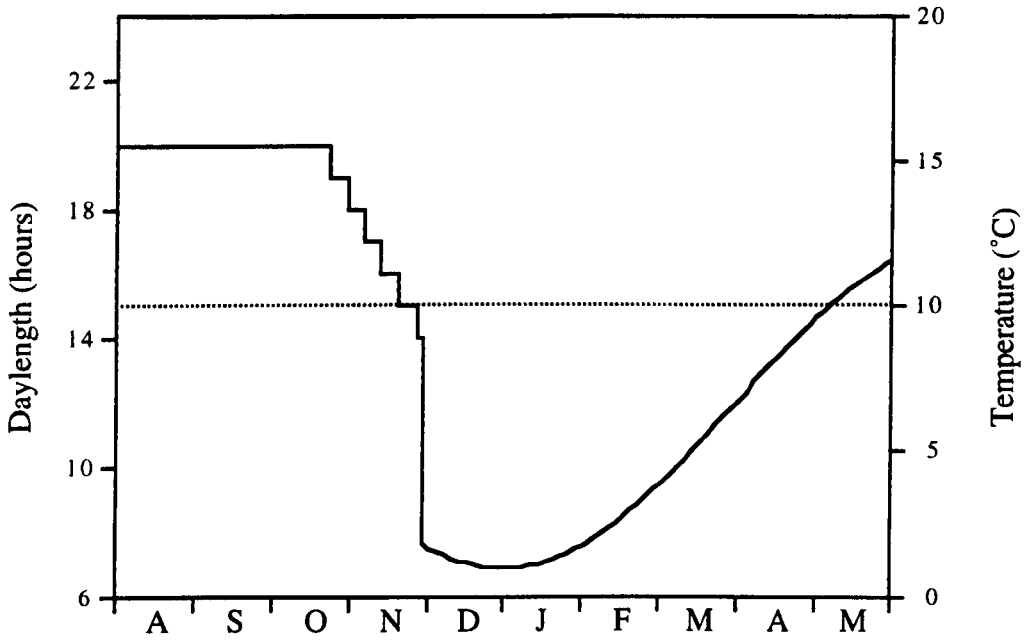


Figure 4.6 Temperature (dotted line) and photoperiod regime (constant line) experienced by the diploid fish transferred into sea water during May (group MayS1D).

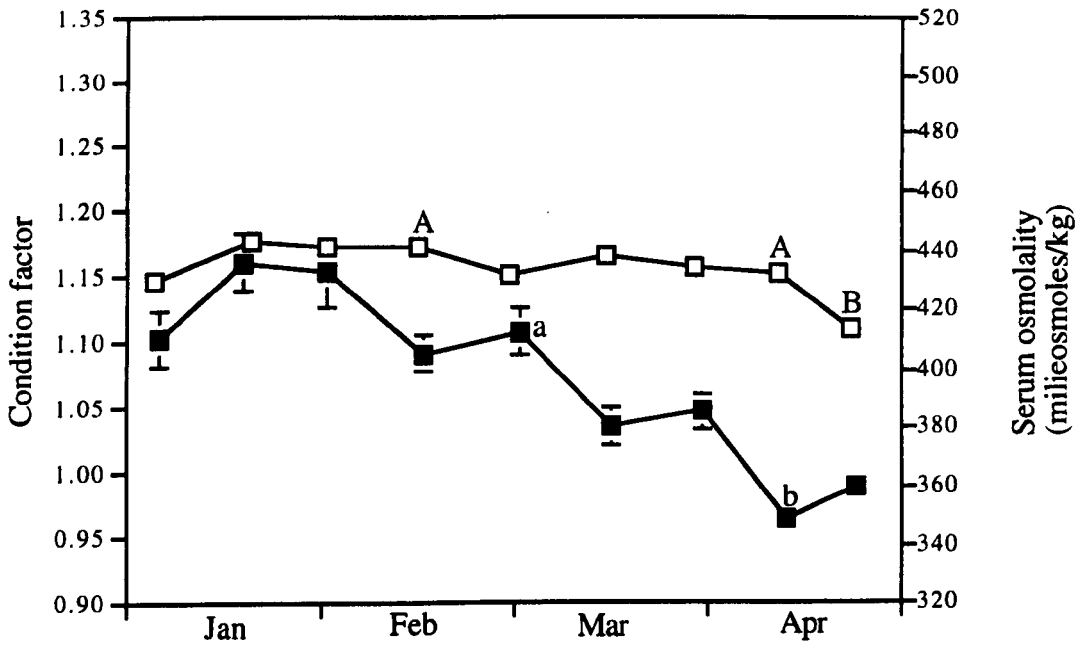


Figure 4.7 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24hr at 28‰) diploid fish transferred into sea water during May (group MayS1D). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

osmolality decreased significantly ($p<0.01$), and remained between 348.9±3.02 and 359.8±4.65 mOsm.kg $^{-1}$ until transfer.

15500 smolts (mean weight 43.4±0.8g) were transferred to a 12m cage at site SW2 (section 2.2.2.2) on the 4th May. Mortalities and monthly farm sample weights were recorded. The fish were counted and divided into two 15m cages on the 2nd Feb. During this operation a random sample of 100 fish was obtained. The fish were batch weighed in 11 batches; each fish was measured for length and assessed for maturity. Growth was monitored in the original 15m cage which was re-stocked with 6700 fish. A grilse grade was carried out on the 4th June, the number and mean weight of the salmon and grilse were recorded. The fish were harvested in August and September, 16-17 months after seawater transfer.

Group MayS1T (transferred in May, potential S1 triploid fish).

20000 of the triploid potential S1 smolts were maintained in 7 m^3 raceways. The fish were subjected to a 1 hour . week^{-1} decreasing photoperiod from the 22nd Oct. to the 10th Dec. On the 10th, 17th and 24th Dec. the photoperiod was reduced to LD 11:13, LD 9.5:14.5 and LD 8:16. The LD 8:16 was held until the 10th Jan. when the fish were transferred to an earth pond, the only illumination of the pond being natural daylight (figure 4.8).

Sampling was initiated on the 5th Jan. The condition factor (figure 4.9) of the fish was observed to initially decrease and then increase significantly ($P<0.01$) to 1.14±0.004 on the 15th Feb. The condition factor then decreased significantly ($P<0.01$) from 1.11±0.006 on the 14th March to 1.07±0.007 on the 22nd April. The serum osmolality of the challenged fish (figure 4.9) remained between 374.5±5.35 and 407.1±11.6 mOsm.kg $^{-1}$ from January to early March. The serum osmolality decreased significantly ($P<0.01$) from the 15th March to 352.7±2.76 mOsm.kg $^{-1}$ on the 23rd April.

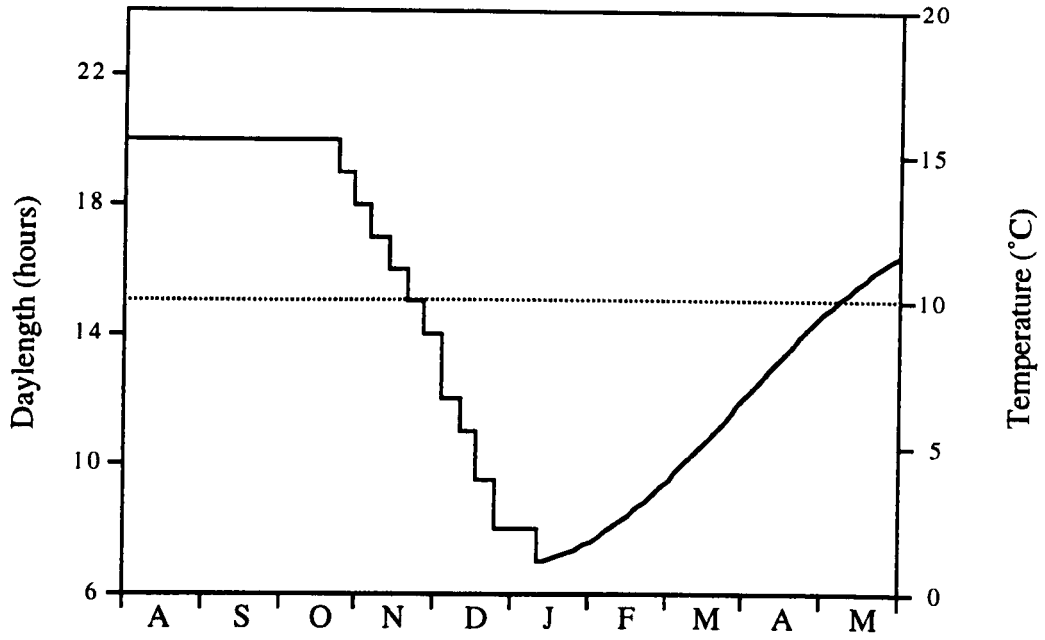


Figure 4.8 Temperature (dotted line) and photoperiod regime (constant line) experienced by the triploid fish transferred into sea water during May (group MayS1T).

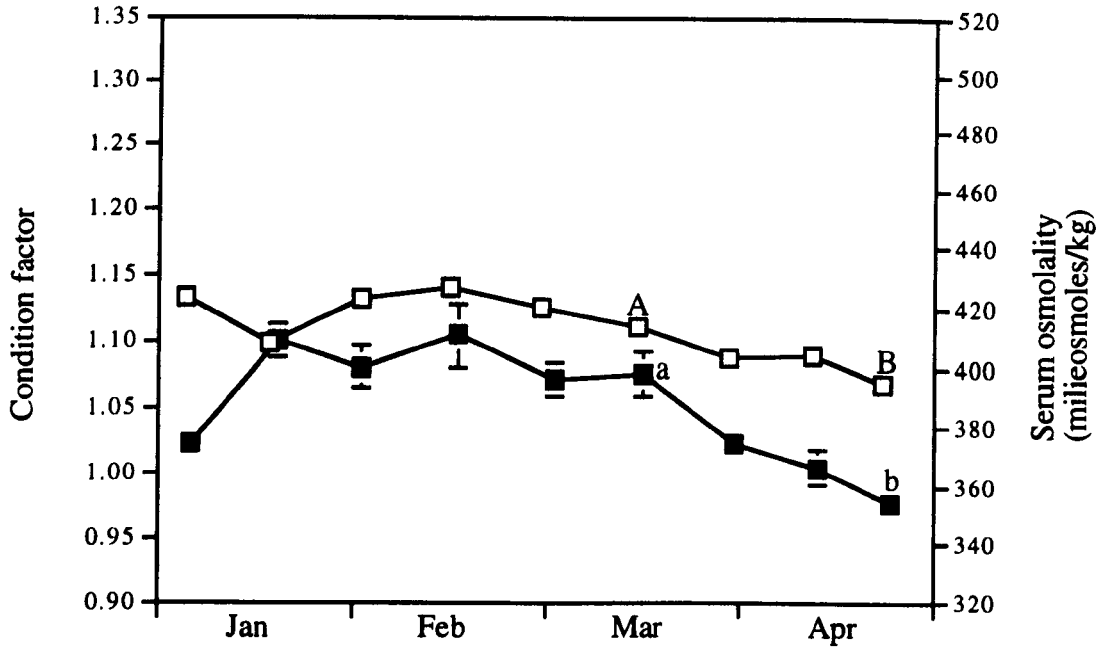


Figure 4.9 Changes in condition factor (open square) and serum osmolality (filled square) for challenged (24h at 28‰) triploid fish transferred into sea water during May (group MayS1T). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

16500 smolts (mean weight 39.7±0.6g) were transferred to a 12m cage at site SW3 (section 2.2.2.3) on the 2nd May. Mortalities and monthly farm sample weights were recorded. In May one year after transfer, due to constraints in the running of the site, the fish were mixed with other fish. Further data collection was not possible.

MayS1D v MayS1T

Prior to transfer on the 23rd April, the osmoregulatory abilities of the triploid and diploid smolts were not significantly different. The diploid group had a significantly higher ($P<0.05$) condition factor than the triploid group on the 23rd April (figures 4.7 and 4.9).

Group NovS2D (transferred in November, potential S2 diploid fish).

40000 potential S2 smolts of the diploid stock were maintained in 7 m^3 raceway under the same photoperiod as the MarS1D (figure 4.2) and MarS1T (figure 4.4) groups. On the 22nd April a 2 hour . $week^{-1}$ decrease from LD 18:6 to LD 8:16 was initiated. The LD 8:16 was held from the 3rd June to the 8th July when a 1 hour . $week^{-1}$ increase was initiated. The increase was terminated on the 23rd Sept. and the photoperiod was held on LD 20:4 (figure 4.10).

Sampling was initiated on the 1st July. The condition factor (figure 4.11) of the fish remained between 1.21±0.008-1.17±0.008 until the 26th Aug. From the 26th Aug. the condition factor decreased significantly ($P<0.01$) over a number of sample points, 26th Aug. to 24th Sept., 10th Sept.. to 7th Oct., 7th Oct.. to 21st Oct. and the 7th Oct. to the 4th Nov. The condition factor prior to transfer was 1.02±0.012. The serum osmolality (figure 4.11) of the challenged fish was between 430.9±11.9 and 455.2±8.91 $mOsm.kg^{-1}$ during July and August. During September the serum osmolality decreased significantly ($P<0.01$) from 443.4±10.40 $mOsm.kg^{-1}$ to 372.7±5.97 $mOsm.kg^{-1}$. In the month before transfer the serum osmolality remained between 372.7±5.97 and 342.3±4.31 $mOsm.kg^{-1}$. During sampling 14% of the total population was found to be

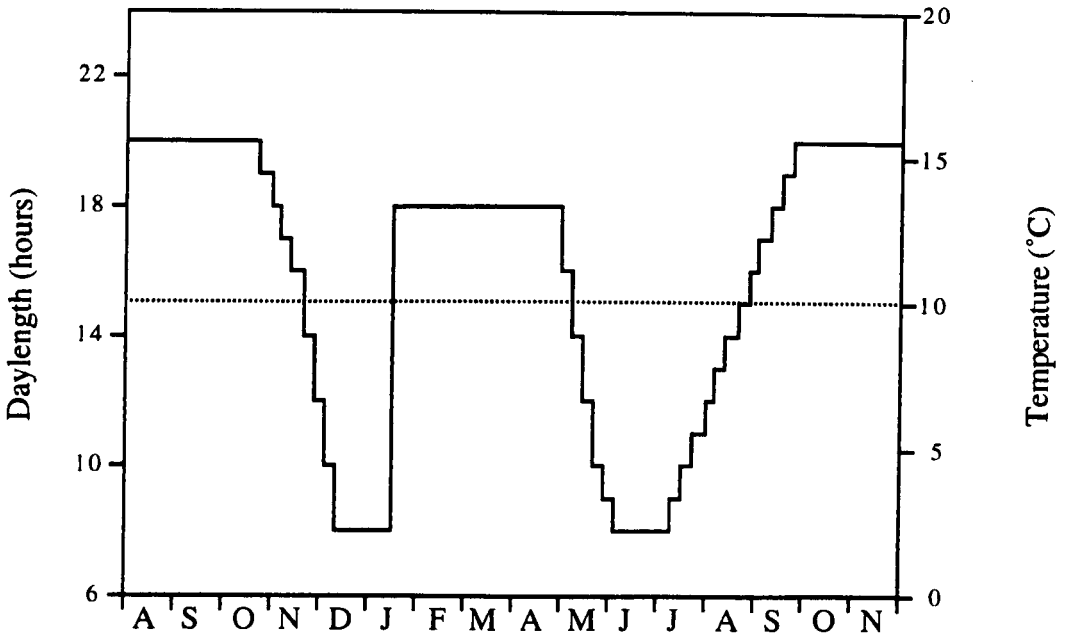


Figure 4.10 Temperature (dotted line) and photoperiod regime (constant line) experienced by the diploid fish transferred into sea water during November (group NovS2D).

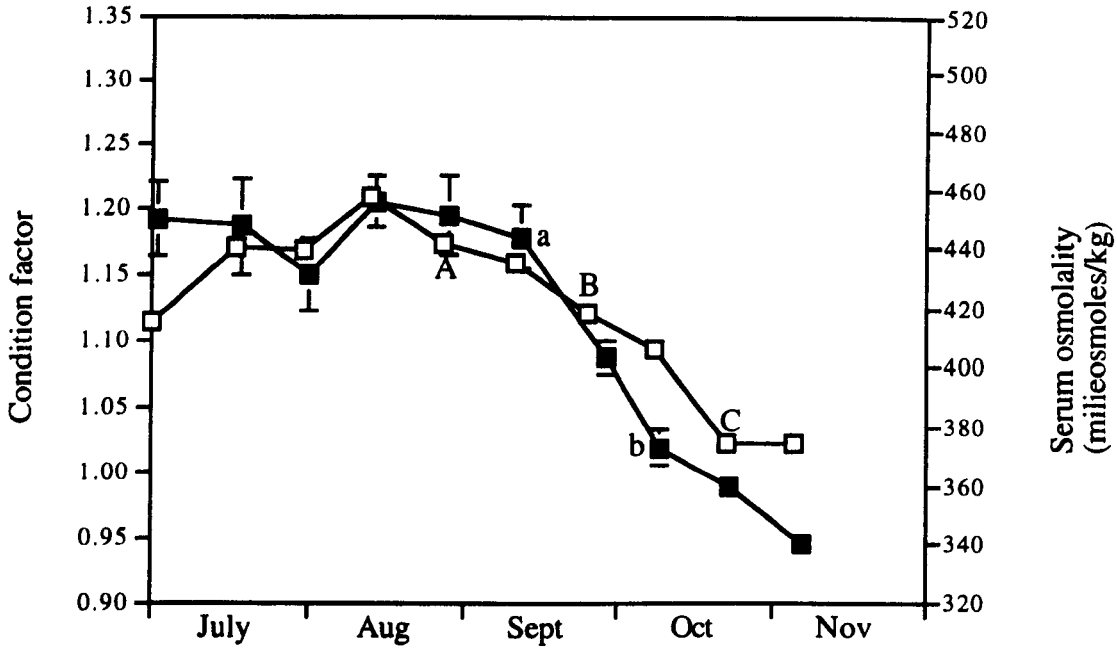


Figure 4.11 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) diploid fish transferred into sea water during November (group NovS2D). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

precocious males. The entire population, including the precocious parr, was transferred to sea water.

23000 smolts (mean weight 33.2±0.8g) were transferred to a 12m cage at site SW2 (section 2.2.2.2) on the 14th Nov. Mortalities and monthly farm sample weights were recorded. The fish were randomly sampled for weight, length and maturation on the 30th Nov., one year after transfer. The fish were crowded, sampled, counted and divided into two 15m cages on the 5th Feb. The fish were harvested in June, 19 months after transfer. During the harvest, weight, length and numbers of mature and immature fish were recorded.

Group NovS2T (transferred in November, potential S2 triploid fish).

40000 potential S2 smolts of the triploid stock were maintained in a 7 m^3 raceway under the same photoperiod as the NovS2D group (figure 4.10 and 4.12).

Sampling was initiated on the 1st July. The condition factor (figure 4.13) of the fish initially increased, increasing significantly from 13th Aug. to the 26th Aug. This increase was followed by a decrease through September and October. The decrease in condition factor was significant ($P<0.01$) from the 10th Sept. to 24th Sept., 10th Sept. to 7th Oct. and 7th Oct. to the 21st Oct. The condition factor prior to transfer was 1.01±0.007. The serum osmolality (figure 4.13) of the challenged fish remained between 397.5±7.69 and 431.7±13.44 $mOsm.kg^{-1}$ during July and August. The serum osmolality decreased significantly ($P<0.01$) from 403.3±8.65 $mOsm.kg^{-1}$ on the 11th Sept. to 332.0±1.9 $mOsm.kg^{-1}$ on the 22nd Oct.

19600 smolts (mean weight 40.0±0.6g) were transferred to a 12m cage at site SW2 on the 8th Nov. Mortalities and monthly farm sample weights were recorded. The fish were randomly sampled for length and weight on the 16th Sept. In February, over one year after transfer, due to constraints in the running of the site, the fish were mixed with

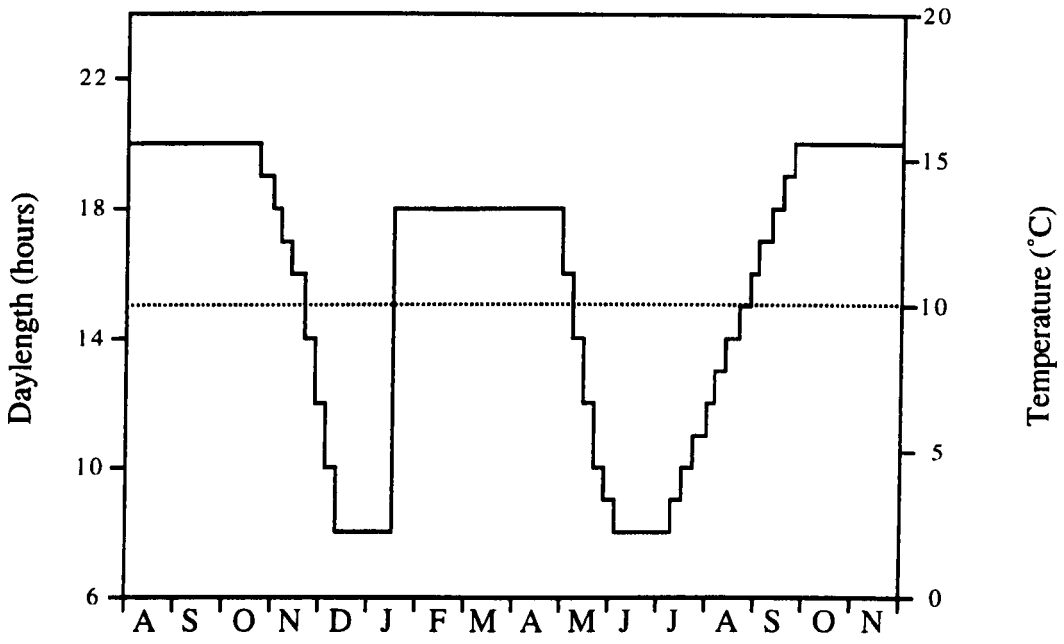


Figure 4.12 Temperature (dotted line) and photoperiod regime (constant line) experienced by the triploid fish transferred into sea water during November (group NovS2T).

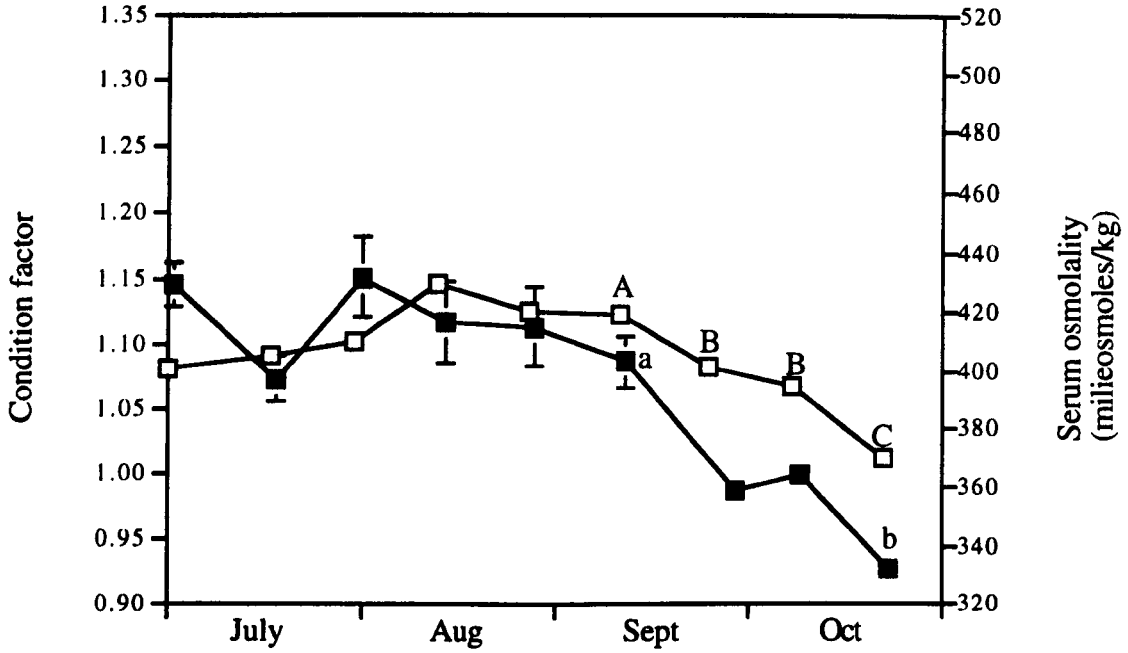


Figure 4.13 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) triploid fish transferred into sea water during November (group NovS2T). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

other fish. Further data collection was not possible.

NovS2D v NovS2T

These groups were transferred on different dates. On the 22nd Oct. the serum osmolalities of the two groups were significantly different ($P<0.05$) while there was no difference in the condition factor between the two groups. The mean weights of the two groups were different at transfer.

Group AprS2D (transferred in April, potential S2 diploid fish).

23000 potential S2 smolts of the diploid stock were held in a 7 m^3 raceway under the same photoperiod as group MayS1T (figure 4.4). On the 14th Jan. a half hour . $week^{-1}$ increase was initiated with a step from LD8:16 to LD8.5:15.5. The increase was terminated on the 25th May and the photoperiod held on LD 18:6 until the 5th Aug. On the 5th Aug. the fish were transferred to an earth pond in which the only illumination was natural daylight (figure 4.14).

Sampling was initiated on the 5th Jan., 2 years after the fish hatched. The condition factor (figure 4.15) of the fish decreased during the sampling period, decreasing significantly ($P<0.01$) from 1.15±0.015 on the 5th Mar. to 1.08±0.01 on the 4th April with a further decrease to 1.04±0.009 on the 17th April. The serum osmolality (figure 4.15) of the challenged fish varied in the range 412.2±11.6 to 384.5±10.1 $mOsm.kg^{-1}$ from January to February. From the 21st Feb. to the 20th March the serum osmolality decreased significantly ($P<0.05$). Before transfer the serum osmolality remained between 364.5±5.8 and 380.4±11.5.

21400 smolts (mean weight 55.4±1.23g) were transferred to a 12m cage at site SW2 (section 2.2.2.2) on the 23rd April. Mortalities and monthly farm sample weights were recorded. In May one year after transfer, due to constraints in the running of the site, the fish were mixed with other fish. Further data collection was not possible.

No triploid potential S2 smolts were reared under a natural photoperiod.

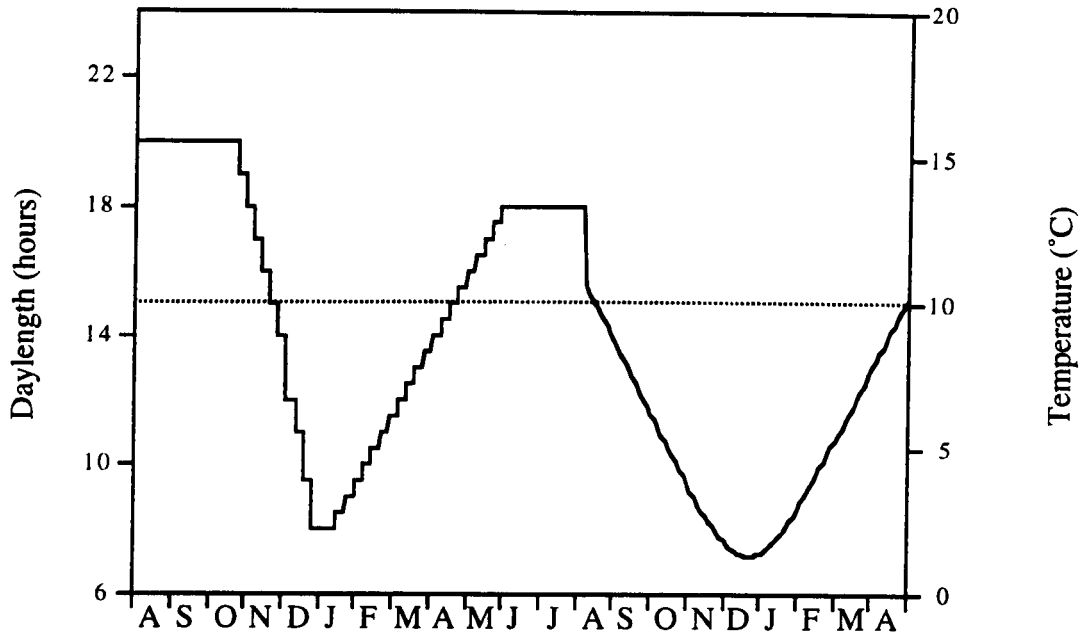


Figure 4.14 Temperature (dotted line) and photoperiod regime (constant line) experienced by the diploid fish transferred into sea water during April (group AprS2D).

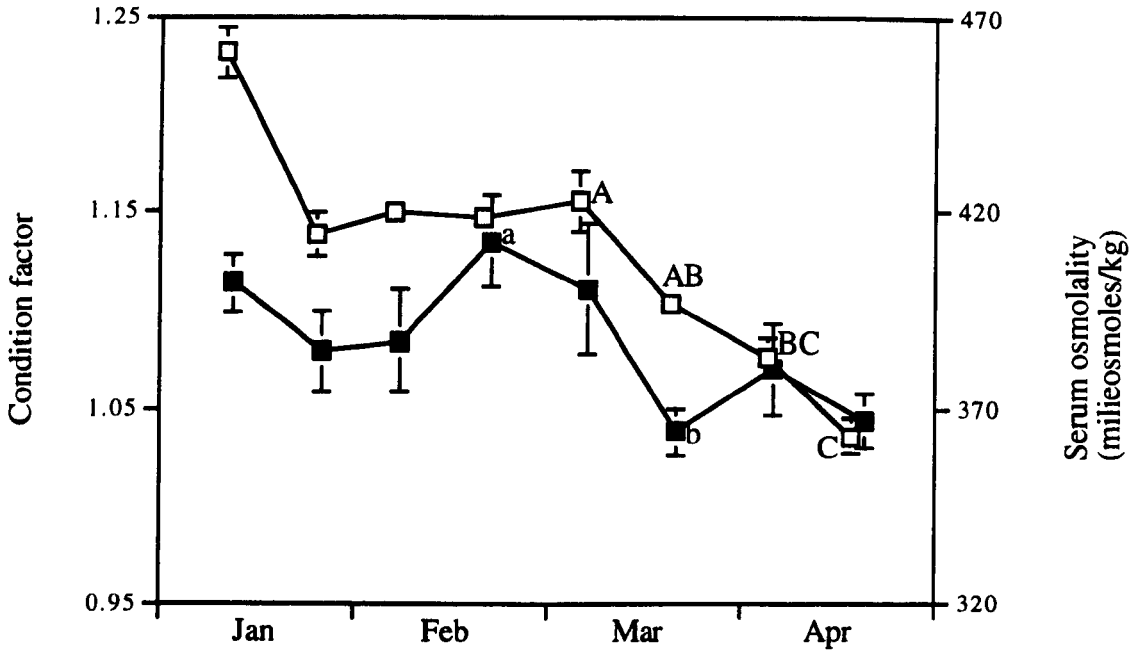


Figure 4.15 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) diploid fish transferred into sea water during April (group AprS2D). Error bars are ± 1 sem, error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

4.2.1.3 Photoperiod regimes, holding conditions and freshwater results for experiment 5.

Diploid eggs were hatched in January. All fish were initially held in 1.5 m^3 concrete raceways. During Aug. and Sept. the parr were transferred into 7 m^3 concrete raceways. During November the parr were graded into two groups: potential S1's and potential S2's. The different grades of diploid fish were divided into several experimental groups which were subjected to different photoperiod regimes (see figure 4.16).

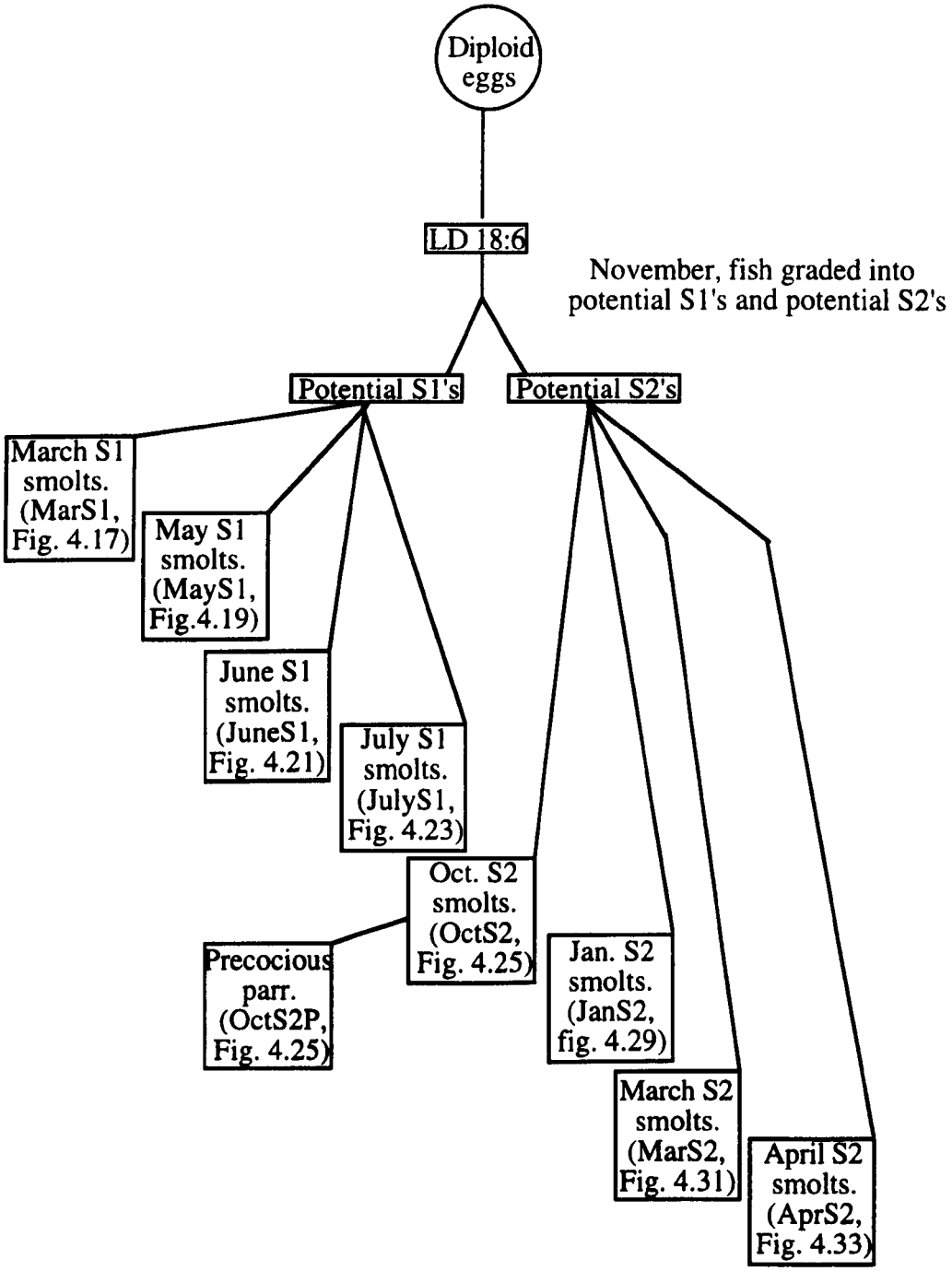
The photoperiod regimes were designed to produce smolts during March, May (S1 control), June, July, October, January, March and April (S2 control). The photoperiod regimes and holding conditions for each group are outlined below. The group name refers to the month of transfer (i.e. May, Mar, June etc.) and whether the fish were potential S1's (S1) or potential S2's (S2). The initial photoperiod for all groups was LD 18:6 from first feeding until the 11th Nov. when a reduction of 2 hours . $week^{-1}$ was initiated.

Group MarS1 (transferred in March, potential S1 fish)

27000 parr were held in 7 m^3 concrete raceways. The fish were subjected to a 2 hour . $week^{-1}$ decreasing photoperiod from the 11th Nov. to the 9th Dec. On the 9th Dec. the daylength was decreased from LD 10:14 to LD 8.5:15.5. The LD 8.5:15.5 daylength was held until 13th Jan. when the daylength was increased directly to LD 18:6. The LD 18:6 daylength was held until the fish were transferred to sea water (figure 4.17).

Sampling was started on the 11th Jan. The condition factor (figure 4.18) of the fish was between 1.12±0.007 and 1.14±0.006 from January to February. In the month prior to transfer the condition factor of the fish decreased significantly ($P<0.01$) from 1.09±0.06 on the 5th March to 1.04±0.006 on the 19th March. There was a further significant decrease ($P<0.01$) from 19th March to 0.97±0.005 on the 5th April. The serum osmolality (figure 4.18) of the challenged fish decreased significantly ($P<0.01$) during

Figure 4.16 The origin and relationship of each group in experiment 5. The month of transfer of each group is stated and the group's name is described as follows: the month of transfer (Mar, May etc.) and whether the fish were potential S1's or S2's (S1 or S2). The figure number quoted for each group refers to the photoperiod which was employed to produce smolts in that particular month.



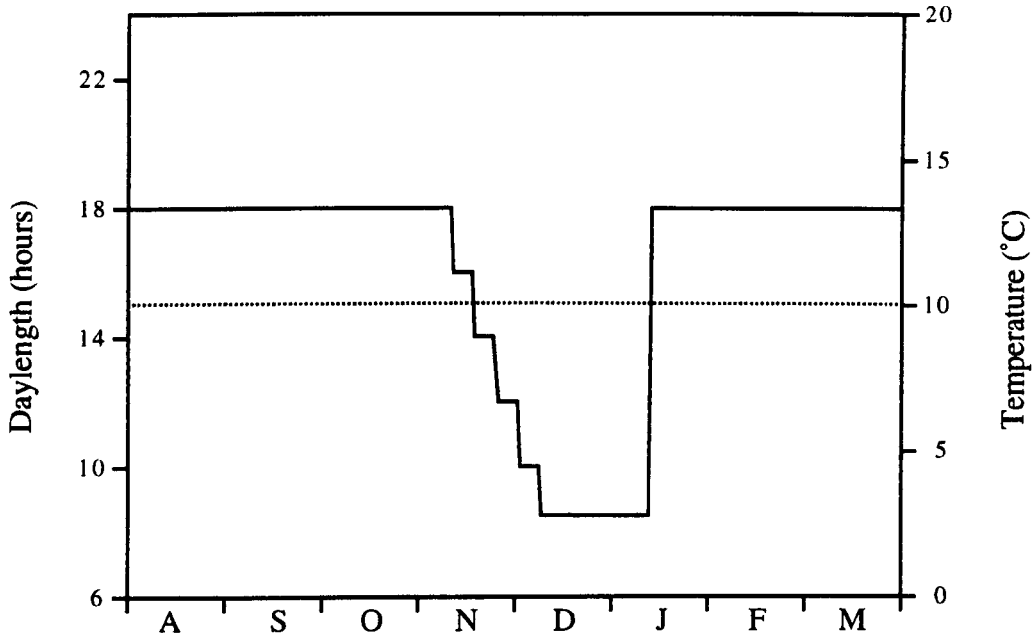


Figure 4.17 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred into sea water during March (group MarS1).

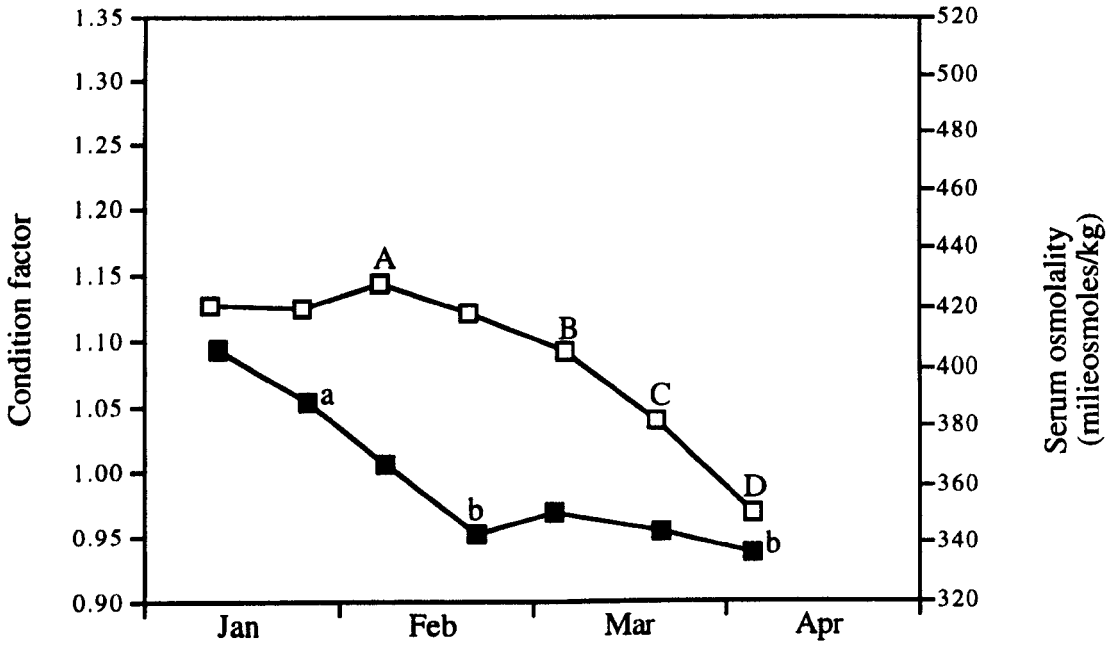


Figure 4.18 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred into sea water during March (group MarS1). Error bars are ± 1 sem; error bars are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

January and February. The serum osmolality remained between 350.93±1.73 and 336.50±2.41 mOsm.kg $^{-1}$ from late February to early April. The osmolality prior to transfer on the 5th April was 336.5±2.41 mOsm.kg $^{-1}$.

The smolts were transferred directly to two different seawater sites on the 30th March and the 8th April. On the 30th March, a group of 19500 smolts (mean weight 39.5±0.7g) was transferred to a 15m cage at site SW3. Mortalities and monthly farm sample weights were recorded. The mean weight and length of 100 randomly selected fish were determined every second month. During sampling fish with signs of maturation were recorded as mature. The fish were contaminated by an oil spillage from the MV Braer on the 5th Jan. Feed input in January was much reduced due to uncertainty over the future of the fish. In February, compensation plans were outlined and feeding resumed according to feed company guidelines. During January and February 4000 dead fish were removed; the mortality was attributed to oil contamination. The entire cage was harvested on 20th Aug., 17 months after the transfer to sea water.

On the 8th April, 500 smolts were transferred into a 3m ($7m^3$) tank at site SW1. The weights and lengths of 50 randomly selected fish were recorded every month. During sampling fish with signs of maturation were recorded as mature. A pump failure on the 3rd Aug. caused the death of all the fish in this group.

Group MayS1 (transferred in May, potential S1 fish)

40000 parr were held in 7 m^3 concrete raceways. The fish were subjected to the 2 hour . week^{-1} decreasing photoperiod from the 11th Nov. to the 9th Dec. On the 9th Dec. the daylength was decreased from LD 10:14 to LD 8.5:15.5. The LD 8.5:15.5 daylength was held until 16th Jan. when the fish were transferred to an earth pond where the only illumination was natural daylight (figure 4.19).

Sampling started on the 11th Jan. The condition factor (figure 4.20) increased significantly ($P<0.01$) during the first month of sampling. From February through to

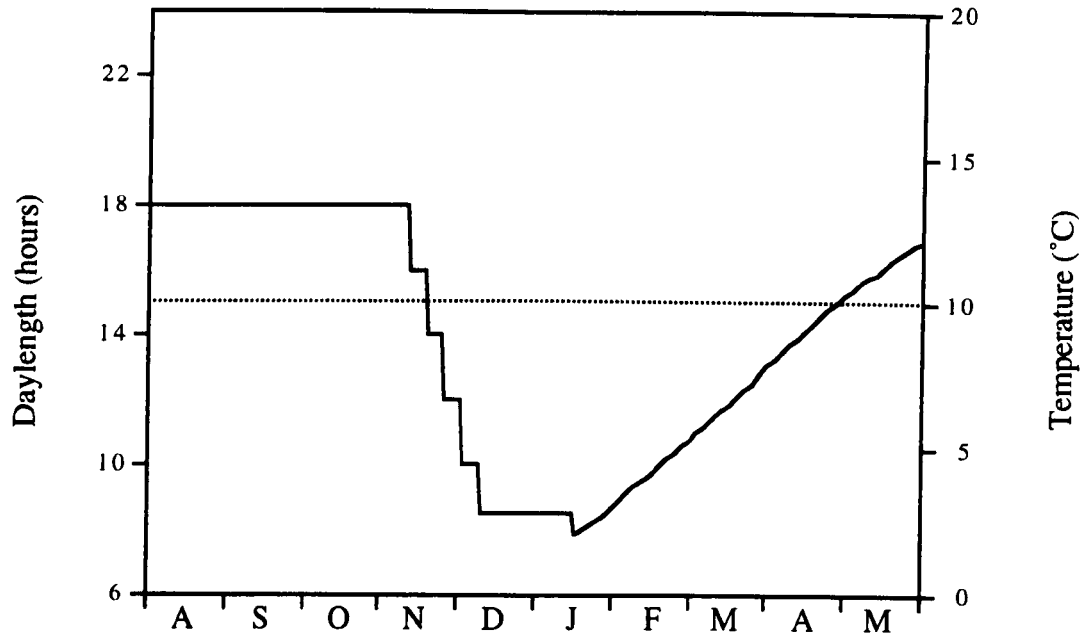


Figure 4.19 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred into sea water during May (group MayS1).

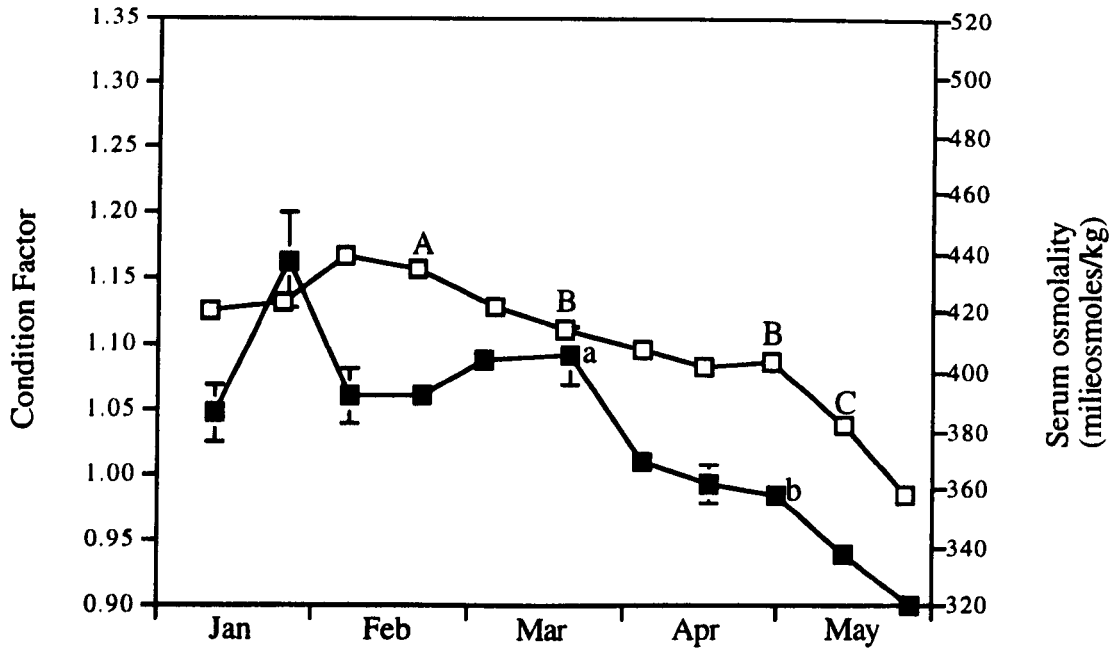


Figure 4.20 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred into sea water during May (group MayS1). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

April the condition factor gradually decreased; the decrease was significant ($P<0.01$) between 29th April and 13th May from 1.09±0.065 to 1.04±0.007. There was a further decrease from the 13th May to 0.99±0.006 on the 25th May. The serum osmolality (figure 4.20) of the challenged fish remained between 385.7±9.57 and 437.2±16.3 from January to March. The serum osmolality decreased significantly ($P<0.01$) from the 20th March to the 13th May resulting in an osmolality of 320.1±2.02 $mOsm.kg^{-1}$ prior to transfer.

The smolts were transferred directly to 2 seawater sites. On the 29th May, one group of 37800 smolts (mean weight 36.1±0.8g) was transferred to a 15m cage at site SW3 (section 2.2.2.3). Mortalities and monthly farm sample weights were recorded. The mean weight and length of 100 randomly selected fish were determined every second month. During sampling, fish with signs of maturation were recorded as mature. The fish were divided into a second 15m cage on the 22nd July. Monitoring was continued on the 17900 fish which remained in the initial 15m cage. The fish were contaminated by an oil spillage from the MV Braer on the 5th Jan. Feed input in January was reduced due to the uncertainty over the future of the fish. In February compensation plans were outlined and feeding resumed according to feed company guidelines. On 26th April over a year after transfer the fish were divided into a third 15m cage. Monitoring was continued on the 7500 fish which remained in the original 15m cage. The entire cage was harvested on 26th Nov., 18 months after the transfer into sea water.

On the 2nd June, a second group consisting of 500 smolts was transferred into a 3m ($7m^3$) tank at site SW1 (section 2.2.2.1). The weights and lengths of 50 randomly selected fish were recorded every month. During sampling fish with signs of maturation were recorded as mature. After 4 months the stocking density was reduced; 240 fish were randomly removed to leave 140 fish in the tank. On the 30th Nov., 18 months after the fish were transferred, 25 of the fish were sacrificed; the gonads were removed and weighed for GSI determination.

Group JuneS1 (transferred in June, potential S1 fish)

21000 parr were held in 7 m^3 concrete raceways. The fish were subjected to the 2 hour . $week^{-1}$ decreasing photoperiod from the 11th Nov. to the 24th Nov. (figure 4.21). The LD 14:10 was held from the 24th Nov. to the 23rd Dec. On the 23rd Dec. a 1 hour . $week^{-1}$ decrease was initiated. The 1 hour . $week^{-1}$ decrease was continued until LD 8:16 on the 24th Feb. The LD 8:16 was held for 2 weeks until the 16th March. On the 16th March a half hour . $week^{-1}$ increase was initiated. The half hour . $week^{-1}$ increase was continued until the 11th May when the daylength was stepped up from LD 12:12 to LD 18:6. The LD 18:6 was held until the fish were transferred to sea water.

Sampling began on the 5th April. During April and early May the condition factor (figure 4.22) of the fish was between 1.09±0.005 and 1.12±0.005. The condition factor decreased significantly ($P<0.01$) from 1.15±0.006 on the 25th May to 1.0±0.005 on the 10th June. The serum osmolality (figure 4.22) of the challenged fish remained between 408.8±29.4 and 369.28±4.47 $mOsm.kg^{-1}$ during April and early May. A significant decrease ($P<0.01$) from the 13th May to the 26th May resulted in an osmolality of 348.14±2.32 $mOsm.kg^{-1}$ on the 26th May. The osmolality remained close to this value for the following month and prior to transfer the osmolality was 351.54±8.54 $mOsm.kg^{-1}$.

On the 19th June, 13800 smolts (mean weight 36.6±0.6g) were transferred to a 12m cage at site SW2 (section 2.2.2.2). Mortalities and monthly farm sample weights were recorded. On the 13th April the fish were transferred to a 15m cage. The fish were grilse graded on the 8th June. During the grade the numbers of maturing and immature salmon were recorded. The growth and mortality of the fish was monitored until September, 15 months after transfer.

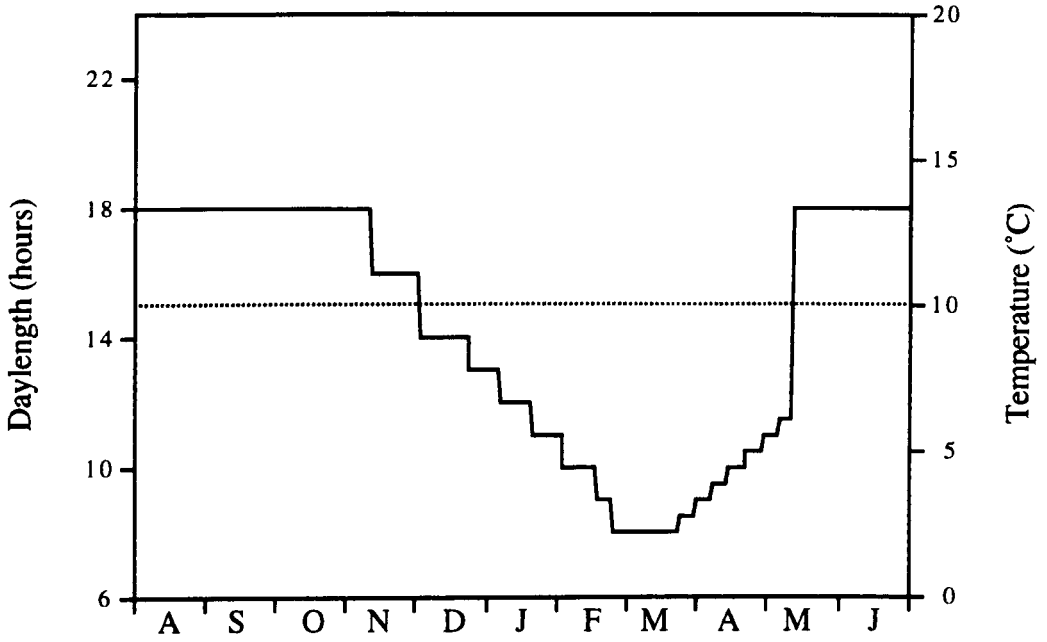


Figure 4.21 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred into sea water during June (group JuneS1).

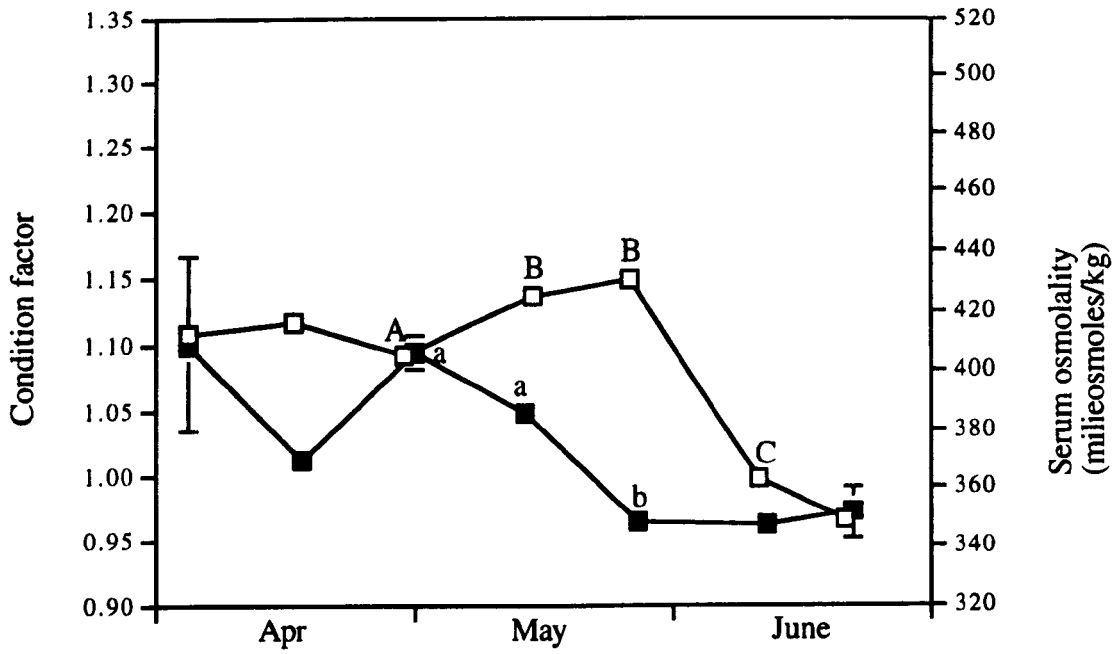


Figure 4.22 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred into sea water during June (group JuneS1). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

Group JulyS1 (transferred in July, potential S1 fish)

24000 parr were held in 7 m^3 concrete raceways. Prior to the 11th May the fish were subjected to the same photoperiod regime as group JuneS1 (figure 4.21). The half hour . $week^{-1}$ increase was continued from LD 12:12 on the 11th May (figure 4.23) until the fish were transferred in to sea water.

Sampling for the condition factor began on the 29th April and sampling for the serum osmolality of challenged fish began on the 11th June. The condition factor (figure 4.24) of the fish initially increased significantly from 1.08±0.009 to 1.17±0.011 on the 15th May. From the 15th May the condition factor decreased. The decrease was significant ($P<0.01$) from 1.13±0.008 on the 10th June to 1.02±0.006 on the 6th July and from 1.1±0.01 on the 20th June to 1.02±0.006 on the 6th July. The condition factor prior to transfer on the 20th July was 0.98±0.005. The serum osmolality (figure 4.24) of the challenged fish increased before decreasing significantly ($P<0.01$) from the 7th July to the 21st July. On the 21st July, prior to transfer, the serum osmolality was 336±2.55 $mOsm.kg^{-1}$.

On the 24th July, 24000 smolts (mean weight 34.7±0.6g) were transferred to a 12m cage at site SW2 (section 2.2.2.2). Mortalities and monthly farm sample weights were recorded. On the 4th Feb. the fish were divided into a second 12m cage. Monitoring continued of 11000 fish which remained in the original 12m cage. The fish were grilse graded on the 7th June. During the grade the numbers of maturing and immature salmon were recorded. The growth and mortality of the fish were monitored until October, 15 months after transfer.

Group OctS2 (transferred in October, potential S2 fish)

33000 parr were held in 7 m^3 raceways until 5th Aug. over 1 year after hatching. On the 5th Aug. the parr were transferred to 18 m^3 raceways. The fish were maintained in the 18 m^3 concrete raceways until transfer to sea water. From 17th Dec. to 29th March, the

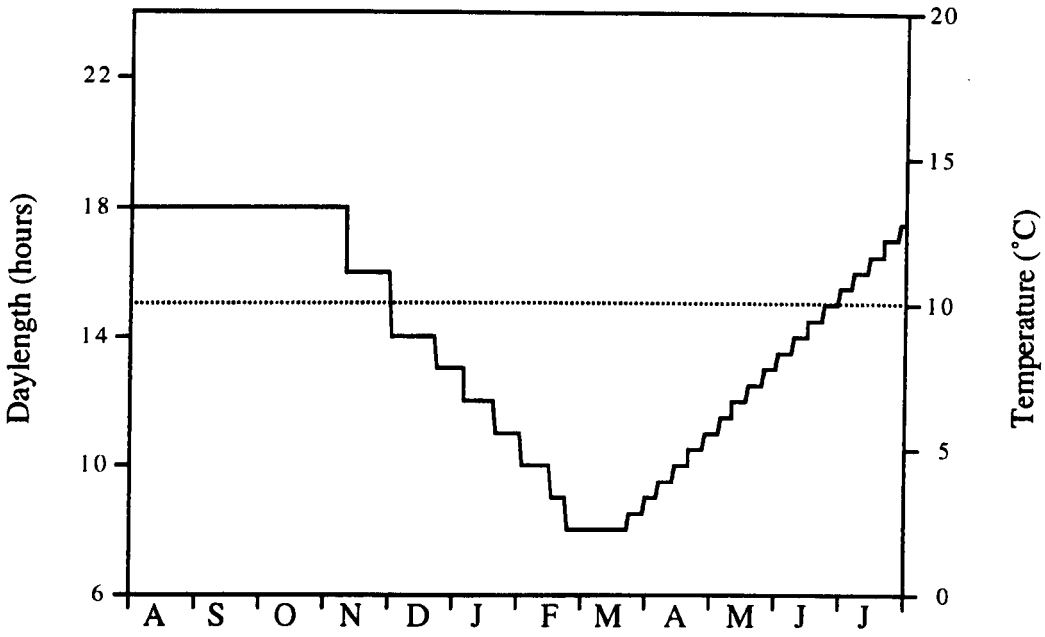


Figure 4.23 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred into sea water during July (group JulyS1).

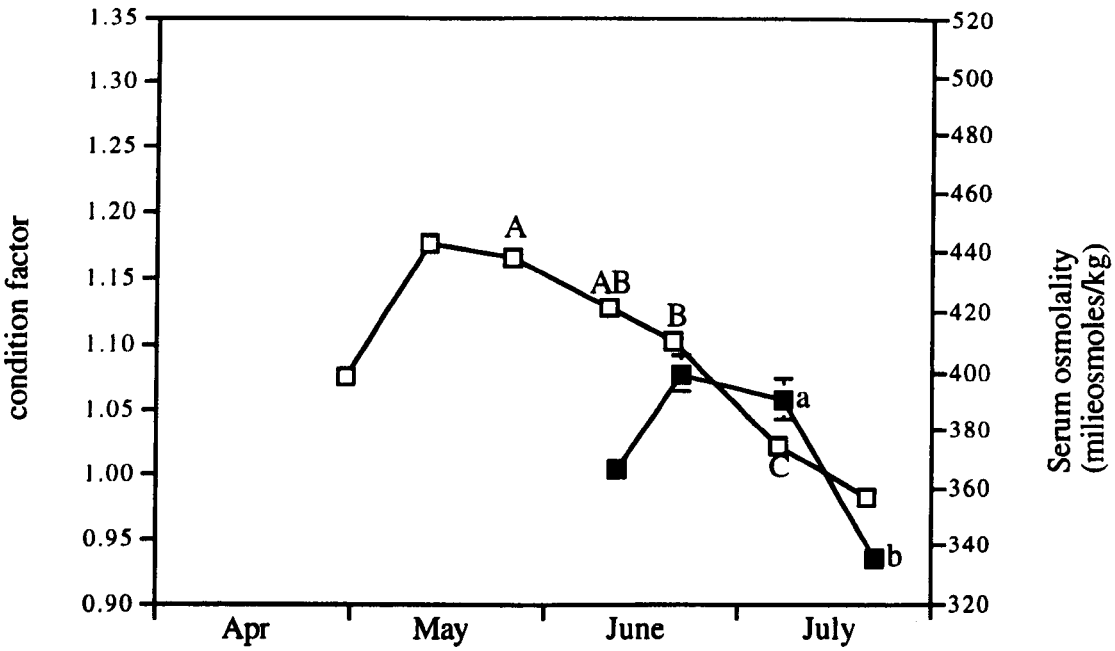


Figure 4.24 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred into sea water during July (group JulyS1). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

fish were subjected to the same photoperiod regime as group MarS1 (figure 4.17). The LD18:6 daylength was held from the 13th Jan. to the 21st May. On the 21st May the daylength was decreased from LD 18:6 to LD 8:16 (figure 4.28). The LD 8:16 was held until 17th Aug. when the daylength was stepped up to LD 18:6. The LD18:6 was held until the fish were transferred to sea water.

Sampling was initiated on the 5th Aug. During July, Aug. and Sept. the condition factor of the fish (figure 4.26) remained between 1.19±0.017 and 1.12±0.01. In the month prior to transfer the condition factor decreased significantly ($P<0.01$) from 1.15±0.008 on the 19th Sept. to 1.03±0.01 on the 1st Oct., the condition factor then increased significantly to 1.12±0.015 on the 12th Oct. The serum osmolality (figure 4.26) of the challenged fish decreased significantly ($P<0.01$) between the 20th Aug. and the 20th Sept. This decrease in serum osmolality was followed by a significant ($P<0.01$) increase from 342.5±5.51 $mOsm.kg^{-1}$ on the 2nd Oct. to 413.5±9.09 $mOsm.kg^{-1}$ on the 13th Oct. During the parr-smolt transformation the fish suffered from a *Aeromonas hydrophila* sp. infection. The infection was diagnosed on the 6th Oct., a 10 day antibiotic treatment was immediately initiated. The infection resulted in 34.9% mortality during the month prior to transfer.

The smolts were transferred directly to 2 seawater sites. On the 26th Oct., one group of 21000 smolts (mean weight 23.1±0.8g) was transferred to a 15m cage at site SW3 (section 2.2.2.3). Mortalities and monthly farm sample weights were recorded. The mean weight and length of 100 randomly selected fish were determined every second month. During sampling the maturity of the fish was assessed and recorded. The fish were contaminated by an oil spillage from the MV Braer on the 5th Jan. Feed input during January was reduced due to uncertainty over the future of the fish. In February compensation plans were outlined and feeding, according to feed company guidelines, resumed. The fish were transferred into a 20m cage on the 15th June. On 8th Dec. over a year after transfer the fish were divided into a second 20m cage. Monitoring of growth

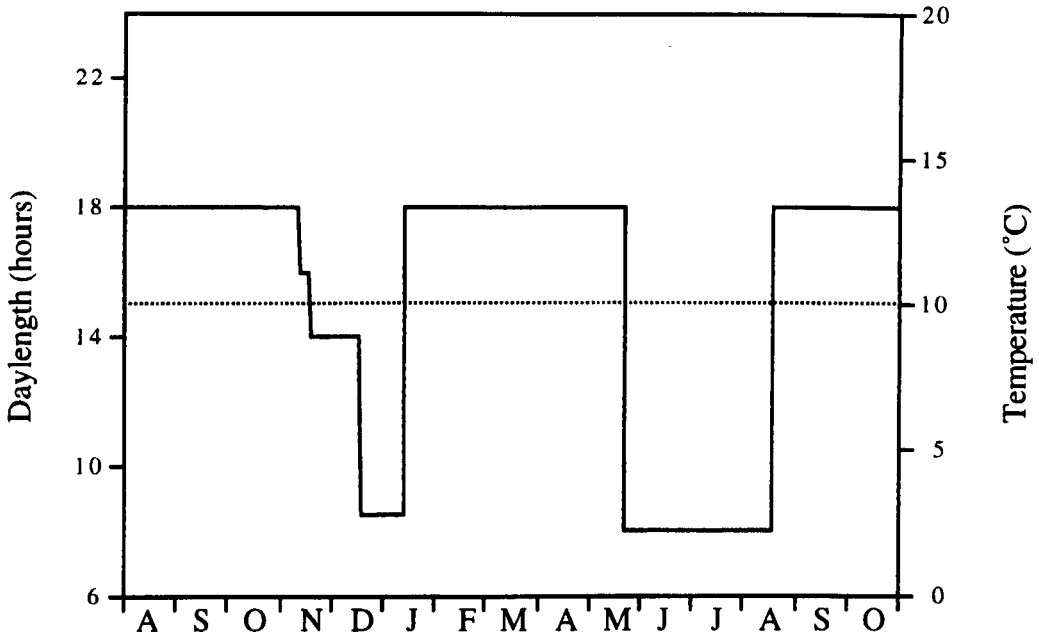


Figure 4.25 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred into sea water during October (group Oct. S2).

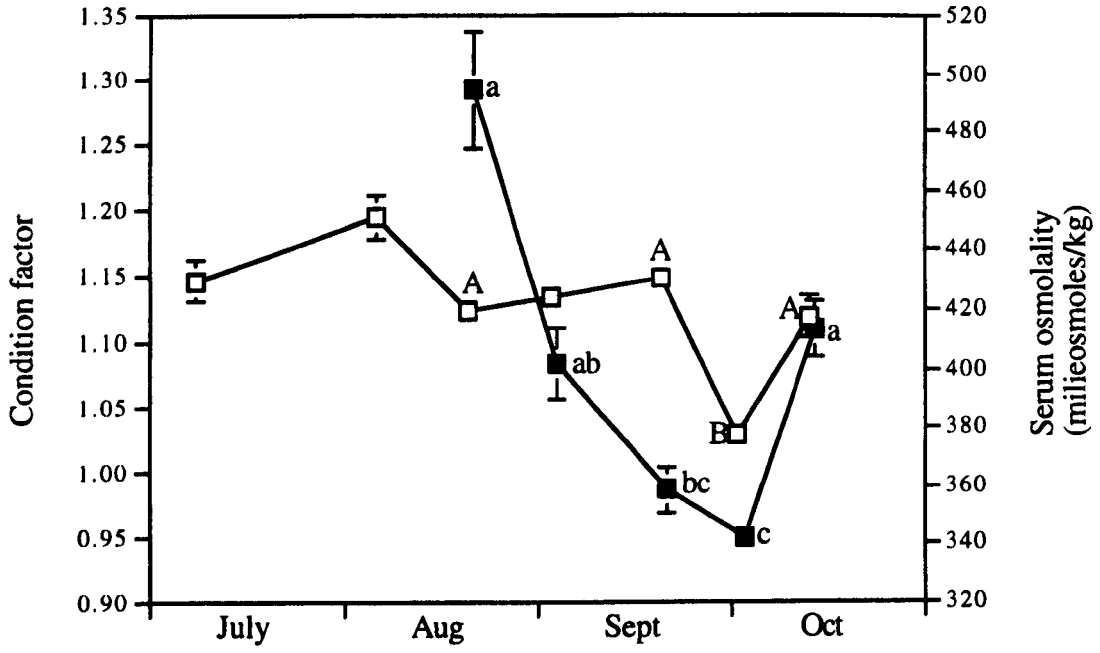


Figure 4.26 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred into sea water during October (group Oct. S2). Error bars are ± 1 sem, error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

and mortality continued on the 14000 fish which remained in the original 20m cage. The entire cage was harvested on 26th Feb., 16 months after the transfer date.

On the 23rd Oct. 700 smolts were transferred into a 3m (7 m^3) tank at site SW1. At the time of transport the fish were in poor health, to account for possible losses 600 fish rather than 500 were transferred. The weights and lengths of 50 randomly selected fish were recorded every month. During sampling fish with signs of maturation were recorded as mature. After 6 months the stocking density was reduced, 150 randomly selected fish were removed to leave 140 fish in the tank. On the 30th Nov., and the 2nd Dec., 13 months and 25 months respectively after the fish were transferred, 25 of the fish were sacrificed; the gonads were removed and weighed for GSI determination. There was a high mortality in the group of fish which matured during the first year in sea water. These mature mortalities were recorded.

Group OctS2P (transferred in October, mature male parr from group OctS2)

On the 18th Aug., over 1 year after hatching, 150 mature male parr were selected from group OctS2. The parr were selected if milt ran from the sperm duct when pressure was applied to the abdomen. These male parr were placed in a 1m (0.25m^3) tank and subjected to the same photoperiod (figure 4.25) and sampling protocol as the main OctS2 group. Challenged fish were killed and the GSI determined.

Sampling was initiated on the 19th Aug. The condition factor (figure 4.27) showed no significant change, remaining between 1.20±0.012 and 1.23±0.007 during the sampling period. The serum osmolality of the challenged fish (figure 4.27) increased significantly ($P<0.01$) from 398.1±10.5 mOsm.kg^{-1} on the 19th Aug. to 522.21±9.29 mOsm.kg^{-1} on the 2nd Sept before decreasing significantly ($P<0.05$). The serum osmolality remained between 464.1±17.0 mOsm.kg^{-1} and 430.8±13.0 mOsm.kg^{-1} until transfer to sea water. During the fresh water period the GSI (figure 4.28) of the parr decreased. The GSI of 2.5% recorded on the 12th oct., 11 days prior to transfer, was significantly

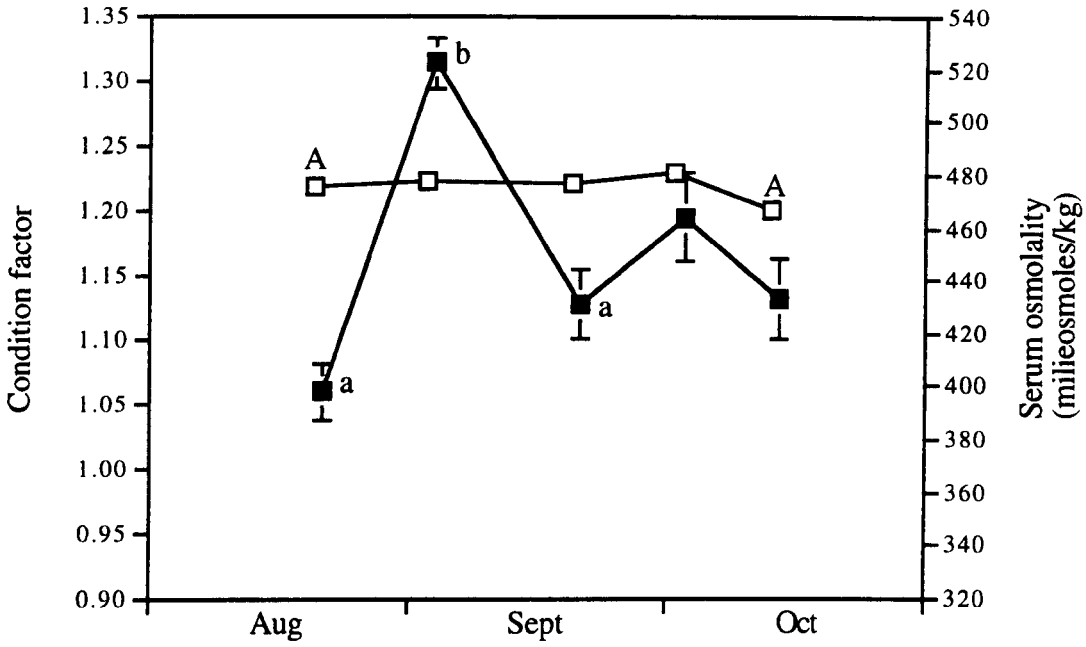


Figure 4.27 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) precocious parr transferred into sea water during October (group OctS2P). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

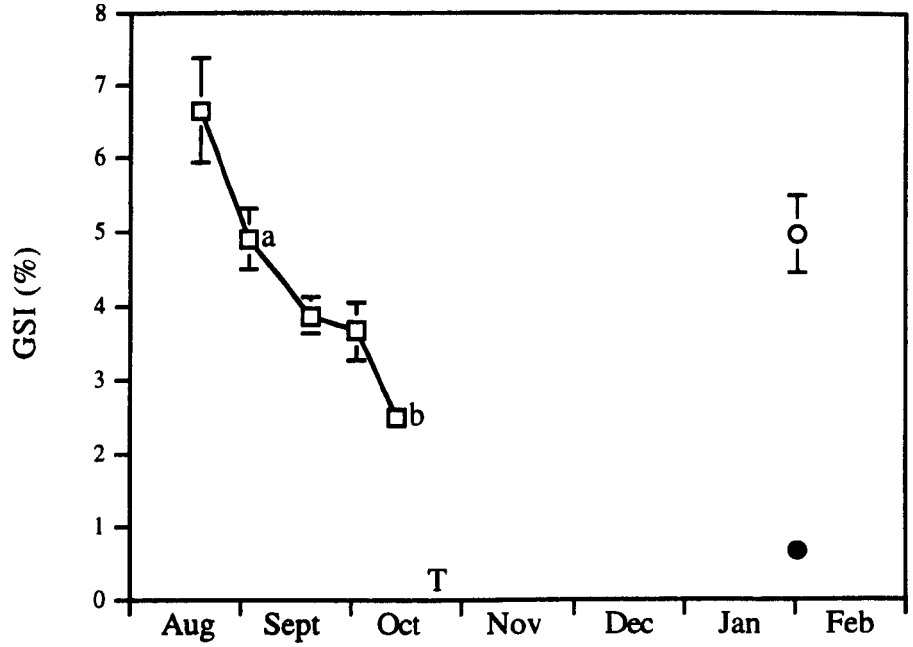


Figure 4.28 Changes in the GSI for the precocious parr transferred into sea water during October (group OctS2P). The square symbols are pre-transfer GSIs taken from challenged fish (n=10). The circular symbols represent the GSIs of surviving mature (open circular symbol) and immature (filled circular symbol) fish sampled on the 1st Feb. T indicates transfer date (23rd Oct.). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period.

($P<0.05$) lower than the GSI recorded on the 19th Aug. and the 2nd Sept. At the time of transfer to sea water 35.8% of the parr produced milt when pressure was applied to the abdomen.

On the 23rd Oct., 99 fish were transferred to site SW1 into the same 3m ($7m^3$) tank as the group OctS2 fish. Daily mortality was recorded. Mortality was high during the first month. On the 2nd Dec. the OctS2P fish were separated from the OctS2 fish and transferred into a 2m ($1.9m^3$) tank. During this transfer the weight and length of the fish were measured. This part of the experiment was terminated on the 1st Feb. when the fish were killed. The weight, length and GSI for each fish was determined.

Group JanS2 (transferred in January, potential S2 fish)

15000 parr were held in 7 m^3 raceways until 3rd Nov. over one year after the fish hatched. On the 3rd Nov. the fish were transferred to $18m^3$ concrete raceways. The fish were subjected to the 2 hour . $week^{-1}$ decreasing photoperiod from the 11th Nov. to the 9th Dec. (figure 4.29). On the 9th Dec. the daylength was decreased from LD 10:14 to LD 8.5:15.5. The LD 8.5:15.5 daylength was held until the 20th Jan. when a half hour . $week^{-1}$ increase was initiated. The half hour . $week^{-1}$ increase was terminated on the 8th June at LD 19:5. The LD 19:5 daylength was held until 10th Aug. when the daylength was stepped down to LD 8:16. The LD 8:16 was held until 19th Oct. when a 1 hour . $week^{-1}$ increase was initiated. This increase was maintained until 12th Dec. when the daylength was held on LD 18:6. The LD 18:6 daylength was maintained until the fish were transferred to sea water.

Sampling was initiated on the 4th Nov. The condition factor (figure 4.30) of the fish decreased throughout the sampling period. Prior to transfer the condition factor decreased significantly ($P<0.01$) from 1.16±0.009 on the 21st Dec. to 1.03±0.008 on the 19th Jan. The serum osmolality (figure 4.30) of the challenged fish decreased significantly ($P<0.01$) from the 17th Nov. to the 4th Dec. and again decreased

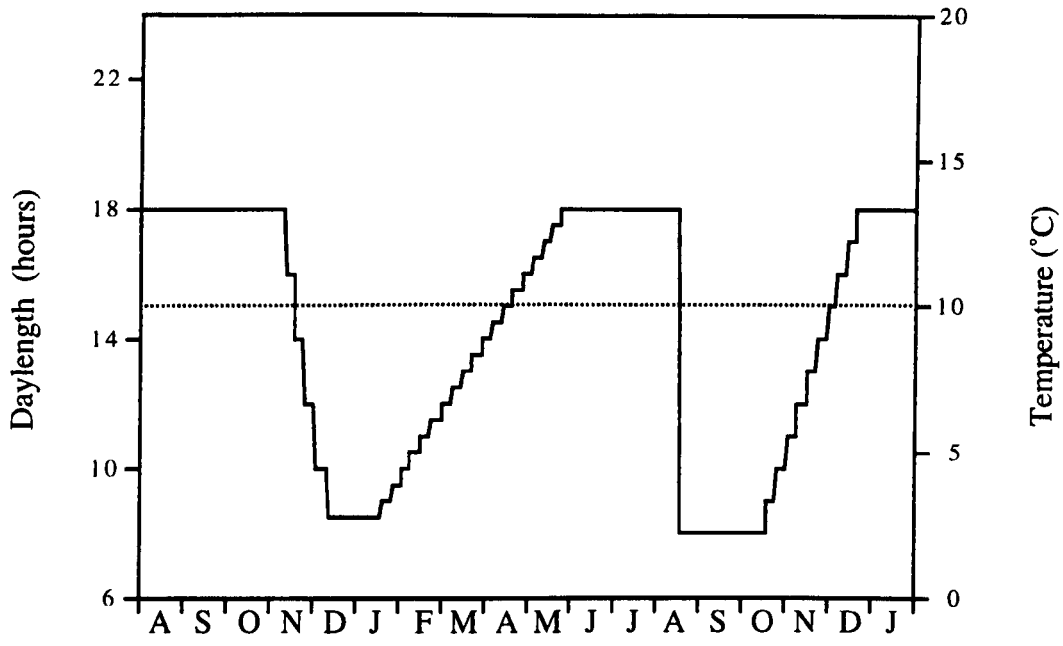


Figure 4.29 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred during January (group JanS2).

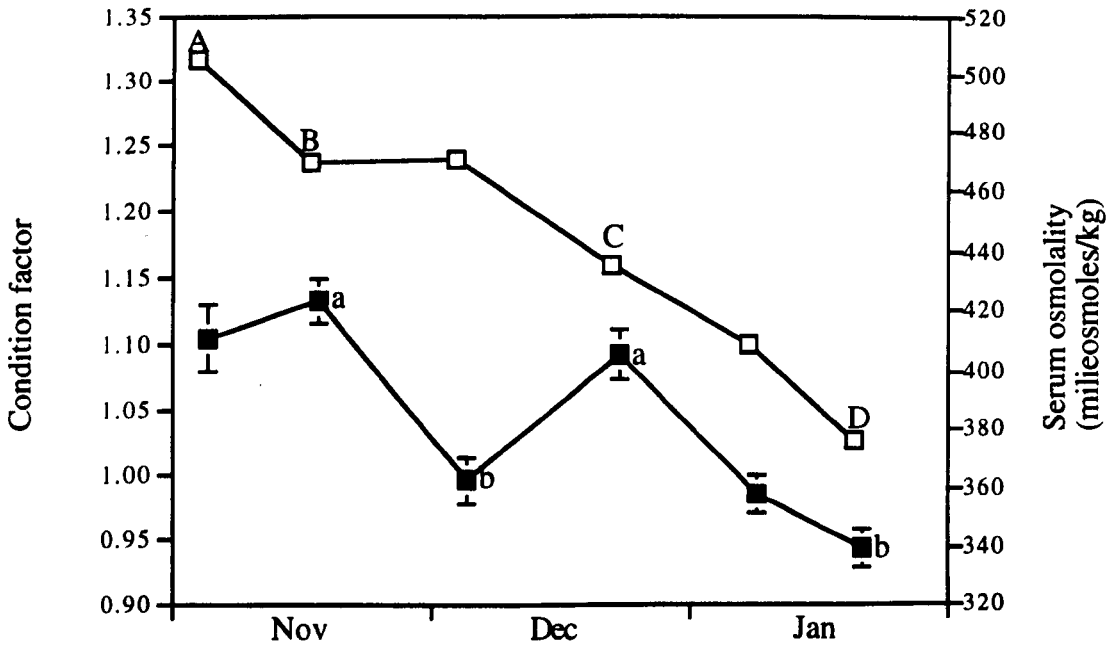


Figure 4.30 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred during January (group JanS2). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

significantly ($p<0.01$) from the 22nd Dec. to the 20th Jan. Prior to transfer of the serum osmolality of the challenged fish was 339.5±6.38 mOsm.kg $^{-1}$ on the 20th Jan.

The smolts were transferred directly to 2 seawater sites. On the 25th Jan. 14000 smolts (mean weight 43.3±1.3g) were transferred to a 12m cage at site SW2 (section 2.2.2.2). Mortalities and monthly farm sample weights were recorded. On 8th Nov. the fish were transferred into a 15m cage. The fish were harvested from the 28th April through to the 23rd May, 15-16 months after the transfer date.

On the 21st Jan. 500 smolts were transferred into a 3m ($7m^3$) tank at site SW1. The weights and lengths of 50 randomly selected fish were recorded monthly. During sampling fish exhibiting signs of maturation were recorded as mature. After 3 and 4 months the stocking density was reduced, 150 fish and 100 fish respectively were randomly selected and removed to leave 100 fish in the tank. On the 30th Nov., and the 2nd Dec., 9 months and 21 months respectively after the fish were transferred, 25 of the fish were sacrificed for GSI determination. There was a high mortality in the group of fish which matured during the first year in sea water. These mature mortalities were recorded.

Group MarS2 (transferred in March, potential S2 fish)

20000 parr were held in $7m^3$ raceways until 10th Feb during the 2nd year of rearing. On the 10th Feb. the fish were transferred to $18m^3$ concrete raceways. Prior to the 8th June, the fish were subjected to the same photoperiod regime as group JanS2 (figure 4.29). The LD 19:5 daylength was held from the 8th June to the 12th Oct. On the 12th Oct. the daylength was decreased to LD 18:6. On the 19th Oct. a 2 hour . week^{-1} decrease was initiated and continued until LD 10:14 on 9th Nov. Further decreases were made on the 16th Nov. to LD 9:15 and on the 23rd Nov. to LD 8:16. The LD 8:16 was held until 28th Dec. when a 1 hour . week^{-1} increase was initiated. The 1 hour . week^{-1} increase was terminated on 22nd Feb. with an increase from LD 16:8 to LD 18:6. The LD18:6 was held until the fish were transferred to sea water (figure 4.31).

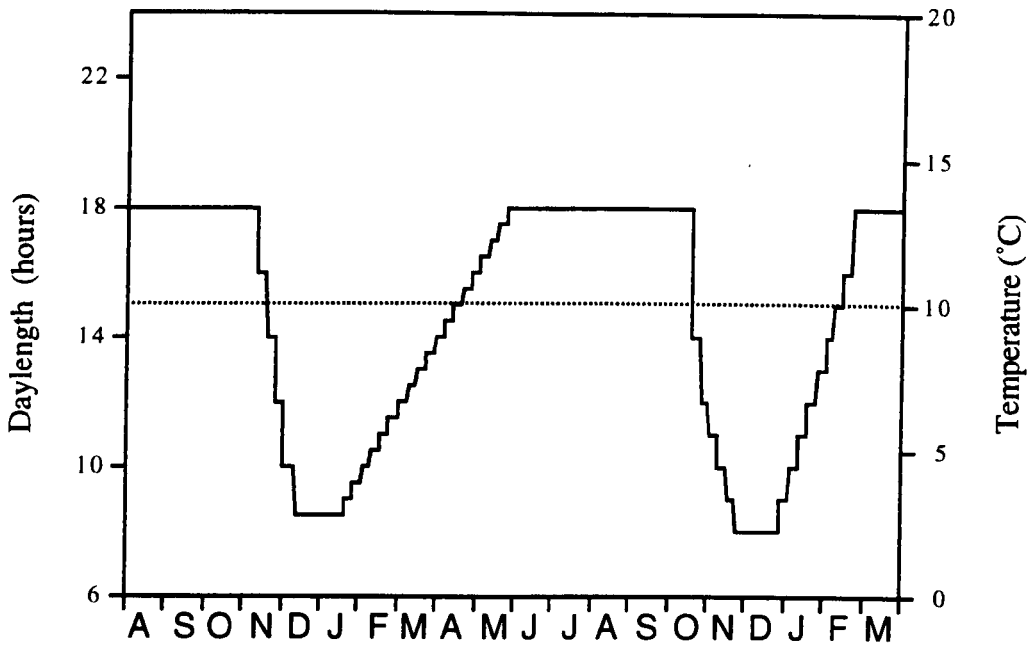


Figure 4.31 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred during March (group MarS2).

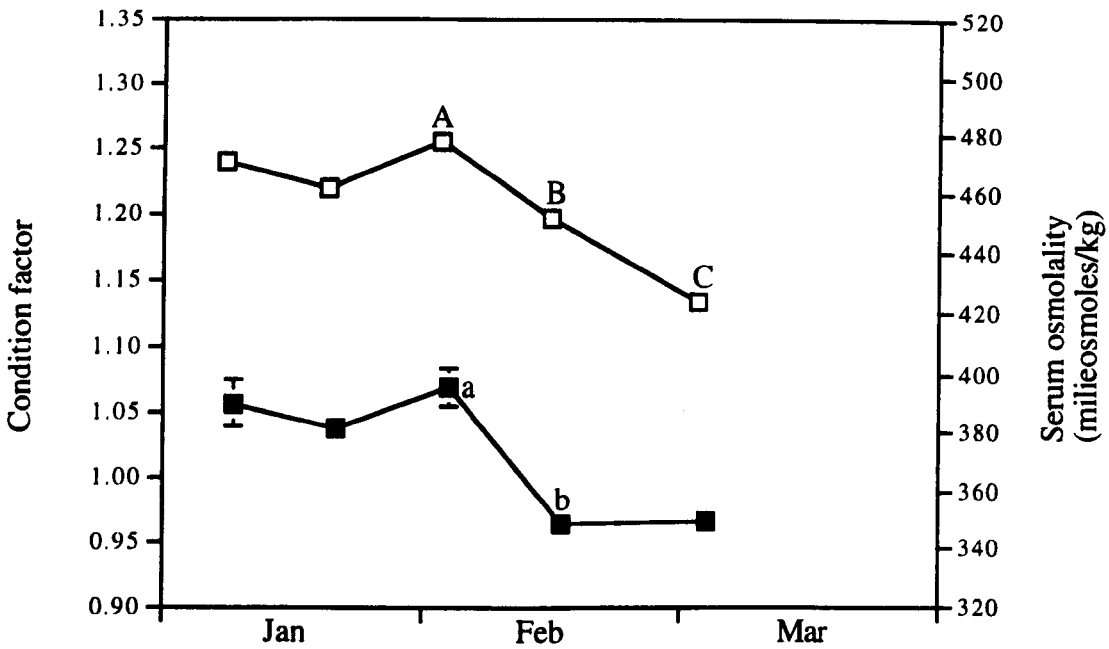


Figure 4.32 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred during March (group MarS2). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

Sampling was initiated on the 8th Jan. During Jan. and early Feb. the condition factor (figure 4.32) of the fish was between 1.22±0.008 and 1.25±0.009. The condition factor then decreased significantly ($P<0.01$) from 1.25±0.009 on the 2nd Feb. to 1.19±0.008 on the 15th Feb. and again from 1.19±0.008 on the 15th Feb. to 1.13±0.007 on the 2nd March. The serum osmolality (figure 4.32) of the challenged fish decreased significantly ($P<0.01$) between the 3rd Feb. and the 16th Feb. to an osmolality of 348.6±2.95 $mOsm.kg^{-1}$; the osmolality remained close to this value and was 349.6±6.32 $mOsm.kg^{-1}$ on 3rd March prior to transfer.

The fish were transferred directly to 2 seawater sites. On the 11th March, 12000 smolts (mean weight 58.3±1.0 g) were transferred to a 12m cage at site SW2 (section 2.2.2.2). Mortalities and monthly farm sample weights were recorded. On 20th Jan. the fish were divided into a second 12m cage. Monitoring was continued on the 4800 fish which remained in the original cage. The fish were harvested from the 6th June through to the 18th July, 15-16 months after the transfer date.

On the 11th March 500 smolts were transferred into a 3m ($7m^3$) tank at site SW1 (section 2.2.2.1). The weights and lengths of 50 randomly selected fish were recorded every month. During sampling fish with signs of maturation were recorded as mature. After 4, 6 and 12 months the stocking density was reduced; 100, 60 and 140 fish respectively, were randomly selected and removed to leave 100 fish in the tank. On the 30th Nov., and the 2nd Dec., 8 months and 20 months respectively after the fish were transferred, 25 of the fish were sacrificed for GSI determination.

Group AprS2 (transferred in April, potential S2 fish).

150 parr were randomly selected from the MarS2 group on the 21st July over one year after the fish hatched. The parr were placed in a 1m ($0.25m^3$) circular tank and subjected to a simulated natural photoperiod (figure 4.33).

Sampling was initiated on 19th Aug. after stocking from the MarS2 group. The condition factor (figure 4.34) of the fish gradually decreased during the sampling period. A significant decrease ($P<0.01$) was observed from 1.23±0.008 on the 15th Feb. to 1.17±0.01 on the 16th March. A slight increase was observed before a further decrease to 1.14±0.011 on the 12th April. The serum osmolality (figure 4.34) of the challenged fish decreased significantly ($P<0.01$) during the sampling period. However, the decrease was over a long time interval. Between the 20th Jan. and the 30th March the serum osmolality decreased from 385.39±6.29 to 342.5±6.38 mOsm.kg $^{-1}$. On the 12th April the condition factor was 1.14±0.011 and on the 13th April the serum osmolality of the challenged fish was 336.6±4.58 mOsm.kg $^{-1}$. After these sample dates the fish were considered to have attained smolt status and sampling was stopped.

There was no seawater facility available for the transfer of this group of fish. It was therefore not possible to continue monitoring these fish after the completion of smoltification.

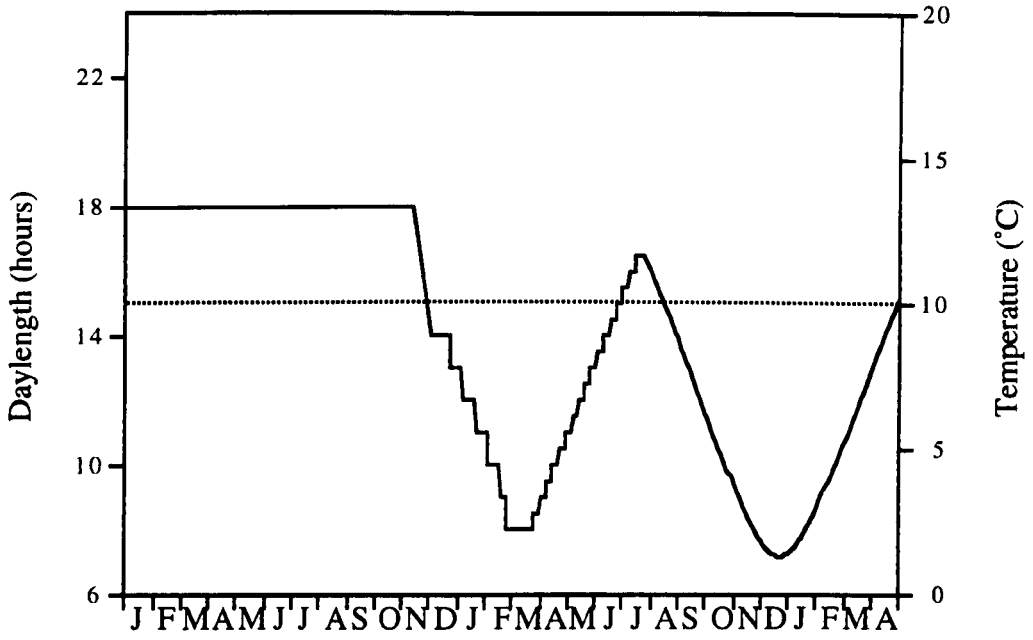


Figure 4.33 Temperature (dotted line) and photoperiod regime (constant line) experienced by the fish transferred during April (group AprS2).

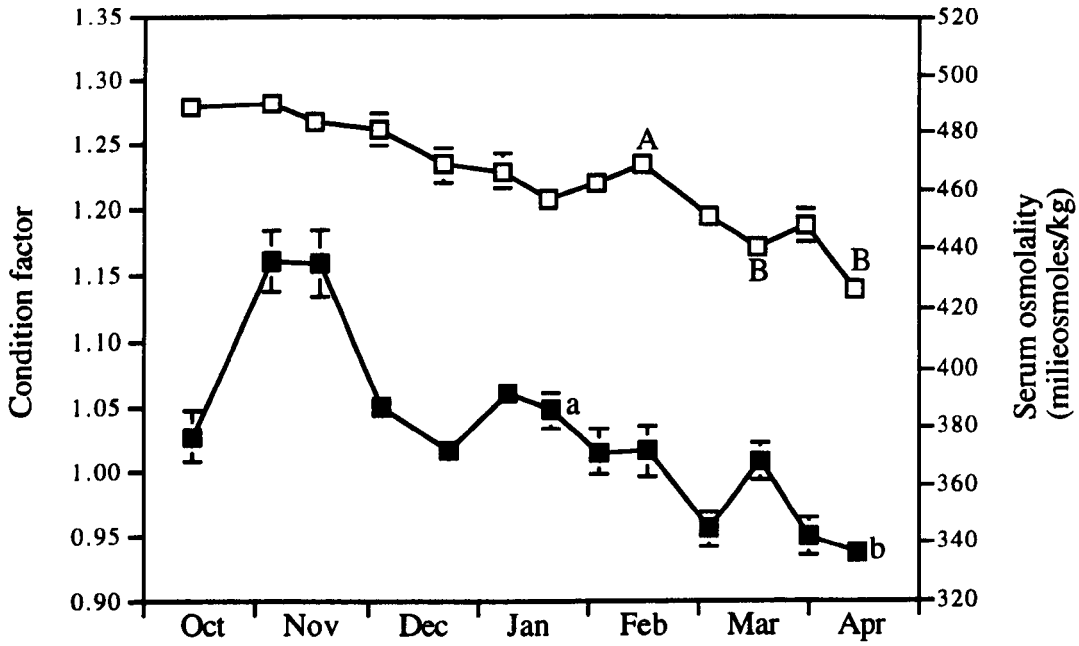


Figure 4.34 Changes in condition factor (open square) and serum osmolality (filled square) for the challenged (24h at 28‰) fish transferred during April (group AprS2). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

4.2.3 Seawater Results for Experiments 4 and 5.

The seawater results for experiments 4 and 5 are presented in 3 subsections: seawater growth in subsection 4.2.3.1: maturation in sea water in subsection 4.2.3.2 and seawater mortality in subsection 4.2.3.3. Within each subsection the results for each experiment are presented separately.

The different photoperiod regimes, to which the groups of fish were subjected, resulted in each group completing smoltification at different times of the year. Each group of fish was transferred directly to sea water as close as possible to what was considered the completion of smoltification. The fish were transferred during January, March, April, May, June, July, October and November.

The different groups in experiments 4 and 5 were transferred to 3 different sea sites (SW1, SW2 and SW3). Each sea site has different environmental conditions (see section 2.2.2) which may have resulted in different growth or rates of maturation in fish of the same stock, a factor which should be taken into account when making a comparison between sites. In particular the fish from experiment 5 were transferred to 3 different seawater sites. Hence, the results for experiment 5 are grouped into results for each sea site.

4.2.3.1 Growth.

Fish from Experiment 4 (Site SW2)

The fish in all groups grew steadily throughout the seawater growing cycle (figure 4.35 and 4.36). After 6 months growth in sea water the fish were 6-8 times larger (Table 4.1) than when transferred. Group MayS1D was harvested from the 19th Aug. to the 10th Sept., 16-17 months after transfer. Average harvest weights for the MayS1D fish ranged from 2.4-2.7kg. Group MarS1D was harvested from the 18th Aug. through to the 10th Sept., 17-18 months after transfer. Average harvest weights for the MarS1D fish ranged from 2.3-2.9kg. The NovS2D group was harvested on the 23rd and 30th June, 19

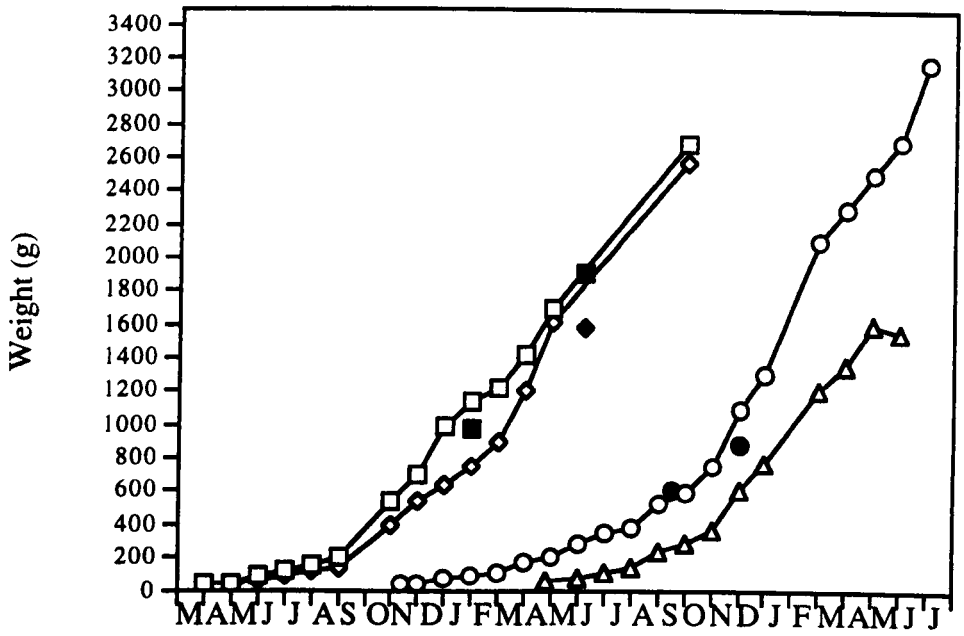


Figure 4.35 Seawater growth for the four diploid groups transferred into sea water (site SW2) during May, diamond symbol (MayS1D); March, square symbol (MarS1D); November, circular symbol (NovS2D) and April, triangular symbol (AprS2D). Open symbols represent farm weights and filled symbols represent mean sample weights ± 1 sem, error bars are obscured by the plot symbol.

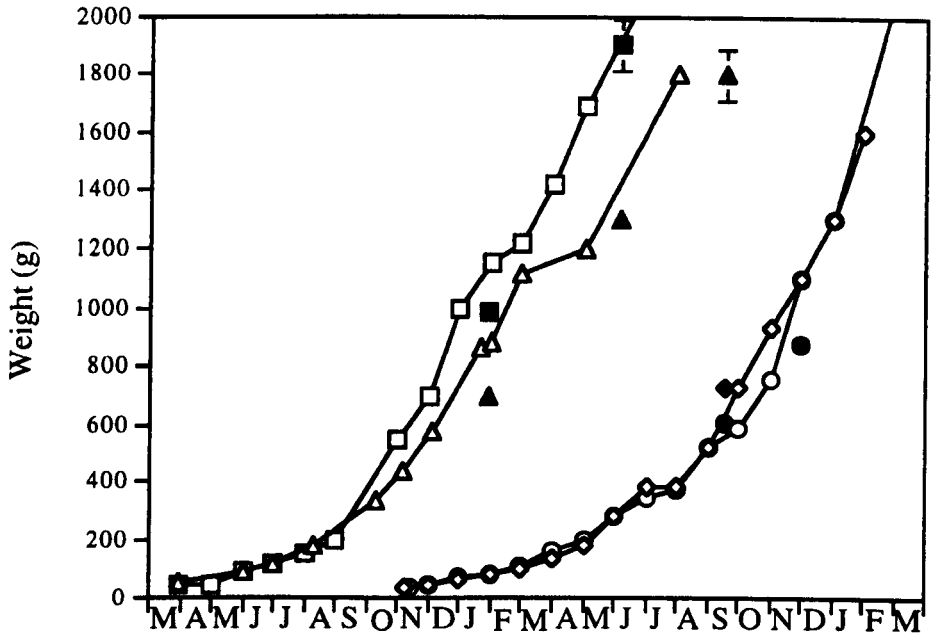


Figure 4.36 Seawater growth of the diploid (group MarS1D, square symbol) and triploid (group MarS1T, triangular symbol) fish transferred into sea water during March and the diploid (group NovS2D, circular symbol) and triploid (group NovS2T, diamond symbol) fish transferred during November. Open symbols represent farm weights and filled symbols represent sample weights ± 1 sem, error bars which are not visible are obscured by the plot symbol.

months after transfer. The average harvest weight was 3.2kg. During the harvest of the NovS2D fish, 35.6% of the fish had the colouring of mature fish. The average weight of these mature fish (grilse) was 3.6kg. All the grilse were processed as first class salmon as flesh quality had not deteriorated.

Table 4.1 Sea water growth data for the 7 groups MarS1D, MarS1T, MayS1D, MayS1T, NovS2D, NovS2T and AprS2D in experiment 4.

	MarS1 D	MarS1 T	MayS1 D	MayS1 T	NovS2 D	NovS2 T	AprS2 D
Sea Site.	SW2	SW2	SW2	SW3	SW2	SW2	SW2
Transfer date	29/3	29/3	4/5	2/5	14/11	8/11	23/4
Pre-transfer weight (g).	42.2 ±0.9	48.0 ±0.7	43.4 ±0.8	39.09 ±0.6	33.2 ±0.8	40.0 ±0.6	55.4 ±1.23
Weight (g) after 6 months sea growth.	375	260	400	270	280	183	370
Specific growth rate during 6 month	1.18	1.06	1.23	1.06	1.08	0.88	0.99
Harvest date	18/8-30/9	-	19/8-10/9	-	23/6-30/6	-	-
Months, transfer date to harvest.	17-18	-	16-17	-	19	-	-
Harvest Weight (g)	2300-2900	-	2400-2700	-	3200	-	-

The two triploid groups transferred to site SW2 produced different growth patterns compared to the diploid groups subjected to the same photoperiod regime (Figure 4.36). Group MarS1T grew slowly compared with group MarS1D giving respective sample weights of 1.3±0.05kg and 1.9±0.09kg on the 4th June, 14 months after transfer. Over the growing period monitored, the two groups NovS2D and NovS2T exhibited similar growth patterns.

Fish from Experiment 5.

Site SW1.

Six groups MarS1, MayS1, OctS2, OctS2P, JanS2 and MarS2 were transferred to site SW1. The sea water growth of 4 of these groups MayS1, OctS2, JanS2 and MarS2 was monitored for 1.5 to 2 years. After the first six months in sea water the fish in the 4 groups exhibited weights from 226±6.67 to 363.7±10.6 g, and specific growth rates

from 0.91±0.11 to 1.18±0.15 (table 4.2). The growth continued until the end of the first year when the growth curve levelled out (figure 4.37), indicating that the growth rate of some or all of the fish had slowed or stopped. The growth in groups MayS1 and OctS2 completely stopped and the mean weight declined. The decline in weight was also illustrated by reduced specific growth rates (figure 4.38). This period of reduced growth coincided with varying proportions of the fish maturing in the different groups.

On all sample dates the mean weight for group MayS1 was significantly different ($P<0.01$) from the other groups transferred to site SW1 (figure 4.37). During the first year on all sample dates except 4th Aug. the mean weights for group OctS2 were significantly different ($P<0.01$) from the other groups. The sample weights of groups JanS2 and MarS2 were similar. The groups mean weights were significantly different ($P<0.01$) on the 1st April, 4th May and the 28th May.

The SGR curves (figure 4.38) for the 4 groups of fish showed the highest specific growth rates after transfer. These rates generally declined during the first year decreasing in the winter months and increasing in the summer months. During the Autumn of the second year the growth rates decreased to 0 or below. Different groups exhibited slightly different growth profiles, higher growth being associated with the different transfer dates and the summer. However, the mean specific growth rates for the entire growing cycle were not significantly different between the four groups MayS1, OctS2, JanS2 and MarS2 (table 4.2).

During the four months over which group MarS1 was monitored, the group exhibited a growth rate comparable with the groups MayS1, OctS2, JanS2 and MarS2 (table 4.2). Group OctS2P exhibited a low post transfer SGR (table 4.2). Prior to transfer, the OctS2P population was normally distributed ($\partial<0.01$). After 3 months seawater growth the population was not normally distributed, the distribution being skewed in the positive direction. On the 1st Feb. when this part of the experiment was terminated the smaller fish in the group, which had shown little or no growth, had higher GSIs in comparison to the fish, which had exhibited growth. GSI had a reciprocal relationship ($r^2= 0.796$,

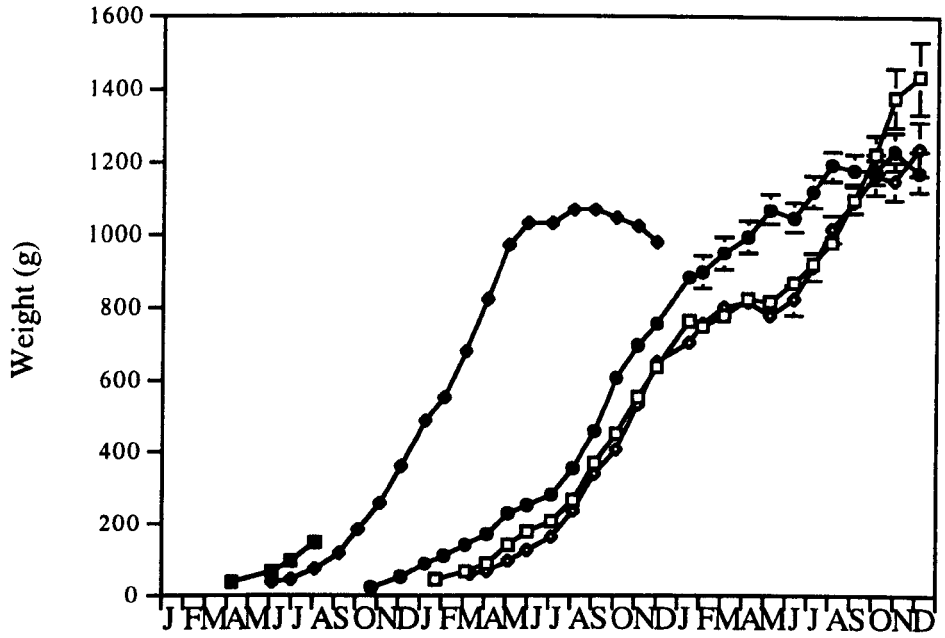


Figure 4.37 Seawater growth for the fish transferred to site SW1. The filled square symbols represent fish transferred into sea water during March (group MarS1), the filled diamond symbols represent fish transferred during May (group MayS1), the filled circular symbols represent fish transferred during October (group OctS2), the open square symbols represent fish transferred during January (JanS2) and open diamond symbols represent fish transferred during March (group MarS2). Mean sample weights are ± 1 sem, error bars which are not visible are obscured by the plot symbol.

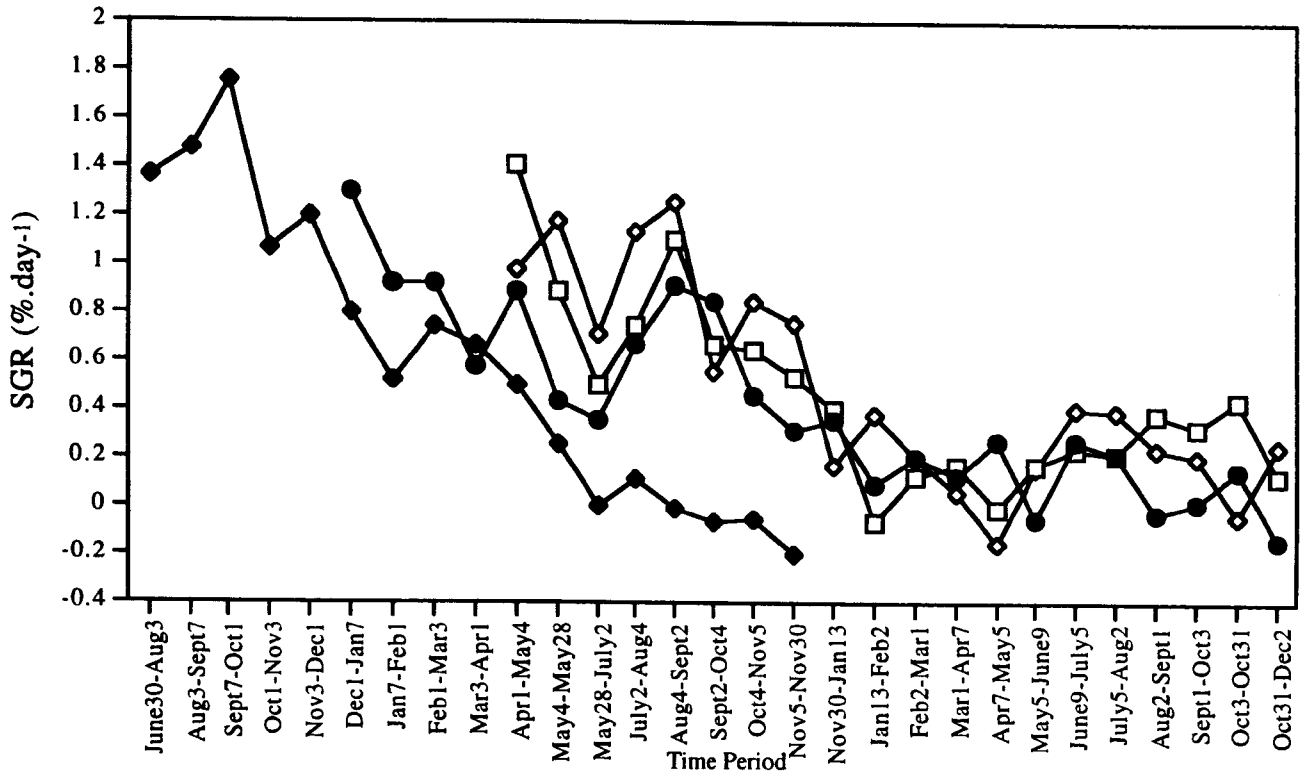


Figure 4.38 Changes in SGR over time periods between consecutive sample dates for 4 groups transferred to sea site SW1 during May (group MayS1, filled diamond symbol), October (OctS2, filled circular symbol), January (group JanS2, open square symbol) and March (MarS2, open diamond symbol).

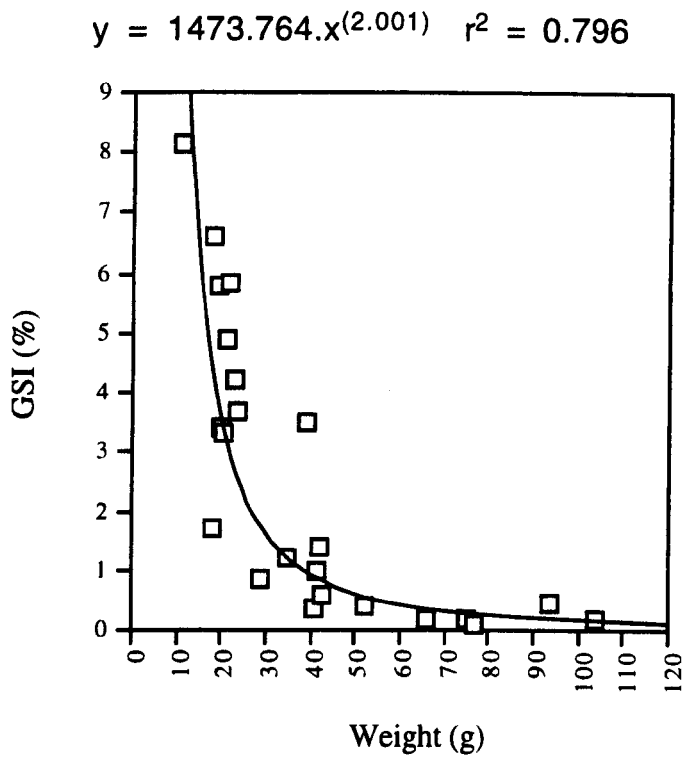


Figure 4.39 Correlation of GSI against weight on the 1st Feb, for precocious parr transferred into sea water during October (OctS2P).

significant at $p<0.05$) with weight on the 1st Feb (figure 4.39), 3 months after the fish were transferred (it should be noted this correlation is in part an auto correlation). Fish with a high GSI exhibited poor seawater growth in comparison to fish with a low GSI.

Table 4.2 Seawater growth for the 6 groups MayS1, MarS1, OctS2, OctS2p, JanS2 and MarS2 transferred to sea site SW1.

	MarS1	MayS1	OctS2	OctS2P	JanS2	MarS2
Pre-transfer weight (g).	40±0.7	36±0.4	21±0.8	19±0.6	43±0.9	58±1.0
Transfer date.	8/4	2/6	23/10	23/10	21/1	11/3
Weight (g) after 6 months sea water growth (g).	146.5± 4.2*	363.7 ±10.6	226.0 ±6.67	38.2 ±4.95^	267.7 ±13.2	342.5 ±11.7
Specific growth rate during 6 month period (%.day^{-1}).	1.08*	1.18± 0.15	1.11± 0.22	0.59^	0.93± 0.13	0.91± 0.11
Final Weight (g)	-	960.6± 94.2	1173.4 ±52.6	-	1433.5 ±97.5	1239.3 ±73
Mean specific growth rate during entire cycle(%.day^{-1}).	-	0.60± 0.14	0.46± 0.10	-	0.47± 0.08	0.49± 0.09

* Weight at 4 months when pump failed; SGR calculated from weight.

^ Weight after 3 months when trial was terminated; SGR calculated from weight.

Site SW3

The 3 groups MarS1, MayS1 and OctS2 of smolts transferred to site SW3 exhibited steady growth over the entire growing cycle (figure 4.40 and 4.41). During the first six months in sea water the fish in the 3 groups exhibited mean specific growth rates from 1.18±0.09 to 1.35±0.25 (table 4.3). The MayS1 and MarS1 fish exhibited similar specific growth rates 1.35±0.25 and 1.25±0.33 respectively (table 4.3). These growth rates during the first six months resulted in similar sample weights after 6 months seawater growth, group MayS1 weighed 328 g and group MarS1 327 g. The fish in the OctS2 group grew at a lower SGR 1.18±0.09 (table 4.3) resulting in a lower mean weight of 213g after 6 months seawater growth.

If the entire seawater growing period is considered, the specific growth rates were highest in group OctS2 which exhibited a mean specific growth rate of 1.13±0.14. The growth rate of group MarS1 was 0.91±0.14 and for group MayS1 0.88±0.14 (table 4.3). There was no significant difference between the mean specific growth rates over t

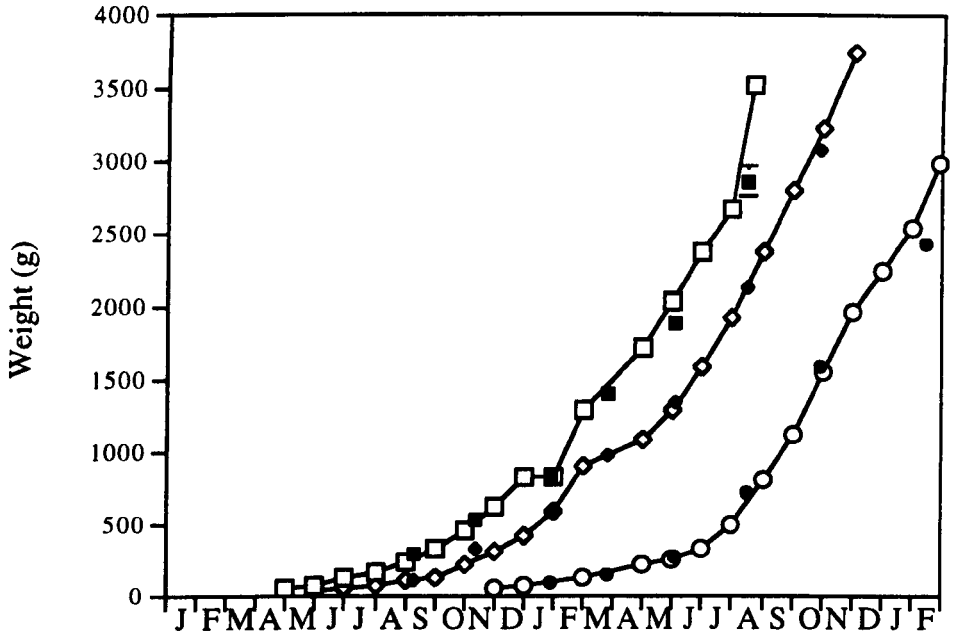


Figure 4.40 Seawater growth, change in weight for the fish transferred to site SW3. The square symbols represent fish transferred into sea water during March (group MarS1), the diamond symbols represent fish transferred during May (group MayS1) and the circular symbols represent fish transferred during October (group OctS2). Open symbols represent farm sample weights (no error bar) and filled symbols represent mean sample weights ± 1 sem, error bars which are not visible are obscured by the plot symbol.

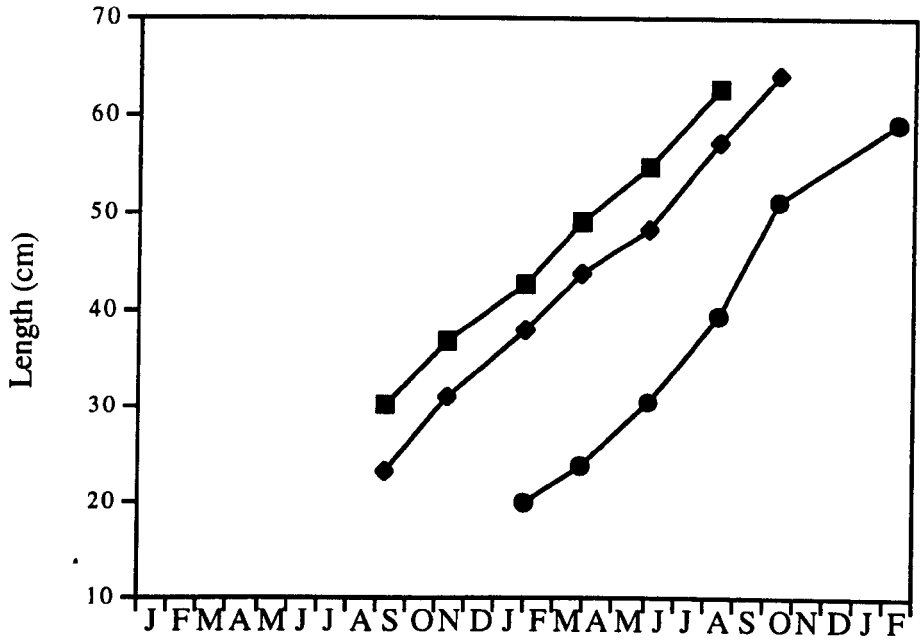


Figure 4.41 Seawater growth, (change in fork length) for the fish transferred to site SW3. The square symbols represent fish transferred into sea water during March (group MarS1), the diamond symbols represent fish transferred during May (group MayS1) and the circular symbols represent fish transferred during October (group OctS2). Mean sample lengths ± 1 sem, all error bars are obscured by the plot symbol.

he entire seawater growing period. The 3 groups were harvested after 16-18 months; average harvest weights ranged from 2979-3750 g (table 4.3). The fish in the 3 groups exhibited significantly different ($P<0.01$) sample lengths on all sample dates (figure 4.41) during the seawater growing period.

Table 4.3 Seawater growth for the 3 groups MayS1, MarS1 and OctS2 transferred to sea site SW3.

	MarS1	MayS1	OctS2
Cage size.	15m	15m	15m
Pre-transfer weight (g).	40±0.7	36±0.4	21±0.8
Transfer date.	30/3	29/5	26/10
Weight (g) after 6 months sea water growth.	327	328.5±6.28	213
Specific growth rate during 6 month period (%.day^{-1}).	1.25±0.33	1.35±0.25	1.18±0.09
Harvest date.	20/8	26/11	26/2
Months from transfer date to harvest date.	17	18	16
Harvest weight (g)	3520	3750	2979
Mean specific growth rate (%.day^{-1}) (n=7)	0.91±0.14	0.88±0.14	1.13±0.14

Site SW2

The fish in the 4 groups, JuneS1, JulyS1, JanS2 and MarS2, transferred to site SW2, showed steady increases in weight during the growing cycle (figure 4.42). The weights after six months growth (table 4.4) were highest in groups JuneS1 and JulyS1. The growth of these two groups was higher in the months immediately after transfer.

Table 4.4 Seawater growth for the 4 groups JuneS1, JulyS1, JanS2 and MarS2 transferred to sea site SW2.

	JuneS1	JulyS1	JanS2	MarS2
cage size.	12m	12m	12m	12m
Transfer date.	19/6	24/7	25/1	11/3
Pre-transfer weight (g).	37±0.6	34.7±0.6	43±0.9	58±1.0
Weight (g) after 6 months sea water growth (g).	435	393	269	250

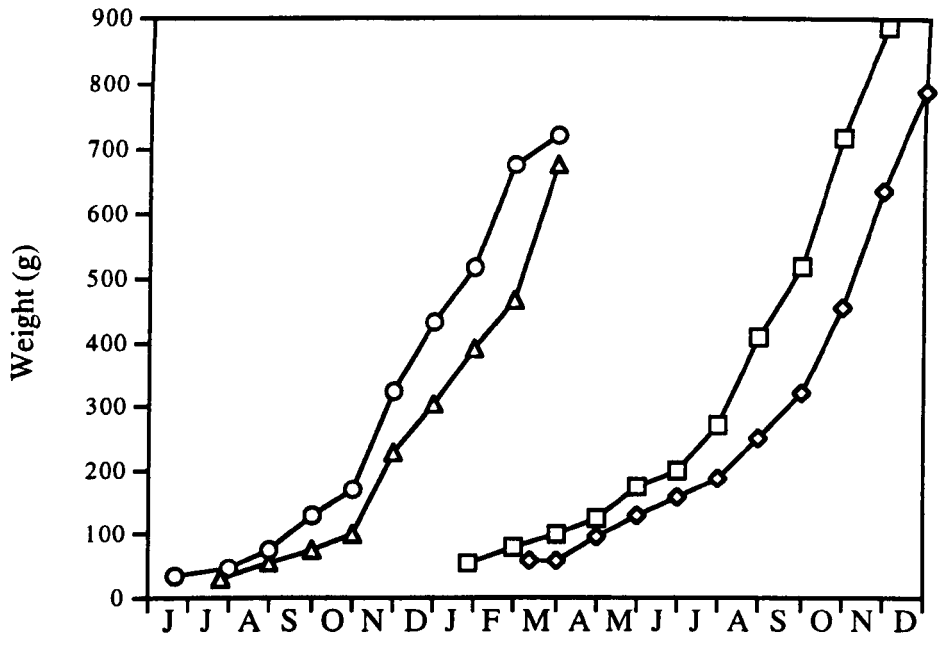


Figure 4.42 Seawater growth, (change in weight) for the fish transferred to site SW2. The circular symbols represent the fish transferred into sea water during June (group JuneS1), the triangle symbols represent the fish transferred during July (group JulyS1), the square symbols represent the fish transferred during January (group Jan. S2) and the diamond symbols represent the fish transferred during March (group MarS2). Sample points are farm sample weights; there are no error bars.

4.2.3.2 Maturation.

Maturation in sea water is defined as follows. Maturation during the first maturation episode refers to maturation during the first year in sea water or post-smolt maturation and maturation in the second maturation episode refers to maturation during the second year in sea water or grilse maturation. For the calculation of time in sea water prior to maturation, the completion of maturation was defined as the 30th November. Fish transferred during October or November, which exhibited maturation during the first year and completed maturation just 12 to 13 months after transfer, were considered to have matured during the first maturation episode as post-smolts. It should be noted that this could also be considered as grilse maturation after one sea winter. However, for the purpose of this account such fish were described as post-smolts.

Fish from Experiment 4 (site SW2).

There was no maturation, in any of the groups, during the first maturation episode (maturation during the year after transfer to sea water, post smolt maturation). During the second maturation episode (maturation during the second year after sea water transfer, grilse maturation) there were varying proportions of maturation (Table 4.5). Each of the 3 groups monitored until the grilse maturation episode, had significantly different proportions of fish maturing, group MayS1D 15.7%, group MarS1D 19% and group NovS2D 35.6%. The grilse from groups MayS1D and MarS1D were harvested in June, 13-14 months after transfer. Grilse weights for groups MayS1D and MarS1D were 2.2kg and 2.9kg respectively. The grilse from the NovS2D group were harvested one year later in June, 19 months after transfer. The average weight of the NovS2D grilse was 3.6kg.

Table 4.5 Sea water maturation data; 1st maturation episode is maturation during the first year in sea water or post smolt maturation; 2nd maturation episode is maturation during the second year in sea water or grilse maturation. Time period is the time in days the smolts were in sea water prior to final maturation ie days from the transfer date to final maturation taken as 30th Nov. Note the 2nd maturation episode was assessed in June when the associated weights were also recorded. Different letters in a row indicate significant differences ($p<0.05$).

	MarS1 D	MarS1 T	MayS1 D	MayS1 T	NovS2 D	NovS2 T	AprS2 D
Sea Site.	SW2	SW2	SW2	SW3	SW2	SW2	SW2
Transfer date	29/3	29/3	4/5	2/5	14/11	8/11	23/4
Pre-transfer weight (g).	42.2 ±0.9	48.0 ±0.7	43.4 ±0.8	39.09 ±0.6	33.2 ±0.8	40.0 ±0.6	55.4 ±1.23
1st maturation episode (%)	**0**	**0** triploid	**0**	**0** triploid	**0**	**0** triploid	**0**
Days in sea water, transfer-maturation.	277	277	241	243	381	381	251
Immature weight (g)	703	577	537	470	1300	1100	600
2nd maturation episode (%)	**19**[a]	-	**15.7**[b]	-	**35.6**[c]	-	-
Days in sea water, transfer-maturation.	642	-	606	-	746	-	-
Mature weight (g)	2900	-	2200	-	3549	-	-
Immature weight (g)	1600	-	1360	-	2519	-	-

Fish from Experiment 5.

Site SW1.

Fish transferred to site SW1 exhibited maturation during the 1st maturation episode (post-smolt maturation). Only the OctS2 and JanS2 groups exhibited post smolt maturation; the percentages maturing were 36% and 12% respectively (table 4.6). There was no significant difference between maturation during the first maturation episode in groups OctS2 and JanS2. However, the maturation in groups OctS2 and JanS2 was significantly greater ($P<0.01$) than the zero rate of maturation observed in groups MayS1, Mar S1 and MarS2. During the second maturation episode all the groups at site SW1 exhibited high levels of maturation. The percentages maturing were 96% in group

MayS1, 88% in OctS2, 48% in JanS2 and 48% in MarS2. During the second maturation episode there was no significant difference between the proportions maturing in groups MayS1 and OctS2. However, the proportions maturing in these two groups were significantly higher ($P<0.01$) than the proportions in groups JanS2 and MarS2, which exhibited the same maturation rate.

Table 4.6 Sea water maturation data for the groups transferred to site SW1; 1st maturation episode is maturation during the first year in sea water or post smolt maturation; 2nd maturation episode is maturation during the second year in sea water or grilse maturation. Time period is the time in days the smolts were in sea water prior to final maturation i.e. days from the transfer date to final maturation taken as 30th Nov. Different letters in a row indicate significant differences ($p<0.05$).

	MarS1	MayS1	OctS2	OctS2P	JanS2	MarS2
Pre-transfer weight (g).	40±0.7	36±0.4	21±0.8	19±0.6	43±0.9	58±1.0
Transfer date.	8/4	2/6	23/10	23/10	21/1	11/3
1st maturation episode	**0 %**'a	**0 %**a	**36 %**b	-	**12 %**b	**0 %**a
Days in sea water, transfer-final maturation.	-	154	403	-	314	264
Weight (g) mature fish.	-	-	696.7± 53.2	-	696.1± 62.0	-
Weight (g) immature fish.	-	363.7± 10.6	796.6± 57.0	-	608.9± 32.6	615.3± 24.3
2nd maturation episode	-	**96 %**a	**88 %**a	-	**48 %**b	**48 %**b
Days in sea water, transfer-final maturation.	-	514	768	-	680	631
Weight (g) mature fish.	-	582.8	1002.7 ±48.2	-	846.6± 96.9	931.0± 78.2
Weight (g) immature fish.	-	953.0± 59.6	1625± 233	-	2031.2 ±99.0	1724± 162

' Maturation assessed from GSIs taken on the 3rd Aug. after the pump failure.

The percentage of fish maturing increased, as the number of days in sea water prior to final maturation (30th November) increased (table 4.6). There was a positive linear relationship between percentage maturing and days in sea water (figure 4.43). The linear regression is represented by the equation.

$$\text{Percentage maturing} = 0.137.(\text{Days in sea water}) - 22.609$$

$$\text{coefficient of determination } r^2 = 0.647$$

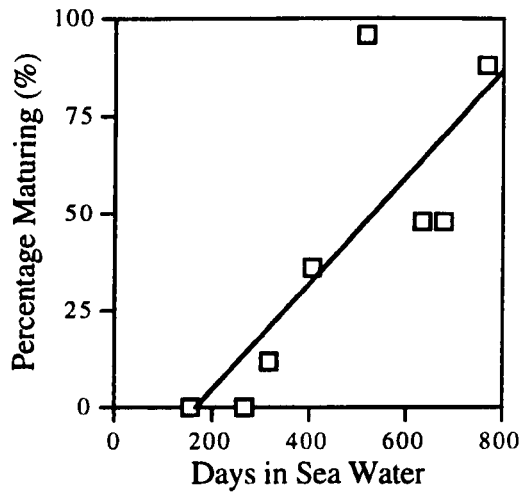


Figure 4.43 Linear regression of the percentage of fish maturing against the number of days in sea water. Each point represents either the 1st or the 2nd maturation episode of the groups of fish transfered to site SW1. The regression equation is $y = 0.137x - 22.609$ and the coefficient of determination $r^2 = 0.647$.

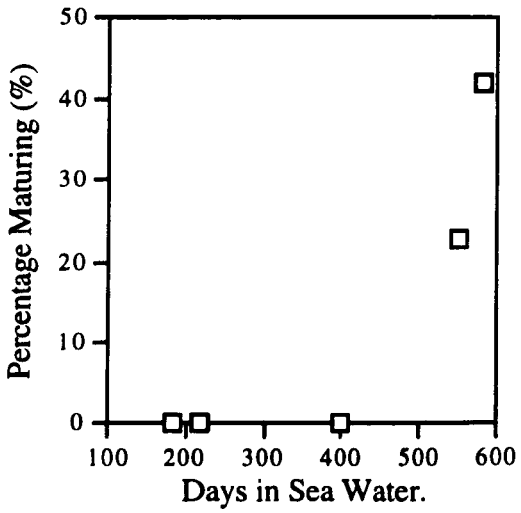


Figure 4.44 The percentage of fish maturing against the number of days in sea water. Each point represents either the 1st or the 2nd maturation episode of the groups of fish transfered to site SW3.

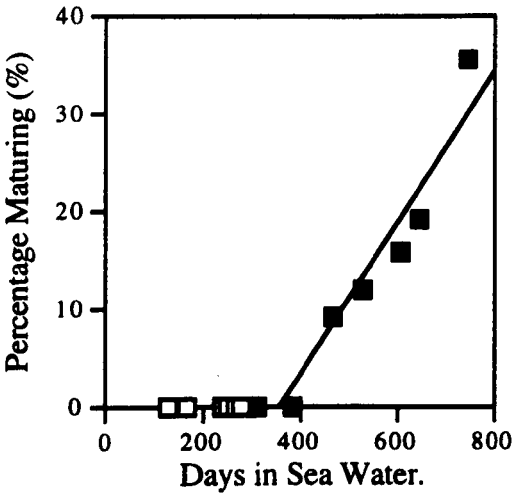


Figure 4.45 Linear regression of the percentage of fish maturing and the number of days in sea water. Each point represents either the 1st or the 2nd maturation episode of the groups of fish transfered to site SW2. The linear regression was applied to the filled symbols. Open symbols were considered to be below a threshold value. The threshold value must be exceeded before a relationship is expressed. The regression equation is $y = 0.077x - 27.499$ and the coefficient of determination $r^2 = 0.920$.

Analysis of variance shows the linear relationship between the number of days in sea water (prior to final maturation) and the percentage within the group maturing was significant ($P<0.05$). The variation in days in sea water accounted for 64.7% of the variation in the percentage of fish maturing.

Site SW3

During the 1st maturation episode there was no maturation in the groups held at site SW3 (table. 4.7). Fish from groups MayS1 and MarS1 exhibited maturation levels of 23% and 42.2% respectively during the 2nd maturation episode (table. 4.7). The maturation rate in MarS1 was significantly higher ($P<0.01$) than in MayS1. Group OctS2 weighed 1597±31.2g at the end of the 1st maturation episode (table 4.7) and was harvested on the 26th Feb. at a mean weight of 2979g. This harvest date did not allow any assessment of maturation during the 2nd maturation episode to be made.

Table 4.7 Sea water maturation data for the groups transferred to site SW2; 1st maturation episode is maturation during the first year in sea water or post smolt maturation; 2nd maturation episode is maturation during the second year in sea water or grilse maturation. Time period is the time in days the smolts were in sea water prior to final maturation i.e. days from the transfer date to final maturation taken as 30th Nov. Note the 2nd maturation episode was assessed in June when the associated weights were also recorded. Different letters in a row indicate significant differences ($p<0.05$).

	MarS1	MayS1	OctS2
Pre-transfer weight (g).	40±0.7	36±0.4	21±0.8
Transfer date.	30/3	29/5	26/10
1st maturation episode	**0 %**	**0 %**	**0 %**
Days in sea water, transfer-final maturation.	217	185	400
Immature weight (g)	528.58±14.3	328.5±6.28	1597±31.2
2nd maturation episode	**42.2 %**^{a}	**23 %**^{b}	**Harvested**
Days in sea water, transfer-final maturation.	582	550	-

In the same manner as site SW1 the percentage of fish maturing increased, as the number of days in sea water prior to final maturation (30th November) increased (table 4.7). However, there were not enough data to apply a linear regression (figure 4.44).

Site SW2.

During the 1st maturation episode there was no maturation in any of the groups held at site SW2 (table. 4.8). JuneS1 and JulyS1 exhibited 11.8% and 9.2% maturation respectively during the 2nd maturation episode (table. 4.8). The maturation rate in JuneS1 fish was significantly higher ($P<0.01$) than in JulyS1. The largest fish in group JanS2 were selected and harvested on 28th May (mean harvest weight 2710g) during the year of the 2nd maturation episode. While in group MarS2 the largest fish were selected on the 26th May and harvested on the 6th June (mean harvest weight 2160g) during the year of the 2nd maturation episode. The harvests was carried out before any assessments of maturation could be made.

The percentage of fish maturing increased, as the number of days in sea water prior to final maturation (30th November) increased (table 4.8). The trend observed at site SW2 in experiment 4 (table 4.5) was similar to the trend observed at site SW2 in experiment 5. The data, for site SW2, from the two experiments were combined and plotted (figure 4.45). There was no maturation in any group which was held in sea water for a period, which was less than 400 days prior to the final maturation date (30th November). Above 300 days there was a positive linear relationship between percentage maturing and days in sea water (figure 4.45). The linear regression was represented by the equation.

$$\text{Percentage maturing} = 0.077.(\text{Days in sea water}) - 27.499$$

$$\text{coefficient of determination } r^2 = 0.92$$

Analysis of variance showed a significant ($P<0.05$) linear relationship between the number of days (above 300) in sea water (prior to final maturation) and the percentage within the group maturing. The variation in days in sea water accounts for 92% of the

variation in the percentage of fish maturing. The values below 300 days appeared to follow a different relationship and were excluded from the regression analysis.

Table 4.8 Sea water maturation data for the groups transferred to site SW3; 1st maturation episode is maturation during the first year in sea water or post smolt maturation, 2nd maturation episode is maturation during the second year in sea water or grilse maturation. Time period is the time in days the smolts were in sea water prior to final maturation i.e. days from the transfer date to final maturation taken as 30th Nov. Note: 2nd maturation episode was assessed in late June. Different letters in a row indicate significant differences ($p<0.05$).

	JuneS1	JulyS1	JanS2	MarS2
Pre-transfer weight (g).	37±0.6	34.7±0.6	43±0.9	58±1.0
Transfer date.	19/6	24/7	25/1	11/3
Episode 1 maturation	**0 %**	**0 %**	**0 %**	**0 %**
Days in sea water, transfer-final maturation.	164	129	309	264
Immature weight (g)	327	231	888	633
Episode 2 maturation	**11.8 %**[a]	**9.2 %**[b]	**Harvested**	**Harvested**
Days in sea water, transfer-final maturation.	529	464	-	-

4.2.3.3 Mortality.

Fish from Experiment 4 (site SW2).

The transfer mortalities were defined as mortalities in transit and during the first month in sea water, these ranged from 13.9 to 44.9% (Table 4.9). The mortalities in the advanced out-of-season smolts were significantly higher ($P<0.01$) than the natural smolts. The two groups of natural smolts MayS1D and AprS2D had transfer mortalities of 13.9% and 14.8% respectively. The out-of-season smolts mortality ranged from 27.5% in group MarS1T to 44.9% in group NovS2D.

Table 4.9 Sea water mortality data in relation to fresh water results; Transfer mortality refers to mortality during transport and the first month in sea water. Precocious maturation refers to maturation as parr. Different letters indicate a significant difference ($p<0.05$).

	MarS1 D	MarS1 T	MayS1 D	MayS1 T	NovS2 D	NovS2 T	AprS2 D
Sea Site.	SW2	SW2	SW2	SW3	SW2	SW2	SW2
Pre-transfer weight (g).	42.2 ±0.9	48.0 ±0.7	43.4 ±0.8	39.1 ±0.6	33.2 ±0.8	40.0 ±0.6	55.4 ±1.23
Pre-transfer condition factor.	1.06 ±0.007	1.02 ±0.008	1.11 ±0.006	1.07 ±0.007	1.02 ±0.012	1.01 ±0.007	1.04 ±0.009
Pre-transfer challenged serum osmo ($mOsm.kg^{-1}$)	340.39 ±2.17	338.11 ±2.67	359.84 ±4.65	352.71 ±2.76	342.33 ±4.31	332.0 ±1.9	367.54 ±6.92
Precocious maturation (%)	0	0 triploid	0	0 triploid	14	0 triploid	18.9
Transfer date	29/3	29/3	4/5	2/5	14/11	8/11	23/4
Advance in transfer date. (months)	1	1	0	0	5	5	0
Transfer mortality (%).	**31.3^{a}**	**27.5^{b}**	**13.9^{c}**	**27.2^{b}**	**44.9^{d}**	**31.8^{a}**	**14.8^{c}**

Fish from Experiment 5.

Site SW1.

Transfer mortality varied between groups MarS1, MayS1, OctS2, OctS2P and MarS2 transferred to site SW1, only MayS1 and MarS2 exhibiting similar levels of mortality.

OctS2P exhibited the highest transfer mortality at 76.8% (table 4.10) while MarS1 exhibited the lowest mortality. Groups OctS2, OctS2P and JanS2 all exhibited significantly ($P<0.01$) higher transfer mortalities than the control MayS1 group. The mortality in MarS1 was significantly lower than in the MayS1 group.

Table 4.10 Sea water mortality data from fish transferred to site SW1. The data are presented in relation to freshwater results; Transfer mortality refers to mortality during transport and the first month in sea water. Precocious maturation refers to maturation as parr. Pre-transfer mortality is the percentage mortality in the month prior to transfer. Different letters indicate a significant difference ($p<0.05$).

	MarS1	MayS1	OctS2	OctS2P	JanS2	MarS2
Pre-transfer weight (g).	40±0.7	36±0.4	21±0.8	19±0.6	43±0.9	58±1.0
Pre-transfer condition factor.	0.968 ±0.005	0.985 ±0.006	1.119 ±0.015	1.20 ±0.184	1.027 ±0.008	1.133 ±0.007
Pre-transfer challenged serum osmo. (mOsm.kg $^{-1}$)	336.46 ±2.41	320.1 ±2.02	408.92 ±11.31	433.2 ±15.1	339.5± 6.38	349.65 ±6.32
Fin damage (%)	0	1	34	0	0	0
Precocious maturation (%).	0	0	20	35.8	2	5
Pre-transfer mortality (%).	0.7	0.2	34.9	0	6.6	1.3
Transfer date.	8/4	2/6	23/10	23/10	21/1	11/3
Advance in transfer date. (months)	1	0	5	5	2	1
Transfer mortality (%).	**7.4**^{a}	**20.6**^{b}	**57.8**^{c}	**76.8**^{d}	**28.2**^{e}	**18.6**^{b}

Site SW3.

The transfer mortalities of the 3 groups MarS1, MayS1 and OctS2 transferred to site SW3 were all significantly ($P<0.01$) different. The control group MayS1 exhibited the lowest transfer mortality at 5.6%, MarS1 exhibited 12.2% and NovS2 the highest mortality at 32.4% (table 4.11).

Site SW2.

The 4 groups June S1, JulyS1, Jan. S2 and MarS2 transferred to site SW2 all showed significantly different ($P<0.01$) transfer mortalities. The two delayed groups JuneS1 and JulyS1 exhibited the lowest transfer mortalities at 4.41% and 5.56% respectively. The

MarS2 and JanS2 groups were significantly higher at 24.9% and 37.85% respectively (table 4.12).

Table 4.11 Sea water mortality data from fish transferred to site SW3. The data are presented in relation to freshwater results. Transfer mortality refers to mortality during transport and the first month in sea water, precocious maturation refers to maturation as parr, pre-transfer mortality is the percentage mortality in the month prior to transfer. Different letters indicate a significant difference ($p<0.05$).

	MarS1	MayS1	OctS2
Pre-transfer weight (g).	40±0.7	36±0.4	21±0.8
Pre-transfer condition factor.	0.968 ±0.005	0.985 ±0.006	1.119 ±0.015
Pre-transfer challenged serum osmo. (mOsm.kg $^{-1}$)	336.46 ±2.41	320.1 ±2.02	408.92 ±11.31
Fin damage (%)	0	1	34
Precocious maturation (%).	0	0	20
Pre-transfer mortality (%).	0.7	0.2	34.9
Transfer date.	30/3	29/5	26/10
Advance in transfer date. (months)	1	0	5
Transfer mortality (%).	**12.2**^{a}	**5.6**^{b}	**32.4**^{c}

Table 4.12 Sea water mortality data from fish transferred to site SW2. The data are presented in relation to freshwater results. Transfer mortality refers to mortality during transport and the first month in sea water, precocious maturation refers to maturation as parr, pre-transfer mortality is the percentage mortality in the month prior to transfer. Different letters indicate a significant difference ($p<0.05$).

	JuneS1	JulyS1	JanS2	MarS2
Pre-transfer weight (g).	37±0.6	34.7±0.6	43±0.9	58±1.0
Pre-transfer condition factor.	0.9645 ±0.007	0.9823 ±0.005	1.027 ±0.008	1.133 ±0.007
Pre-transfer challenged serum osmo. (mOsm.kg $^{-1}$)	351.54 ±8.54	336.0 ±2.55	339.5 ±6.38	349.65 ±6.32
Fin damage (%)	0	0	0	0
Precocious maturation (%).	0	0	2	5
Pre-transfer mortality (%).	1.94	4.07	6.6	1.3
Transfer date.	19/6	24/7	25/1	11/3
Advance in transfer date. (months)	-1	-2	2	1
Transfer mortality (%).	**4.41**^{a}	**5.65**^{b}	**37.85**^{c}	**24.9**^{d}

Correlation of transfer mortality with the freshwater parameters.

All the data from experiments 4 and 5 displayed in tables 4.9-4.12 were assessed for any correlation between transfer mortality and the following freshwater parameters: pre-transfer weight, pre-transfer condition factor, pre-transfer serum osmolality of challenged fish, fin damage at transfer, percentage precocious maturation in the year of smoltification, pre-transfer mortality and advance in the timing of the transfer.

There was a significant correlation between transfer mortality and percentage precocious maturation, pre-transfer mortality and the number of months the transfer date was advanced compared to the transfer date of smolts reared under a natural photoperiod.

Table 4.13 Pearson correlation coefficients and the significance of the correlation between transfer mortality and the freshwater parameters displayed in tables 4.9-4.12. Where a group was transferred to 2 seawater sites, an average of the two transfer mortalities was used in the correlation.

Freshwater parameter.	Pearson correlation coefficient (r).	Significance of correlation. ($p<0.05$)
Weight.	-0.301	Not significant.
Condition factor.	0.455	Not significant.
Serum osmolality.	0.328	Not significant.
Fin damage.	0.577	Not significant.
Precocious maturation.	0.754	**Significantly correlated.**
Pre-transfer mortality.	0.805	**Significantly correlated.**
Advance in transfer.	0.857	**Significantly correlated.**

Summary of Results from Experiments 4 and 5.

1) Out-of-season smolts, produced with photoperiod, exhibited similar seawater growth rates as smolts produced under a natural photoperiod.

2) The different transfer dates of out-of-season smolts and the similar seawater growth patterns resulted in different harvest periods.

3) A longer growing period prior to maturation resulted in a higher percentage of the fish maturing.

4) There was a significant correlation between the time, from transfer to maturation, and the percentage of fish maturing for sea sites SW1 and SW2.

5) There was a significantly higher mortality in out-of-season smolts compared to natural smolts.

6) There was a significant correlation between transfer mortality and the parameters: time of transfer, mortality prior to transfer and percentage of precocious parr.

4.3 Experiment 6. The seawater growth, maturation and survival of diploid, out-of-season Atlantic salmon smolts produced using artificial photoperiods and ambient temperature conditions.

This experiment has been reported in the same format as experiments 4 and 5.

4.3.1 Materials and Methods and Freshwater Results.

The experiment was carried out at site FW1 (section 2.2.2.1) using different year classes of the same stock. The two year classes formed different groups.

4.3.1.1 Sampling Procedures and Data Analysis.

Condition factor (section 2.3.3.1) was determined and seawater challenge tests (section 2.3.3.2) carried out at intervals of 2 weeks. Sampling in each group was initiated before the start of the 'spring' increase in the photoperiod and continued until the fish were transferred to a seawater holding facility. The appearance of the fish and the maturity (presence of milt when pressure was applied to the abdomen) were also assessed during sampling. The serum osmolality of the challenged fish, the condition factor and the appearance of the fish were assessed to establish when smoltification was considered complete.

Weight, condition factor data and serum osmolality were compared using DUNN'S multiple comparison test and the t-test (section 2.4.2). Pair wise comparisons of condition factor, serum osmolalities and weight data were made using the Mann-Whitney test (section 2.4.1). Confidence limits for the proportions of seawater mortality were calculated and compared (section 2.4.3).

4.3.1.2 Photoperiods, holding conditions and freshwater results for experiment 6.

Potential S2 fish from one year class formed a group named PS2 and potential S1 fish from the following year class formed a group named PS1. A group of natural smolts was also monitored for seawater growth and survival.

Group PS2.

The eggs, alevins and fry were reared in heated water (8.4±0.2°C) until the 23rd April. The eggs and alevins were held in darkness, until first feeding. The fry were subjected to LD 23:1 from first feeding to the 23rd April. The hatchery's main stock was transferred to a natural photoperiod and water at ambient temperature, on the 23rd April. The largest fish from the top 50% of the hatchery's stock were divided into 2 groups. One of these groups consisted of 50000 parr. This group of parr was placed onto an artificial photoperiod (group PL, experiment 1) on the 4th June. The artificial photoperiod began with a decrease from natural daylength to LD 14:10; this was continued at 4 hours . $week^{-1}$ to LD 6:18. The LD 6:18 was held for 2 months before beginning an increase of 1 hour . $week^{-1}$ to LD 18:6 (figure 4.46). The fish were stocked from 4-20 $kg.m^{-3}$ while in fresh water. The fish were transferred from 2m (2-4m^3) to 5m (25m^3) tanks on the 5th Nov. During September the fish were size graded; the potential S1 parr were maintained as group PL (experiment 1) and 44000 potential S2 parr were maintained as group PS2. The group PS2 fish were held under LD 18:6 from the 19th Oct. to the 22nd Feb. During the 7 months following the 22nd Feb. the fish were subjected to a 7 month compressed photocycle (figure 4.46). The fish were held in 5m (25 m^3) tanks. From the 1st Nov. to the 5th Nov. the fish were vaccinated against furunculosis and any parr removed. The parr removed accounted for 0.7% of the population. Sampling to determine condition factor and serum osmolality of challenged fish was initiated on the 26th June.

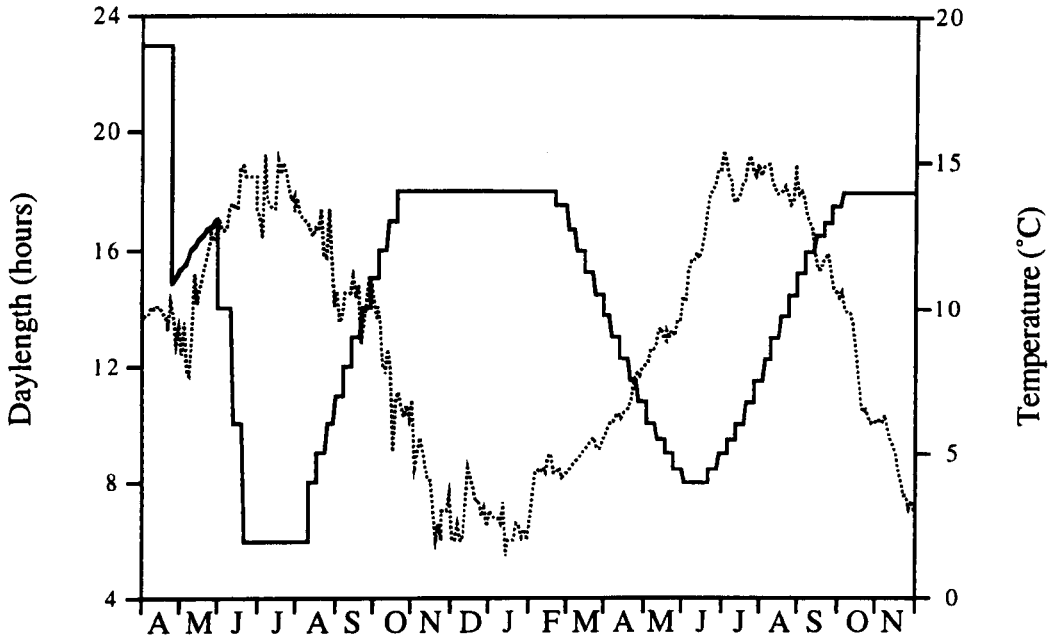


Figure 4.46 The temperature (dotted line) and photoperiod regime (continuous line) experienced by the fish transferred into sea water during November (group PS2).

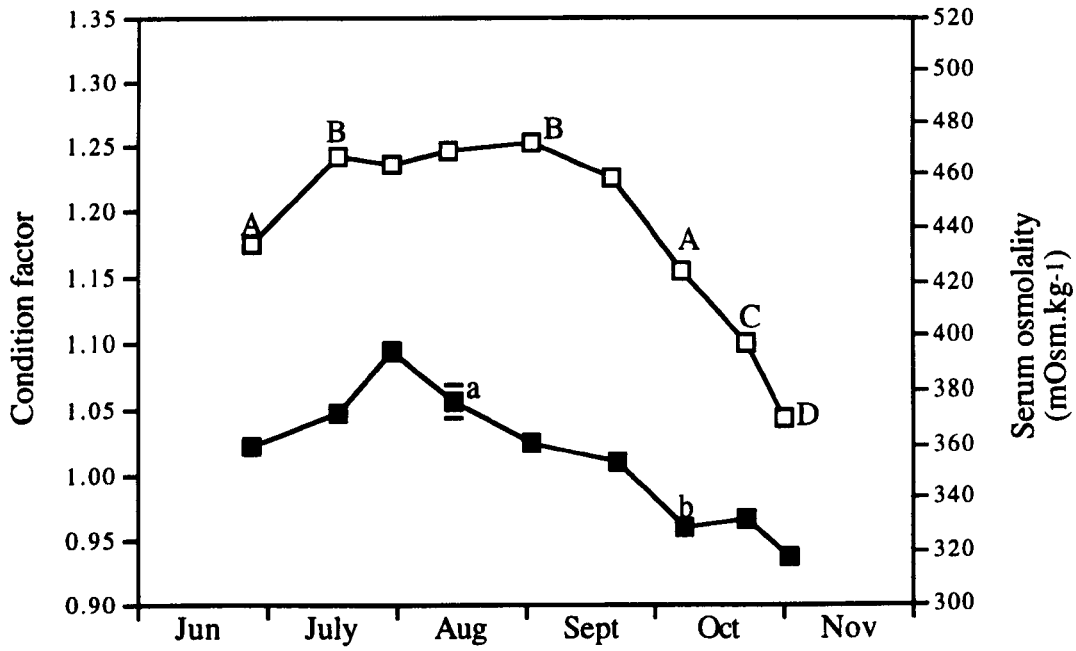


Figure 4.47 Changes in condition factor (open square) and challenged (24hr at 28‰) serum osmolality (filled square) for the fish transferred into sea water during November (group PS2). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

The condition factor increased significantly ($p<0.01$) from the 26th June to the 16th July (figure 4.47). During July, August and September the condition factor remained level between 1.22±0.009 and 1.25±0.006 before decreasing significantly ($p<0.01$) over the consecutive sampling dates 6th Oct., 21st Oct. and the 31st Oct. Prior to seawater transfer the condition factor was 1.04±0.006. The serum osmolality of the challenged fish peaked at 394±3.02 $mOsm.kg^{-1}$ on the 29th July (figure 4.47). From this peak there was a significant decrease ($p<0.01$) to 353.1±4.32 $mOsm.kg^{-1}$ on the 21st Sept. The decrease continued resulting in a serum osmolality of 317.7±2.4 $mOsm.kg^{-1}$ on the 1st Nov., 14 days prior to seawater transfer.

27000 smolts were transferred to site SW4 (section 2.2.2.4) on the 15th Nov. Mortalities and monthly farm sample weights were recorded. The fish were maintained in the 18.5m ($1000m^3$) tank until the 2nd July. From the 2nd to the 4th July the fish were divided into two 18.5m ($1000m^3$) tanks where the fish were maintained until harvested. The fish were harvested from the 21st Nov. through to the 31st Dec. 1 year after transfer to sea water. During the harvest the number of mature and immature fish were recorded.

Group PS1

The eggs, alevins and fry were reared in heated water 10.6±0.3°C until the 20th May. The eggs and alevins were held in darkness until first feeding and the fry were subjected to LL from first feeding to the 20th May. On the 20th May a sample of 60000 (group PS1) of the largest 50% (mean weight 3g) of the hatchery's stock were transferred to 5m ($25m^3$) tanks which were supplied with water at ambient temperature and illuminated by natural daylight. The group PS1 fish were maintained under the NL photoperiod from the 25th May to the 9th Aug. (figure 4.48). On the 9th Aug. the photoperiod was stepped down to LD 8:16, which was held until the 4th Oct. when the photoperiod was stepped up to LD 18:6. The LD 18:6 was held until the fish were transferred to sea water. The water temperature was ambient. The fish were held in 5m ($25\ m^3$) tanks.

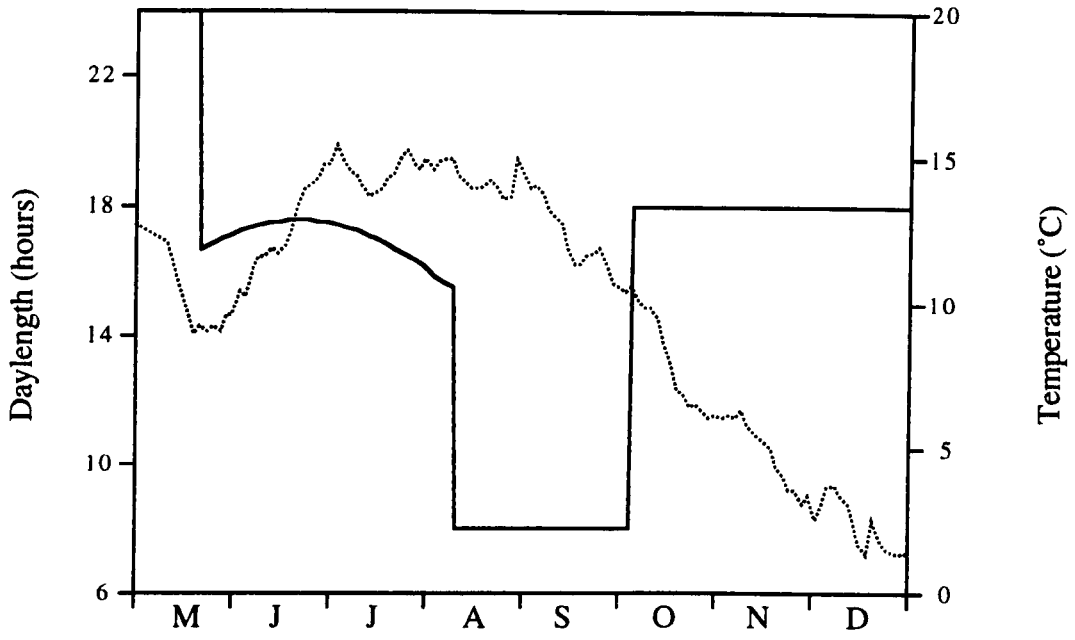


Figure 4.48 Temperature (dotted line) and photoperiod regime (continuous line) experienced by the fish transferred into sea water during December (group PS1).

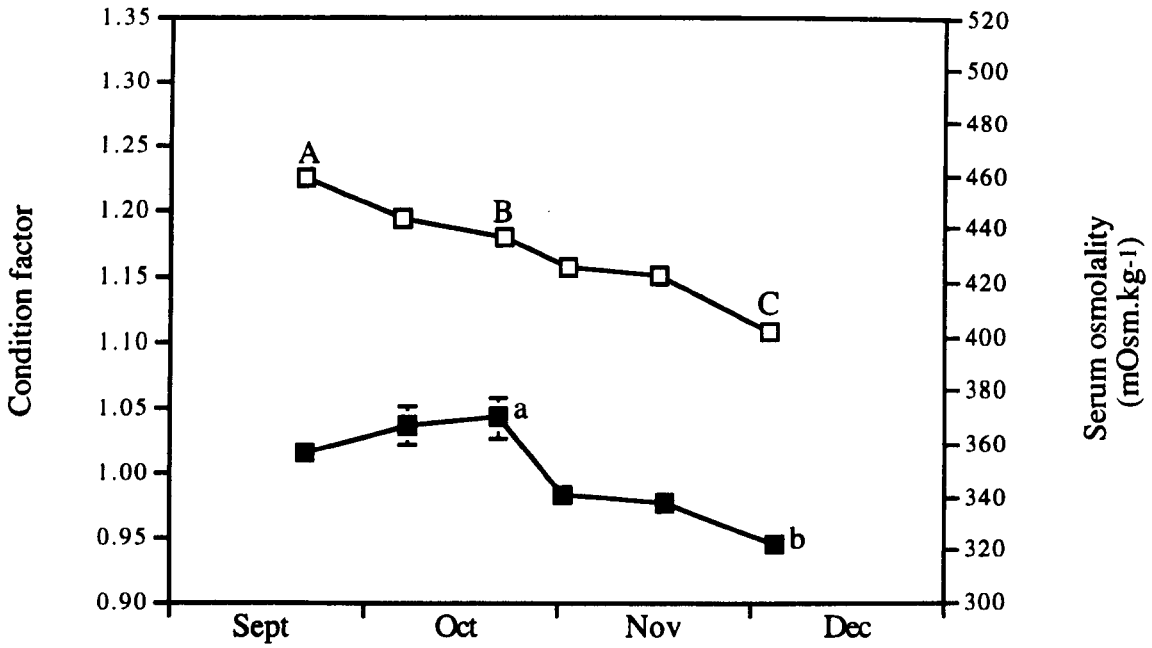


Figure 4.49 Changes in condition factor (open square) and challenged (24hr at 28‰) serum osmolality (filled square) for the fish transferred into sea water during December (group PS1). Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

From the 25th Oct. to the 29th Oct. the fish were vaccinated against furunculosis and any parr were removed. The 945 parr removed accounted for 3.3% of the population. Sampling to determine the condition factor and serum osmolality of challenged fish was initiated on the 21st Sept.

The condition factor decreased throughout the sampling period (figure 4.49) with a significant decrease from the 16th Nov. to the 12th Dec. resulting in a condition factor of 1.10±0.006 prior to transfer. The serum osmolality of the challenged fish decreased significantly through November to 322.55±2.63 $mOsm.kg^{-1}$ on the 4th Dec., the sample date prior to seawater transfer (figure 4.49).

54000 fish were transferred to site SW4 (section 2.2.2.4) on the 21st Dec. Mortalities and monthly farm sample weights were recorded. The fish were maintained in 18.5m ($1000m^3$) tanks during the growing cycle. From the 21st June to the 24th June 40000 fish were transferred from the original tank into a second 18.5m ($1000m^3$) tank. On the 19th October 22000 fish were transferred from the second tank into a third 18.5m ($1000m^3$) tank. The fish in the original and the second tank were harvested from the 13th March through to the 17th April and 6541 fish from the third tank were harvested from 27th March to the 30th March over one year after transfer to sea water. During the harvest mature and immature fish were recorded. The mature fish harvested prior to the 30th March were recorded as maturing during the first maturation episode. The remainder of the third tank was harvested from the 15th May to the 7th Sept. The mature fish recorded in the harvests during August and September were recorded as maturing during the second maturation episode.

Natural smolts.

A group of natural smolts (S1) from the same year class as group PS1 was also transferred to sea site SW4. The growth and mortality of this natural smolt group was monitored.

4.3.2 Seawater Results.

4.3.2.1 Growth.

The 3 groups grew steadily over the entire growing cycle (figure 4.50). However the growth patterns were different. During the first six months the fish in group PS2 grew to 350g and exhibited SGR's in the range 0.85-1.01 %.day^{-1} (table 4.14, figure 4.51,a and b.). The fish in group PS1 grew to 190g and exhibited SGR's in the range 0.47-0.79 %.day^{-1} (table 4.14, figure 4.51,a and b.). The natural smolts grew to 480g during the first 6 months exhibiting SGR's in the range 0.74-2.33 %.day^{-1} (table 4.14, figure 4.51,a and b.). During the period 8-11 months after transfer, groups PS1 and PS2 exhibited higher growth rates than the natural smolts (figure 4.51b.). In each case the periods of high growth in the 3 groups coincided with the summer/autumn (figure 4.51a). The growth over the entire growing cycle was similar in the 3 groups. The SGR of the 3 groups over the first 12 months, ranging from 0.9 to 0.99 %.day^{-1}.

Groups PS2 was harvested after 12 months in sea water, the average harvest weight ranging from 2748-2850g (table 4.14). Group PS1 was harvested during the period from

Table 4.14. Sea water growth data for the potential S2 fish transferred to sea site SW4 on the 15th Nov. (group PS2), the potential S1 fish transferred to sea site SW4 on the 21st Dec. (group PS1) and the natural smolts transferred to sea site SW4 on 20th Apr.

	Group PS2	Group PS1	Natural
Transfer weight (g).	109±3.1	59.8±1.4	50.9
Transfer date.	15th Nov.	21st Dec.	20th Apr.
Weight (g) after 6 months sea water growth.	350	190	480
Specific growth rate during 6 month period. (%.day $^{-1}$)	0.70	0.61	1.38
Specific growth rate during 12 month period. (%.day $^{-1}$)	0.90	0.93	0.99
Harvest Date.	21/11-31/12	27/3-7/9	-
Months from transfer date to harvest date.	12-13	15-21	-
Mean harvest weights (g) superior class salmon.	2889	2331-4149	-
Mean harvest weights (g) mature fish	1623	1281-4152	-

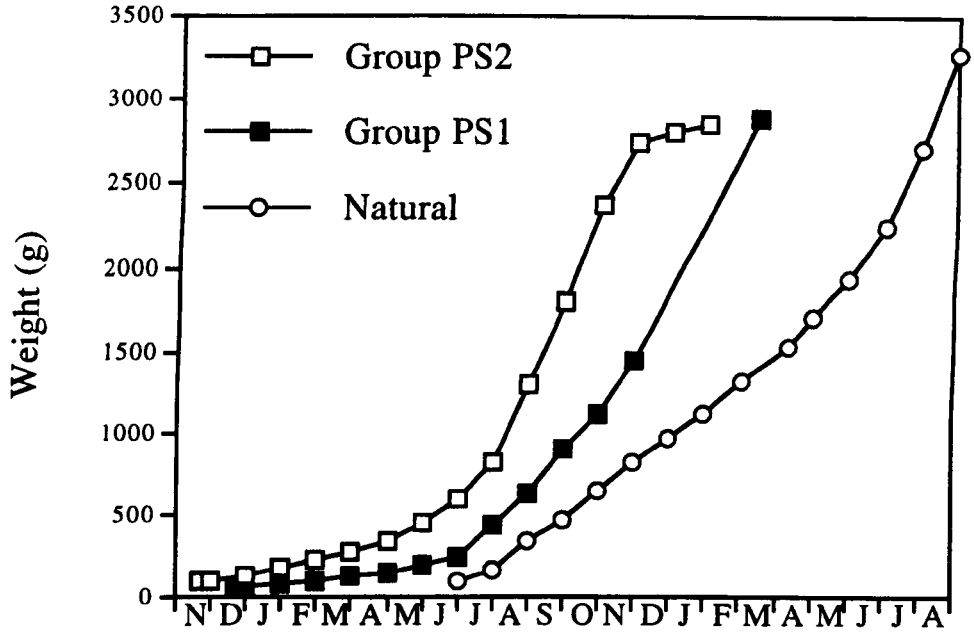


Figure 4.50 Seawater growth for the three groups in experiment 6: open square represents fish transferred to sea during November (group PS2), filled square represents fish transferred during December (group PS1) and open circle represents fish transferred April (natural smolts).

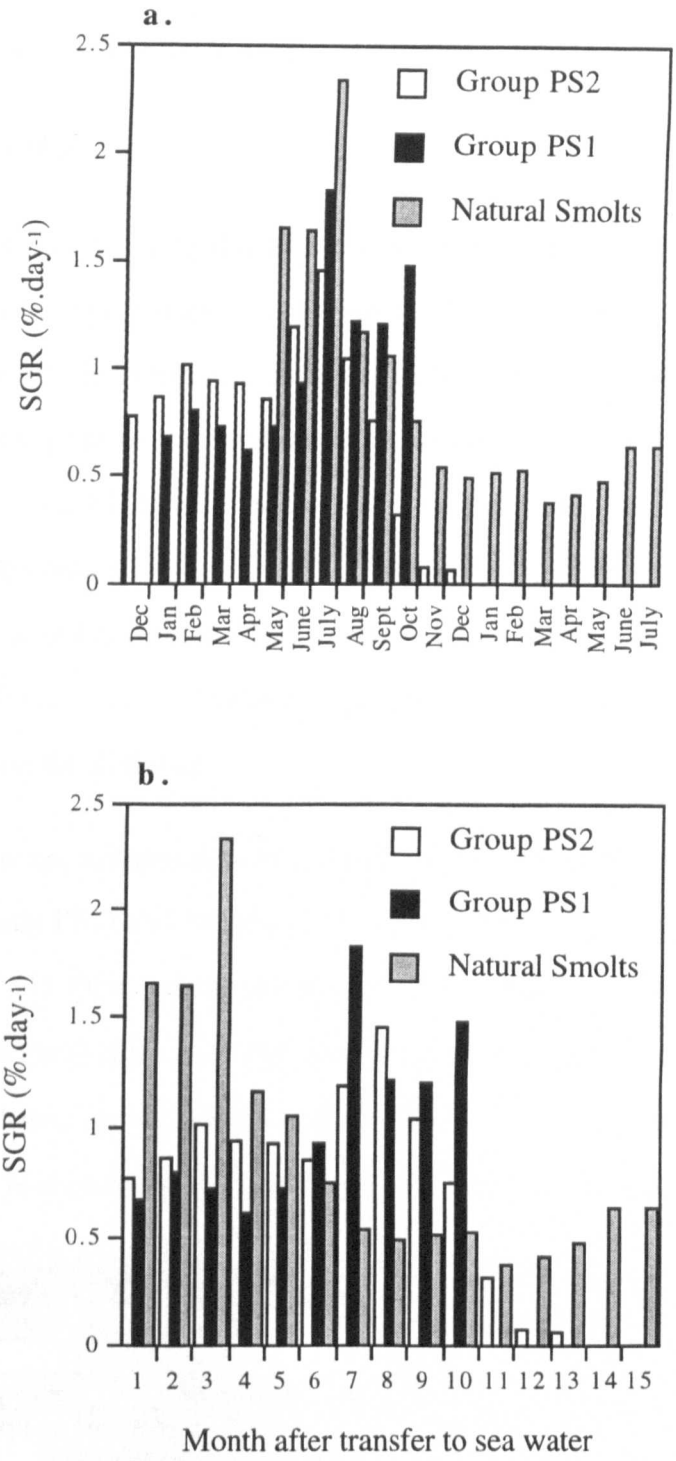


Figure 4.51. Specific growth rate for the time periods between sampling plotted against the actual month (a.) and the number of the month after transfer (b.): open bars represent group PS2 transferred to sea on the 15th Nov. (group 1+), filled bars represent group PS1 transferred to sea on the 21st Dec. (group 0+) and speckled bars represent natural smolts transferred to sea on the 20th April.

15 months to 20 months in sea water. The average harvest weight during this period ranged from 2331g to 4149g (table 4.14).

4.3.2.2 Maturation.

The proportion of fish maturing during the first maturation episode was significantly different between the two groups. There was 0.4% maturation in the group PS1 fish while 6.5% of the fish in group PS2 matured (table 4.15). The PS2 fish had a longer period in sea water prior to the final stages of maturation (30th November) and the immature fish weighed 2873g compared to the 1444g in group PS1. The fish in group PS2 were harvested before any assessment of the second maturation episode could be made. The fish remaining in group PS1 exhibited 1.1% maturation during the second maturation episode (table 4.15). The immature and mature fish had respective weights of 4149g and 4152g on the 21st Aug.

Table 4.15. Percentage maturation in the potential S2 fish transferred to sea site SW4 on the 15th Nov. (group PS2) and the potential S1 fish transferred to sea site SW4 on the 21st Dec. (group PS1). First maturation episode refers to maturation during the first year in sea water or post smolt maturation and second maturation episode refers to maturation during the second year in sea water or grilse maturation. First maturation episode weights are from November and second maturation episode weights and maturation percentage are from the 21st Aug. to 7th Sept.

	PS2	PS1
Pre-transfer weight (g).	109±3.1	59.8±1.4
Transfer date.	15/11	21/12
Episode 1 maturation.	**6.5 %**	**0.4 %**
Days in sea water, transfer-final maturation.	380	344
Immature weight (g)	2873	1444
Mature weight (g)	1623	-
Episode 2 maturation.	**Harvested**	**1.1 %**
Immature weight (g)	-	4149
Mature weight (g)	-	4152

4.3.2.3 Mortality.

The transfer mortality between the three groups was significantly different ($P<0.01$). However the percentage mortality was below 1% in both of the PS2 and PS1 groups (table 4.16). The natural smolts exhibited a transfer mortality of 5.3%, which was significantly greater than the photoperiod groups.

Table 4.16. Sea water mortality and fresh water data for the three groups transferred to sea site SW4. The potential S2 fish were transferred on the 15th Nov. (group PS2) and the potential S1 fish were transferred on the 21st Dec. (group PS1). The natural smolts were transferred on the 20th April.

	PS2	PS1	Natural
Pre-transfer weight (g).	109±3.1	59.8±1.4	50.9
Pre-transfer condition factor.	1.0906 ±0.0073	1.1078 ±0.006	-
Pre-transfer challenged serum osmo. ($mOsm.kg^{-1}$)	317.7 ±2.4	322.55 ±2.63	-
Fin damage (%)	0	0	-
Precocious maturation (%).	removed	removed	-
Pre-transfer mortality (%).	0.05	0.04	-
Transfer date.	15/11	21/12	20/4
Advance in transfer date. (months)	5	4	0
Transfer mortality (%).	**0.07**	**0.28**	**5.3**

Summary of Results from Experiment 6.

1) Out-of-season smolts, produced with photoperiod, exhibited similar seawater growth rates as smolts produced under a natural photoperiod.

2) The different transfer dates of out-of-season smolts and the similar seawater growth patterns resulted in different harvest periods.

3) A longer growing period prior to maturation resulted in a higher percentage of the fish maturing.

4) The transfer mortality in the out-of-season smolts was below 1%; this was significantly lower than the transfer mortality observed in natural smolts.

4.4 Discussion.

4.4.1 Seawater Growth.

The results from experiments 4, 5 and 6, described in this chapter, demonstrate that the seawater growth of out-of-season smolts, produced using photoperiod manipulation, is comparable to that achieved by naturally produced smolts. Out-of-season smolts transferred into sea water during March, June, July, October, November and December exhibited similar growth rates as natural smolts transferred during April and May (experiments 4, 5 and 6). Out-of-season smolts produced in March, October and November were harvested after 16-19 months rearing in sea water (experiments 4 and 5) and smolts produced in November and December were harvested after 12-15 months (Experiment 6). Natural smolts transferred to sea water in May were harvested after 16-18 months (Experiment 4 and 5). The similar growth over the production cycle, in combination with the different transfer dates, resulted in the groups of fish exhibiting different sample weights on all sample dates during the seawater growing period (experiments 4, 5 and 6). Where statistics could be applied to the data, the sample weights were significantly different ($P<0.01$) at all times (experiment 5). In the 3 experiments the greater the growing period prior to the sample date, the greater the sample weight (experiment 4, 5 and 6). These results clearly show that out-of-season smolts grow at the same rate as naturally-produced smolts, all groups achieving market size over a similar time period. The different transfer dates of out-of-season smolts therefore result in different harvest times, compared to the harvest period associated with naturally-produced smolts (figure 4.52). These observations agree with the observations made by Duston and Saunders (1995).

However, the growth profiles of out-of -season and in-season smolts are different during the growing cycle. In experiment 4 and 5 smolts transferred between October to January exhibited lower growth during the first six months than smolts transferred between May to July. The first six months in sea water for the smolts transferred

during the spring coincided with the warm summer temperatures, which contributed to the observed high growth rates. The out-of-season smolts transferred during the autumn or winter also exhibited high growth rates in the summer. A similar situation was also observed in experiment 6. The highest growth in all 3 groups was observed during the summer/autumn. In groups PS2 and PS1 (transferred during November and December respectively) this was 8 months after transfer while the natural smolts (transferred during May) exhibited highest growth during the first months after transfer. These observations are probably related to both temperature and photoperiod. The warm summer/autumn sea temperatures will have promoted growth with slower growth during the winter. A decrease in photoperiod has been observed to suppress growth (Saunders, et al. 1985; McCormick, et al. 1987). The groups in experiment 4 and 5 transferred during the autumn and winter will has experienced a decrease from the freshwater photoperiod (LD18:6 or LD20:4) to a natural photoperiod. This decrease in photoperiod may have suppressed growth during the first six months in sea water.

It is unclear whether the fish transferred to sea water during October and November in experiment 6 experienced a decrease in photoperiod when transferred. The seawater photoperiod was a dual photoperiod combining a natural photoperiod and night lighting which increased the daily illumination to LD 18:6. It is unclear weather the fish responded to the natural photoperiod or constant LD 18:6 night illumination. It is possible the fish experienced a decrease in photoperiod when transferred to sea water.

The results from the triploid and diploid groups in experiment 4 are contradictory. The diploid and triploid groups transferred during November, displayed similar growth patterns whilst the groups transferred during March exhibited different growth patterns. The growth curves of the groups transferred during March diverged, the diploid group exhibiting a higher growth rate. Triploid salmon exhibited the same growth rate as immature diploid salmon (Benfey & Sutterlin 1984, Johnstone 1992). However, during the spring after the onset of maturation, maturing diploid salmon exhibited

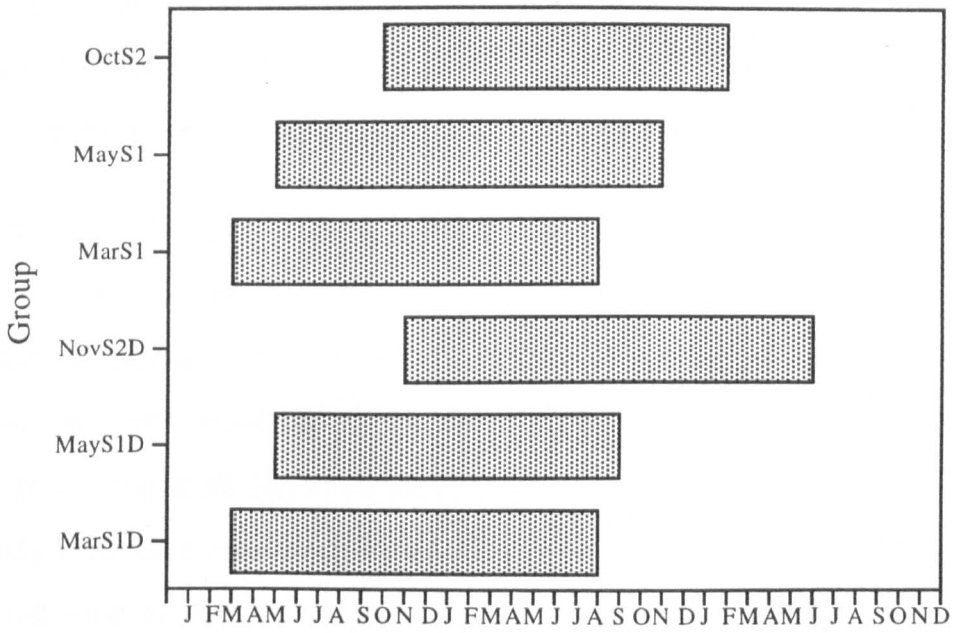


Figure 4.52 Growing period for 6 groups grown through to harvest OctS2, MayS1, MarS1, NovS2D, MayS1D and MarS1D. The bar represents the growing period starting on the month of transfer and finishing on the initial month of harvesting.

higher growth rates than triploid salmon (Johnstone 1992) resulting in larger diploid salmon during the summer. In experiment 4, the diploid group transferred during March exhibited 19% grilse maturation and therefore showed a higher growth rate than the triploid fish. The diploid group transferred during November displayed 0% maturation during the first year and consequently exhibited similar growth rates as the triploid group during the first year of the growing cycle. These results therefore agree with the observations made by Johnstone (1992), that maturing diploid salmon exhibit higher growth rates than triploid salmon and immature diploid salmon.

The precocious parr (group OctS2P), transferred during October, exhibited very poor seawater growth compared to the immature fish which were reared under the same photoperiod (group OctS2) and transferred during October (Experiment 5). Approximately 50% of the precocious parr (group OctS2P) exhibited little or no growth in the sea. After three months growth in sea water the fish showed a significant ($P<0.05$) reciprocal correlation between weight and GSI; fish with a high GSI exhibited little or no growth (experiment 5). Fish, which have not completed smoltification (Saunders *et al.* 1985, McCormick *et al.* 1987) or parr (Duston 1994) exhibit poor growth and stunting when transferred directly to sea water. Duston (1994) suggested that parr employ a different mechanism of hypo-osmoregulation which has a high energetic cost compared to the mechanism employed by smolts and as a consequence this high energetic cost leads to reduced growth and stunted fish. In agreement with the present study Lundqvist *et al.* (1986) found precocious parr had a reduced ability to osmoregulate compared with immature fish and Berglund *et al.* (1991) demonstrated that the ability to osmoregulate was negatively correlated with the GSI. It is possible that the fish with a high GSI in group OctS2P (experiment 5) employed a hypo-osmoregulatory mechanism which had a high energetic cost, resulting in the seawater growth of the fish being stunted. In contrast the fish with a low GSI possibly will have employed a smolt mechanism of osmoregulation which would have enabled the fish to use acquired energy for growth.

The growth in a number of the groups of fish in experiment 5 reduced or ceased during the summer period after the first year of the seawater growing cycle. During this period maturing Atlantic salmon have been observed to cease feeding and mobilise body reserves for gonadal growth (Aksnes *et al.* 1986). In experiment 5 different proportions of fish matured in each group. These maturing fish resulted in the growth rate of the group decreasing. In one group where 96% of the fish were observed to mature, the fish lost weight. It would appear that the reduced growth rates observed in experiment 5 were the result of maturing fish mobilising body reserves for gonadal growth.

4.4.2 Maturation in Sea Water.

The different groups in experiments 4, 5 and 6 showed a large variation in the percentage maturation at a particular age. The same groups, transferred to different seawater sites, showed different percentages of maturation at a particular age (experiment 5) and different groups, transferred to the same site, also showed different percentages of maturation at a particular age (experiment 4, 5 and 6).

The differences in the age at maturity, observed in groups transferred to the same site, were related to the length of time from seawater transfer to final maturation (experiment 4 and 5). There was a positive relationship between percentage maturation at a particular age and the time period from transfer to final maturation (experiment 4 and 5). The variation in time period was the direct result in variation of the transfer date (date of final maturation was fixed), the earlier the transfer date the longer the time period and the higher the percentage of maturation (figure 4.53). An early transfer date also resulted in a longer growing period prior to a particular date and a greater sample weight on a particular date (experiment 4, 5 and 6). Absolute size or a threshold size, which must be exceeded before the initiation of maturation has been suggested as possible determinants of maturation (Bailey *et al.* 1980, Saunders *et al.* 1982, Berglund 1992). However, Thorpe (1986) pointed out that fish are not aware of their size and suggested that fish were physiologically aware of their rate of acquisition of surplus

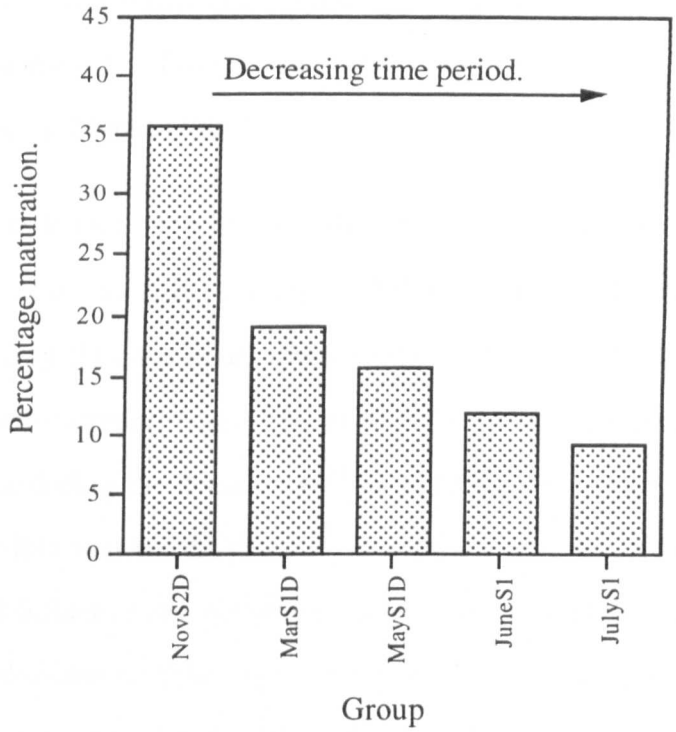


Figure 4.53 Percentage maturation during the second maturation episode (grilse maturation) for 5 groups NoveS2D, MarS1D, MayS1D, JuneS1 and JulyS1 transferred to sea site SW2. The groups are arranged so that the time period, from transfer to final maturation, decreases from left to right.

energy and provided that during the spring, this rate is above a genetically determined threshold, the fish would proceed to mature. When out-of-season smolts are considered, the earlier transfer date and hence longer growing period before final maturation, has enabled a higher percentage of fish to attain the hypothesised physiological threshold. Therefore a higher percentage of fish proceeded with maturation in the groups which had a longer growing period prior to the decision to proceed with maturation. These results and conclusions are in agreement with observations made by Thrush (1994).

In addition to the importance of growth opportunity, one should consider the photoperiod history of the fish. Photoperiodic manipulation has been observed to affect age at maturity (Hansen *et al.* 1992, Krakenes *et al.* 1991, Taranger 1993) of salmon in the marine environment. The photoperiod either affected growth rate or energy acquisition during the decision period or altered the timing of the decision period. It is possible that the freshwater photoperiod and the change to a natural photoperiod could influence age at maturity in sea water. However, the length of the period of natural photoperiod from seawater transfer to maturation would suggest that the endogenous rhythm controlling maturation had become synchronised with the natural photoperiod. However, it can not be discounted that the freshwater photoperiod and the change to a natural photoperiod suppressed maturation in the 1st maturation episode (post-smolt maturation).

Maturation during the 1st maturation episode (post-smolt maturation) is uncommon in smolts reared under natural conditions although it has been observed in association with high maturing stocks (Herbinger & Newkirk 1990) and with specific environmental factors which enhance freshwater development resulting in large smolts (Saunders & Henderson 1965, Sutterlin *et al.* 1978, Solbakken *et al.* 1994). In experiment 5 the environmental conditions of site SW1 and the longer period of growth opportunity resulted in an early age at maturity.

Environmental variation affects biological factors which in turn affect the age at maturity (Chadwick *et al.* 1986). Sites with different environmental conditions result in different age at maturity in fish of the same sibling group (Naevdal 1983) or stock (Thrush 1994, experiment 5). Different environmental conditions associated with different sites can have a pronounced effect on the age at maturity. In experiment 5 during the 2nd maturation episode, the group MayS1 exhibited 96% maturation in fish held at site SW1 but only 23% maturation in fish held at site SW3.

The immature fish (group OctS2) and the precocious parr (group OctS2P), transferred to site SW1 during October, received the same photoperiod. Maturation and aspects of smoltification are probably controlled by endogenous rhythms timed by photoperiod. Smoltification was advanced by 6 months by the artificial photoperiod, whilst the timing of precocious maturation was advanced perhaps 1-2 months by the artificial photoperiod. Smoltification was not observed in the precocious parr (group OctS2P). It has been suggested that smoltification and maturation are conflicting physiological processes (Thorpe 1986, 1989). The results, presented here, would suggest that fish can not mature and smolt at the same time. The maturing fish were presented with photoperiod cues to initiate smoltification. However, the fish continued to mature and showed no indications of smoltification. These observations suggest that either maturation must be completed before smoltification can proceed or that maturation inhibits the mechanism(s) controlling smoltification.

4.4.3 Seawater mortality.

Mortality during and after transfer was variable. The transfer mortalities observed in experiments 4 and 5 all exceeded levels which would be acceptable to the salmon farming industry and an explanation would be expected. Mortalities were higher in the groups in which smoltification was advanced by photoperiodic manipulation. By contrast the groups in experiment 6 were advanced 5 and 4 months and exhibited mortalities below 1% which are levels to which the salmon farming industry would normally aspire.

The transfer mortalities, in experiments 4 and 5, indicated that the out-of-season smolts which were advanced were not prepared for a direct transfer to sea water (figure 4.54). There was a positive significant correlation between transfer mortality and each of the 3 freshwater parameters; advance in transfer date, precocious maturation and pre-transfer mortality.

Plasma cortisol has been observed to increase during smoltification (Patino & Schreck 1986) suggesting that the process increases susceptibility to stress. In this study, the transfer of the fish from freshwater to seawater sites involved long journeys (section 2.33 and 2.32) and the direct transfer of fish from fresh water to sea water. The transport and transfer incurred additional physiological and handling stress. The stress caused by transfer may have a greater effect on survival depending on the conditions encountered during the 1st month in sea water, the health status of the fish and or the ability of the fish to survive in a hypo-osmotic environment.

Many of the transfer mortalities from the smolts transferred during March in experiment 4 (group MarS1D and group MarS1T) were reported to have abrasions to the head. These abrasions were possibly due to fish burrowing into the net in an attempt to escape the change in light intensity from artificial (20 lux) to natural (>30000 lux) illumination. Fish subsequently transferred from the freshwater site FW2 were given additional illumination which increased the light intensity in the holding facility from 20 lux to 600 lux. This rectified the problem of abrasions to the head.

Correlation between transfer mortality and advance in transfer date.

The changing seasons result in a cycle of change in the environmental conditions encountered by the fish during the 1st month after transfer into sea water. These environmental changes are often difficult to quantify. The general trend in mortality, observed in experiment 4 and 5, was high in the winter months October, November and January and low in the summer months May, June and July. This trend might be related to changes in the photoperiod and/or the environment of the holding facility.

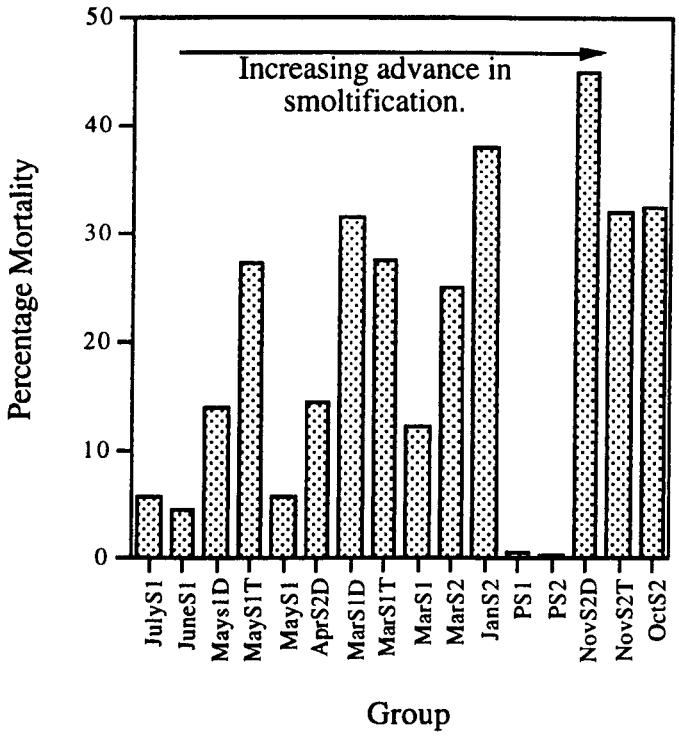


Figure 4.54 Percentage mortality for all the groups (JulyS1, JuneS1, MayS1D, MayS1T, MayS1, AprS2D, MarS1D, MarS1T, MarS1, MarS2, JanS2, NovS2D, NovS2T and OctS2) in experiments 4 and 5 and the two groups (PS1 and PS2) in experiment 6. The groups have been arranged so that the period of time that smoltification was advanced, in relation to naturally produced smolts, increases from left to right

During the transfer to sea water, the fish experience a change in photoperiod from a long daylength in fresh water to a natural daylength in sea water. During the winter months the fish experienced a decrease in photoperiod whilst in the summer months the change in photoperiod was negligible. Although the decrease in photoperiod appeared not to cause any mortality, the decrease may have caused additional stress.

The seawater cages used at sites SW2 and SW3 are open to changes in the weather and storms can have serious effects on fish held in exposed cages. The winter period can produce very stormy conditions which result in a harsh environment for the transfer of out-of-season smolts. Group JanS2 in experiment 5 was transferred in January soon after the MV Braer sank. This month experienced particularly stormy conditions. The transfer mortality for group JanS2 was 37.85%.

These observations possibly explain the correlation between transfer mortality and advance in transfer date.

Correlation between transfer mortality and precocious maturation.

The positive correlation between precocious parr and transfer mortality would suggest that precocious parr do not transfer successfully to a marine environment. At transfer to sea water, the precocious parr (group OctS2P) in experiment 5 had not completed smoltification and if pressure was applied to the abdomen, milt was produced. These precocious parr (OctS2P) exhibited a transfer mortality of 76.8%. Mature parr have poor survival if transferred to sea water (Lundqvist *et al.* 1989, Duston & Knox 1992, Bjerknes *et al.* 1992, experiment 5) possibly due to high blood levels of gonadal steroids impairing osmoregulatory ability (Lundqvist *et al.* 1989). Precocious parr were not a problem in groups transferred during the spring and summer. Precocious parr have been shown to complete smoltification in the spring following maturation (Saunders *et al.* 1982, Berglund *et al.* 1992a, 1992b, Saunders *et al.* 1994). These observations possibly explain the correlation between transfer mortality and precocious maturation.

Correlation between transfer mortality and pre-transfer mortality.

The positive correlation between pre-transfer mortality (percentage mortality in the month prior to transfer) and transfer mortality would suggest that, as one would expect, smolts in a poor state of health prior to transfer do not transfer successfully. The group transferred during October in experiment 5 (group OctS2) exhibited a pre-transfer mortality of 34.9% due to a bacterial infection and recorded a transfer mortality of 57.8% at site SW1 and 32.4% at site SW3. As stated above, the transfer of smolts from fresh water to sea water is a stressful transition for the fish, any fish in a poor state of health are unlikely to survive. Site FW2 appeared to have an underlying gill problem (section 2.22) possibly related to water quality.

There was no correlation between serum osmolality of challenged fish or condition factor and transfer mortality. This would suggest that the hypoosmoregulatory ability or the ability to survive in a hypo-osmotic environment was not a factor related to the transfer mortality. The smolt parameters which were followed for each group suggested that smoltification was complete when the groups were transferred. A number of groups exhibited a degree of dissociation between condition factor and serum osmolality of challenged fish. In all cases the decrease in serum osmolality was observed in advance of the decrease in condition factor. A short winter period or the direct increase from a short to long photoperiod has been observed to dissociate condition factor and hypoosmoregulatory ability (Bjornsson *et al.* 1989, chapter 3). However, dissociation appeared unrelated to transfer mortality. The fish transferred during March in experiment 4 (group MarS1D), exhibited dissociation of condition and hypoosmoregulatory ability and a transfer mortality of 31.3% whereas the fish transferred during March in experiment 5 (group MarS1), had a similar dissociation of the 2 parameters but a transfer mortality of 5.6%. Furthermore fish transferred during November in experiment 4 (group NovS2D), exhibited no dissociation of smolt parameters but a transfer mortality of 44.9%.

It would appear that transfer mortality was not related to the smolt status of the fish. The fish in all groups, which survived, grew well in the sea suggesting that these fish were smolts. Poorly adapted smolts (Saunders *et al.* 1985, McCormick *et al.* 1987) or parr (Duston & Knox 1992, Bjerknes *et al.* 1992, Duston 1994) generally exhibit high mortality and stunting or poor growth in sea water. It is probable that the mortalities observed in experiment 4 and 5 were not the result of incomplete smoltification, but a combination of the health problems related to site FW2, the presence of precocious parr and the sea condition in the month after transfer. This conclusion is supported by the sea water performance of out-of-season smolts produced at site FW1 in experiment 6. Smoltification, in these fish, was advanced 5 and 4 months and the fish exhibited transfer mortalities below 1%. It should also be remembered that high mortalities in the commercial rearing of salmon are not uncommon. Canadian cage production has reported 25% mortality during the first 4 months in sea water (Saunders *et al.* 1985) and in Scottish production, the seawater production of salmon has recorded mortalities (including all causes of mortality) over the entire growing cycle which range from 10.2% in 1993 to 42.1% in 1989 (The Scottish Office Agriculture, Environment and Fisheries Department 1995).

The results show that photoperiod can be used to produce out-of-season smolts. These out-of-season smolts have the same growth potential as smolts reared under a natural photoperiod. The growth advantage gained from early transfer was maintained throughout the growing cycle and resulted in a different harvest period. Increased maturation was observed in association with a longer growing period prior to maturation. This resulted in large mature fish which could be harvested at market size (2-4 kg) prior to maturation associated deterioration of flesh quality. Increased mortality was observed in association with certain groups of out-of-season smolts (experiments 4 and 5) and although production is possible with low mortalities (experiment 6), this aspect is of concern. The producer must determine whether these possible draw backs are out-weighed by the benefits. The production of out-of-season smolts could increase the production of both smolt producers and producers of market

sized salmon. The stocking of out-of-season smolts could enable the salmon farmer to target production and if managed properly could help stabilise market prices.

Chapter 4 Conclusions.

1) Photoperiod was used to manipulate the timing of smoltification with smolts being produced in January, March, April, May, June, July, October, November and December.

2) Out-of-season smolts have the same growth pattern and rates as natural smolts.

3) The age at maturity in out-of-season smolts was positively related to the length of the seawater growing period prior to the completion of maturation.

4) Out-of-season smolts only exhibit increased maturation in association with an increased size (compared to natural smolts) for a particular time of year. These large mature fish can be harvested as superior salmon prior to flesh deterioration.

Chapter 5. Maturation and photoperiodic control in Atlantic salmon retained in fresh water.

5.1 Introduction

Maturation follows a seasonal cycle for many temperate species. The Atlantic salmon is no exception with maturational development occuring during the summer and autumn. This development takes approximately 8-10 months from the time of the decision to proceed with maturation to the completion of maturation in late autumn. It is important that the maturation process is timed to provide optimum conditions for growth and survival of the offspring which are subsequently produced. A reliable seasonal indicator often used to time biological processes is the photoperiod. Photoperiod has been identified as a probable environmental cue in the timing of the maturational process in a number of temperate fish species, including the rainbow trout a species in which maturation has been extensively studied (Bromage *et al.* 1992).

Photoperiod has been observed to affect the age at maturity in rainbow trout and Atlantic salmon. It was shown that the earlier the increase to a long daylength was applied between October and March the lower the percentage of fish which matured (Duston & Bromage 1988, Hansen *et al.* 1992, Randall 1992, Taranger 1993, Campbell 1995). It would appear that photoperiod controls the timing of the decision period (Thorpe 1986, see section 4.1) or a gating mechanism (Duston & Bromage 1988); the fish must attain a threshold stage of development before they are capable of responding to the photoperiodic stimuli and as a consequence proceeding with maturation.

There is an extensive body of information concerning the control of maturation and photoperiod in the rainbow trout. Maturation has been advanced and delayed using accelerated and decelerated photoperiod cycles (Whitehead *et al.* 1978). Abrupt changes between short and long daylengths have been shown to alter the timing of maturation.

Abrupt changes from a short daylength to a long daylength close to the winter solstice and through to the summer solstice advanced maturation (Bromage *et al.* 1982, 1984, Duston and Bromage 1986, 1987, 1988) and changes to a long daylength after the summer solstice delayed maturation (Bourlier & Billard 1984, Campbell 1995). While abrupt changes from a long daylength to a short daylength close to the summer solstice through to the winter solstice advanced maturation (Whitehead & Bromage 1980) and changes to a short daylength after the winter solstice delayed maturation (Bromage *et al.* 1984, Duston & Bromage 1986, 1987). Further studies on the effect of photoperiod on maturation have shown, over a 5 year period of constant LD 6:18, that maturation cycles with a periodicity of approximately 1 year (Duston & Bromage 1986, 1991) and phase response curves have been generated using abrupt decreases in daylength (Duston & Bromage 1988) and 2 month periods of continuous light (Randall 1992). From these observations Duston and Bromage (1988) and Randall (1992) concluded that maturation in the rainbow trout is controlled by an endogenous circannual rhythm timed by photoperiod. This rhythm appears to time all aspects of maturation in the rainbow trout. This includes the timing of the decision to proceed with maturation, the timing of the completion of maturation and probably the progression of the different developmental stages during the maturational process. It should be noted that the involvement of photoperiod in the timing of the decision to proceed with maturation suggests that photoperiod and the endogenous rhythm play an important role in determining the age at maturity.

Considerably fewer studies have been carried out on photoperiod and maturation in the Atlantic salmon. In the following paragraph the studies on Atlantic salmon are compared with studies on rainbow trout which used similar photoperiod regimes.

Atlantic salmon kelts subjected to two 6 month accelerated photoperiods did not spawn until returned to a natural photoperiod (Johnston *et al.* 1990, 1992). The kelts spawned in June, exhibiting an advance of 5 months. A similar 6 month accelerated photoperiod regime advanced spawning, after 1 cycle, by 3-4 months in rainbow trout (Bromage *et*

al. 1982, Elliott *et al.* 1984). Taranger (1993) examined the response of Atlantic salmon to both abrupt changes in daylength and accelerated / decelerated photoperiods. Maturation was advanced 1 month under a 9 month accelerated photocycle and delayed 2 months under an 18 month decelerated photocycle. In the rainbow trout a 9 month accelerated cycle advanced spawning by 6 weeks (Bromage *et al.* 1982) and an 18 month decelerated photoperiod delayed spawning by 3 months (Bromage *et al.* 1984). Atlantic salmon appeared not to respond to the accelerated photocycles of 6 months. A maximum of a 1 month advance/delay was achieved with accelerated/decelerated photocycles in the Atlantic salmon. This compares to a maximum advance of 4 months and delay of 3 months in the rainbow trout subjected to similar accelerated/decelerated photocycles. Taranger (1993) also observed that an abrupt increase to a long daylength in March and an abrupt decrease to LD 8:16 in July advanced ovulation by 1 month and an abrupt increase from a natural photoperiod to LL in July delayed ovulation by 1 month. Hansen *et al.* . (1992) demonstrated that a long daylength applied from October through to July, followed by a natural photoperiod advanced the timing of ovulation by 19 days. Abrupt increases to a long daylength advanced spawning in the rainbow trout by 6 weeks (increase on the 19th Feb.) (Bromage *et al.* 1984) and 8 weeks (increase on 19th Jan.) (Duston & Bromage 1988), whilst an LD 16:8 from February to the 21st June when the photoperiod was decreased to LD 8:16 advanced spawning by 12-14 weeks (Whitehead & Bromage 1980, Bromage *et al.* 1982). Using artificial photoperiods which incorporated abrupt changes in daylength resulted in advances ranging from 6-14 weeks in rainbow trout compared to a maximum of 4 weeks in the Atlantic salmon.

These studies would suggest that similar mechanisms control maturation in the Atlantic salmon. However, artificial photoperiods do not appear to manipulate the timing of spawning in the Atlantic salmon by the same magnitude as in the rainbow trout. Atlantic salmon do not respond to similar photoperiods with a similar advance / delay as has been observed in rainbow trout. There are a number of factors which could account for these differences such as the different natural spawning times of the fish species or the effects of salinity or temperature.

The effect of temperature on reproduction has not been fully investigated but, it has been observed to affect both the rate of development of aspects of maturation and the timing of spawning. A temperature dependence of the up-take of vitellogenin into rainbow trout ovarian follicles (*in vitro*) has been reported (Tyler *et al.* 1987) suggesting that temperature regulates the rate of development of aspects of maturation. Delays of spawning in trout held at low temperatures (Morrison & Smith 1986, Nakari *et al.* 1987) and the advances of spawning in fish held at high temperatures (Titarev 1975) have also been reported. A delay or inhibition of spawning in salmon held at temperatures greater than 13°C has also been described (Taranger & Hansen 1993). Similar results were also observed with rainbow trout held at ambient temperatures compared with constant 9±1°C water (Campbell 1995). These elevated temperatures also resulted in reduced egg quality (Taranger & Hansen 1993, Campbell 1995) and atresia (Campbell 1995). Although temperature can influence the timing of spawning, Campbell (1995) suggested that the effect of temperature on the timing of reproduction in rainbow trout was marginal; temperature was not a controlling mechanism. It would appear that over a specific temperature range, which is different at different stages of maturation, the maturational process continues normally and is temperature compensated to enable the timing of maturation by photoperiod to continue, despite changes in the rate of development. However, exposure to temperatures outwith this 'normal' temperature range affects development, reducing egg quality and in extreme cases maturation is inhibited and eggs retained. Such a 'normal' temperature range would vary between species. The photoperiodic manipulation of maturation, which results in the various stages of the maturation process occurring at unfavourable temperatures, could explain the different photoperiod responses described for rainbow trout and Atlantic salmon.

The effect of salinity on maturation in the Atlantic salmon has also received little attention, but is a third environmental variable which may be important in the timing of maturation in the Atlantic salmon. A range of life history strategies have been observed in the Atlantic salmon. Generally the males mature as either precocious parr or after 1 sea winter, whilst the females mature after 1 or 2 sea winters. However, there are examples

of female anadromous salmon which mature as parr (Bagliniere & Maisse 1985, Hindar & Nordland 1989). It has been suggested, that this was due to high growth rates resulting from low population densities and/or a productive freshwater environment (Thorpe 1986, 1994).

Freshwater maturation in anadromous females is uncommon but, there are numerous populations of landlocked salmon which do not leave the freshwater environment. These landlocked populations are often characterised by a younger and smaller size of fish at maturity (Sutterlin & MacLean 1984) and have been referred to as dwarf salmon. These differences in maturation strategies have been attributed to genetic differences between anadromous and non-anadromous populations (Birt *et al.* 1991a, 1991b). There are examples of landlocked populations which mature at a similar size and age as anadromous populations (Graynoth 1995, Trepanier *et al.* 1996).

Juvenile landlocked salmon have been reported to exhibit both full smoltification (Birt *et al.* 1991a) and reduced hypoosmoregulatory ability compared to anadromous salmon (Burton & Idler 1984, Birt & Green 1993). It would appear that anadromous populations have additional adaptations which are absent in some populations of landlocked salmon and that these adaptations allow acclimation to sea water. Studies of anadromous smolts retained in fresh water have reported increased condition factor (Johnston 1983, Komourdjian *et al.* 1976) and loss of sea water tolerance or decline in Na^+ - K^+ ATPase activity (Johnston 1983, Boeuf *et al.* 1985, Virtanen & Soivio 1985, Duston *et al.* 1991). Temperature (Duston *et al.* 1991) and photoperiod (Kurokawa 1990) have both been implicated as mediators in the rate of loss of certain smolt characteristics.

After the loss of smolt characteristics the life history pattern followed by anadromous salmon retained in fresh water has not been studied. Experiment 7 examines both smoltification and maturation of salmon retained in fresh water. The experiment was carried out using constant 10°C borehole water to avoid the complications of maturation and temperature (Taranger 1993). The fish were divided into two groups, one group

being manipulated using photoperiod. The management of photoperiod manipulation, temperature and the isolation of a water source from disease are relatively easy to achieve in fresh water compared to sea water.

5.2 Experiment 7 Atlantic salmon (*Salmo salar*) smolts retained in fresh water as broodstock and the photoperiodic production of out-of-season eggs.

5.2.1 Materials and Methods.

Experiment 7 was carried out at site FW 2. A 7 m^3 raceway of 10000 (23g) potential S1 smolts was selected on the 4th Jan. The raceway was supplied with constant 10±1°C borehole water. The fish were subjected to a photoperiod (Figure 5.1) which increased 0.5 hour.$week^{-1}$ from LD 8:16 on the 14th Jan. to LD 18:6 on the 20th May. The fish were retained under the LD 18:6 daylength until the 4th Nov. On the 1st June 7000 fish were transferred from the raceway to sea site SW2. On the 4th Nov. the daylength to which the remaining 3000 fish were subjected was decreased by 2 hour . $week^{-1}$ to LD 10:14 on the 2nd Dec. On the 9th Dec. the daylength was decreased from LD 10:14 to LD 8.5:15.5.

On the 14th Jan. the fish were graded and 400 small fish (mean 274g) which included mature male fish were removed from the group. The remaining fish were divided into 2 groups. Group NLb (1400 fish) was transferred into a pond, whose only illumination was natural daylight. Group Pb (750 fish) was retained in the raceway.

On the 14th Jan. the daylength to which group Pb was exposed was increased from LD 8.5:15.5 to LD 18:6 (Figure 5.1) which was maintained until the 21st May when the daylength was decreased to LD 8:16. The LD 8:16 was maintained until the 13th July when a reciprocal 12 month seasonal photocycle was initiated, weekly changes of 0.5 hours were used to apply the reciprocal artificial photoperiod.

On the 8th June during the 3rd year of the experiment, 100 maturing fish were randomly selected from group NLb and placed in a separate raceway (group Jb). Group Jb fish were maintained under LD 8:16 from 8th June until the completion of maturation. As a result of a farm management error the groups Jb and NLb were combined on the 26th

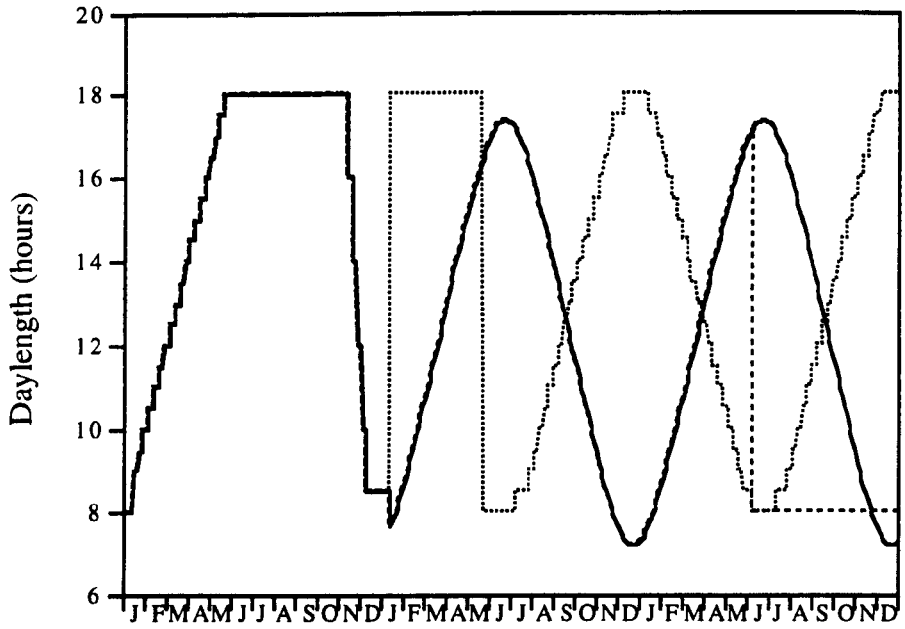


Figure 5.1 Photoperiod regimes applied to group NLb (solid line), group Jb (dashed line) and group Pb (dotted line).

October. Prior to the groups being combined, 47 fish had ovulated in group Jb and 1 fish had ovulated in group NLb, no fish were marked for identification prior to the error. For all statistical comparisons the data for group Jb and group NLb were combined and treated as one group.

The 2 groups were sampled for weight and length at bi-monthly intervals during the first year and at monthly intervals during the second and third years. The fish were seawater challenged at bi-monthly intervals during the first year. During spawning periods each fish was examined at two weekly intervals. The production of milt when pressure was applied to the abdomen was recorded and fish which had ovulated were stripped. The eggs obtained were fertilised and incubated in hatching trays, egg data and survival to first feeding were recorded. Mature female fish were killed prior to stripping the eggs from the abdomen. Any surviving mature males and unovulated mature females were removed from the experimental groups when the spawning period had finished.

The eggs obtained from group NLb during the second year of the experiment were incubated through to hatch. The fry and parr were held in $1.5m^3$ and $7m^3$ raceways under a LD 20:4 until the 26th Nov., when a 2 hour . $week^{-1}$ decrease was initiated. On the 24th Nov., the fish were graded into 2 groups, potential S1 and S2 fish, 21000 potential S1 fish were transferred from an LD 12:12 photoperiod into a pond, the only illumination the pond received was natural daylight (figure 5.2). The fish were sampled for weight, length and serum osmolality of challenged fish at bi-monthly intervals. Sampling was initiated on the 7th January, one year after the fish hatched and continued until the fish were transferred to sea water on the 26th May. Furunculosis (*Aeromonas salmonicida*) was isolated on the 19th April and a 13 day antibiotic treatment was initiated. The furunculosis infection resulted in a 2.6% mortality in the month prior to transfer. The fish were transferred to sea site SW3 on the 26th May.

Condition factor and serum osmolality data were compared over the sampling period using DUNN'S multiple comparison test (section 2.62). Pair wise comparisons of the

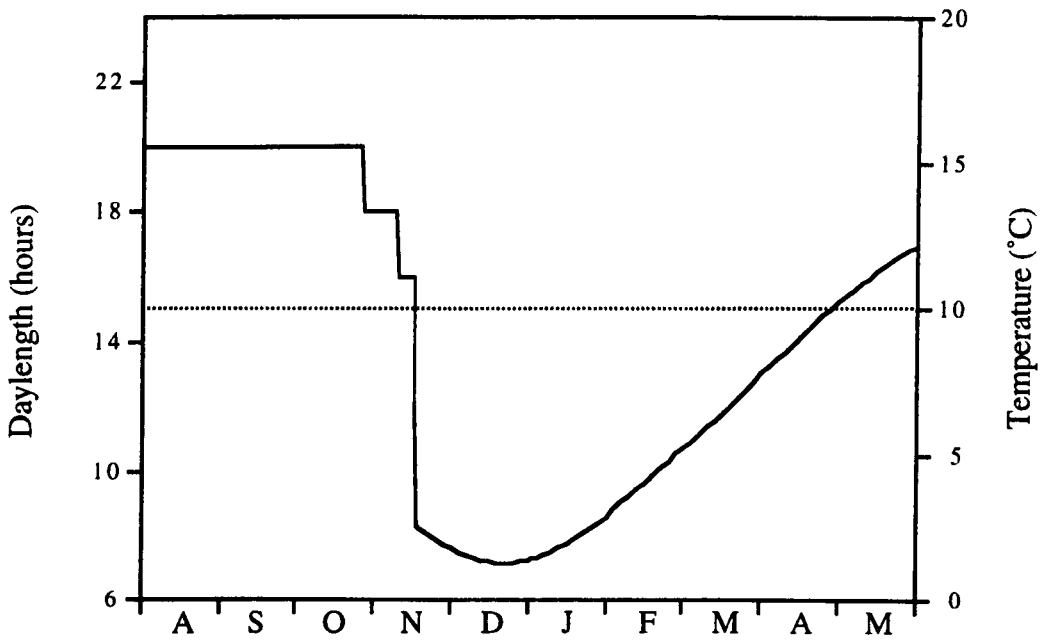


Figure 5.2 Photoperiod (solid line) and temperature (dotted line) regime experienced by the smolts produced from the eggs obtained from group NLb during the second year of the experiment.

spawning profiles, weight data, egg size and fecundity were made using the Mann-Whitney test (section 2.61). Confidence limits for the proportions of egg, alevin and first feeding mortalities were calculated and compared (section 2.63).

5.2.2 Results

5.2.2.1 Smoltification.

During the spring period of the first year condition factor and serum osmolality of challenged fish (figure 5.3) decreased. Condition factor remained at a low level during July and then gradually increased during the Autumn. Serum osmolality of the challenged fish reached a low point on the 7th June and then increased to values ranging between 351-378 $mOsm.kg^{-1}$.

The condition factor decreased significantly ($P<0.01$) from the 1.22±0.005 on 11th April to 1.16±0.006 on the 7th May, from 1.20±0.006 on the 22nd April to 1.11±0.005 on the 23rd May and from 1.16±0.006 on the 7th May to 1.08±0.007 on the 6th June (figure 5.3). The condition factor increased significantly ($P<0.01$) from 1.03±0.008 on the 30th June to 1.12±0.02 on the 26th Aug. and from 1.1±0.01 on 24th Sept. to 1.21±0.02 on the 21st Oct.

The serum osmolality of the challenged fish decreased significantly ($P<0.01$) from 401.7±8.9 $mOsm.kg^{-1}$ on the 29th March to 340.8±2.8 $mOsm.kg^{-1}$ on the 25th May (figure 5.3). Serum osmolalities of the challenged fish recorded from the 25th May to the 18th June were significantly different from all values recorded prior to the 29th March. After the 7th June the serum osmolality increased to values between 351.8±5.1 $mOsm.kg^{-1}$ and 378.9±6.3 $mOsm.kg^{-1}$ from the 1st July to the 9th Jan. However, only the serum osmolality recorded on the 9th Jan. (378.8±3.4 $mOsm.kg^{-1}$) was significantly greater ($P<0.01$) than the low point on the 7th June (335.3±2.2 $mOsm.kg^{-1}$).

The changes in serum osmolality and condition factor suggested that smoltification was complete during late May / early June. The 7000 fish transferred to sea site SW2

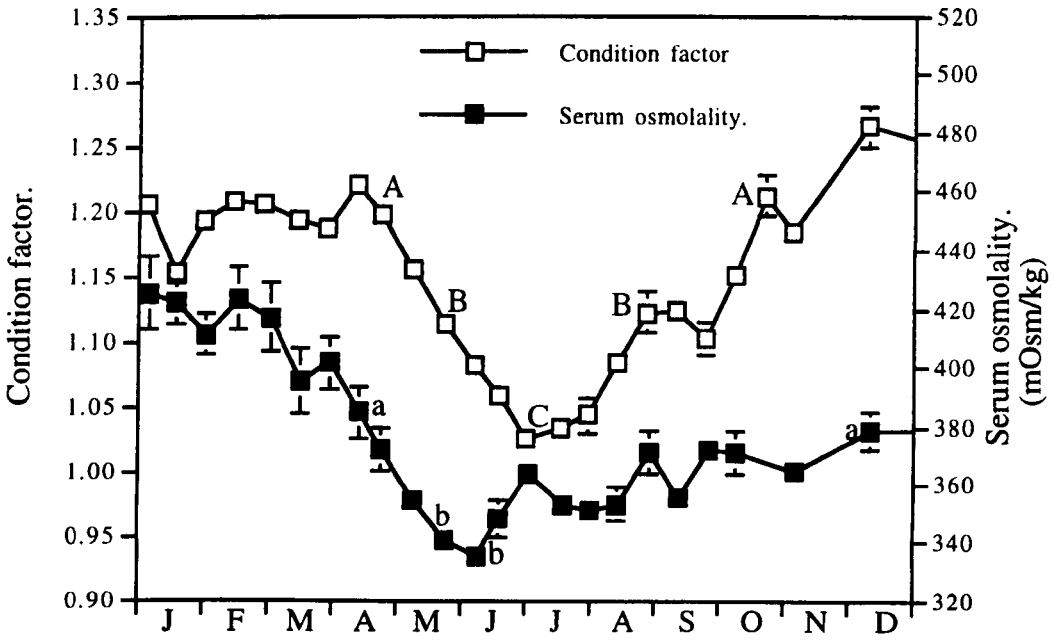


Figure 5.3 Changes in condition factor (open square) and serum osmolality of challenged fish (filled square) during the first year of the experiment. Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

exhibited a transfer mortality of 3.3% and grew to 500g during the first 6 months; this represented an SGR of 1.4 %.day^{-1}. There was no post-smolt maturation.

The majority of the mortality observed during the first year period was during the period from July to September. The cumulative mortality increased from less than 1% in May to over 10 % in September (Figure 5.4). The cumulative mortality for the entire year was 11.7%. The increased mortality in the period from July to September was the result of a fungal infection. Twice weekly malachite green baths were administered during this period. During September the fish responded to the treatments and fungal infection decreased.

5.2.2.2 Maturation and Spawning.

During the first year of the experiment 22% of the population matured. All the mature fish were males. The mature males were removed from the experimental group during the grade on the 14th January.

During the 2nd year of the experiment 71.8% of the fish in group NLb and 76.7% of the fish in group Pb matured (Table 5.1 and Figure 5.6). The percentages of mature females and males were similar (Table 5.1 and Figure 5.6). A number of the mature female fish did not ovulate, 39.6% in group NLb and 82.9% in group Pb.

Table 5.1 Percentage of fish and sex ratios in each group which matured or remained immature during the second and third years of the experiment. The percentages of the mature fish which were males and females in each group and the percentage of the females which ovulated or remained unovulated a the end of the spawning period. All fish were maiden spawners.

	NLb (2nd) (n=1549)	Pb (2nd) (n=693)	NLb/Jb (3rd) (n=254)	Pb (3rd) (n=134)
% Immature	28.2	23.4	22.8	14.9
% Mature	71.8	76.7	77.2	85.0
Mature fish % males	44.4	53.8	29.1	11.4
Mature fish % females	55.6	46.1	70.9	88.6
Females % ovulated	60.4	17.1	95.7	82.2
Females % unovulated	39.6	82.9	4.3	17.8

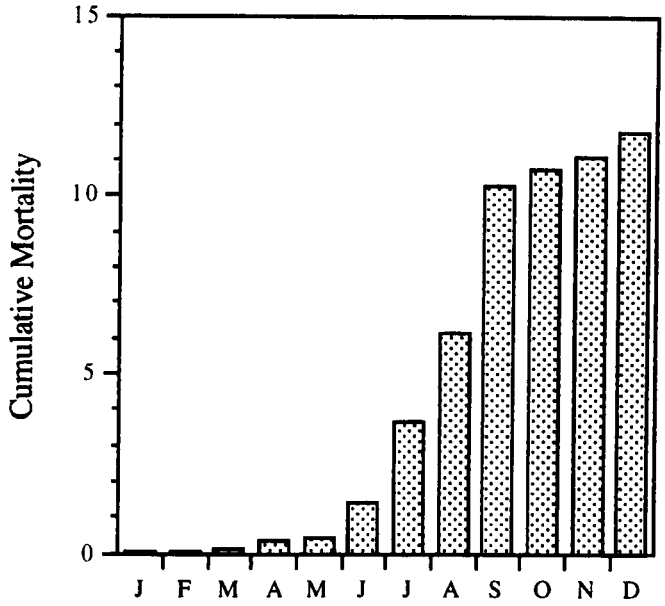


Figure 5.4 Percentage cumulative mortality during the first year of the experiment.

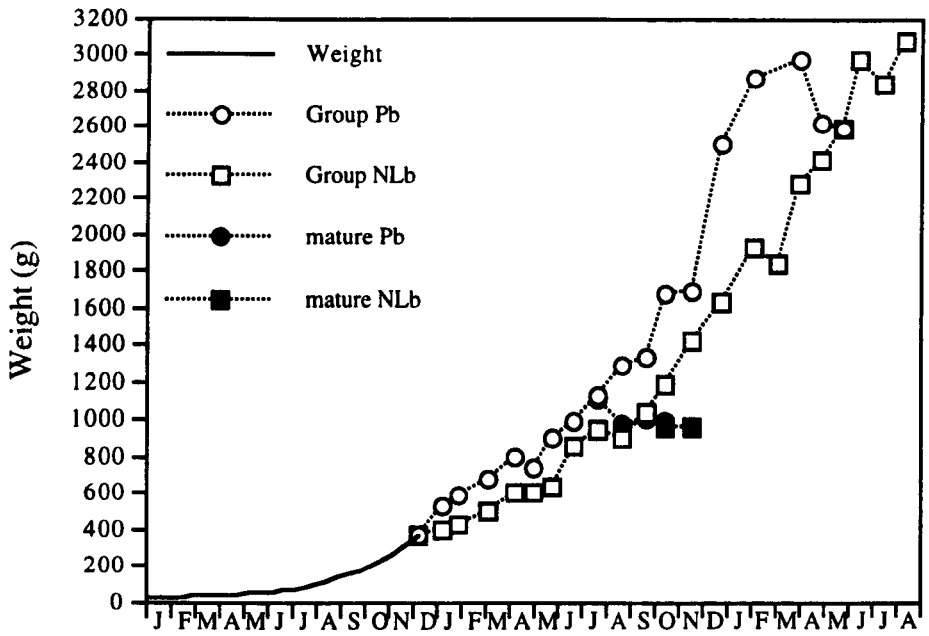


Figure 5.5 Changes in weight against time over the entire experimental period. The continuous line represents the fish prior to division into groups. Following separation group Pb (artificial photoperiod) and group NLb (natural photoperiod) are represented by circular and square symbols respectively; open symbols represent immature fish and filled symbols represent mature fish.

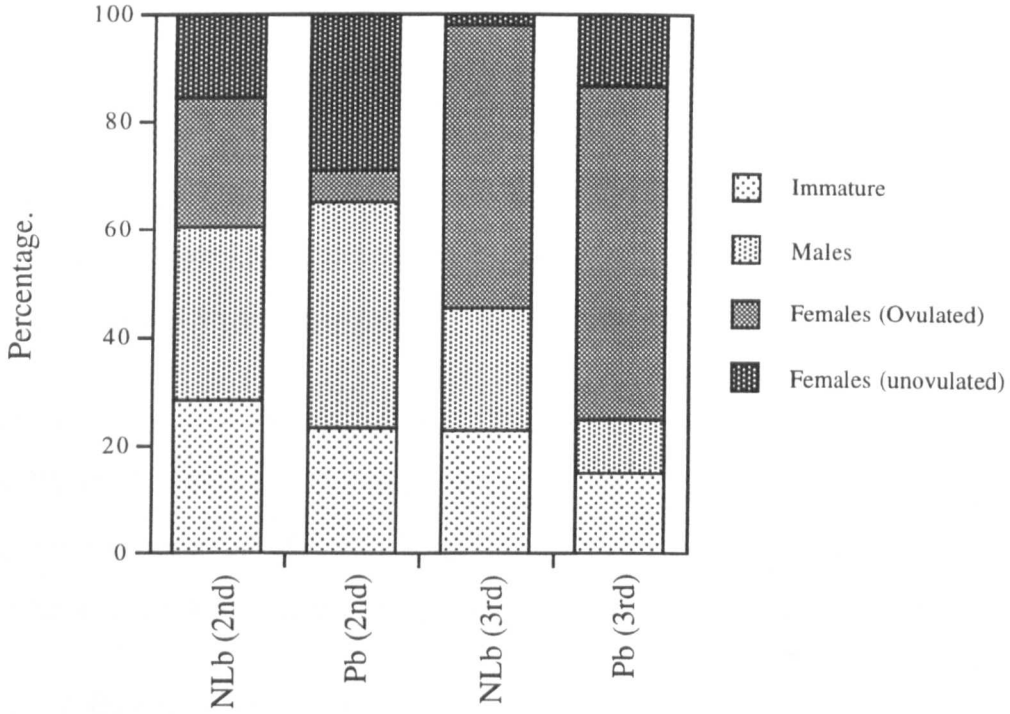


Figure 5.6 Proportions of immature fish, mature male fish, mature female fish which ovulated and mature female fish which did not ovulate presented for the two groups; group Pb which was subjected to an artificial photoperiod and group NLb(/Jb) which was subjected to a natural photoperiod for the 2nd and the 3rd year of the experiment.

During the 3rd year of the experiment 77.2% of the fish in group NLb/Jb matured and 85% of the fish in group Pb matured (Table 5.1 and Figure 5.6). Both groups of mature fish contained predominately females, 70.9% in group NLb/Jb and 88.6% in group Pb. A number of females in each group did not ovulate, 4.3% in group NLb/Jb and 17.8% in group Pb.

Males.

During the 2nd year of the experiment, milt flow was first observed in group Pb on the 21st of July, 12 weeks in advance of the date (12th Oct.) when milt flow was first observed in group NLb (Table 5.2).

During the 3rd year of the experiment, milt flow was first observed on the 13th April in group Pb, on the 14th Sept. in group Jb and on the 13th Oct. in group NLb. Milt flow in group Pb was observed 22 weeks in advance of group Jb and 26 weeks in advance of group NLb. Milt flow in group Jb was observed 4 weeks in advance of group NLb.

Table 5.2 Earliest date in each group when milt flow was observed following the application of pressure to the abdomen of the mature male fish.

Group	Date milt observed.
Pb (2nd)	21st July
NLb (2nd)	12th Oct.
Pb (3rd)	13th April
Jb (3rd)	14th Sept.
NLb (3rd)	13th Oct.

Females.

During both years a significant ($P<0.01$) advance was observed in the timing of spawning (Figure 5.7 and 5.8). During the 2nd year of the experiment the median of the spawning profile in group Pb was advanced 12 weeks earlier than the median in group NLb (figure 5.7).

During the 3rd year the median of the spawning period for group Pb was 23 weeks in advance of the median spawning period of the combined NLb and Jb groups (Figure

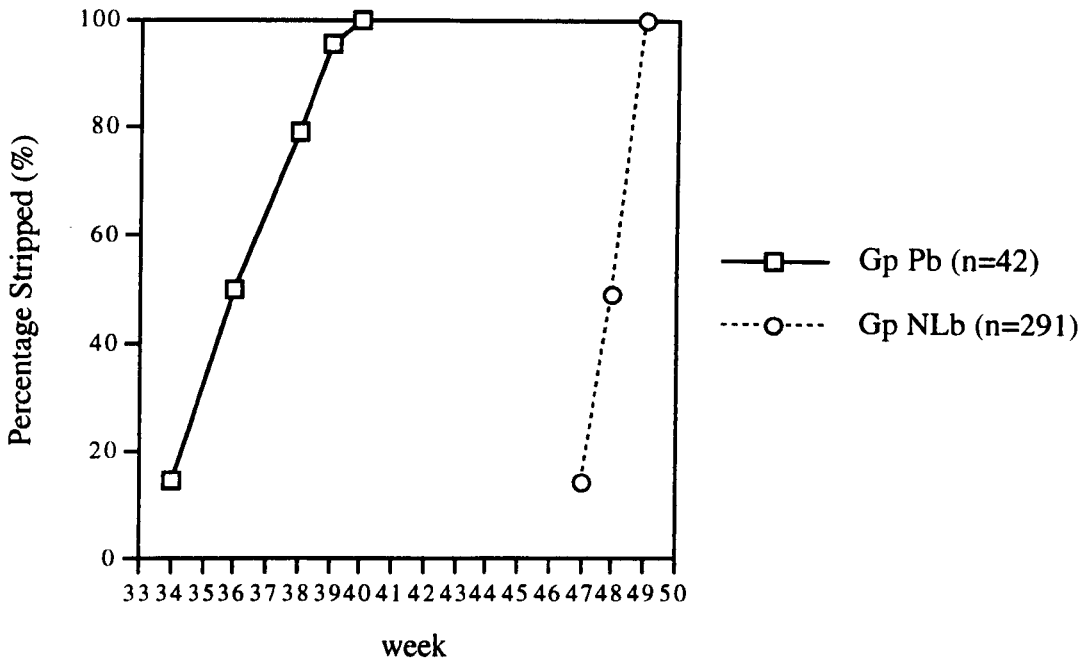


Figure 5.7 Cumulative percentage of fish stripped each week for the 2 groups, group Pb (square symbol) which was subjected to an artificial photoperiod and group NLb (circular symbol) which was subjected to a natural photoperiod. Week 1 represents the first week of the year.

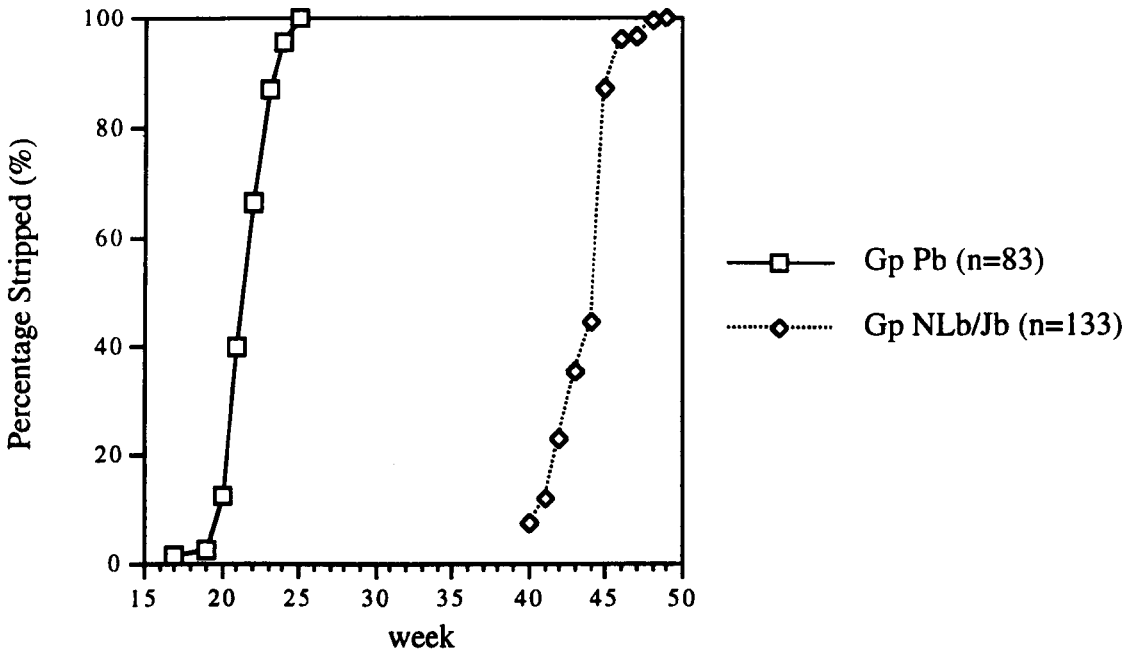


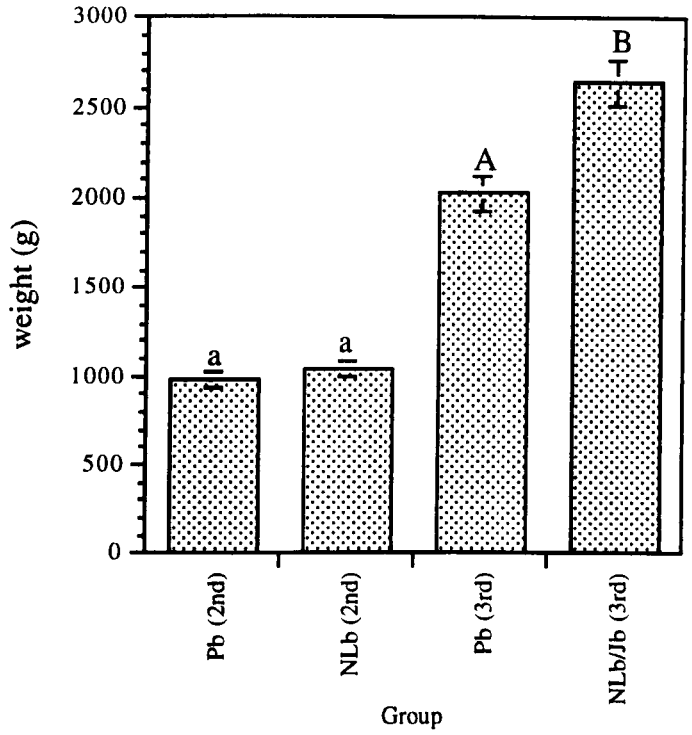
Figure 5.8 Cumulative percentage of fish stripped each week for group Pb (square symbol) which was subjected to an artificial photoperiod and the combined group NLb/Jb (diamond symbol) which was subjected to a natural photoperiod. Week 1 represents the first week of the year.

5.8). 10 fish in group Jb were stripped on the 8th Oct.; this was 2 weeks in advance of the 1 fish from group NLb which was stripped on the 25th Oct. The two groups were combined on the 25th Oct. A total of 47 fish were stripped from group Jb and 1 fish from group NLb prior to the groups being combined.

5.2.2.3 Egg size, total and relative fecundity and egg quality.

During the 2nd year of the experiment, egg data were collected from 20 fish in group Pb and 55 fish in group NLb. The mean pre-strip weight of the fish in the 2 groups was not significantly different. The fish in group Pb had a mean weight of 949±41g being slightly smaller than the fish in group NLb which had a mean weight of 1039±150g (Figure 5.9). The mean egg diameter of the eggs from group NLb was 5.4±0.02 mm. The eggs from group NLb were significantly ($P<0.05$) larger than the eggs produced by group Pb which were 4.7±0.04 mm (Figure 5.10). The mean total fecundity of the group NLb fish was 2052±59 eggs.fish^{-1} which was significantly ($P<0.05$) lower than the mean total fecundity of the fish in group Pb which was 2594±171 eggs.fish^{-1} (Figure 5.11). There was no significant ($P<0.05$) difference in the mean relative fecundity between the groups of fish: the mean relative fecundity from group NLb was 3239±90 eggs.kg^{-1} whilst the mean for group Pb was 3623±278 eggs.kg^{-1} (Figure 5.12).

During the 3rd year of the experiment, egg data were collected from 46 fish in group Pb and 75 fish from the combined groups NLb and Jb. The fish in the group NLb/Jb were significantly larger than the fish in group Pb (Figure 5.9). The pre-strip mean weights were 2259±71g for group NLb/Jb and 2024±70g for group Pb. The eggs from group NLb/Jb were significantly ($P<0.05$) larger than the eggs obtained from group Pb. The mean egg diameter for group NLb/Jb was 5.95±0.03 mm and 5.49±0.04 mm for group Pb (Figure 5.10). There was no significant difference in the mean total fecundities of the 2 groups; the mean total fecundity for group NLb/Jb was 4096±129 eggs.fish^{-1} and for group Pb was 3973±153 eggs.fish^{-1} (Figure 5.11). There was a significant ($P<0.01$)



Group 5.9 Mean pre-strip weight (±sem) for the groups Pb and NLb during the 2nd year of the experiment and for group Pb and the combined group NLb/Jb during the 3rd year of the experiment. Group Pb was subjected to an artificial photoperiod and group NLb was subjected to a natural photoperiod. Different letters indicate significant differences ($p<0.05$) between goups, lower case refers to second year and upper case refers to the third year of the experiment.

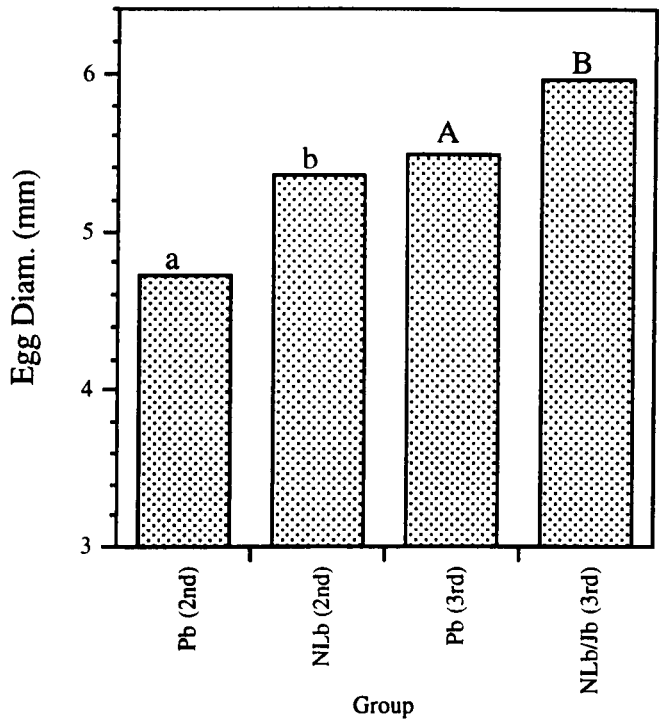
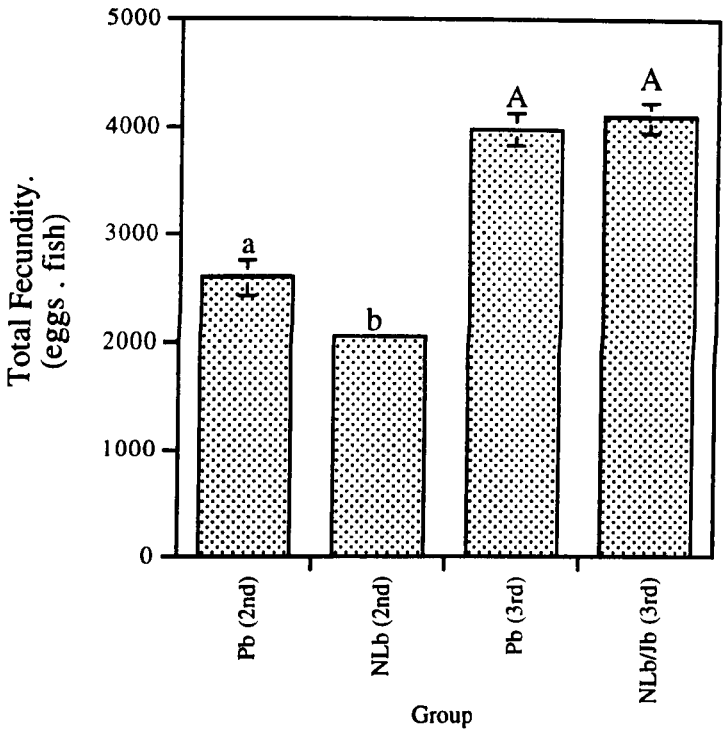
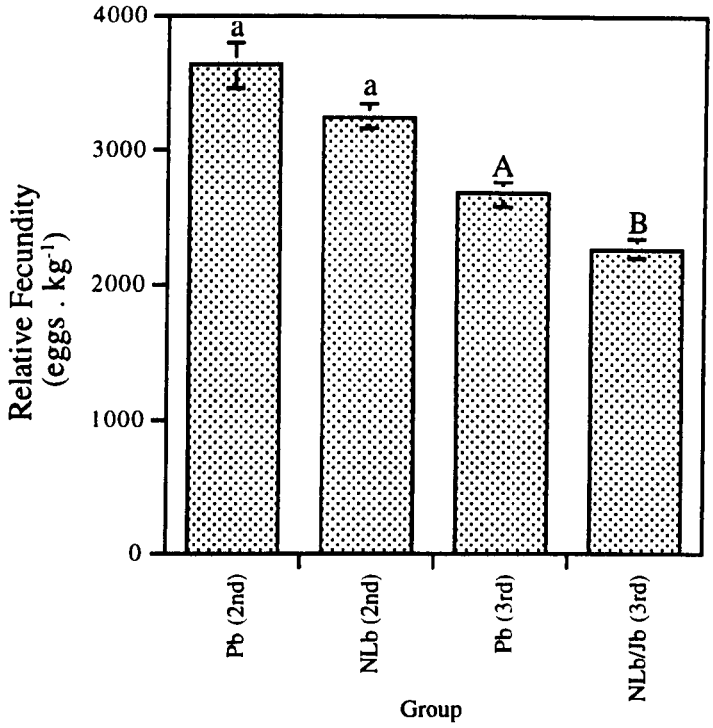


Figure 5.10 Mean egg diameter (±sem) for the groups Pb and NLb during the 2nd year of the experiment and for group Pb and the combined group NLb/Jb during the 3rd year of the experiment. Group Pb was subjected to an artificial photoperiod and group NLb was subjected to a natural photoperiod. Different letters indicate significant differences ($p<0.05$) between goups, lower case refers to second year and upper case refers to the third year of the experiment.



Group 5.11 Mean total fecundity for the groups Pb and NLb during the 2nd year of the experiment and for group Pb and the combined group NLb/Jb during the 3rd year of the experiment. Group Pb was subjected to an artificial photoperiod and group NLb was subjected to a natural photoperiod. Error bars are ± sem; error bars which are not visible are obscured by the histogram bar. Different letters indicate significant differences ($p<0.05$) between goups, lower case refers to second year and upper case refers to the third year of the experiment.



Group 5.12 Mean relative fecundity (±sem) for the groups Pb and NLb during the 2nd year of the experiment and for group Pb and the combined group NLb/Jb during the 3rd year of the experiment. Group Pb was subjected to an artificial photoperiod and group NLb was subjected to a natural photoperiod. Different letters indicate significant differences ($p<0.05$) between goups, lower case refers to second year and upper case refers to the third year of the experiment.

difference between the mean relative fecundity for the 2 groups. The relative fecundity for group NLb was 2259±72 eggs.kg^{-1} and for group Pb was 2664±89 eggs.kg^{-1} (Figure 5.12).

Survival of eggs alevins and fry.

During the two years in which the female fish were spawned the eggs, alevins and fry from the photoperiod group Pb exhibited higher rates of mortality than the natural group NLb (table 5.3 and figure 5.13). During the 2nd year of the experiment, 97.6% of the group Pb eggs did not hatch. This was significantly higher than the 36.5% unhatched eggs observed in group NLb. The eggs stripped from group Pb were of poor quality, with an estimated 35% dead (white eggs) after the eggs were laid out in the hatching baskets. A further 50% of the surviving eggs did not develop to the neural tube stage. Further losses were observed during shocking and hatching, resulting in just 2.4% of the eggs hatching.

Table 5.3 Percentage survival of photoperiod (Pb) and natural (NLb) groups up to the first month of feeding and the percentage mortality during the stages, egg incubation, the alevin stage and the first month of first feeding. Different letters indicate significant differences ($p<0.05$) between goups, lower case refers to second year and upper case refers to the third year of the experiment.

	Percentage unhatched eggs.	Percentage alevin mortality.	Percentage mortality in the 1st month of first feeding.	Percentage survival up to the 1st month of first feeding.
Pb (2nd)	97.6^{a}	2.4	-	-
NLb (2nd)	36.5^{b}	14.3	6.3	42.9
Pb (3rd)	51.9^{A}	25.4^{A}	13.7^{A}	8.9^{A}
NLb/Jb (3rd)	34.8^{B}	7.5^{B}	7.3^{B}	50.3^{B}

During the 3rd year of the experiment the photoperiod group again exhibited higher rates of mortality (table 5.3 and figure 5.12). The egg, alevin and first feeding mortalities were significantly ($P<0.05$) higher in group Pb and consequently the survival up to the

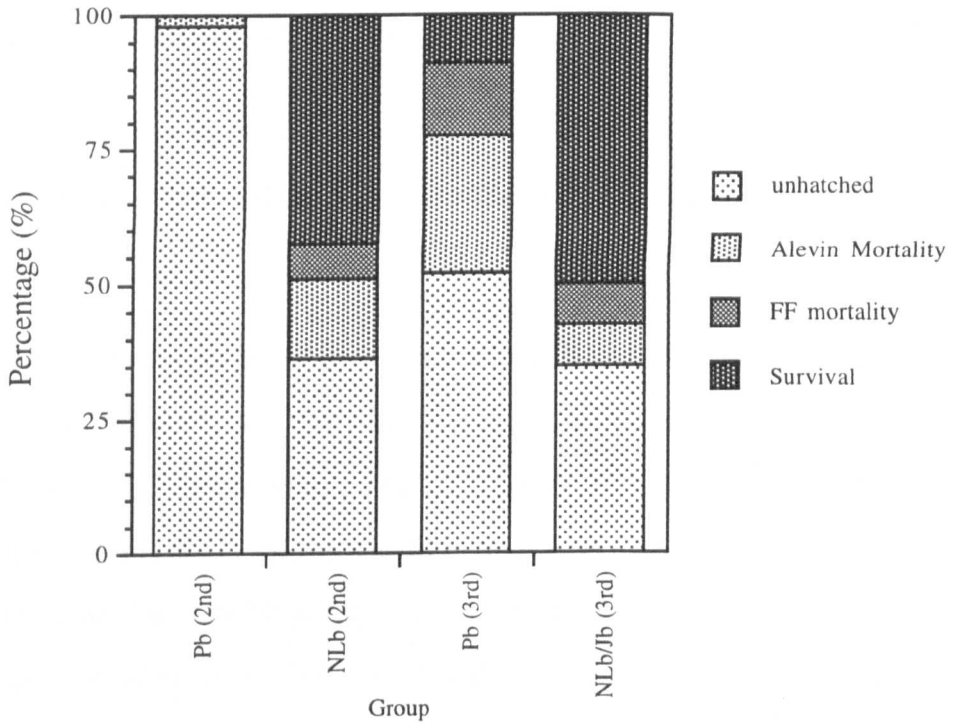


Figure 5.13 Percentage of eggs not hatching, alevin mortality, first feeding mortality and overall survival up to first feeding. Group Pb was subjected to an artificial photoperiod and group NLb to a natural photoperiod.

end of the first month of first feeding was significantly ($P<0.05$) lower in group Pb than in the group NLb/Jb. The major cause of mortality in group Pb, particularly during egg incubation, was fungal infections. Malachite treatments were used. However, the fungus was established and treatments were ineffective.

5.2.2.4 Performance of the progeny (F1 generation).

The parr from the eggs stripped from group NLb during the second year of the experiment were monitored through smoltification and during the first four months in sea water.

The condition factor remained between 1.90±0.005 and 1.80±0.006 from the 7th Jan. to the 21st Mar. (figure 5.14). The condition factor decreased significantly ($P<0.01$) from 1.80±0.006 on the 21st Mar. to 1.14±0.006 on the 18th April, from 1.16±0.006 on the 5th April to 1.11±0.008 on the 3rd May and from 1.14±0.006 on the 18th April to 1.08±0.012 on the 16th May, the sample date 10 days prior to transfer. The serum osmolality of challenged fish decreased significantly ($P<0.01$) from 383.1±9.1 $mOsm.kg^{-1}$ on the 21st Feb. to 335.1±3.5 $mOsm.kg^{-1}$ on the 19th April and from the 21st Feb. to 338.4±2.3 $mOsm.kg^{-1}$ on the 4th May. The serum osmolality increased to 373.0±8.3 $mOsm.kg^{-1}$ on the 16th May, the sample date 10 days prior to transfer. 20000 fish were transferred to sea site SW3 on the 26th May.

The mean weight when the fish were transferred to seawater was 44.2g. The fish exhibited a transfer mortality of 18.1% and grew to 382g during the first 4 months; this represented an SGR of 1.7 $\%.day^{-1}$.

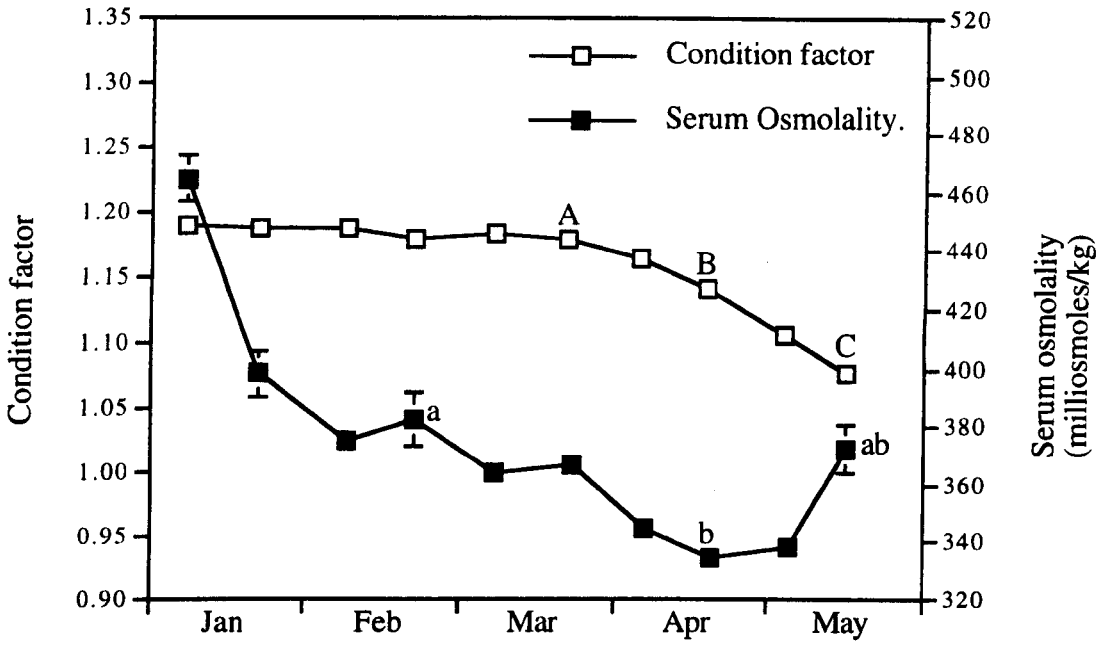


Figure 5.14 Change in condition factor (open square) and serum osmolality of challenged fish (filled square) for the smolts produced from the eggs obtained from group NLb (natural photoperiod) during the second year of the experiment. Error bars are ± 1 sem; error bars which are not visible are obscured by the plot symbol. Different letters indicate significant ($p<0.05$) differences over the sample period, lower case refer to serum osmolality and upper case refer to condition factor.

Summary of Results from Experiment 7.

1) The artificial photoperiods which were subjected to the fish advanced spawning by 12 and 22 weeks compared to fish held under a natural photoperiod.

2) An increased percentage of maturing fish were observed in the groups of fish maintained in fresh water.

3) The eggs produced under the artificial photoperiods exhibited significantly higher mortalities.

4) Smoltification and the loss of smolt characteristics was observed in the fish maintained in fresh water as broodstock.

5) After smoltification, a period of mortality was observed in the fish maintained in fresh water.

6) Smoltification was observed in the progeny of the broodstock reared in fresh water.

5.2.3 Discussion.

Smolts held in fresh water grew steadily and matured producing eggs and sperm both in- and out-of-season. There was, however, a period of increased mortality after smoltification and egg quality was reduced especially in fish subjected to photoperiod manipulation.

Photoperiod manipulation.

The artificial photoperiod, comprising of an increase to long days in January followed by a decrease to a short days in May and a second increase to long days in July, advanced the spawning time by 12 weeks compared to fish held under a natural photoperiod (group NLb). Under ambient temperature conditions a 4 weeks advance in spawning was achieved using an abrupt increase to a long daylength in March followed by an abrupt decrease in July (Taranger 1993). Spawning in rainbow trout was advanced by 6-14 weeks when fish were subjected to an increase to a long daylength in February followed by a decrease to a short daylength in either May, June or July (Whitehead & Bromage 1980, Bromage *et al.* 1982, Bromage *et al.* 1984, Elliott *et al.* 1984). The photoperiods in these studies were not identical but, the response in the timing of spawning in salmon under constant temperature conditions, was comparable to the response in trout.

In the present study the abrupt changes in the artificial photoperiod were followed by a reciprocal seasonal photoperiod, 6 months out of phase with the natural photocycle. Spawning, during this year, was advanced by 22-23 weeks compared to the fish which experienced a natural photoperiod (group NLb). This is only the second time Atlantic salmon spawning has been recorded during June. Johnston *et al.* (1990, 1992) advanced maturation of salmon kelts to June using a combination of two 6 month accelerated photoperiods followed by a simulated natural photoperiod. A similar combination of photoperiod cycles was used by Taranger (1993). A 9 month accelerated photocycle followed by a three month advanced 12 month photocycle which was applied to Atlantic salmon, under ambient temperature conditions, appeared to advance spawning by 12-16

weeks (Taranger 1993). In rainbow trout spawning was advanced by 24 weeks using a reciprocal photoperiod which was applied after 2 compressed cycles which had advanced spawning by 16 weeks (Elliott *et al.* 1984). In these studies the salmon in constant temperature conditions responded with similar changes in the timing of spawning as were observed in trout. In comparison, the salmon under ambient temperature conditions showed a different response but, perhaps this was related to the slightly different photoperiod.

These results support the suggestion that the mechanisms controlling maturation in the Atlantic salmon are similar to those previously described for the rainbow trout. Furthermore the results observed in this study, under constant temperature conditions, appear to support the suggestion that the different results observed in salmon (manipulated under ambient temperature conditions) and rainbow trout subjected to similar photoperiods (see section 5.1) were the result of a part of the maturation process coinciding with ambient temperatures which were outwith the temperature range normally experienced by that part of the maturation process. High temperatures during the final stages of maturation have delayed or inhibited maturation in the Atlantic salmon (Taranger 1993) and caused atresia and the termination of maturation during final maturation in rainbow trout (Campbell 1995). Low temperatures during vitellogenesis have inhibited maturation in Atlantic salmon (Johnston *et al.* 1992), whilst high temperatures during vitellogenesis have delayed maturation in rainbow trout (Campbell 1995).

Maturation.

Maturation of 22% (all males) of the fish held in fresh water was observed during the post-smolt year (first year following smoltification). In contrast no post-smolt maturation was observed in the proportion of the group which was transferred to sea water. Previous studies on smolts retained in fresh water have observed 100% post-smolt maturation in previously precocious male parr (Lundqvist & Fridberg 1982) and no post-smolt maturation (Duston & Saunders 1992) in normal smolts. It has been

suggested that smoltification and sexual maturation are biologically opposed (Thorpe 1986, Saunders *et al.* 1994) and post-smolt maturation (maturation during the 1st maturation episode) is considered uncommon in smolts reared under natural conditions and transferred to sea water. However, post-smolt maturation has been observed (Saunders *et al.* 1994), particularly in association with high maturing stocks (Herbinger & Newkirk 1990) and with specific environmental factors which enhanced freshwater development resulting in large smolts (Saunders & Henderson 1965, Sutterlin *et al.* 1978, Solbakken *et al.* 1994). Environmental influences are important in determining age at maturity. Different seawater sites, with different environmental conditions have resulted in different ages at maturity in fish of the same sibling group (Naevdal 1983) or stocks (Thrush 1994, and the current work experiment 5).

These observations of post-smolt maturation would suggest that some aspect of the freshwater environment experienced by the smolts in this study provided the exceptional conditions which resulted in a high post-smolt maturation. Salinity, photoperiod, temperature, nutrition and holding facility were the environmental variables which differed between the freshwater and seawater groups. To suggest that fresh water *per se* caused the increased maturation would contradict Duston and Saunders (1992) who observed no post-smolt maturation in smolts retained in freshwater. The differences in photoperiod in this study may have been a contributing factor. The fish retained in fresh water were maintained on a long daylength whilst the fish transferred to sea water were exposed to a natural photoperiod. Maintaining a long daylength after the summer solstice has been observed to increase percentage maturation in populations of rainbow trout (Whitehead 1979). However, the cause of the increased maturation in fresh water is unclear. The results suggest that a combination of the different holding conditions caused an early age at maturity in the fish retained in fresh water. Hence the effects of fresh water *per se* can not be discounted.

Maturation of female fish was observed during the 2nd and 3rd year after smoltification (i.e. the 2nd and 3rd year of experiment 7) both in group NLb held under natural

photoperiod and group Pb which was subjected to a artificial photoperiod. This was the equivalent of 1 sea winter (SW) maturation (maturation in the second maturation episode or grilsing) and 2 SW maturation (maturation in the third maturation episode). Under the natural photoperiod (group NLb) 71.6% of the remaining population matured during the second maturation episode (grilse). This again was higher than would be expected in the marine environment. Fish of the same stock exhibited maturation levels of 15.7 and 23% (experiment 4 and 5) in sea water. This again suggests that either the holding conditions or fresh water *per se* caused an early age at maturity. High percentages of maturation would be expected during the third maturation episode (i.e. as 2SW fish) but, the fish in this study were comparatively small for fish maturing at the equivalent age of 2 SW. Thrush (1994) examined the relationship between fecundity, egg size and fish size in four stocks of farmed Atlantic salmon. 2 SW fish were observed to mature at post-strip weights ranging from 2.6-12.6 kg; this compares to a pre-strip mean weight of 2.2±0.7 kg in the present study (group NLb). Fecundity was also low in this study compared to between 11000-15000 in fish reared in sea water (Thrush 1994). However, fecundity has been shown to increase with fish size in Atlantic salmon (Thorpe *et al.* 1984, Randall 1989, Thrush 1994). The relative fecundity and egg size observed in the fish reared under a natural photoperiod were comparable to similarly aged fish maintained in sea water (Thrush 1994). The broodstock maintained in fresh water, in this study, matured at a smaller size, producing a similar size and number of ova per kg of somatic tissue as broodstock maintained in seawater. The difference in size of fish resulted in a lower number of ova per fish from broodstock maintained in fresh water.

Egg and Juvenile Survival.

Egg mortality from the freshwater broodstock maintained under a natural photoperiod was 36.5 % during the second year of the experiment and 34.8 % during the third year of the experiment. Egg mortalities ranging from 7 % (Peterson *et al.* 1977) to 20 % (Foda and Henderson 1977) have been reported, at a constant 10°C, suggesting that the mortalities observed in this study were higher than would be expected. However,

mortalities in excess of 60 % have been reported during the first few months in the hatchery (Bromage *et al.* 1992). There are many different factors which can contribute to poor egg quality, such as environment or holding conditions: temperature, salinity, water quality, husbandry, nutrition and disease (Bromage 1995). It is difficult to say which factor or combination of factors contributed to the poor egg quality in this experiment. The problem did not affect alevin and fry quality which were both within a reasonable range; alevin mortality was 14.3 and 7.5 and fry mortality was 6.3 and 7.3 during the second and third years, respectively. At a constant 10°C alevin mortalities have been reported in the range from 0 % (Peterson *et al.* 1977) to 26 % (Gunnes 1979) and fry mortalities generally are close to 0 % (Koss 1994).

The development of parr, smoltification and the early seawater performance of the F1 generation produced from the natural group (NLb), in the second year of the experiment, was comparable to the smolts produced from the offspring of broodstock reared in sea water (experiments 4 and 5).

Egg mortality was significantly higher in the eggs produced under the artificial photoperiod (group Pb) compared to the fish reared under a natural photoperiod (group NLb). The eggs obtained from the photoperiod group (Pb) were significantly smaller than the eggs obtained from the natural group (NLb). During the 2nd year of the experiment there was no significant difference in the size of the fish and as the relationship between egg size and fish size is said to be poor (Thrush 1994), the difference in egg size is unlikely to be attributable to the size of the fish. Photoperiod has also been observed to affect egg size in rainbow trout. Advances in spawning induced by photoperiod manipulation have resulted in smaller eggs (Duston & Bromage 1988, Randall 1992), probably because the maturation process is compressed, reducing the time for egg development and consequently the eggs were smaller. However, Springate and Bromage (1985) demonstrated that egg size was not related to survival of the egg or fry in rainbow trout. It would appear that the increased egg mortality can not be attributed to differences in egg size.

A larger proportion of females in group Pb compared to group NLb did not ovulate and were therefore not stripped. This was a particular problem during the 2nd year of the experiment when 82.9 % of the mature females in the photoperiod group (Pb) did not ovulate compared to 39.6% in the natural group (NLb). The eggs obtained from the group Pb fish during the 2nd year showed a percentage hatch of just 2.4%; 35% of the eggs were dead after fertilisation and a further 50% did not develop to the neural tube stage. The non-ovulation of eggs would suggest that the maturation process had been arrested at a very late stage in development. The process of atresia, the arrest of oocyte development with oocytes being resorbed before full maturity, has been described (Cumaranatunga 1985, Bromage & Cumaranatunga 1988) and atresia of all developing oocytes has been observed after starvation or hormonal changes (Springate *et al.* 1985) and high temperatures during final maturation (Campbell 1995). The non-ovulated fish in this study were not examined for indications of atresia but, this would explain the high proportion of non-ovulated fish and the very poor egg quality. A similar situation was observed in rainbow trout maintained at high temperatures during final maturation (Campbell 1995). A large proportion of the fish did not ovulate and those which did produced poor quality eggs.

The temperature in this study was a constant 10±1°C for both the photoperiod and natural groups, suggesting that the poor egg quality in the photoperiod group was not the result of temperature. The only environmental differences between the two groups were holding facility and photoperiod. Although the stocking densities of the 2 groups were similar the photoperiod fish were in a smaller facility and therefore may have experienced a greater degree of confinement. However, these fish were maintained in the same facility during the second year when egg survival improved. This suggests that the holding facility was not the cause of poor egg quality.

It would appear that the artificial photoperiod experienced by the fish in the photoperiod group (Pb) was the cause of the poor egg quality during the second year of the experiment. The artificial photoperiod to which the photoperiod fish (group Pb) were

subjected during the second year advanced the endogenous clock by 12 weeks over a period of 8 months. The fish were stripped under a long daylength which was initiated in July after a 3 month period of short days. Photoperiod cues during the early stages of maturation have been observed to stop maturation from proceeding (Duston & Bromage 1988, Hansen *et al.* 1992, Taranger 1993, Campbell 1995). There are no examples of photoperiod causing maturation to be stopped or abandoned during the final months of the maturational process. However, this would appear to be the case in the present study. The salmon appeared not to be able to respond to the rapidly changing photoperiod (a driving zeitgeber). Possibly, the short period of time available for egg development was not sufficient to allow full maturational development. This may have resulted in poor egg quality and the arrest of the maturation process in the final stages.

A similar suggestion was made in chapter 3 to explain the dissociation of smolt parameters and incomplete smoltification. A driving zeitgeber such as photoperiod could result in a limited time period for the biological process to development. This limited time period may be insufficient to allow the biological process to be completed. Gwinner (1986) observed incomplete moulting in European starlings held under a compressed 2, 1.7 and 1.5 month photoperiod cycles and suggested that this was due to insufficient time to complete the moult.

This again highlights differences in the mechanisms controlling maturation in the rainbow trout and the Atlantic salmon. Rainbow trout have been observed to respond to a rapidly changing photoperiod and successfully spawn under a long daylength (Duston & Bromage 1988, and Randall 1992). In the present study Atlantic salmon subjected to a similar rapidly changing photoperiod (a driving zeitgeber) appeared unable to successfully complete maturation. A large proportion of fish did not spawn and those that did, produced poor quality eggs.

During the third year of the experiment egg mortality was again significantly higher in the eggs produced under the artificial photoperiod (group Pb) compared to the fish reared under a natural photoperiod (group NLb). However, a significant proportion of the

mortality observed in the eggs produced under the artificial photoperiod (group Pb) was attributable to fungal (*Saprolegnia*) infections. Once the fungal infection was established it was difficult to treat. Other studies incubating eggs at 10°C and above have stated that the development of fungus at higher temperatures was responsible for mortalities (Peterson *et al.* 1977, Foda & Henderson 1977).

Smoltification and Loss of Smolt Characteristics.

The smolt parameters condition factor and serum osmolality of challenged fish and the seawater performance of the fish which were transferred, indicated that the fish completed smoltification during May. The fish retained in fresh water were observed to lose hypoosmoregulatory ability and exhibited an increase in condition. Studies of anadromous smolts retained in fresh water have reported increased condition factor (Johnston 1983, Komourdjian *et al.* 1976) and loss of seawater tolerance or decline in Na^+ - K^+ ATPase activity (Johnston 1983, Boeuf *et al.* 1985, Virtanen & Soivio 1985, Duston *et al.* 1991). The loss of smolt characteristics has been referred to as de-smoltification.

In the present study (experiment 7) de-smoltification appeared to take place over 3-4 months. Temperature (Duston *et al.* 1991) and photoperiod (Kurokawa 1990) have both been implicated as mediators in the rate of loss of smolt characteristics. The fish in experiment 7 were subjected to constant photoperiod and temperature and consequently it is possible that the fish did not receive a cue for de-smoltification. Smolts retained under ambient temperature conditions and natural photoperiod have been reported to lose hypoosmoregulatory ability over a 1 month period (Duston *et al.* 1991). At temperatures greater than 10°C hypoosmoregulatory ability was lost in less than 1 month (Duston *et al.* 1991). In masu salmon, photoperiod was considered the most important factor in the loss of morphological smolt characteristics and a decrease to a short daylength (LD 8:16) during July increased the rate of de-smoltification compared to fish held on a summer solstice LD 16:8 (Kurokawa 1990).

The period over which the condition factor and serum osmolality of challenged fish increased was associated with a fungal infection which resulted in the mortality of 10% of the fish. *Saprolegnia* is present in most freshwater bodies and fish under stress are open to fungal infections (Pickering & Duston 1983, Schreck *et al.* 1993). Smolts pre-adapt for the hyper-osmotic environment, reduced sodium and chloride ion concentrations (Houston & Threadgold 1963) and increased body moisture content (Komourdjian *et al.* 1976) have been observed in smolts prior to transfer into sea water. Retaining smolts pre-adapted for a hyper-osmotic environment in a hypo-osmotic environment may be stressful for the fish. Plasma cortisol has been observed to increase during smoltification (Barton *et al.* 1985, Patino & Schreck 1986) suggesting that the process caused and increased stress. The association of a period of mortality with a period when the fish is reverting back to a hyper-osmoregulatory physiology whilst in a hyper-osmotic environment would suggest that during this period the fish were susceptible to *saprolegnia* infections.

Chapter 5 Conclusions.

1) Atlantic salmon responded to an artificial photoperiod, resulting in advances of 12 weeks and 22 weeks.

2) The maturation process in the Atlantic salmon would appear to be controlled by similar mechanisms to those described for the rainbow trout.

3) Freshwater holding conditions decrease the age at maturity.

4) Freshwater holding conditions appeared to decrease egg quality.

5) The artificial photoperiod appeared to decrease egg quality.

6) The rapidly changing photoperiod (a driving zeitgeber) to which the fish were subjected appeared to decrease egg quality.

Chapter 6 General Conclusions.

The present study has addressed each of the aims; 1) To investigate the mechanisms involved in the photoperiodic control of smoltification, establishing a photoperiod technique for the production of out-of-season smolts; 2) To examine the potential seawater performance of out-of-season smolts; and 3) To investigate the use photoperiod to manipulate reproduction and produce out-of-season eggs.

Chapter 3 examined the mechanisms involved in the photoperiodic control of smoltification. The different smoltification perimeters were closely examined under both constant and square waves photoperiods. The results suggested that the different aspects of smoltification: the development of bimodality, hypoosmoregulatory ability, decrease in condition and smolt coloration were controlled by different independent mechanisms. The decrease in condition factor appeared to be in direct response to changes in the photoperiod requiring a period of short days followed by an increase to a long daylength. The development of bimodality, hypoosmoregulatory ability and possibly coloration are probably controlled by independent endogenous rhythms. The dissociation of these smolt parameters was observed under constant and rapidly changing photoperiods. Further work is required to establish how and which environmental cues are involved in the mechanisms controlling the different smoltification parameters. This work should show how the environmental variables affect each aspect of smoltification; for example, the decrease in daylength (at a certain temperature and applied at a certain time in the smoltification cycle) may advance hypoosmoregulatory ability by X weeks, coloration by Y weeks whilst not effecting the timing of the decrease in condition factor. Such work should provide artificial photoperiods which produce out-of-season smolts which develop the different smolt parameters in association with each other. Although the dissociation of smolt parameters observed in this study appeared not to effect smolt quality; the dissociation of the smolt parameters at temperatures above 12°C has resulted in problems in predicting the time of smolt transfer and has possibly affected smolt

quality (personal observation). The examination of smoltification in a dissociated form may also allow the investigator to tease out the endocrine functions involved in the control of different smolt parameters; for example, the development of hypoosmoregulatory ability in isolation from other changes (Duston & Saunders 1990) should allow the associated endocrine changes to be identified and monitored.

Chapter 4 also examined the development of smoltification under artificial photoperiods. Both chapters 3 and 4 showed that a 2-3 month period of short days followed by a period of long days could be used to advance the smoltification process, producing out-of-season smolts from October through to March. The production of out-of-season smolts could and is being used to increase the annual output of smolt facilities. A smolt facility with the capacity to hold 250,000 smolts used to produce one batch of natural smolts will only operate at full capacity in the months prior to the sale of the single batch of natural smolts. During much of the year the facility will have unused tanks. During the autumn the potential S1 parr would be expected to attain a mean weight of 20-30g. This would represent using approximately 50% of the smolt units holding capacity (assuming 50-60g smolts are produced by the unit). The unused 50% of the smolt unit could be used to produce 125,000 0+ smolts during September and October. This could be achieved without any further investment in facilities. However, additional heating and oxygenation would be required during early rearing, increasing the production cost per smolt. This additional cost should be offset by the shortened production time. If this argument is extended, a hatchery could have two intakes of eggs, one in June and one in November. This would allow the smolt unit illustrated above to produce 4 batches of 125,000 smolts a year (this assumes the smolts are produced at 50-60g and the fish double their weight during the final 3 months in the smolt unit using heated or spring supplies during the winter). This would increase the smolt units annual production by 100%. Using the 2 batch production scenario, outlined above, one of the smolt units involved in this study has increased its annual production of smolts by 20%. Other farms have reported increases in production of 200-300% (personal communication). However, this was in combination with installing new heating and oxygenation systems.

The benefits of such an expansion balanced against the increased capital cost may not appear clear cut. The farm must also consider the benefits of out-of-season smolts in the seawater production of market sized fish.

The increased use of the smolt units facilities and the production of multiple batches of smolts throughout the year will in turn result in the increased use of the facilities at the sea site. As was seen with the freshwater facilities, the sea site facilities would be fully stocked for a greater period of time. When the fish were harvested, the cages could be immediately restocked, rather than waiting of the spring smolt input. For example, the pre-Christmas period is traditionally a period of high market demand for salmon. The harvesting of large numbers of salmon leaves cages empty. Some of these cages are restocked with fish from highly stocked cages but, a number of cages remain unused until the spring input of natural smolts. These cages could be moved to another site and stocked with November or December out-of-season smolts. This would increase the productivity per cage and reduce capital costs per tonne produced.

Chapter 4 examined the seawater performance of out-of-season smolts and clearly demonstrated that out-of-season smolts have the same growth potential as natural smolts. Natural smolts transferred to sea during May were harvested after 16-18 months and smolts transferred to sea during March, October and November were harvested after 16-19 months. By timing the input of smolts the production of market-sized fish could be targeted for a particular time of year; the regular input of smolts throughout the year resulting in the production of market-sized fish throughout the year.

A major problem for the profitability of the salmon farming industry is the price instability associated with the product. The salmon price collapsed during 1989, 1991, 1993 and 1994. Price instability is almost always supply induced (Chamberland 1994) and one solution would be an 'evening out' of fluctuations in the supply of market-sized fish. The seasonal fluctuations in supply can be attributed principally to the seasonal supplies if eggs and smolts. The management of both natural and out-of-season smolt

production should enable the industry to control the seasonal fluctuation in supply of market-sized fish.

The 'smoothing' of seasonal fluctuations in supply, combined with the a producer organisation co-ordinating the industry's production should enable a more stable pricing structure to be achieved. The trout industry was afflicted by similar problems of fluctuation in supply. The use of out-of-season eggs in conjunction with producer organisations and centralised processing facilities, has lead to a more stable pricing structure for the trout industry.

The effect of the timing of seawater transfer on maturation and mortality were not so clear. In this study transfer mortality was correlated with mortality prior to transfer, time of transfer and the percentage of precocious males in the population. Mortality appeared not to be related to smolt parameters such as hypoosmoregulatory ability and condition factor. The mortalities resulting from different freshwater sites suggested that mortality was related to the environmental characteristics of the freshwater site and their husbandry procedures. Different smolt units with different environmental variables and operating practices may result in different transfer mortalities, these mortalities may be elevated in out-of-season smolts due to the problems associated with identifying the optimal transfer time in out-of-season smolts and seasonal changes in sea conditions. Different farming operations must consider whether the transfer mortalities which they experience with out-of-season smolts are acceptable compared with the benefits of improved use of freshwater and seawater facilities and the controllable production of market-sized fish.

The age at maturity in out-of-season smolts was positively related to the length of the seawater growing period prior to the completion of maturation. Advances of smoltification to provide early out-of-season smolts decreased age at maturity and delays of smoltification to provide late out-of-season smolts increased age at maturity. In this study the increased maturation observed in the second maturation episode (grilse) was in association with weights in excess of 3kg and these fish were harvested as superior salmon prior to any deterioration in flesh quality. These increased grilse rates did not

affect production as the harvesting period resulting from the out-of-season smolts began before the fish matured as grilse. Unlike naturally-produced smolts the harvest period was not dependant on grilse maturation because of the longer growing period before maturation .

However, it should be noted that changing farming practices could and are resulting in different ages at maturity and the above situation will not be the case for all farms. Different ration levels (Jones & Bromage 1987, Thorpe *et al.* 1990) and sites (experiments 4 and 5) have resulted in different ages at maturity. Changes in farming practices such as diet improvements may result in a lower age at maturity. Maturation levels below 5% during the first maturation episode (post-smolt) have been observed (experiment 6, Duncan unpublished) in post-smolts which were transferred to a commercial sea site during November. Under these different growing conditions the same positive relationship between age at maturity and seawater growing period would be expected. The age at maturity would again increase as the seawater growing period prior to maturation lengthened. Therefore farms with historically high rates of maturation might expect to obtain, during the first year in seawater, a low percentage of small (1kg) mature fish from out-of-season smolts transferred during November. Such farms must again consider if this is acceptable compared with the benefits of improved use of freshwater and seawater facilities and the controllable production of market-sized fish.

An aspect of out-of-season smolt production which has received little investigation is the photoperiodic control of growth and maturation when the smolts have been transferred to sea water. In this study the out-of-season smolts transferred during the autumn or winter experienced a decrease in photoperiod from the long daylength in the hatchery to a short natural daylength at the sea site. The majority of these groups exhibited no maturation during the first year in sea water. It can not be discounted that the decrease from a long daylength to a short natural daylength inhibited maturation during the first year in sea water. A number of groups did exhibit maturation during the first year in sea water with the completion of maturation approximately 1 year after transfer. The timing of

completion of maturation in these groups was similar to groups reared under a natural photoperiod. This suggests that the maturation process in these out-of-season groups of smolts had entrained to the natural photoperiod. Preliminary investigations on December out-of-season smolts maintained on a long daylength after transfer to sea water has shown that growth was enhanced by 100% compared to smolts transferred onto a natural photoperiod (Duncan unpublished). The smolts maintained on the long daylength exhibited 2% maturation (milt production in males) after 6 months. These observations suggest that photoperiod can be used to influence growth and maturation in sea water. This is an area which requires further work.

Chapter 5 examined the photoperiodic manipulation of broodstock which had been reared in constant 10°C fresh water. The fish clearly responded to the photoperiodic manipulation and eggs were produced during August after an increase to a long daylength in January followed by a decrease to a short daylength in May and in June from fish which were subjected to a reciprocal photocycle. This represented advances of 12 and 22 weeks compared to fish maintained under a natural photoperiod. It was suggested that the maturation process in the Atlantic salmon is controlled by similar mechanisms as described for the rainbow trout. However, questions remain over the quality of the eggs which appeared to be compromised by some aspect of the holding environment, possibly the rapidly changing photoperiod or the freshwater holding conditions.

Clearly, the mechanisms involved in the photoperiodic control of reproduction in the Atlantic salmon require further research. Particular attention should focus on the involvement of endogenous processes and the mechanisms by which information on the daily and seasonal changes in photoperiod are transmitted to the pituitary-gonadal axis. The importance of the interactions of both temperature and salinity with the photoperiodic control of reproduction must also be addressed. Temperature in particular would appear to have adverse effects on the maturation process in Atlantic salmon. High temperatures (>13°C) appear to interfere with ovulation and diminish egg quality (Taranger 1993).

Further investigation of the photoperiodic control of reproduction in the Atlantic salmon is also important to the salmon farming industry. The industry favours 0+ smolt production. However, advances in the smolt transfer date of 0+ smolts are restricted by the time required for the parr to grow from eggs to a size capable of undergoing the parr-smolt transformation. Earlier eggs are required to provide the time necessary to rear the parr to a size capable of undergoing the parr-smolt transformation. This requirement is perhaps illustrated by the increasing importation of eggs from Australia, which increased from 240,000 in 1994 to 600,000 in 1995. With growing restrictions on imports of ova, it is essential that the problems of photoperiodic control of reproduction in the Atlantic salmon are addressed. This would allow the salmon farming industry to have full control over the timing of both smolt and market-sized fish production.

This thesis has increased the understanding of out-of-season smolt and egg production and clearly demonstarted how out-of-smolts preform in sea water. The potential of the use of out-of-season smolts in the farming industry has been highlighted.

References.

Aksnes, A., Gjerde, B., and Roald, S. O., 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. Aquaculture, 53:7-20.

Bagliniere, J. L., and Maisse, G., 1985. Precocious maturation and smoltification in wild Atlantic salmon in the Armorican massif, France. Aquaculture, 45:249-264.

Bailey, J. K., Saunders, R. L., and Buzeta, M. I., 1980. Influence of parental smolt age and sea age on growth and smolting of hatchery-reared Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 37:1379-1386.

Barton, B. A., Schreck, C. B., Ewing, R. D., Hemmingsen, A. R., and Patino, R., 1985. Changes in plasma cortisol during stress and smoltification in coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 59:468-471.

Benfey, T. J., Dye, H. M., Solar, I. I., and Donaldson, E. M., 1989. The growth and reproductive endocrinology of adult triploid Pacific salmonids. Fish Phys. Biochem., 6: 113-120.

Berge, A.I., Berg, A.B., Ffhn H.J., Barnung, T., Hansen, T. and Stefansson, S.O., 1995. Development of salinity tolerance in underyearling smolts of Atlantic salmon (*Salmo salar*) reared under different photoperiods. Can. J. Fish. Aquat. Sci., 52:243-251.

Bergheim, A., Kroglund, F., Vatne, D. E., and Rosseland, B. O., 1990. Blood plasma parameters in farmed Atlantic salmon (*Salmo salar* L.) transferred to sea cages at eight to ten months. Aquaculture, 84:159-165.

Berglund, I., 1992. Growth and early sexual maturation in Baltic salmo (*Salmo salar*) parr. Can. J. Zool., 70:205-211.

Berglund, I., Hansen, L. P., Lundqvest, H., Jonsson, B., Eriksson, T., Thorpe, J. E., and Eriksson, L.-O., 1991. Effects of elavated winter temperature on seawater adaptability, sexual rematuration, and downstream migratory behaviour in mature male Atlantic salmon parr (*Salmo salar*). Can. J. Fish. Aquat. Sci., 48:1041-1047.

Berglund, I., Schmitz, M., and Lundqvist, H., 1992. Seawater adaptability in Baltic salmon (*Salmo salar*): A Bimodal smoltification pattern in previously mature males. Can. J. Fish. Aquat. Sci., 49:1097-1106.

Birt, T. P., and Green, J. M., 1993. Acclimation to seawater of dwarf nonanadromous Atlantic salmon, *Salmo salar*. Can. J. Zool., 71:1912-1916.

Birt, T. P., Green, J. M., and Davidson, W. S., 1991a. Contrasts in development and smolting of genetically distinct sympathric anadrmous and nonanadromous salmon, *Salmo salar*. Can. J. Zool., 69:2075-2084.

Birt, T. P., Green, J. M., and Davidson, W. S., 1991b. Mitochondrial DNA variation reveals genetically distinct sympatric populations of anadromous and nonanadromous Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci., 48:577-582.

Bjerknes, V., Duston, J., Knox, D., and Harmon, P., 1992. Importance of body size for acclimation of underyearling Atlantic salmon parr (*Salmo salar* L.) to seawater. Aquaculture, 104: 357-366.

Bjornsson, B. T., Thorarensen, H., Hirano, T., Orasawara, T., and Kristinsson, J. B., 1989. Photoperiod and temperature affect plasma growth hormon levels, growth, condition factor and hypoosmoregulatory ability of juvenile Atlanic salmon (*Salmo salar*) during par-smolt transformation. Aquaculture, 82:77-91.

Blackburn, J., and Clarke, W. C., 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci., 1515:1-35.

Boeuf, G., le Roux, A., Gaignon, J. L., and Harache, Y., 1985. Gill (Na^+-K^+)-ATPase activity and smolting in Atlantic salmon (*Salmo salar* L.) in France. Aquaculture, 45:73-82.

Boeuf, G., le Bail, P.Y. and Prunet, P., 1989:. Growth hormone and thyroid hormones during Atlantic salmon, *Salmo salar* L., smolting, and after transfer to seawater. Aquaculture, 82: 257-268.

Boeuf, G., and Prunet, P., 1985. Measurements of gill (Na+-K+)-ATPase activity and plasma thyroid hormones during smoltification in Atlantic salmon (*Salmo salar* L.). Aquaculture, 45:111-120.

Bolger, T., and Connolly, P. L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. J. Fish. Biol., 34:171-182.

Bourlier, A., and Billard, R., 1984. Delayed gametogenesis and spawning in rainbow trout (*Salmo gairdneri*) kept under permanent light during the first and second reproductive cycle. Aquaculture, 43:259-268.

Bromage, N., 1995. Broodstock management and seed quality-general considerations. In: N. Bromage and R. J. Roberts (Editors), Broodstock management and egg and larval quality. Blackwell Science Ltd, Oxford, pp. 1-25.

Bromage, N., and Cumaranatunga, R., 1988. Egg production in the rainbow trout. In: J. F. Muir and R. J. Roberts (Editors), Recent Advances in Aquculture. Croom Helm/Timber Press, London and Sydney/Portland, Oregan, IV, pp. 63-138.

Bromage, N. and Duston, J., 1986. The control of spawning in the rainbow trout (*Salmo gairdneri* Richardson) using photoperiod techniques. Inst. Freshwater Res. Drottingholm Rep., 63:26-35.

Bromage, N., Elliott, J., Springate, J. R., and Whitehead, C., 1984. The effects of constant photoperiods on the timing of spawning in the rainbow trout. Aquaculture, 43:213-223.

Bromage, N., Jones, J., Randall, C., Thrush, M., Springate, J., Duston, J., and Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 100:141-166.

Bromage, N., Randall, C., Davies, B., Trush, M., Duston, J., Carillo, M., and Zanuy, S., 1993. Photoperiodism and the control of reproduction and development in farmed fish. In: B. Lahlou and P. Vitiello (Editors), Aquaculture: Fundmental and Applied Research. American Geophysical Union., Washington D.C.

Bromage, N., Whitehead, C., Elliott, J., and Breton, B., 1982. Investigations into the importance of daylength on the photoperiod control of ovarian development in the

rainbow trout. In: C. J. J. Richter and H. J. T. Goos (Editors), Reproductive Physiology of Fish, Pudoc Press, Wageningen.

Brown, M. E., 1946. The growth of brown trout (*Salmo truta*). II. The growth of 2-year-old trout at a constant temperature of 11.5C. J. Exp. Biol., 22:130-144.

Burton, M. P., and Idler, D. R., 1984. Can Newfoundland landlocked salmon, *Salmo salar*, adapt to seawater? J. Fish Biol., 24:59-64.

Cadwick, E. M. P., Randall, R. G., and Leger, C., 1986. Ovarian devlopment of Atlantic salmon (*Salmo salar*) smolts and age at first maturity. In: D. J. Meerburg (Editor), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 15-23.

Campbell, B., 1995. Environmental and hormonal control of reproduction in the female rainbow trout, *Oncorhynchus mykiss*. PhD Thesis. University of Stirling, UK.

Chamberland, D., 1994. Finding a way out of 'boom and bust' cycles. Fish Farmer,17 (5, September/October): 45-48.

Clarke, W. C., 1989. Photoperiod control of smolting: A review. Physiol. Ecol. Japan Spec., 1:497-502.

Clarke, W. C., and Blackburn, J., 1977. A seawater challenge test to measure smolting of juvenile salmon. Fish. Mar. Serv. Res. Dev. Tech. Rep., 705:1-11.

Clarke, W. C., Lundqvist, H., and Eriksson, L.-O., 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon, *Salmo salar* L. J. Fish Biol., 26:29-35.

Clarke, W. C., Shelbourn, J. E., and Brett, J. R., 1981. Effects of artificial photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon. Aquaculture, 22:105-116.

Clarke, W. C., Shelbourn, J. E., Ogasawara, T., and Hirano, T., 1989. Effect of initial daylength on growth, seawater adaptability and plasma growth hormone levels in underyearling coho, chinook, and chum salmon. Aquaculture, 82:51-62.

Cornell, S. C., Portesi, D. M., Veillette, P. A., Sundell, K., and Specker, J. L., 1994. Cortisol stimulates intestinal fluid uptake in Atlantic salmon (*Salmo-salar*) in the post-smolt stage. Fish Phys. Biochem., 13:183-190.

Cumaranatunga, R., 1985. The ovarian cycle of rainbow trout with special reference to atresia. PhD Thesis. University of Aston, Birmingham, UK.

Cunjak, R. A., Saunders, R. L., and Chadwick, E. M. P., 1990. Seasonal variations in the smolt characteristics of juvenile Atlantic salmon (*Salmo salar*) from estuarine and riverine environments. Can. J. Fish. Aquat. Sci., 47:813-820.

Dally, E. L., Andrews, C. W., and Green, J. M., 1983. Precocious male Atlantic salmon parr (*Salmo salar*) in insular Newfoundland. Can. J. Fish. Aquat. Sci., 40:647-652.

de Vlaming, V. L., 1972. Environmental control of teleost reproduction cycles: a brief review. J. Fish. Biol., 4:131-140.

Dempson, J. B., Myers, R. A., and Reddin, D. G., 1986. Age at first maturity of Atlantic salmon (*Salmo salar*) - influnces of the environment. In: D. J. Meerburg (Editor), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 79-89.

Dickoff, W. W., Folmar, L. C., Mighell, J. L., and Mahnken, C. V. W., 1982. Plasma thyroid hormones during smoltification of yearling and underyearling coho salmon and yearling chinook salmon and steelhead trout. Aquaculture, 82:39-48.

Duncan, N., Bromage, N., and Robertson, D., 1995. An out-of-season chance to stabilise salmon prices. Fish Farmer,18 (4, July/August): 18, 51.

Duston, J., 1994. Effect of salinity on survival and growth of Atlantic salmon (*Salmo salar*) parr and smolts. Aquaculture, 121:115-123.

Duston, J., and Bromage, N., 1986. Photoperiod mechanisms and rythms of reproduction in the female rainbow trout. Fish Physiol. Biochem., 2:35-51.

Duston, J., and Bromage, N., 1987. Constant photoperiod regimes and the entrainment of the annual cycle of reproduction in the female Rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol., 65:373-384.

Duston, J., and Bromage, N., 1991. Circannual rhythms of gonadal maturation in female rainbow trout (*Oncorhynchus mykiss*). J. Biol. Rhythms, 6:49-53.

Duston, J., and Knox, D., 1992. Acclimation of Atlantic salmon (*Salmo salar*) parr to seawater in Autumn: stimulatory effect of a long photoperiod. Aquaculture, 103:341-358.

Duston, J., and Saunders, R. L., 1990a. The entrainment role of photoperiod on hypoosmregulatory and growth-related aspects of smolting in Atlantic salmon (*Salmo salar*). Can. J. Zool., 68:707-715.

Duston, J., and Saunders, R. L., 1990b. Control of the timing of smoltification in Atlantic salmon: endogenous rhythms and environmental factors. In: R. L. Saunders (Editor), Proceedings of Canada-Norway finfish aquaculture workshop., Can. Tech. Rep. Fish. Aquat. Sci., 1761.: 99-105. pp.

Duston, J., and Saunders, R. L., 1992. Effect of 6-, 12-, and 18-month photoperiod cycles on smolting and sexual maturation in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 49:2273-2280.

Duston, J., and Saunders, R. L., 1995. Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. Aquaculture, 135:295-309.

Duston, J., Saunders, R. L., and Knox, D. E., 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 48:164-169.

Eddy, F. B., and Talbot, C., 1985. Urine production in smolting Atlantic salmon, *Salmo salar* L. Aquaculture, 45:67-72.

Elliott, J. A. K., Bromage, N., and Springate, J. R., 1984. Changes in reproductive function of three strains of rainbow trout exposed to constant and seasonally-changing light cycles. Aquaculture, 43:23-34.

Elson, P. E., 1957. The importance of size in the change from parr to smolt in Atlantic salmon. Can. Fish. Cult., 21:1-6.

Eriksson, L.-O., and Lundqvist, H., 1982. Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). Aquaculture, 82:113-122.

Evans, G. T., Rice, J.C. and Chadwick, E.M.P., 1984. Patterns in growth and smolting of Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci., 41:783-797.

Farmer, G. J., Ritter, J. A., and Ashfield, D., 1978. Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can., 35:93-100.

Fishmongers Company, 1989-1995. Annual reports of arrivals and averge prices of salmon on Billingsgate Market. Fishmongers Company.

Foda, A., and Henderson, T. K., 1977. Effect of water temperature on rate of embyonic development, growth and survival of Atlantic salmon (*Salmo salar*). ICES C.M. 1977/M:, 31:7.

Folmar, L. C., and Dickhoff, W. W., 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aqaculture, 21:1-37.

Foskett, J. K., and Scheffey, C., 1982. :The chloride cell: Definitive identification as the salt-secreting cell in teleosts. Science, 251:

Fowler, J., and Cohen, L., 1987. Statistics for Ornithologists. British Trust for Ornithology.

Gardner, M. L. G., 1976. A review of factors which may influence the sea-age and maturaion of Atlantic salmon *Salmo salar* L. J. Fish Biol., 9:289-327.

Gjerde, B., 1984. Responce to individual selection for age at sexual maturity in Atlantic salmon. Aquaculture, 38:229-240.

Glebe, B. D., and Saunders, R. L., 1986. Genetic factors in sexual maturity of cultured Atlantic salmon (*Salmo salar*) parr and adults reared in sea cages. In: D. J. Meerburg (Editor), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 24-29.

Grau, E. G., Specter, R. S., Nishioka, R. S., and Bern, H. A., 1982. Factors determining the occurrence of the surge in thyroid activity in salmon during smoltification. Aquaculture, 82:49-58.

Graynorth, E., 1995. Spawning migrations and reproduction of landlocked sockeye salmon (*Oncorhynchus-nerka*) in the waitaki catchment, New Zealand. New Zealand J. Marine Fresh. Res., 29:257-269.

Gunnes, K., 1979. Survival and development of Atlantic salmon (*Salmo salar*) eggs and fry at three different temperatures. Aquaculture, 16:211-218.

Gwinner, E., 1986. Circannual Rhythms. Springer-Verlag, Berlin.

Hansen, T., Stefansson, S., and Taranger, G. L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aqua. and Fish. Management, 23:275-280.

Herbinger, C. M., and Newkirk, G. F., 1990. Sources of family variability for maturation incidence in cultivated Atlantic salmon (*Salmo salar*). Aquaculture, 85:153-162.

Higgins, P. A., and Talbot, C., 1985. Growth and feeding in juvenile Atlantic salmon *Salmo salar* L. In: C. B. Cowey, A. M. Mackie and J. G. Bell (Editors), Nutrition and Feeding in Fish. Academic Press, London, pp. 243-263.

Higgins, P. J., 1985. Metabolic differences between Atlantic salmon (*Salmo salar*) parr and smolts. Aquaculture, 45:33-53.

Hindar, K., and Nordland, J., 1989. A female Atlantic salmon, *Salmo salar* L maturing sexually in the parr stage. J. Fish Biol., 35:461-463.

Hoar, W. S., 1939. The weight-length relationship of the Atlantic salmon. J, Fish Res. Bd. Can., 4:441-459.

Hoar, W. S., 1988. The physiology of smolting salmonids. In: Hoar, W. S., and Randall, D. J. (Editors), Fish Physiology. Academic Press, London, 6B:275-341 pp.

Hoar, W. S., and Randall, D. J., 1984. Fish Physiology., Academic Press, London, 10A and 10b.

Houston, A. H., and Treadgold, L. T., 1963. Body fluid regulation in smolting Atlantic salmon. J. Fish. Res. Board Can., 20:1355-1369.

Hunt, S. M. V., Simpson, T. H., and Wright, R. S., 1982. Seasonal changes in the levels of 11-oxotestosterone and testosterone in the serum of male salmon, *Salmo salar* L., and their relationship to growth and maturation cycle. J. Fish Biol., 20:105-119.

Jaffa, M., 1994. Ritson report on Europe's salmon market has no easy answers. Fish Farmer,17 (2, March/April): 11,54.

Johnston, C. E., 1983. Seasonal changes in gill (Na+K)-ATPase activity in Atlantic salmon retained in fresh water after smolting. Trans. American Fish. Soc., 112:720-724.

Johnston, C. E., and Eales, J. G., 1967. Purines in the integument of the Atlantic salmon (*Salmo salar*) during parr-smolt transformation. J. Fish. Res. Bd. Can., 24:953-964.

Johnston, C. E., and Eales, J. G., 1968. Influence of temperature and photoperiod on guanine and hypoxanthine levels in skin and scales of Atlantic salmon (*Salmo salar*) during parr-smolt transformation. J. Fish. Res. Bd. Can., 25:1901-1909.

Johnston, C. E., and Eales, J. G., 1970. Influence of body size on silvering of Atlantic salmon (*Salmo salar*) at parr-smolt transformation. J. Fish. Res. Bd. Can., 27:983-987.

Johnston, C. E., Farmer, S. R., Gray, R. W., and Hambrook, M., 1990. Reconditioning and reproductive responces of Atlantic salmon kelts (*Salmo salar*) to photoperiod and temperature manipulation. Can, J. Fish. Aqat. Sci., 47:701-910.

Johnston, C. E., Hambrook, M., Gray, R. W., and Davidson, K. G., 1992. Manipulation of reproductive functionin Atlantic salmon (*Salmo salar*) kelts with controlled photoperiod and temperature. Can, J. Fish. Aqat. Sci., 49:2055-2061.

Johnston, R., 1992. Production and performance of triploid Atlantic salmon in Scotland. Scottish Aquaculture Research Report. The Scottish Office Agriculture and Fisheries Department., 2: 1-10.

Jones, J., and Bromage, N., 1987. The influence of ration size on the reproductive performance of female rainbow trout. In: D. Idler, L. Crim and J. Walsh (Editors), Reproductive Physiology of Fish 1987, Marine Science Laboratory, St johns, newfoundland. pp.

Knutsson, S., and Grav, T., 1976. Seawater adaptation in Atlantic salmon (*Salmo salar* L.) at different experimental temperatures and photoperiods. Aquaculture, 8:169-187.

Komourdjian, M. P., Saunders, R. L., and Fenwick, J. C., 1976. Evidence for the role of growth hormone as a part of a 'light-pituitary axis' in the growth and smoltification of Atlantic salmon (*Salmo salar*). Can. J. Zool., 54:544-551.

Koss, D. R., 1994. The effects of temperature and first feeding on egg and fry development in Atlantic salmo, *Salmo salar* L. PhD Thesis. University of Stirling, UK.

Krakenes, R., Hansen, T., Stefansson, S. O., and Taranger, G. L., 1991. Continuous light increases growth rate of Atlantic salmon (*Salmo salar* L.) postsmolts in sea cages. Aquaculture, 95:281-287.

Kristinsson, J. B., Saunders, R. L., and Wiggs, A. J., 1985. Growth dynamics during the development of bimodal length-frequency distribution in juvenile Atlantic salmon (*Salmo salar* L.). Aquaculture, 45:1-20.

Kurokawa, T., 1990. Influence of the date and body size at smoltification and subsequent growth rate and photoperiod on desmoltification in underyaerling masu salmon (*Oncorhynchus masou*). Aquaculture, 86:209-218.

Kutty, M. N. a. S., R.L., 1973. Swimming performance of young Atlantic salmon (*Salmo salar*) as affected by reduced ambient oxygen concentration. J. Fish Res. Bd. Can., 30:223-227.

Langdon, J. S., 1983. Development physiology of gill choride cells in juvenile Atlantic salmon *Salmo salar*. PhD Thesis. University of Stirling, UK.

Langdon, J. S., 1985. Smolification phsiology in the culture of salmonids. In: J. F. Muir and R. J. Roberts (Editors), Recent Advances in Aqaculture. 2, pp. 79-118.

Langdon, J. S., and Thorpe, J. E., 1985. The ontogeny of smoltification: developmental patterns of gill Na+/K+-ATPase, SDH, and choride cells in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture, 45:83-95.

Lubin, R. T., Rourke, A. W., and Saunders, R. L., 1991. Influence of photoperiod on the number and ultrastructure of gill chloride cells of the Atlantic salmon (*Salmo salar*) before and during smoltification. Can. J. Fish. Aquat. Sci., 48:1302-1307.

Lundqvist, H., Borg, B., and Berglund, I., 1989. Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). Can. J. Zool., 67:1733-1736.

Lundqvist, H., and Fridberg, G., 1982. Sexual maturation versus immaturity: different tactics with adaptive values in Baltic salmon (*Salmo salar* L.) male smolts. Can. J. Zool., 60:1822-1827.

Mayer, I., Burg, G., and Plisetskaya, L. M., 1994. Plasma-levels of insulin and liver-glycogen contents in one-year and two-year old Atlantic salmon (*Salmo salar* L) during the period of parr-smolt transformation. Fish Physio. Biochem., 13:191-197.

McCartney, T. H., 1976. Sodium-potassium dependent adenosine triphosphatase activity in gills and kidneys of atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol., 53A:351-353.

McCormick, S. D., Saunders, R. L., Henderson, E. B., and Harmon, P., 1987. Photoperiod cotrol of parr-smolt transformation in Atlantic salmon (*Salmo salar*): Changes in salinity tolerance, gill Na+, K+ -ATPase activity and plasma thyroid hormones. Can. J. Fish. Aquat. Sci., 44:1462-1468.

McCormick, S.D., Moyes, C.D. and Ballantyne, J.S., 1989. Influence of salinity on the energies of gill and kidney of Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem., 6(4):243-254.

Metcalfe, N. B., and Thorpe, J. E., 1990. Determinants of geographical variation in the age of seaward-migrating salmon, *Salmo salar*. J. Animal Ecol., 59:135-145.

Metcalfe, N. B., and Thorpe, J. E., 1992. Anorexia and defended energy levels in over-wintering juvenile salmon. J. Animal Ecol., 61:175-181.

Morrison, J. K., and Smith, C. E., 1986. Altering the spawning of rainbow trout by manipulating water temperature. Prog. Fish Cult., 48:52-54.

Myers, R. A., 1986. Game theory and the evolution of Atlantic salmon (*Salmo salar*) age at maturity. In: D. J. Meerburg (Editor), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 53-61.

Naevdal, G., 1983. Genetic factors in connection with age at maturation. Aquaculture, 33:97-106.

Nakari, T., Soivio, A., and Pesonen, S., 1987. Effects of an advanced photoperiod cycle on the gonadal development and spawning time of 2-year-old *Salmo gairdneri* R. reared in earth ponds under extreme annual water temperatures. Aquaculture, 67:369-384.

Nicieza, A. G., Brana, F. and Toledo, M.M., 1991. Development of length-bimodality and smolting in wild stocks of Atlantic salmon, *Salmo salar* L., under different growth conditions. J. Fish Biol., 38:509-523.

Okumoto, N., Ikuta, K., Aida, K., and Hirano, T., 1989. Effects of photoperiod on smolting and hormonal secretion in masu salmon. *Oncorhynchus masou*. Aquaculture, 82:63-76.

Patino, R., and Schreck, C. B., 1986. Sexual dimorphism of plasma sex steroid levels in juvenile coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol., 61:127-133.

Peterson, R. A., Spinney, H. C. E., and Sreedharan, A., 1977. Development of Atlantic salmon eggs and alevins under varied temperature regimes. J. Fish. Res. Board Can., 34:33-43.

Pickering, A. D., and Duston, J., 1983. Administration of cortisol to brown trout, *Salmo trutta* L., and its effect on the susceptability to sprolegnia infection and furunculosis. J. Fish Biol., 23:163-175.

Power, G., 1986. Physical influences on age at maturity of Atlantic salmon (*Salmo Salar*): A synthesis of ideas and questions. In: D. J. Meerburg (Editor), Salmonid Age at Maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 97-101.

Prunet, P., and Boeuf, G., 1985. Plasma prolactin levels during transfer of rainbow trout (*Salmo gairdneri*) and Atlantic salmon (*Salmo salar*) from fresh water to sea water. Aquaculture, 45:167-176.

Prunet, P., and Boeuf, G., 1989. Plasma prolactin levels during smolting in Atlantic salmon, *Salmo salar*. Aquaculture, 82:297-306.

Randall, C. F., 1992. Photoperiodic control of reproduction and patterns of melatonin secretion in the rainbow trout, *Oncorhyhchus mykiss*. PhD Thesis. University of Stirling, UK.

Randall, R. G., 1989. Effect of sea age on reproductive potential of Atlantic salmon (*Salmo salar*) in eastern Canada. Can. J Fish. Aquat. Sci., 46:2210-2218.

Ritson, C., 1994. Causes of instability in salmon prices. Fish Farmer,17 (3, May/June): 8-9.

Ritter, J. A., Farmer, G. J., Misra, R. K., Goff, T. R., Bailey, J. K., and Baum, E. T., 1986. Parental influences and smolt size and sex ratio effects on sea age at first maturity of Atlantic salmon (*Salmo salar*). In: D. J. Meerburg (Editors), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 30-38.

Rowe, D. K., and Thorpe, J. E., 1990. Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reduction in feeding and growth during spring months. Aquaculture, 86:291-313.

Saunders, R. L., 1965. Adjustment of buoyancy in young Atlantic salmon and brook trout by changes in swimbladder volumn. J. Fish. Res. Bd. Can., 22:335-352.

Saunders, R. L., 1986. The scientific management implications of age and size at sexual maturity in Atlantic salmon (Salmo salar). In: D. J. Meerburg (Editor), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89, pp. 3-6.

Saunders, R. L., 1994. Special Issue-Salmonid Smoltification .4. Prologue and Acknowledgements. Aquaculture, 121:R9-R10.

Saunders, R. L., and Harmon, P. R., 1990. Influence of photoperiod on growth of juvenile Atlantic salmon and development of salinity tolerance during winter-spring. Trans. American Fish. Society, 119:689-697.

Saunders, R. L., Harmone, P. R., and Knox, *D. E., 1994. Smolt development* and subsequent sexual maturity in previously mature male Atlantic salmon (*Salmo salar*). Aquaculture, 121:79-93.

Saunders, R. L., and Henderson, E. B., 1965. Precocious sexual development in male post-smolt Atlantic salmon reared in the laboratory. J. Fish Res. Bd. Canada, 22:1567-1570.

Saunders, R. L., and Henderson, E. B., 1970. Influence of photoperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada, 27:1295-1311.

Saunders, R. L., and Henderson, E. B., 1978. Changes in gill ATPase activity and smolt status of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can., 35:1542-1546.

Saunders, R. L., Henderson, E. B., and Glebe, B. D., 1982. Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture, 28:211-229.

Saunders, R. L., Henderson, E. B., Glebe, B. D., and Loudenslager, E. J., 1983. Evidence of a major environmental component in determination of the grilse:larger salmon ratio in Atlantic salmon (*Salmo salar*). Aquaculture, 33:107-118.

Saunders, R. L., Henderson, E. B., and Harmon, P. R., 1985. Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. Aquaculture, 45:55-66.

Saunders, R. L., and Schom., C. B., 1985. Importance of the Variation in life history parameters of the Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 42:615-618.

Saunders, R. L., Specker, J. L., and Komourdjian, M. P., 1989. Effects of photoperiod on growth and smolting in juvenile Atlantic salmon (*Salmo salar*). Aquaculture, 82:103-117.

Schaffer, W. M., and Elson, P. F., 1975. The adaptive significance of variations in life history among local populations of Atlantic salmon in North America. Ecology, 56:577-590.

Schmitz, M., Berglund, I., Lundqvist, H., and Bjornsson, B. T., 1994. Growth hormone responce to seawater challenge in Atlantic salmon (*Salmo salar* L.). Aquaculture, 121:209-222.

Schreck, C., Maule, A. G., and Kaattari, S. L., 1993. Stress and disease resistance. Blackwell Scientific Publications, London, pp.

Sedgwick, S. D., 1990. Who is buying farmed salmon, and why ? Fish Farmer,13 (2, March/April): 10,21.

Shapiro, S. S., and Wilk, M. B., 1965. An analysis of variance test for normality (complete samples). Biometrika, 52:591-611.

Sheridan, M. A., 1989. Alterations in lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture, 82:191-203.

Sigholt, T., Jarvi, T., and Lofthus, R., 1989. The effect of constant 12-hour light and simulated natural light on growth, cardiac-somatic index and smolting in the Atlantic salmon (*Salmo salar*). Aquaculture, 82:127-136.

Sigholt, T., Stautnes, M., Jakobsen, H. J., and Asgard, T., 1995. Effects of continuous light and short-day photoperiod on smolting, seawater survival and growth in Atlantic salmon (*Salmo salar*). Aquaculture, 130:373-388.

Skilbrei, O. T., 1991. Importance of threshold length and photoperiod for the development of bimodal length-frequency distribution in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 48:2163-2172.

Snedecor, G. W., and Cochran, W. G., 1980. Statistical Methods. Iowa State University Press, Ames.

Soivio, A., Muona, M., and Virtanen, E., 1989. Temperature and daylength as regulators of smolting in cultured Baltic salmon, *Salmo salar* L. Aquaculture, 82:137-146.

Solbakken, V. A., Hansen, T., and Stefansson, S. O., 1994. Effects of photoperiod and temperature on growth and parr-smolt transformation in Atlantic salmon (*Salmo salar* L.) and subsequent performance in seawater. Aquaculture, 121:13-28.

Specker, J. L., Whitesel, T. A., Parker, S. J., and Saunders, R. L., 1989. Thyroidal responce of Atlantic salmon to seawater challenge: predictor of growth in seawater. Aquaculture, 82:307-318.

Springate, J., Bromage, N., and Cumaranatunga, R., 1985. The effects of different rations on fecundity and egg quality in the rainbow trout (*Salmo gairdneri*). In: C. B. Cowey, A. M. Mackie and J. A. Bell (Editors), Nutrition and Feeding in Fish. Academic Press, London, pp. 371-391.

Stefansson, S. O., Bjornsson, B. T., Hansen, T., Haux, C., Taranger, G. L., and Saunders, R. L., 1991. Growth, parr-smolt transformation, and changes in the growth hormone of Atlantic salmon (Salmo salar) reared under different photoperiods. Can. J. Fish Aquat. Sci., 48:2100-2108.

Stefansson, S. O., and Hansen, T. J., 1989. The effect of spectral composition on growth and smolting in Atlantic salmon (*Salmo salar*) and subsequent growth in sea cages. Aquaculture, 82:155-162.

Stefansson, S. O., Hanson, T., and Taranger, G. L., 1990. The influence of light on growth and smolting of Atlantic salmon (*Salmo salar*): effects of spectral composition, intensity and photoperiod. In: Canada-Norway Finfish Aqua. Workshop 11-14 Sept. 89, Can. Tech. Report Fish. Aqu. Sci., 1761, 79-84 pp.

Sutherland, R., 1990. Cobwebs and cycles in the salmon market ? Scottish Agricultural Economics Review., 5:99-107.

Sutterlin, A. M., Harmon, P., and Young, B., 1978. Precocious sexual materation in Atlantic salmon (*Salmo salar*) postsmolts reared in a seawater impoundment. J. Fish Res. Bd. Canada, 35:1269-1272.

Sutterlin, A. M., and MacLean, D., 1984. Age at first Maturity and the early expression of oocyte recruitment processes in two forms of Atlantic salmon (*Salmo salar*) and their hybrids. Can. J. Fish. Aquat. Sci., 41:1139-1149.

Sutterlin, A. M., and MacLean, D., 1984. Age at first maturity and the early expression of oocyte recruitment processes in two forms of Atlantic salmon (*Salmo salar*) and their hybrids. Can. J. Fish. Aquat. Sci., 41:1139-1149.

Sweeting, R. M., G.F., W., and McKeown, B. A., 1985. Changes in plasma glucose, amino acid nitrogen and growth during smoltification and sea water adaptation in coho salmon, *Oncorhynchus kisutch*. Aquaculture, 45:185-197.

Sweeting, R. M., and McKeown, B. A., 1989. Changes in plasma growth hormone and various metabolic factors during smoltification of coho salmon, *Oncorhynchus kisutch*. Aquaculture, 82:279-295.

Taranger, G. L., 1993. Sexual maturation in Atlantic salmon, *Salmo salar* L.; aspects of environmental and hormonal control. *PhD Thesis. University of Bergen, Norway.*

Taranger, G. L., and Hansen, T., 1993. Ovulation and egg survival following exposure of Atlantic salmon, *Salmo salar* L., broodstock to different water temperatures. Aquaculture Fish. Mangement, 24:151-156.

The Scottish Office, Agriculture, Environment and Fisheries Department., 1988-1995. Scottish fish farms annual production survey. Marine Laboratory Aberdeen.

Thorpe, J. E., 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. J. Fish Biol., 11:175-184.

Thorpe, J. E., 1986. Age at first maturity in Atlantic salmon, *Salmo salar*: Freshwater period influences and conflicts with smolting. In: D. J. Meerburg (Editor), Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci., 89,, pp. 7-14.

Thorpe, J. E., 1987. Environmental regulation of growth patterns in juvenile Atlantic salmon. In: R. C. Summerfelt and G. E. Hall (Editors), Age and growth of fish. The Iowa State University Press.

Thorpe, J. E., 1989. Developmental variation in salmonid populations. J. Fish Biol., 35 (sup. A):295-303.

Thorpe, J. E., 1994. An alternative view of smolting in salmonids. Aquaculture, 121:105-113.

Thorpe, J. E., Adams, C. E., Miles, M. S., and Keay, D. S., 1989. Some influences of photoperiod and temperature on opportunity for growth in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture, 82:119-126.

Thorpe, J. E., Miles, M. S., and Keay, D. S., 1984. Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture, 43:123-132.

Thorpe, J. E., and Morgan, R. I. G., 1978. Periodicity in atlantic salmon (*Salmo salar*) smolt migration. J. Fish Biol., 12::541-548.

Thorpe, J. E., and Morgan, R. I. G., 1980. Growth-rate and smolting-rate of progeny of male Atlantic salmon parr, (*Salmo salar*). J. Fish. Biol., 17:451-459.

Thorpe, J. E., Morgan, R. I. G., and Miles, M. S., 1983. Inheritance of developmental rates in Atlantic salmon, *Salmo salar* L. Aquaculture, 33:119-128.

Thorpe, J. E., Morgan, R. I. G., Ottaway, E. M., and Miles, M. S., 1980. Time of divergence of growth groups between potential 1+ and 2+ smolts amonge sibling Atlantic salmon. J. Fish Biol., 17:13-21.

Thorpe, J. E., Talbot, C., Miles, M. S., and Keay, D. S., 1990. Control of maturation in cultured Atlantic salmon, *Salmo salar*, in pumped seawater tanks, by restricting food intake. Aquaculture, 86:315-326.

Thorpe, J. E., Talbot, C., and Villarreal, C., 1982. Bimodality of growth and smolting in Atlantic salmon, *Salmo salar* L. Aquaculture, 28:123-132.

Thrush, M. A., 1994. Photoperiodic control of smoltification and aspects of broodstock management in Atlantic salmon, *Salmo salar*. PhD Thesis. University of Stirling, UK.

Thrush, M. A., Duncan, N. J., and Bromage, N. R., 1994. The use of photoperiod in the production of out-of-season Atlantic salmon (*Salmo salar*) smolts. Aquaculture, 121:29-44.

Titarev, F., 1975. Acceleration of maturation in the rainbow trout (*Salmo gairdneri*) under the influence of increased water temperature. J. Ichthyol., 15:507-509.

Trepanier, S., Rodriguez, M. A., and Magnan, P., 1996. Spawning migrations in landlocked Atlantic salmon: time series modelling of river discharge and water tempearture effects. J. Fish Biol., 48:925-936.

Tyler, C., Sumpter, J. P., and Bromage, N., 1987. Uptake of vitellogenin into cultured ovarian follicals of rainbow trout. In: D. R. Idler, L. W. Crim and J. M. Walsh (Editors), 3rd International Symposium on the Reproductive Physiology of Fish, Memorial University of Newfoundland, St John's, Newfoundland. 221 pp.

Veillette, P. A., Sundell, K., and Specker, J. L., 1995. Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr smolt transformation. Gen. Comp. Endo., 97:250-258.

Veillette, P. A., White, R. J., and Specker, J. L., 1993. Changes in intestinal fluid transport in Atlantic salmon (*Salmo salar* L.) during parr-smolt transformation. Fish Physiol. Biochem., 12:193-202.

Villarreal, C. A., and Thorpe, J. E., 1985. Gonadal growth and bimodality of length frequency distribution in juvenile Atlantic salmon (*Salmo salar*). Aquaculture, 45:265-288.

Villarreal, C. A., Thorpe, J. E., and Miles, M. S., 1988. Influence of photoperiod on growth changes in juvenile Atlantic salmon, *Salmo salar* L. J. Fish Biol., 33:15-30.

Virtanen, E., and Soivio, A., 1985. The patterns of T3, T4, cortisol and Na+-K+-ATPase during smoltification of hatchery-reared *Salmo salar* and comparison with wild smolts. Aquaculture, 45:97-109.

Von Bayer, 1950. A method of measuring fish eggs. Prog. Fish Cult., 2:105-107.

Wagner, G. F., and McKeown, B. A., 1985. Cyclical growth in juvenile rainbow trout, *Salmo gairdneri*. Can. J. Zool., 63:2473-2474.

Wagner, H. H., 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool., 52: 219-234.

Wankowski, J. W. J., and Thorpe, J. E., 1979. Spatial distribution and feeding in atlantic salmon, *Salmo salar* L. juveniles. J. Fish Biol., 14: 239-247.

Wedemeyer, G. A., Saunders, R. L., and Clarke, W. C., 1980. Environmental Factors affecting smoltification and early marine survival of anadromous salmoids. Marine fisheries review., 42:1-15.

Whitehead, C., 1979. The control of reproduction in rainbow trout-commercial and technical aspects. PhD Thesis. University of Aston, Birmingham, UK.

Whitehead, C., and Bromage, N. R., 1980. Effects of constant long- and short-day photoperiods on the reproductive physiology and spawning time in rainbow trout. J. Endocrinol., 87:6p-7p (abstract).

Whitehead, C., Bromage, N. R., Forester, J., and Matty, A., 1978. The effects of alterations in photoperiod on the ovarian development and spawning time in the rainbow trout (*Salmo gairdneri*). Ann. Biol. Anim. Biochem. Biophys., 18:1035-1043.

Winans, G. A., and Nishioka, R. S., 1987. A multivarite description of change in body shape of coho salmon (*Oncorhynchus kisutch*) during smoltification. Aquaculture, 66:235-245.

Woo, N. Y. S., Bern, H. A., and Nishioka, R. S., 1978. Changes in body composition associated with smoltification and premature transfer to seawater in coho salmon (*Oncorhynchus kisutch*). J. Fish Biol., 13:421-428.

Zar, J. H., 1984. Biostatisical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ.

Zaugg, W. S., and H.H., W., 1973. Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comp. Biochem. Physiol., 45B:955-965.

Appendix A.

Definitions of photoperiod terminology used in this thesis.

A photoperiod is the daily change *in the number of hours of daylight.* A natural photoperiod is the daily change in the number of hours the sun is visible. Over the year a natural photoperiod, if plotted *(hours of daylight against time)* forms a sine or cosine wave. Certain terms are used to describe sine and cosine waves, the wavelength is the distance from crest to crest or trough to trough and the amplitude is half the vertical distance from the trough to the crest i.e. half the height of the wave.

The wavelength of a natural photoperiod is the time from one summer solstice to the next summer solstice or from one winter solstice to the next winter solstice. All natural photoperiods have the same wavelength, which is 365.25 days. The amplitude of a natural photoperiod is half the time difference, between the daylength of the summer solstice and the daylength of the winter solstice. The amplitude of a natural photoperiod is dependant on the latitude at which the photoperiod is experienced. The latitudes further from the equator have a greater amplitude, for example, the amplitude of the photoperiod experienced in London (51°30'N) is 4 hours 25 minutes while in Glasgow (55°52'N) it is 5 hours and 18 minutes. Under experimental condition the natural photoperiod is often simulated using artificial illumination, the amplitude of a simulated natural photoperiod (SNP) can be altered to give the natural photoperiod for a particular latitude.

Figure 1.

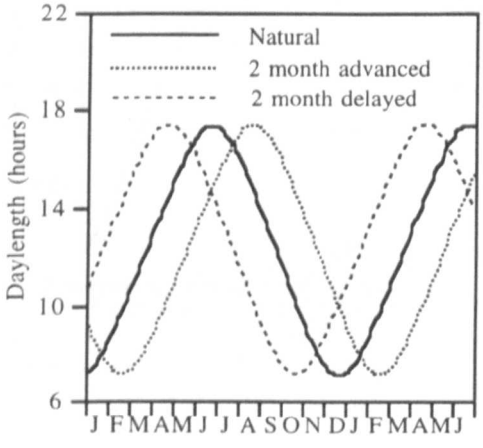
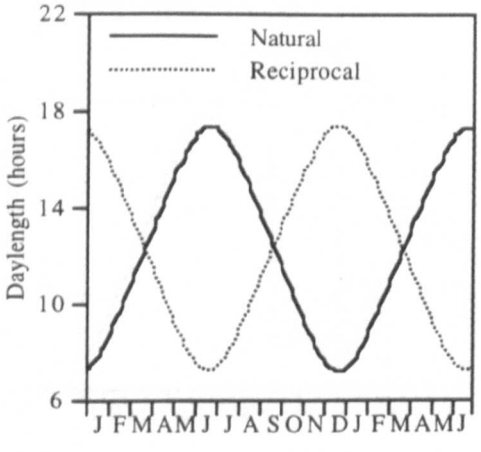


Figure 2.

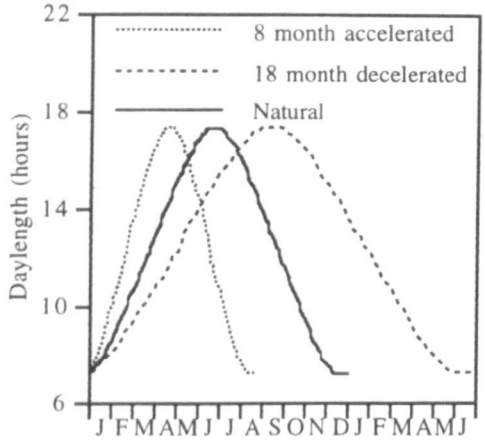


Departures from the natural photoperiod are termed artificial photoperiods. Experimental fish have been subjected to wide range of different artificial photoperiods. The different types of artificial photoperiods are described and illustrated here. All the natural photoperiods illustrated are for 54°59' north, the artificial photoperiods are based on the same natural photoperiod (54°59'N).

An advanced or delayed photoperiod has the same wavelength and amplitude as the natural photoperiod it is based on, the natural photoperiod is shifted forward, advanced, or shifted back, delayed. For example in figure 1 the natural photoperiod has been advanced and delayed by 2 months. The advance of 2 months causes the summer solstice to fall on the 21st August instead of the 21st June, whilst the delay of 2 months causes the summer solstice to fall on the 21st April.

A special case of the advanced or delayed photoperiod is the reciprocal photoperiod, this is the mirror image of the natural photoperiod and equates to a 6 month advanced (or delayed) photoperiod (see figure 2).

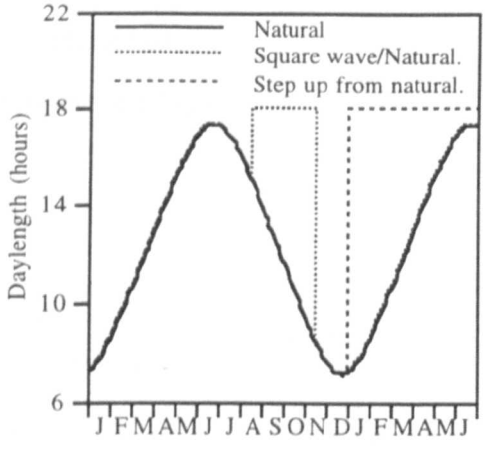
Figure 3.



An accelerated or decelerated photoperiod has the same amplitude as the natural photoperiod on which it is based, but the wavelength is reduced in the case of an accelerated photoperiod, or extended in the case of a decelerated photoperiod. Figure 3 shows an accelerated photoperiod with a wavelength of 8 months (an 8 month accelerated photoperiod) and a decelerated photoperiod with a wavelength of 18 months (a 18 month decelerated photoperiod).

A third type of artificial photoperiod is termed a square wave photoperiod. The square wave photoperiod, departs from the gradually changing daylength, associated with the natural photoperiod and links periods of constant daylength with a direct step from one daylength to another. The periods of constant daylength are termed LD (Light/Dark) followed by the number of light and dark hours, for example a 16 hour day or a 16 hour light period and 8 hour dark period is expressed LD 16:8. This notation is extended to a natural photoperiod which is termed LDN.

Figure 4.



A square wave photoperiod can be used in conjunction with a natural photoperiod or on its own. Figure 4. shows two square wave departures from the natural photoperiod. The dashed line shows a photoperiod where the natural photoperiod was followed until the 1st January when the daylength was stepped up to LD 18:6, the photoperiod was then held on LD 18:6. The dotted line, square wave/natural shows a square wave which has been applied during the Autumn of the natural photoperiod. On the 15th August the natural photoperiod was stepped up to LD 18:6, this was held for 3 months, on the 15th November the photoperiod was stepped down, back on to the natural photoperiod.

Figure 5.

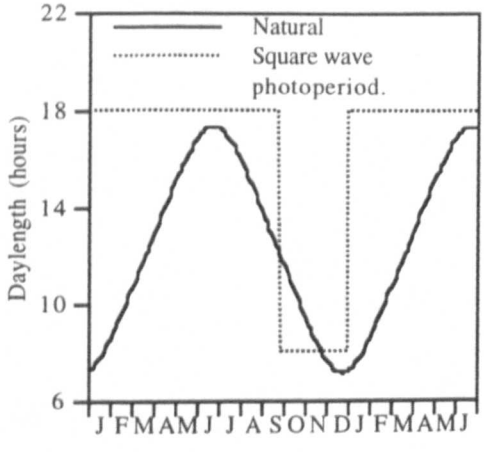


Figure 5 shows a square wave photoperiod, LD 18:6 was maintained from the start of the year until the 1st October when the LD 18:6 was stepped down to LD 8:16, this was maintained for 3 months, on the 1st January the LD 8:16 is stepped up to LD 18:6, where it was held.

Appendix B.

Glossary of common and latin names used in this thesis.

Common name	Latin name
Atlantic salmon	*Salmo salar.*
brown trout	*Salmo trutta.*
rainbow trout	*Oncorhynchus mykiss.*
steelhead trout	*Oncorhynchus mykiss.*
coho salmon	*Oncorhynchus kisutch.*
masu salmon	*Oncorhynchus masou.*
pink salmon	*Oncorhynchus gorbuscha*
chum salmon	*Oncorhynchus keta*
chinook salmon	*Oncorhynchus tshawytcha*
European starling	*Sturnus vulgaris.*

Appendix C.

Work presented in this thesis has been published in the following papers:

Thrush, M.A., Duncan, N.J., and Bromage, N.R. 1994. The use of photoperiod in the production of out-of-season Atlantic salmon (*Salmo salar*) smolts. Aquaculture, 121: 29-44.

Duncan, N.J., Thrush, M.A. and Bromage, N.R., 1994. Comparison of growth, seawater tolerance and mortality for Atlantic salmon (*Salmo salar*) parr and photoperiod smolts transferred to sea water in December. In: Proceeding of an International Fish Symposium, High Performance Fish., Mackinlay D.D. (Editor) July 16-21 1994, University of British Columbia, Vancouver, Canada.

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Aquaculture 121 (1994) 29–44

Aquaculture

The use of photoperiod in the production of out-of-season Atlantic salmon (*Salmo salar*) smolts

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Abstract

Accelerated seasonally-changing and "square-wave" (direct change from short to long day) photoperiods advanced the timing of potential S1 (1+) and S2 (2+) smolts by a maximum of 3 and 7 months respectively. Pre-smolts underwent body silvering, fin darkening and a decrease in condition factor in response to changes from short to long day-lengths. Seawater challenge (24 h, 28 ppt) tests showed these fish to develop strong hypo-osmoregulatory ability. Smolts were transferred to full strength seawater in small land-based facilities and to commercial on-growing sites. S1 groups, which were advanced by up to 3 months, gained a growth advantage from early seawater transfer that was maintained throughout their first year in seawater. Potential S2 smolts transferred in October–November grew well over their first winter. Advanced mixed sex S1s did not show any post-smolt maturation, but did show increased rates of maturation (up to 19%) after one winter at sea. Advanced S2 smolts showed an increased post-smolt maturation.

1. Introduction

Smoltification in juvenile Atlantic salmon is a seasonal occurrence which culminates in seaward migration during late spring (Langdon, 1985; Hoar, 1988). In culture this event must be completed before hatchery-reared smolts can be transferred to seawater on-growing sites, as their premature transfer may result in greatly reduced growth or even death (Clarke and Nagahama, 1977). At present, the restricted temporal availability of smolts leads to a seasonal supply of marketable salmon and as a consequence an unstable price.

The synchronous onset of smoltification in a population is mediated by envi-

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ronmental cues, predominantly photoperiod (Baggerman, 1960; Clarke, 1989), whereby daylength changes play a key role in the entrainment of an endogenous circannual rhythm which is thought to control the development of this event (Conte and Wagner, 1965; Eriksson and Lundqvist, 1982; Duston and Saunders, 1990). The timing of smoltification as well as maturation in salmonids has been significantly altered by modifying daylength changes (Komourdjian et al., 1976; Clarke et al., 1985; Saunders et al., 1985; Bromage and Duston, 1986; McCormick et al., 1987; Duston and Saunders, 1992). However, although the application of artificial photoperiod regimes has provided trout producers with valuable supplies of out-of-season eggs (Bromage and Cumaranatunga, 1988; Bromage et al., 1992), this technique has not been readily accepted to benefit the salmon industries with a similar flexibility in smolt production.

The reluctance of farmers to adopt such an approach for salmon culture stems from two questions: (a) how does photoperiod manipulation affect survival and growth following seawater transfer? and (b) what are the effects on maturation as post-smolt and grilse? Both questions have profound implications on the commercial viability of such methods. This paper summarises work pursued over a number of years, in collaboration with commercial smolt producers and on-growers, with the aim of using photoperiod manipulation to achieve a "year-round" supply of Atlantic salmon smolts.

2. Materials and methods

All trials were conducted at one of two commercial UK smolt units, situated at latitudes 52°N and 55°N, supplied by spring or borehole waters of constant temperature (10 ± 0.5°C). Experimental groups of 1000–40 000 parr (mixed stock origin) were reared from hatch and fed commercially pelleted dry diets at rates according to manufacturers' tables (B.P. Nutrition UK Ltd.). The fish were maintained in either 1000-l glassfibre tanks contained in light-proof boxes; or 7000-l concrete raceways housed in a light-proof shed. Light was provided by standard 60-W and 100-W tungsten filament bulbs providing 300–450 lx and 10–20 lx at the water surface in tanks and raceways respectively. Daylengths were controlled by 24 h time clocks, which were adjusted at weekly intervals where seasonally-changing photoperiods were required.

Length and weight measurements, from samples of 100 fish, were made at bimonthly intervals in all groups prior to smoltification and condition factors calculated as $(\text{weight (g)} \cdot 100)/\text{length (cm)}^3$. The development of hypo-osmoregulatory ability was assessed by determination of either serum sodium or serum osmolality following 24 h (28 ppt) seawater challenge with at least 10 fish per treatment group (Clarke and Blackburn, 1978). Following seawater challenge, serum osmolalities or sodium concentrations close to that of unchallenged smolts (330 mOsmol kg^{-1} or 155 mmol l^{-1} respectively), in conjunction with the development of body silvering, fin darkening and decreases in condition factor, were

considered to be good indications that the fish had achieved smolt status and transfers to seawater were made accordingly.

2.1. Potential S1 smolts (under-yearling parr)

The effect of photoperiod treatment on the timing of parr–smolt transformation in under-yearling fish was investigated in a series of trials. See Figs. 1a–4a.

Trial 1

Parr were grown from hatch on an extended daylength (LD20:4) until 22 October. The photoperiod was then decreased by 1 h each week until 27 November when the fish were transferred to a simulated natural photoperiod (Fig. 1a).

Trial 2

Parr were reared under the same light regime as those in trial 1 until 12 November, after which they were subjected to a 2-h per week decrease in daylength. The daylength was held at LD8:16 from 10 December to 14 January before being changed directly to a long day (LD18:6) (Fig. 2a).

Trial 3

Parr were reared on an extended daylength (LD:20:4) until mid-July. The fish were then graded and the largest fraction (those over 1.8 g) placed on a light cycle approximating to a 6 month seasonally compressed photoperiod until 12 December, after which they were held on a constant long day (LD18:6) (Fig. 3a).

Trial 4

Two batches of medium grade under-yearling parr (including both upper and lower mode fish with mean weights of 5.5 g and 13.2 g) were transferred from a short day (LD7.25:16.75) directly to a long day (LD16.75:7.25) on 22 December and 21 January (Fig. 4a) to provide groups A and B respectively.

2.2. Potential S2 smolts (1+ year-old parr)

Fish used in these experiments were drawn from the S2 (lower mode) fractions graded from farm stock between November and December of their first year. These were reared from hatch on ambient photoperiods in trials 5 and 6. Fish used in trials 7 and 8 were hatched on an extended day (LD20:4) on which they were maintained until mid-July and early-November respectively before being returned gradually to a short day (LD8:16) by late December (winter solstice). Figs. 5a–8a show the experimental photoperiods under which the fish were reared in their second year.

Trial 5

Two groups of mixed sex fish were transferred to compressed seasonal 6-month and 10-month photoperiods respectively on 18 December (Fig. 5a).

Trial 6

A group of diploid and triploid all-female parr were transferred from an ambient daylength (LD14:10) to LD18:6 on 23 April and then subjected to a photoperiod approximating a 6-month compressed seasonal cycle which was held at a long day (LD18:6) from 25 October (Fig. 6a).

Trial 7

A group of parr which had been reared on a photoperiod identical to that in trial 2 was held on a long day (LD18:6) from 14 January until 29 May, after which they were subjected to a photoperiod approximating a 6-month compressed seasonal cycle until 23 September after which they were held on a constant long day (LD20:4) (Fig. 7a).

Trial 8

A group of mixed sex fish was transferred from an ambient daylength (LD8:16) to LD13:11 on 23 January and reared under a photoperiod approximating a 5-month compressed seasonal cycle (Fig. 8a).

To assess their survival and growth in a marine environment, fish reared in these trials were transferred to seawater and grown under ambient conditions of photoperiod and temperature. A number of seawater sites was used in the trials, ranging from small on-shore tanks to commercial size sea cages holding groups of up to 20 000 fish. A summary of the facilities used is provided in Table 1. The survival of the fish after seawater transfer was recorded and their growth monitored.

Data presented for condition factor, serum sodium/osmolality, and fish weight represent sample means ± 1 standard error of the mean (1 s.e.m.). Sample distributions were tested for normality by an adaptation of the Shapiro–Wilk test

Table 1
Summary of rearing conditions at the seawater facilities used for on-growing smolts

Sea site	Location	Holding facility	Number of smolts transferred	Salinity[a] (ppt)	Annual temperature range (°C)
A	West Scotland (Latitude 57°N)	15-m cages	15–20 000	32	7–14
B	Southern England (Latitude 52°N)	20-m cages	2500[b]	35–37	4–18
C	Southern England (Latitude 52°N)	300-l on-shore tanks	100–150	35	2–24
D	West Wales (Latitude 53°N)	5-m cages	850–1000	28–34(32)	4–19
E	West Scotland (Latitude 56°N)	7000-l on-shore tanks	500	25–35(33)	7–15

[a]Values in brackets indicate prevailing salinity.
[b]Incomplete transfer (trial 3 only).

(Shapiro and Wilk, 1965) and for homogeneity of variance by Bartlett's test (Snedecor and Cockran, 1980). Comparisons between means where assumptions of normality and homogeneity were satisfied were provided by one-way analysis of variance (ANOVA) followed by a parametric multiple comparisons *t*-test using the residual mean square from the ANOVA to provide a pooled estimate of the variance (Snedecor and Cockran, 1980). If sample distributions departed significantly from normality ($P<0.01$), or if variances were found to be significantly heterogeneous ($P<0.05$), comparisons were made using the Kruskal–Wallis test followed by Dunn's multiple range procedure (Zar, 1984). In some of the trials repeated sample weighing of individual fish once transferred to sea cages was not possible because the fish formed part of commercial stock.

3. Results

3.1. Potential S1 smolts

The development of morphological (condition factor) and physiological (hypo-osmoregulatory ability) smolt characteristics among different groups prior to seawater transfer is presented in Figs. 1b–4b. The times of peak smolt status and seawater transfer are indicated on the respective photoperiod Figs. 1a–4a, by downward facing arrows. The growth (weight) for groups monitored in seawater is also shown (Figs. 1c, 2c and 4c). Mortalities in the first month following seawater transfer and details of various maturation episodes are presented in Table 2.

Trial 1

Smolts with a mean weight of 43.4 g (± 0.78) were transferred to a commercial on-grower (site A, see Table 1) on 5 May. Prior to transfer, the fish had already developed, between the beginning of March and mid-April, a silver colouration, well-defined black fin margins and a significant increase ($P<0.001$) in hypo-osmoregulatory ability (Fig. 1b). However, condition factor in this stock remained high from January until mid-April, with a small, but significant ($P<0.001$) decrease during the 2 weeks before seawater transfer. Post-transfer mortality was high (13.9%) in the first 2 weeks, although surviving smolts grew well over the following 16 months reaching a mean weight of 2.5 kg. No post-smolt maturation was observed. Maturation after one sea-winter (grilse fraction) was 15.7%.

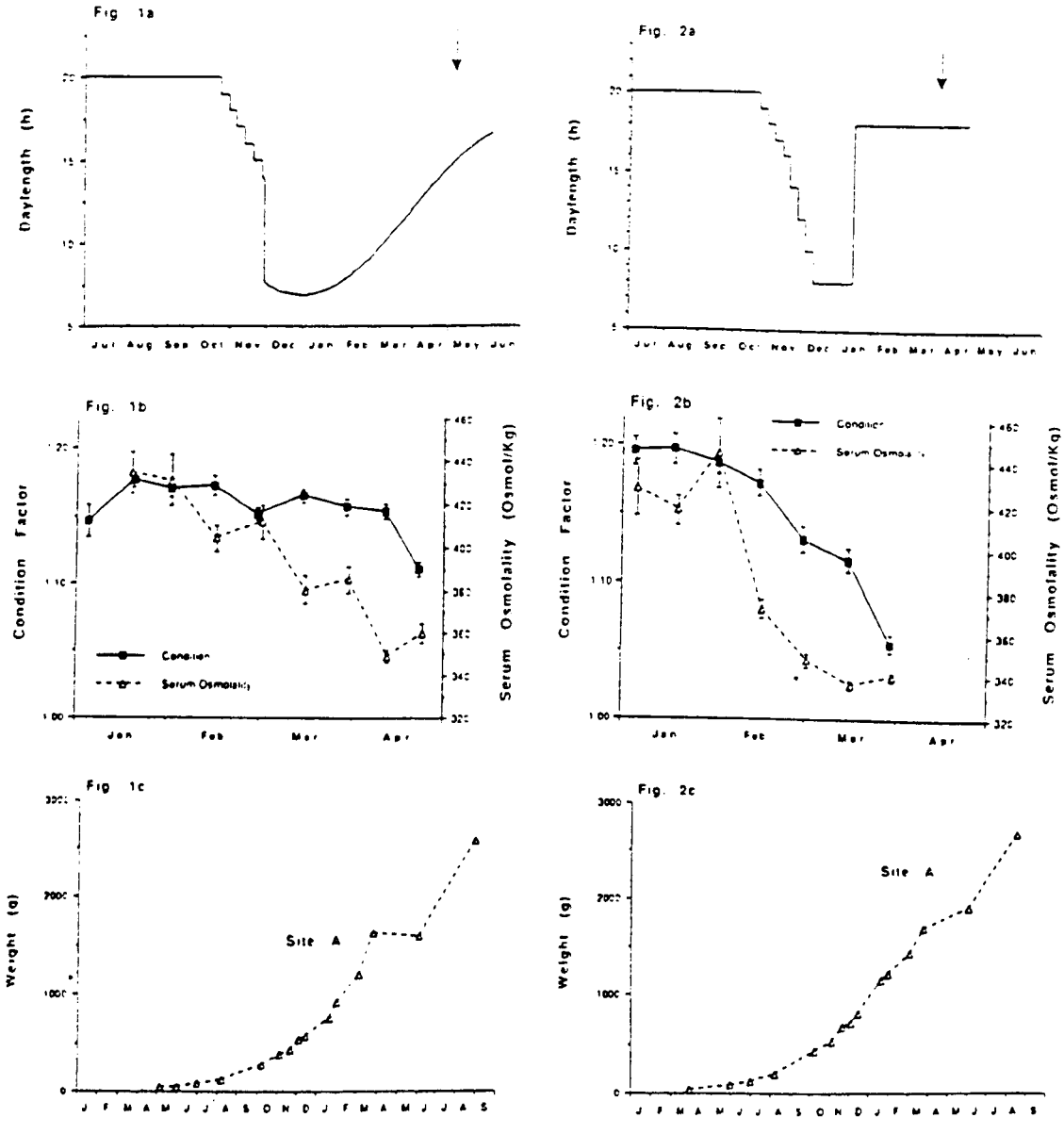
Trial 2

These fish showed a strong smolt morphology and were considered to have achieved smolt status at the end of March, when they were moved to site A. As pre-smolts they had shown a significant ($P<0.001$) increase in hypo-osmoregulatory ability between the beginning and end of February, which remained ele-

Table 2
Summary of dates of seawater transfer and seawater performance of smolts reared under different photoperiod regimes

Group		Date of seawater transfer	Advance in smoltification	Weight at transfer (g ± 1 s.e.m.)	Mortality after 1 month in seawater	First maturation episode	Second maturation episode
Potential S1 (1+) smolts							
Trial 1		5 May	no advance[a]	43.4(±0.78)	13.9%	0% (ps)	15.7% (1sw)
Trial 2		29 March	2 months	42.4(±0.93)	31.3%	0% (ps)	19% (1sw)
Trial 3		18 Feb	3 months	40.0(±0.87)	43%	–	–
Trial 4	(Group A)	28 March	7 weeks	31.0(±0.65)	3.0%	–	–
	(Group B)	5 April	5 weeks	30.5(±0.62)	–	–	–
Potential S2 (2+) smolts							
Trial 5	(6-month photoperiod)	3 Dec	5 months	35.0(±0.85)	< 1.0%	6.5% (1sw)	63% (2sw)
	(10-month photoperiod)	3 March	2 months	48.2(±0.98)	< 1.0%	24% (ps)	–
Trial 6	(Triploid)	21 Nov	6 months	44.0(±0.92)	1.5%	0% (1sw)	–
	(All-female)	15 Dec	5 months	48.3(±0.97)	1.7%	0% (1sw)	–
Trial 7	(Site E)	7 Nov	6 months	48.1(±1.18)	4.6%	5–12% (1sw)[b]	–
	(Site A)	3 Nov	6 months	33.2(±0.77)	44.9%	0% (1sw)	–
Trial 8		30 Sep	7 months	27.3(±0.87)	2.5%	0% (1sw)	–

[a]Smolts transferred prematurely.
[b]Maturation assessed by GSI data 3 months prior to expected spawning.
ps, post-smolt maturation (fish maturing the same year as seawater transfer); 1sw, maturation after 1 sea-winter (grilse); 2sw, maturation after 2 sea-winters.

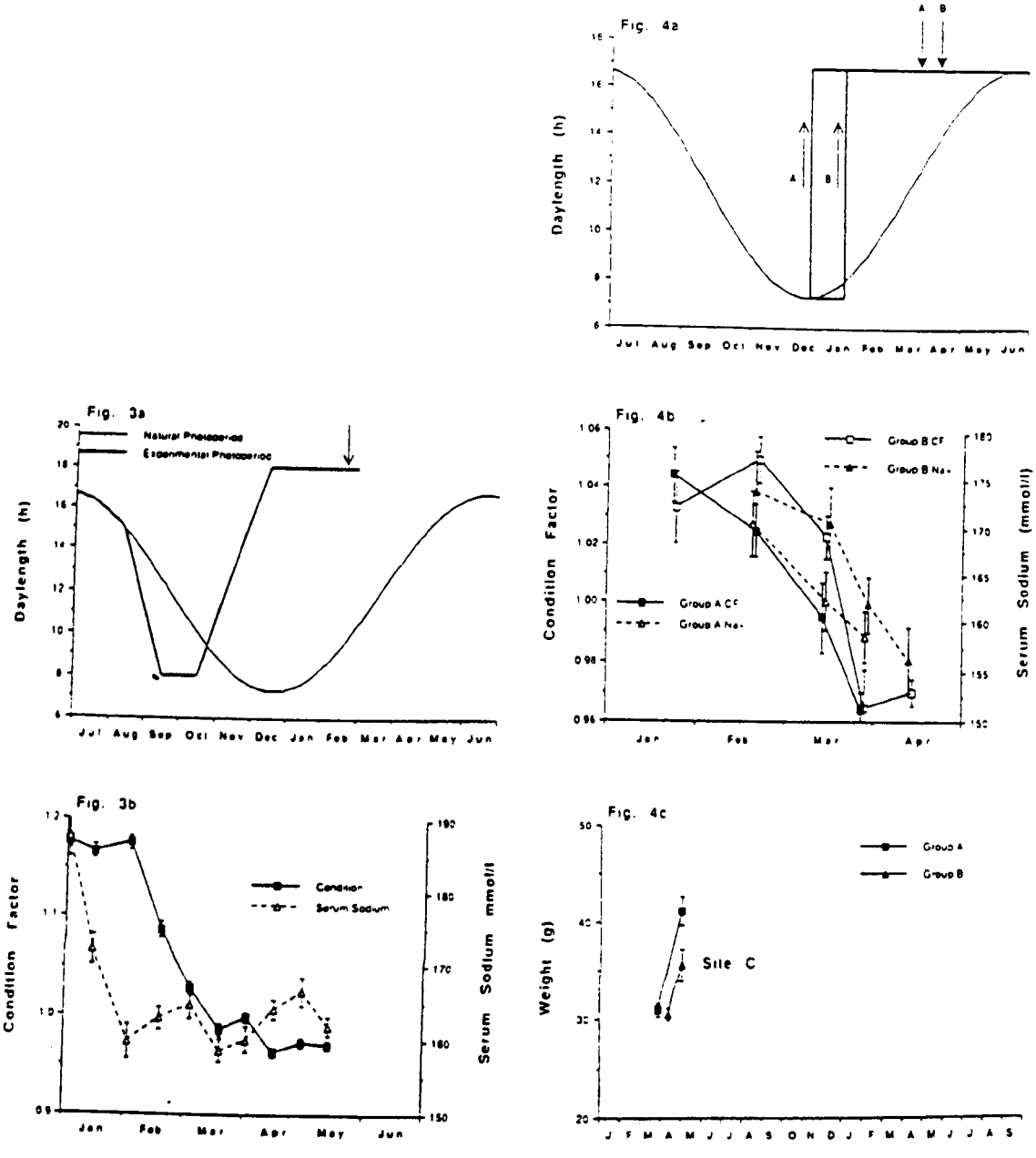


Figs. 1 and 2. Figs. 1a, 1b and 1c represent fish in trial 1; Figs. 2a, 2b and 2c represent fish in trial 2. Figs. 1a and 2a, photoperiod regime, downward facing arrows in the photoperiod figures indicate times of seawater transfer. Figs. 1b and 2b, the development of the smolt parameters: condition factor and challenged serum osmolarity (mean ±s.e.m.). Figs. 1c and 2c, seawater growth; open symbols (without error bars) represent mean weights determined by batch weighing.

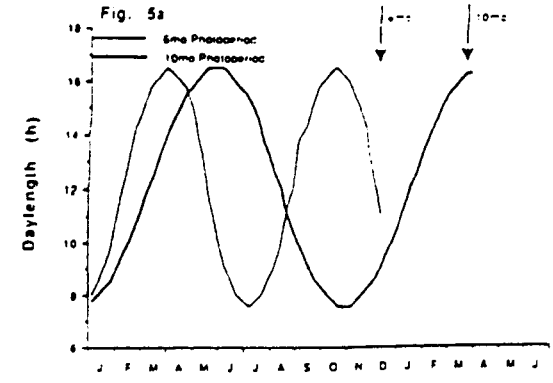
vated throughout March. This was slightly in advance of a significantly ($P<0.001$) decreased condition factor, which was not evident until early March (Fig. 2b). Transfer mortality was high and 29% died within 2 weeks, although surviving fish grew well throughout the following year and achieved a mean weight of 2.7 kg. Again there was no post-smolt maturation, but 19% matured as grilse the following year.

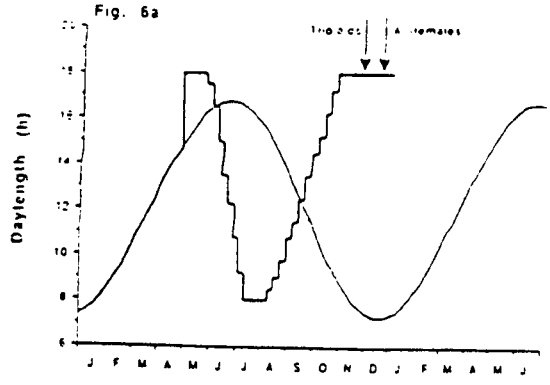
Trial 3

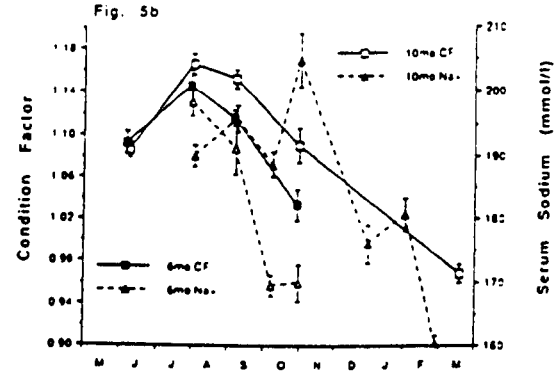
These fish were fully silvered in early February, and were transferred to seawater (site B) on 18 February, 3 months in advance of fish reared under ambient photoperiod conditions. At this time, the fish had shown a significantly ($P<0.01$) reduced condition factor and increased ($P<0.001$) hypo-osmoregulatory ability (serum sodium following seawater challenge in early February was 159 mmol

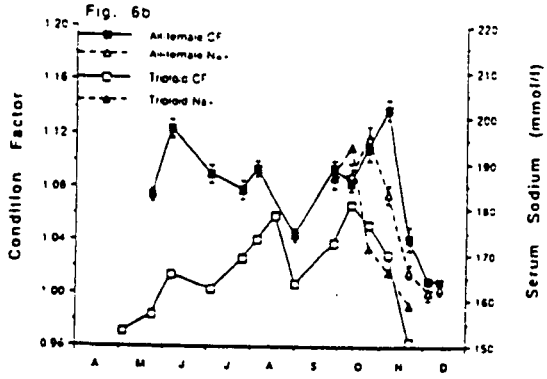


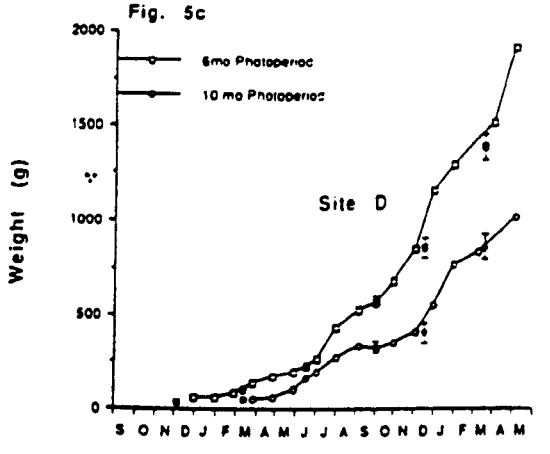
Figs. 3 and 4. Figs. 3a and 3b represent fish in trial 3; Figs. 4a, 4b and 4c represent fish in trial 4. Figs. 3a and 4a, photoperiod regime, downward facing arrows in the photoperiod figures indicate times of seawater transfer. Figs. 3b and 4b, the development of the smolt parameters: condition factor and challenged serum sodium. Fig. 4c, seawater growth; mean weight ± 1 s.e.m.

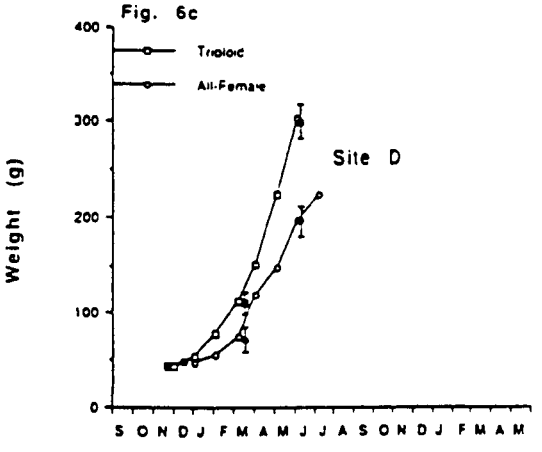












Figs. 5 and 6. Figs. 5a, 5b and 5c represent fish in trial 5; Figs. 6a, 6b and 6c represent fish in trial 6. Figs. 5a and 6a, photoperiod regime, downward facing arrows in the photoperiod figures indicate times of seawater transfer. Figs. 5b and 6b, the development of the smolt parameters: condition factor and challenged serum sodium. Figs. 5c and 6c, seawater growth; open symbols (without error bars) represent mean weights determined by batch weighing, filled symbols indicate mean weight ± 1 s.e.m.

l^{-1}, see Fig. 3b). High losses (43%) were incurred shortly after seawater transfer. Although surviving fish grew well, it was not possible to collect any growth data. A large group of this stock was kept in fresh water until May under a long day photoperiod (LD18:6); and further samples taken. During this time osmoregulatory ability remained elevated and condition factor continued to decrease.

Trial 4

Groups A and B developed full smolt colouration and were transferred to seawater (site C) on 28 March and 11 April respectively, 7 and 5 weeks earlier than farm stock (reared on an ambient photoperiod). Smolt status was confirmed in both groups by significantly ($P<0.001$) reduced condition factors and elevated hypo-osmoregulatory abilities (Fig. 4b). Both groups averaged 31 g at transfer, increasing to 41.2 g (± 1.5) and 35.7 g (± 1.6) on 30 April for groups A and B respectively when the experiment ended. Mortality in seawater was less than 4%.

3.2. Potential S2 smolts

Trial 5

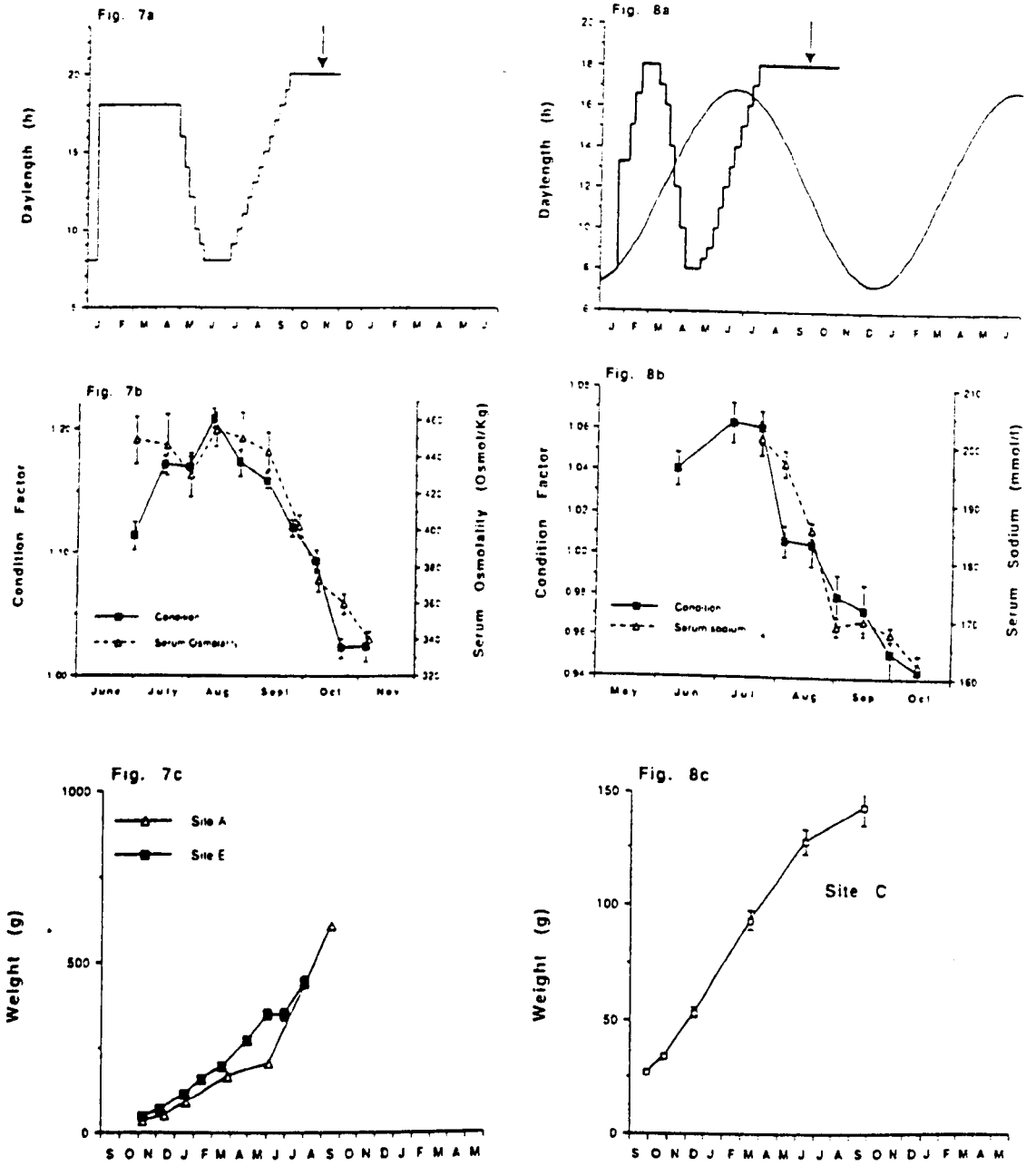
Parr reared on 6- and 10-month compressed seasonal cycles completed smoltification 5 and 2 months earlier than control fish as judged by colouration, reduced condition factor and elevated hypo-osmoregulatory ability (Fig. 5b).Smolts with a mean weight of 35.0 g (± 0.85) and 48.2 g (± 0.98) were transferred to sea site D on 3 December and 3 March respectively. Mortality following entry to seawater was less than 1%. Of the smolts transferred in December, 6.5% matured after one sea-winter, as determined by secondary sexual characteristics (breeding colouration and kype development among males). After a further sea-winter 63% of this stock were estimated to be showing signs of maturation in July when the experiment was terminated. The fish introduced to seawater in March had a post-smolt maturation of 24%. No further maturation data were collected for this group.

Trial 6

Triploid fish showed significantly decreased condition factor ($P<0.05$) and increased osmoregulatory ability ($P<0.01$) approximately 1 month ahead of the all-female stock (Fig. 6b) reared under the same accelerated photoperiod. The two groups were transferred to sea site D on 21 November and 15 December respectively and at transfer the triploids weighed 44.0 g (± 0.92) and the all-females 48.3 g (± 0.97). Mortality following seawater transfer was less than 2% for each of the groups. The triploids showed exceptional growth from March onwards and had reached 300 g by June (the ambient smolt transfer time). In July, when the trial was terminated, neither of the groups was showing any external signs of maturation.

Trial 7

Parr–smolt transformation in this group was judged to be complete by early November, 6 months earlier than in control fish. A significant increase in hypo-osmoregulatory ability ($P<0.01$) and a decrease in condition factor ($P<0.001$) was observed between mid-September and early October (Fig. 7b). Transfers were made to two seawater facilities: site E received a group of 500 smolts, which were on-grown in 7000-l circular tanks; and 20 000 were moved to site A and grown on in 15-m cages. The mean size of smolts transferred to sites A and E was



Figs. 7 and 8. Figs. 7a, 7b and 7c represent fish in trial 7; Figs. 8a, 8b and 8c represent fish in trial 8. Figs. 7a and 8a, photoperiod regime, downward facing arrows in the photoperiod figures indicate times of seawater transfer. Figs. 7b and 8b, the development of the smolt parameters: condition factor and challenged serum osmolality/sodium. Figs. 7c and 8c, seawater growth; site A weighings (without error bars) represent mean weights determined by batch weighing, sites E and C are mean weights ± 1 s.e.m.

33.1 g (±0.77) and 48 g (±1.18) respectively. The mortality during the first month of seawater residence was less than 5% at site E but a much greater loss was incurred at site A with 44.9% of the smolts dying in the first month following transfer. Surviving fish at site A and those at site E grew well throughout the

winter, although both went through a period of slow growth during May and June when growing conditions were optimum. The determinations of GSI indicated a degree of maturation after one sea-winter at site E, although there was no clear bimodality in either male or female data; the maturing fraction was estimated to be between 5 and 12% (secondary sex characteristics were not apparent). There was no maturation at site A after one sea-winter.

Trial 8

Fish reared in this trial showed a steadily decreasing ($P<0.001$) condition factor from July through to October. This was accompanied by a significantly ($P<0.001$) increased hypo-osmoregulatory ability over the same period (Fig. 8b). These fish were fully silvered and considered to have achieved smolt status by late September, 7 months in advance of control fish. At this time a group of smolts was transferred to a small-scale experimental facility at site C. Although these fish were very small at transfer (27 ± 0.87 g) and the ambient seawater temperature was high (19°C), the survival after 1 month exceeded 97%. The transfer weight was doubled within 6 weeks, but continued growth at this facility was slower than that of smolts from other trials held at different seawater sites, particularly during the summer months when water temperatures exceeded 20°C. The mean weight of this stock the following September was 147 g. No signs of maturation were evident in either males or females after 1 year in seawater when the experiment was terminated.

4. Discussion

In the current work, potential S1 smolts (under-yearling parr) were advanced by up to 3 months compared to fish reared under conditions of natural photoperiod. Advances of up to 7 months were achieved with potential S2 smolts (1+ year-old parr). Fish in all groups exhibited significantly increased hypo-osmoregulatory abilities and a significantly decreased condition factor in response to increases in daylength as previously observed by Farmer et al. (1978), McCormick et al. (1987) and Duston and Saunders (1990).

In trials 2, 3 and 5 (6 month) the photoperiod manipulations produced a dissociation in the development of hypo-osmoregulatory ability and decreased condition factor, a phenomenon previously observed in other studies (Björnsson et al., 1989; Duston and Saunders, 1990, 1992). In all these trials, hypo-osmoregulatory ability was evident approximately 1 month in advance of a condition factor which is characteristic of smolts. This dissociation suggests that the two parameters either develop at different rates or are controlled by different mechanisms (Duston and Saunders, 1990). Fish in trial 5 subjected to a compressed 10-month photoperiod showed no dissociation of these two smolt parameters. This would suggest that the increased rate of change in daylength associated with a 6-month compressed photoperiod was the cause of the dissociation. However, fish subjected to compressed 5-month and 6-month cycles, in trial 8 and trials 6 and 7

respectively, completed smoltification with no dissociation of these smolt parameters. A possible explanation is to be found in the mechanisms which control smoltification. It is thought that changes in daylength serve to entrain an endogenous circannual rhythm which controls the development of smoltification (Conte and Wagner, 1965; Eriksson and Lundqvist, 1982; Duston and Saunders, 1990, 1992). It would appear that the timing of a rapid decrease and increase in daylength in relation to the endogenous circannual rhythm caused the increase in hypo-osmoregulatory ability to develop in advance of the decrease in condition factor; while the same rapid decrease and increase in daylength in relation to a different period of the endogenous circannual rhythm allowed all smolt parameters to develop at equivalent, albeit accelerated, rates.

In agreement with Duston and Saunders (1992), all groups which were exposed to compressed photoperiodic cycles demonstrated degrees of phase delay in the appearance of smolt characteristics, i.e. the smolt characteristics appeared later in the photoperiodic cycle than would be expected under a natural photoperiod. This was particularly evident in the 6-month cycle in trial 5, where the fish completed smoltification on a decreasing photoperiod. This phase delay in response to a forcing zeitgeber is a typical feature of the behaviour of endogenous rhythms (Bromage and Duston, 1986; Duston and Saunders, 1992).

Initial survival after seawater transfer was variable. All transfers in trials 4, 5, 6 and 8 resulted in survivals to 1 month in excess of 95%, and in each case these smolts were of good quality. The mortality in the first month following transfer to seawater in trial 1 was 14%, which is considered high by commercial standards, although high losses after transfer to seawater of apparently healthy smolts grown under ambient photoperiod and temperature are not uncommon. Assessment of the condition factor, which first decreased significantly, only two weeks prior to seawater transfer, suggests that these smolts were transferred prematurely, possibly before smoltification was completed.

In general, higher mortalities were incurred when smolts were produced in greater numbers for larger cage sites. These fish were reared at higher stocking densities, sometimes reaching 60 kg m^{-3}, compared to 20–30 kg m^{-3} in the small-scale trials. High rearing density has been shown to reduce Na^+/K^+-ATPase activity in coho salmon (Sower and Fawcett, 1991), to increase serum cortisol levels in coho salmon and rainbow trout (Leatherland and Cho, 1985; Patino et al., 1986) and to reduce growth following seawater transfer in Atlantic salmon. Fagerlund et al. (1981) demonstrated that smaller fish were more sensitive to population density; this could explain the pattern of mortality in trial 7. In the present work smolts reared under the same experimental photoperiod were transferred to two sea sites: those moved to site A had a mean weight of 33 g and suffered a mortality of 44.9%, whereas a group of larger smolts (48 g) taken to site E incurred a loss of only 4.6%. Circulating cortisol has been reported to increase during smoltification (Patino and Schreck, 1986) making smolts particularly susceptible to stress. Site E is situated at much greater distance from the smolt units than any other of the sea rearing facilities and the transport of smolts to this lo-

cation involved journeys by road of up to 10 h and then transfer to cages by helicopter, thus incurring additional handling stress.

The majority of the mortalities in trial 2, recovered from cages in the first week after seawater transfer, showed severe abrasions to the head and ventral surfaces, consistent with the fish burrowing to try to escape conditions of high light intensity. The lighting used in the photoperiod facilities for larger scale smolt production (trials 1, 2 and 3) provided very low light intensities (10–20 lx). Considering that natural daylight under cloud cover is of the order of 30000 lx, these groups will have received a substantial increase in light intensity when transferred to seawater. "Light shock" is now considered by smolt producers to be a particularly severe stress, and the light intensity in photoperiod facilities used for these trials has now been increased to alleviate this problem. The performance of smolts in seawater challenge tests prior to transfer and the fast growth of surviving smolts (especially in trials 1 and 2) indicated these individuals to be physiologically well adapted to a marine environment. It would appear that the mortalities incurred resulted from cumulative stresses during transfer rather than any inherent poor smolt quality.

The data collected from the sea sites showed that the smolts produced out-of-season by photoperiod manipulation had a good capacity for growth in a marine environment. S1 smolts introduced to seawater up to 3 months early, benefitted from their extended period in seawater and were growing well by the time conventionally reared smolts were introduced to seawater in May and June. Potential S2 smolts transferred to sea sites between late September and mid-December grew well over the winter and were usually triple their transfer weight by the following spring. Different environmental conditions (especially with respect to temperature conditions, see Table 1) and the scale of the rearing facilities will have had a marked effect on the growth achieved by groups reared at different sea sites. Smolts grown from late September in trial 8 at site C, achieved a 5-fold increase in weight over 9 months, whereas smolts reared on a large scale by experienced salmon farmers under more favourable environmental conditions increased their weight more than 10 times over the same period of time.

Maturation in seawater has considerable economic consequences in the farming of Atlantic salmon, as it reduces both growth rate and flesh quality (Tveranger, 1985; Aksnes et al., 1986). Potential S1 smolts transferred to seawater between March and May showed no signs of post-smolt maturation, which is a relatively uncommon event in Atlantic salmon (Herbinger, 1987). However, S2s transferred to seawater in March underwent a post-smolt maturation of 24%. S1 smolts introduced to seawater in March showed a higher rate of maturation after one sea-winter than those moved to seawater in May. This was most likely due to enhanced growth among the earlier smolts, particularly during the winter months when the "decision to mature" is thought to be taken (Thorpe et al., 1989). Potential S2 mixed-sex smolts transferred to seawater between November and December showed up to 12% maturation after one sea-winter, although maturation at this time was not seen among all-female or triploid all-female stocks. Mixed-sex potential S2 smolts transferred to seawater in September showed no matura-

tion after one sea-winter, but this was most probably due to their relatively slow growth.

These results show clearly that the use of photoperiod and the production of "out-of season" smolts can make a valuable contribution to the farming of Atlantic salmon. Application of these techniques would increase the efficiency of smolt units. The availability of smolts at times other than the spring would improve cash flow for smolt producers and on-growers alike and, by providing a more consistent supply of harvest size salmon, would help to stabilise market prices.

5. References

Aksnes, A., Gjerde, B. and Roald, S.O., 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. Aquaculture, 53: 7–20.

Baggerman, B., 1960. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (*Oncorhynchus*). J. Fish Res. Board Can., 17: 295–322.

Björnsson, B.T., Thorarensen, H., Hirano, T., Ogasawara, T. and Kristinsson, J.B., 1989. Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypoosmoregulatory ability of juvenile Atlantic salmon (*Salmo salar*) during parr–smolt transformation. Aquaculture, 82: 77–91.

Bromage, N.R. and Cumaranatunga, R., 1988. Egg production in rainbow trout. In: J.F. Muir and R.J. Roberts (Editors), Recent Advances in Aquaculture, Vol. 3. Croom Helm, London, pp. 63–138.

Bromage, N. and Duston, J., 1986. The control of spawning in the rainbow trout (*Salmo gairdneri* Richardson) using photoperiod techniques. Inst. Freshwater Res. Drottingholm, Rep. 63: 26–35.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 100: 141–166.

Clarke, W.C., 1989. Photoperiod control of smolting: a review. Physiol. Ecol. Japan, Spec. Vol. 1: 497–502.

Clarke, W.C. and Blackburn, J., 1978. Sea water challenge tests performed on hatchery stocks of chinook and coho salmon in 1977. Fish. Mar. Serv. Tech. Rep., No. 761: 18 pp.

Clarke, W.C. and Nagahama, Y., 1977. Effect of premature transfer to sea water on growth and morphology of the pituitary, thyroid, pancreas and interrenal in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Zool., 55: 1620–1630.

Clarke, W.C., Lundqvist, H. and Eriksson, L.O., 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon, *Salmo salar* L. J. Fish Biol., 26: 29–35.

Conte, F. and Wagner, H.H., 1965. Development of osmotic and ionic regulation in juvenile steelhead trout, *Salmo gairdneri*. Comp. Biochem. Physiol., 14: 603–620.

Duston, J. and Bromage, N.R., 1986. Photoperiodic mechanisms and rhythms of reproduction in the female rainbow trout. Fish Physiol. Biochem., 2(1–4): 35–51.

Duston, J. and Saunders, R.L., 1990. The entrainment role of photoperiod on hypo-osmoregulatory and growth-related aspects of smolting in Atlantic salmon (*Salmo salar*). Can. J. Zool., 68: 707–715.

Duston, J. and Saunders, R.L., 1992. Effect of 6-, 12-, and 18-month photoperiod cycles on smolting and sexual maturation in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 49: 2273–2280.

Eriksson, L.-O. and Lundqvist, H., 1982. Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). Aquaculture, 28: 113–121.

Fagerlund, U.H.M., McBride, J.R. and Stone, E.T., 1981. Stress-related effects of hatchery rearing density on coho salmon. Trans. Am. Fish. Soc., 110: 644–649.

Farmer, G.J., Ritter, J.A. and Ashfield, D., 1978. Seawater adaptation and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can., 35: 93–100.

Herbinger, C.M., 1987. A study of Atlantic salmon (*Salmo salar*) maturation using individually identified fish. PhD Thesis, Dalhousie University, Halifax, N.S., 285 pp.

Hoar, W.S., 1988. The physiology of smolting salmonids. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. XIB. Academic Press, New York, pp. 275–343.

Komourdjian, M.P., Saunders, R.L. and Fenwick, J.C., 1976. Evidence for the role of growth hormone as a part of a "light-pituitary axis" in growth and smoltification of Atlantic salmon (*Salmo salar*). Can. J. Zool., 54: 544–551.

Langdon, J.S., 1985. Smoltification physiology in the culture of salmonids. In: J.F. Muir and R.J. Roberts (Editors), Recent Advances in Aquaculture, Vol. 2. Croom Helm, London, pp. 79–118.

Leatherland, J.F. and Cho, C.Y., 1985. Effect of rearing density on thyroid and interrenal gland activity and plasma and hepatic metabolite levels in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol., 27: 583–592.

McCormick, S.D., Saunders, R.L., Henderson, E.B. and Harmon, P.R., 1987. Photoperiod control of parr–smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill Na^+,K^+-ATPase activity and plasma thyroid hormones. Can. J. Fish. Aquat. Sci., 44: 1462–1468.

Patino, R. and Schreck, C.B., 1986. Sexual dimorphism of plasma sex steroid levels in juvenile coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol., 61: 127–133.

Patino, R., Schreck, C.B., Banks, J.L. and Zaugg, W.S., 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc., 115: 828–837.

Saunders, R.L., Henderson, E.B. and Harmon, P.R., 1985. Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. Aquaculture, 45: 55–66.

Shapiro, S.S. and Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika, 52: 591–611.

Snedecor, G.W. and Cochran, W.G., 1980. Statistical Methods, 7th edn. Iowa State University Press, Ames.

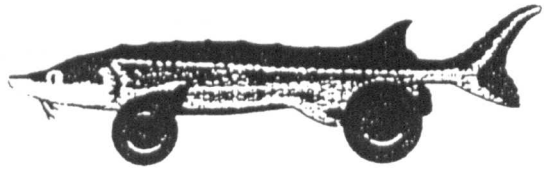
Sower, S.A. and Fawcett, R.S., 1991. Changes in gill Na^+,K^+-ATPase, thyroxine and triiodothyronine of coho salmon held in two different rearing densities during smoltification. Comp. Biochem. Physiol., 99A (1–2): 85–89.

Thorpe, J.E., Talbot, C. and Miles, M., 1989. Research suggests a simple answer to grilsing control. Fish Farmer, 12(1): 10–11.

Tveranger, B., 1985. Variation in growth rate, liver weight and body composition at first sexual maturity in rainbow trout. Aquaculture, 49: 89–99.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ.

High Performance Fish



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COMPARISON OF GROWTH, SEA WATER TOLERANCE AND MORTALITY FOR ATLANTIC SALMON (*SALMO SALAR*) PARR AND PHOTOPERIOD SMOLTS TRANSFERRED TO SEA WATER IN DECEMBER.

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Abstract.
In June a group of Atlantic salmon (*Salmo salar*) parr with a mean weight of 2g were divided into two groups. Group NL was maintained under natural illumination. Group PL was subjected to a LD 6:18 for a period of 2 months followed by a 1 hour/week increase to LD 18:6. In September both groups were graded, the potential S1 fish from each group being selected. Sea water tolerance tests indicated the PL group had the ability to osmoregulate during November while the NL fish showed no osmoregulatory ability at this time. Both the NL and PL groups were transferred to sea water on the 7 Dec weighing 40g and 25g respectively. The groups showed different growth patterns. The PL group in comparison to the NL group, grew slowly during July and August (the short day period), but exhibited higher growth rates during early Autumn (under an increasing photoperiod) and in the first 3 month after sea water transfer. The mortality in the NL group was significantly higher during the first 2 months after sea water transfer.

Introduction.
A major constraint to the Atlantic salmon farming industry is the seasonal variation in availability of market-size fish for harvest. It has been suggested that increasing the window of smolt transfer would lead to an extended harvesting period. The practice of transferring large parr to sea cages in the Autumn is a common method used to increase the window of sea water transfer. This study was undertaken to compare both fresh water and sea water performance of large autumn parr and autumn photoperiod smolts

Materials and Methods.
At a Scottish (54°N) salmon hatchery, supplied with ambient water, 50000 Atlantic salmon (*Salmo salar*) parr from the hatcheries main stock were placed into an artificial photoperiod winter (group PL). The photoperiod began on the 4 June with a decrease from natural daylength to LD14:10, this was continued at 4hr/week to LD6:18. The LD6:18 was held for 2 months before an increase of 1hr/week to LD18:6. The remaining stock was maintained under a natural photoperiod (group NL). The fish were stocked at 4-20kg/m^3. During September both groups were size graded, the potential S1 parr being retained. On the 7 Dec, 1000 fish from each group were transferred to sea water tanks and held at stocking densities of 5-10kg/m^3, daily mortality was recorded. Throughout the experiment, fish were fed in accordance to feed tables and the weights and lengths of 50-100 fish recorded at 2-4 weekly intervals. Both NL and PL fish were sea water tolerance tested at 37.5ppt for 96hrs beginning on 25 Sept, 15 Oct, 6 Nov, 20 Nov and 2 Dec. The tolerance tests were carried out at ambient temperature.

Specific growth rate (SGR) was calculated using the equation SGR=ln(wt2-wt1) . 100/t, wt1-inital weight, wt2-final weight, t-time interval. The weight data was compared using DUNN'S multiple comparison test, tolerance test data and sea water mortality was compared using the standard error of sample proportions.

Results and Discussion.

During the photoperiod winter the PL fish exhibited a lower SGR (9/6-31/7, SGR=1.24g/day) than the NL fish (9/6-31/7, SGR=2.97g/day). Due to the growth restricting affect of the LD6:18 the PL fish were significantly ($p<0.01$) smaller on the 31 July (fig.1).This situation was reversed in October, with the PL group now under an increasing photoperiod, exhibiting a higher SGR (14/10-5/11, SGR=2.28g/day) than the NL fish (14/10-5/11, SGR=0.19g/day). Despite the increased growth, promoted by the increasing photoperiod the PL fish were still significantly ($p<0.01$) smaller before transfer to sea water.

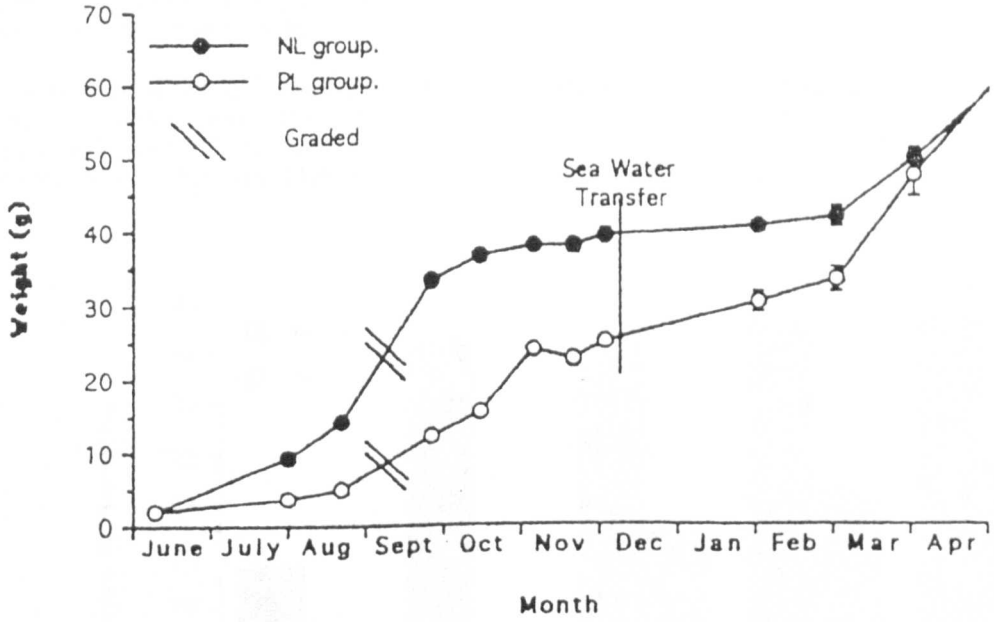

Figure 1. Graph of the changes in weight (g) against time for two groups PL-fish held under an artificial photoperiod and NL-fish held under a natural photoperiod.

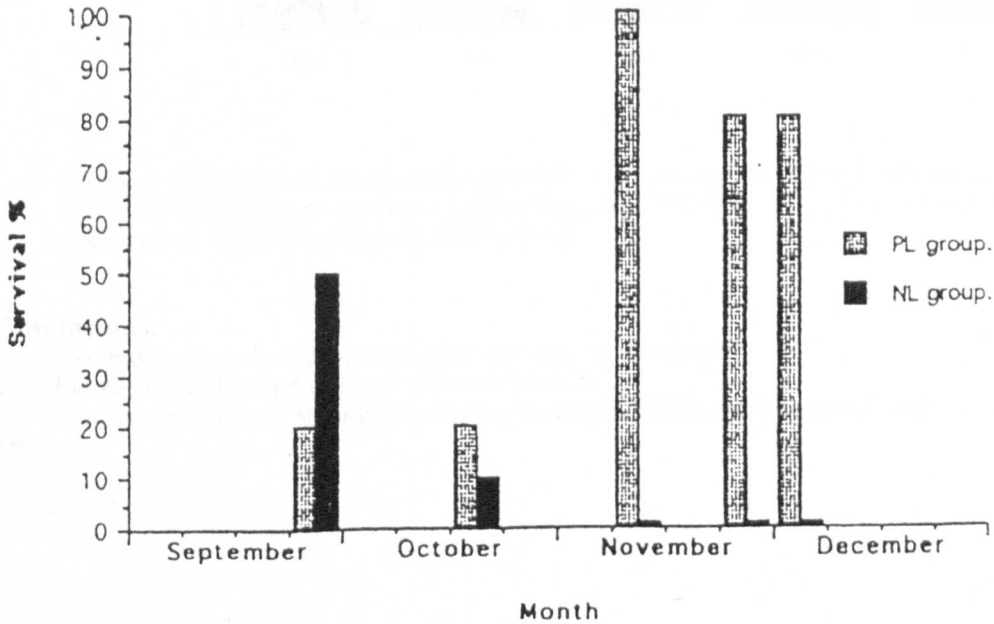

Figure 2. Graph of the percentage of fish surviving 96hrs in 37.5ppt sea water, histograms represent two groups PL-fish held under an artificial photoperiod and NL-fish held under a natural photoperiod, tested on 5 occasions, 25 Sept, 15 Oct, 6 Nov, 20 Nov and 2 Dec.

The sea water tolerance test in September and October showed no significant difference in sea water survival (fig. 2) between the two groups. The tolerance tests in November and December showed a significantly ($p<0.01$) higher survival in the PL fish, indicating that the fish had developed the osmoregulatory ability of smolts which is normally associated with an increasing photoperiod.

The sea water growth of the PL group was consistently higher (SGR=0.31-1.17g/day) than the NL group (SGR=0.04-0.58g/day). However, after 2 months in sea water there was no significant difference between the mean weight of the two groups (fig.1). This situation remained untill the end of the experiment in April.

Sea water mortality (fig.3) during the first two months was significantly ($p<0.01$) higher in the NL group. This agreed with the tolerance test results (fig. 2). During February-April mortality was significantly ($p<0.01$) higher in the PL group (fig.3) which suffered from a vibrio infection. Vibriosis was identified on March 10 and treated with a course of antibiotic.

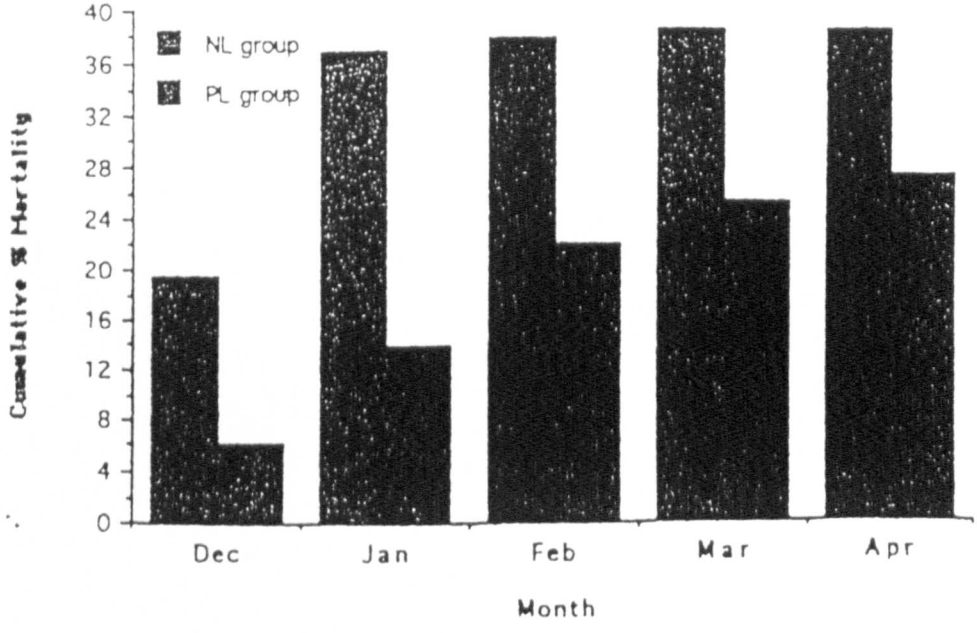

Figure 3. Histogram of the monthly cumulative percentage mortality of two groups PL-fish held under an artificial photoperiod and NL-fish held under a natural photoperiod, transferred directly to sea water.

Conclusions.

1. Decreasing and short daylength photoperiods restrict growth.
2. Increasing and long daylength enhance growth.
3. Smolts significantly smaller in size than parr will exhibit better growth and survival in sea water