PHOTOPERIODIC CONTROL OF SMOLTIFICATION AND ASPECTS OF BROODSTOCK MANAGEMENT IN 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: _____

Signature of supervisor: N. Bromage

Date: 25 May 1994

ABSTRACT

At present, the restricted temporal availability of Atlantic salmon smolts leads to a seasonal supply of marketable salmon and as a consequence an unstable price. The development of smoltification appears to be controlled by the seasonal changes in daylength. The primary aim of this thesis, in collaboration with commercial smolt producers and on-growers, was to achieve a "year-round" supply of Atlantic salmon using photoperiod techniques.

Potential S1 (1+) and S2 (2+) Atlantic salmon smolts were reared in freshwater under compressed and extended seasonally-changing photoperiods and under 'square wave' (direct change from short to long) light regimes. Pre-smolts underwent body silvering and fin darkening, a reduction in condition factor and developed strong hypo-osmoregulatory ability in response to increasing daylengths. In this study, the completion of smoltification was advanced by a maximum of 3- and 7- months among potential S1 and potential S2 smolts respectively, and was delayed by 1-month among potential S2 smolts.

The results were consistent with the hypothesis that changes in daylength entrain an endogenous circannual timing mechanism which controls the development of smoltification. Results were also consistent with the hypothesis that a 'decision to smolt' is made under the influence of a decreasing daylength in the year preceding seaward migration. Furthermore, it was apparent that this decision period may extend into the winter and that recruitment into the smolting fraction is probably terminated by the increasing daylength after the winter solstice. Parr reared under a compressed photoperiod with low intensity night-time illumination failed to complete smoltification at the same time as those without night-time illumination. Serum melatonin was elevated throughout the period of the low intensity phase among fish maintained on the dual light intensity regime, although peak levels were attenuated compared to fish reared under light-dark cycles in other published studies.

Temporally advanced S1 smolts gained a growth advantage from an early transfer to seawater, and potential S2 smolts transferred to seawater between September and December grew well over the winter. Mixed sex S1 smolts transferred to seawater early did not mature

as post-smolts, but advanced S2s did show post-smolt maturation and an elevated incidence of maturation after 1-sea winter. None of the all-female and triploid all-female stocks transferred to seawater in November and December showed any signs of maturation the following year, after 1-sea winter.

Another important aspect of salmon culture includes the supply of eggs, however little attention has been given to the suitability or superiority of various broodstocks currently maintained by the industry for this task. This thesis provides an assessment of various aspects of the reproductive performance among a number of farmed Atlantic salmon stocks with the aim of identifying important criteria which may be used in future stock selection.

Total egg number (fecundity), egg size and total egg volume data were collected from individual 2 and 3-sea winter (SW) spawning females of four farmed salmon stocks. Relationships between these parameters and post-strip fish weight were analysed using regression and analysis of covariance (ANCOVA) techniques to evaluate differences in reproductive performance between the broodstock groups. Egg size was found to be poorly related to fish weight, with coefficients of determination (r^2) of between 0% and 19%. The regression of all data combined provided an r^2 value of only 18.2%. Total fecundity increased with increasing post-strip weight in 2-SW and 3-SW females in all four stocks and r^2 values ranged from 12% to 62%. Total egg volume provided the best measure of reproductive performance. All regressions of this parameter on weight were positive and highly significant ($P<0.001$) with individual r^2 values ranging from 31% to 77%. With data for all stocks combined, ANCOVA showed the rate of increase of both fecundity and total egg volume, with increasing fish weight, to be significantly greater in 3-SW than in 2-SW females. Significant slope differences were also found between ages within stocks, and between different stocks of the same age. Within groups of common slope, after adjustment to a common log weight, significant differences were found between elevations of both fecundity and total egg volume regressions. Within 2-SW groups, differences in fecundity on weight and total egg volume of up to 9% and 20% were found respectively; within 3-SW groups, differences of up to 35% and 15% were found respectively.

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GLOSSARY OF SCIENTIFIC AND COMMON FISH NAMES

Scienctifc	Common
Anguilla rostrata	American eel
Carassius auratus	goldfish
Clupea harengus	herring
Cyprinus carpio	common carp
Esox lucius	pike
Gadus morhua	Atlantic cod
Ictalurus punctatus	channel catfish
Leuciscus leuciscus	dace
Oncorhynchus gorbuscha	pink salmon (humpback)
Oncorhynchus keta	chum salmon
Oncorhynchus kisutch	coho salmon (silver)
Oncorhynchus masou	masu salmon (cherry, gamame)
Oncorhynchus mykiss	rainbow trout
Oncorhynchus mykiss	steelhead trout
Oncorhynchus nerka	sockeye salmon (red, blueback)
Oncorhynchus rhodurus	amago salmon
Oncorhynchus tshawytsch	chinook salmon (king, spring)
Oreochromis niloticus	Nile tilapia
Pleuronectes platessa	plaice
Salmo gairdneri	see *Oncorhynchus mykiss*
Salmo salar	Atlantic salmon
Salmo trutta	brown trout
Salvelinus alpinus	Arctic char
Salvelinus fontinalis	brook trout
Salvelinus malmo	dolly varden

Chapter 1

General Introduction

The Atlantic salmon, *Salmo salar* (Linnaeus, 1758) is distributed throughout the Atlantic coasts of Europe, from the Barents Sea (74°N), northern Norway and the Baltic southward to northern Portugal (41°N), Iceland, southern Greenland and the east coasts of Canada and the United States from Ungava Bay, Quebec (60°N) south to Connecticut River, New England (40°N) (Figure 1.1). Historically the areas of greatest abundance were eastern Canada, the UK and Scandinavia. However, their numbers have decreased substantially over the past century due to over-fishing, pollution and obstruction to migration runs, and they are no longer present in many industrially important rivers, particularly in central Europe. A number of introductions of this species to stock rivers in New Zealand were made between 1868 and 1911. A large self sustaining population was established, but the later introduction of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) resulted in the decline of these salmon stocks, such that few fish remain today (Netboy, 1980). Many attempts have been made since 1905 to introduce spawning runs into the Pacific northwest and British Columbia, but these together with later attempts to introduce this species to Argentina and Tasmania proved unsuccessful (Dore, 1990).

The Atlantic salmon is believed to be descended from a freshwater ancestor. Evidence for this includes its obligate requirement for freshwater to breed (Tchernavin, 1939), and that salmon and closely related species, like the brown trout and the rainbow trout, can complete their life histories entirely in freshwater. It is generally accepted that the Atlantic salmon is the ancestor of the 6 species of salmon indigeneous to the Pacific coasts of North America and Asia belonging to the genus *Oncorhynchus*, which are believed to have evolved since the closure of the Arctic link between the North Atlantic and Pacific oceans around one million years ago (Neave, 1958; Netboy, 1980).

Atlantic salmon undergo a complex and variable anadromous life history (Haslar, 1971; McDowall, 1987) (Figure 1.2). Wild adults naturally spawn in freshwater, generally fast running streams, in gravel nests (redds) excavated by the female, between October and January. The eggs develop over the winter and hatch after 500°-days. The alevins remain in the redds for a further 300°-days until yolk sac absorption is almost complete before emerging into the streams to feed from April onwards. The juvenile freshwater

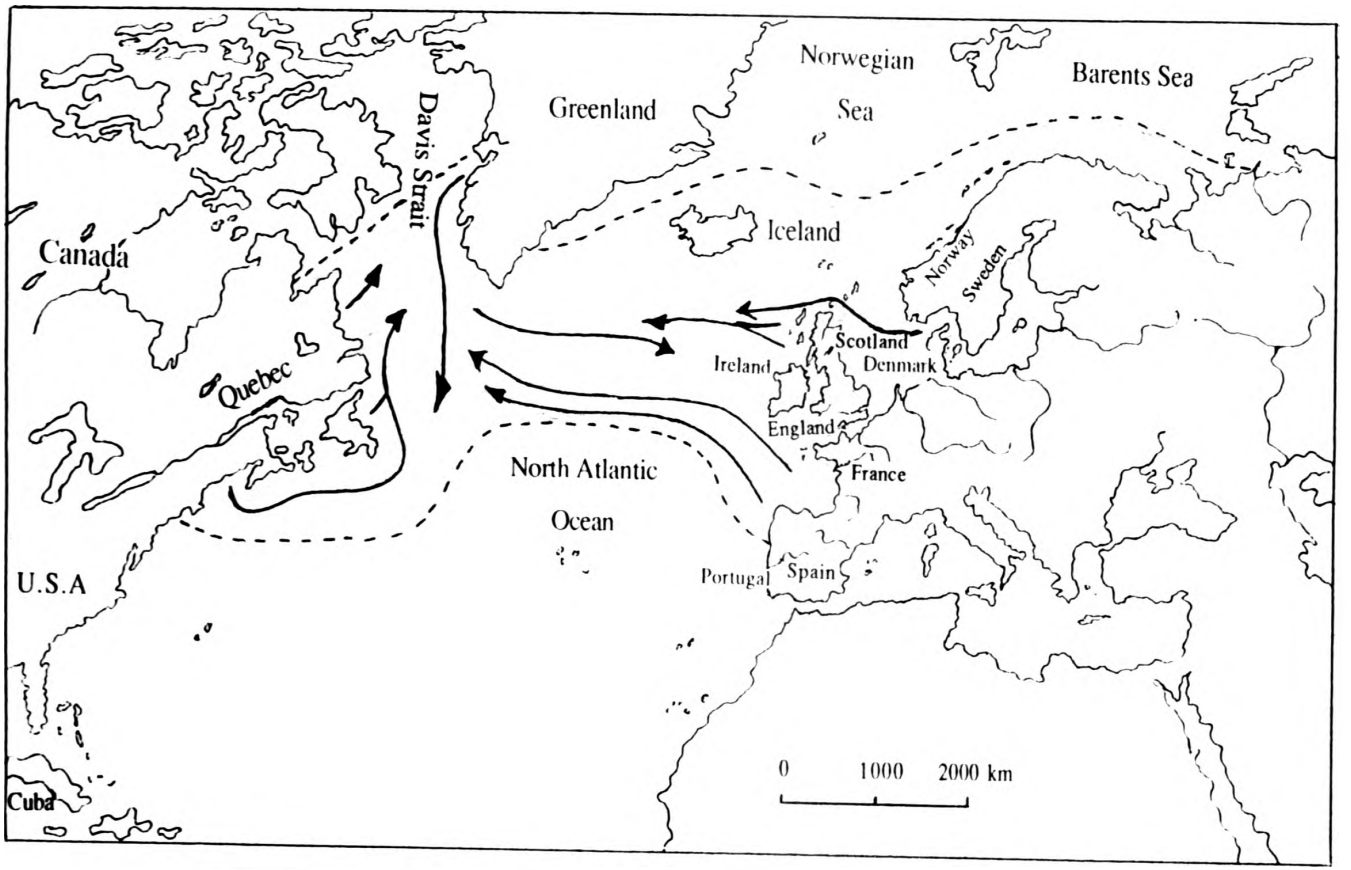
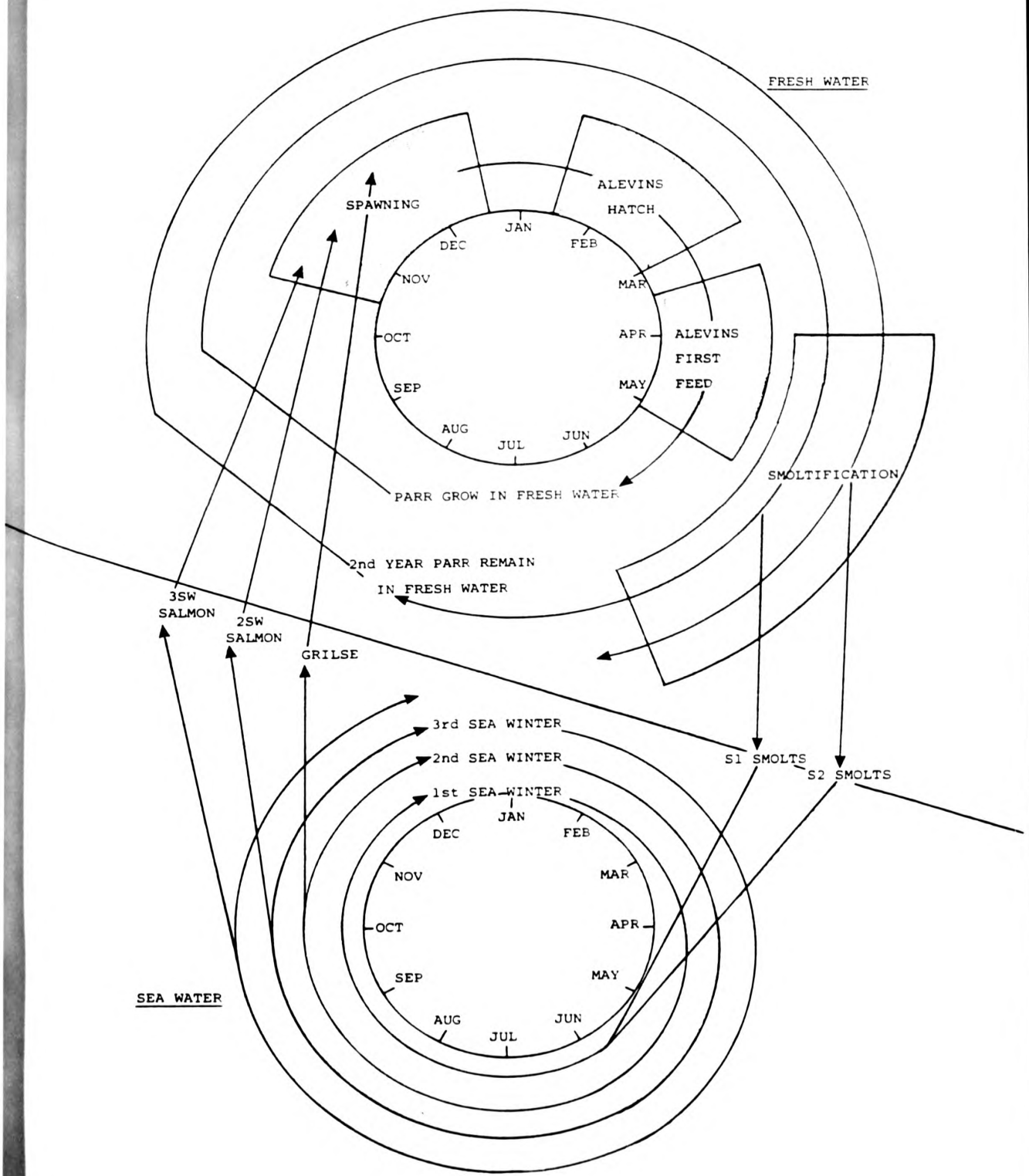


Figure 1.1

Distribution of the Atlantic salmon and oceanic migrations of European and North American stocks (Adapted from Netboy, 1980).

Figure 1.2

The Life cycle of the Atlantic salmon, showing patterns of freshwater and seawater residence


FRESH WATER
ALEVINS
HATCH
SPAWNING
JAN
DEC
FEB
NOV
MAR
OCT
APR
ALEVINS
FIRST
FEED
SEP
MAY
AUG
JUL
JUN
PARR GROW IN FRESH WATER
SMOLTIFICATION
2nd YEAR PARR REMAIN
IN FRESH WATER
3SW
SALMON
2SW
SALMON
GRILSE
3rd SEA WINTER
2nd SEA WINTER
1st SEA WINTER
S1 SMOLTS
S2 SMOLTS
JAN
DEC
FEB
NOV
MAR
OCT
APR
SEP
MAY
AUG
JUL
JUN
SEA WATER

residents (parr) have a cryptic green-brown colouration and characteristic dark bars on their flanks (parr marks). Freshwater residence in this species may last little over a year, but in some extreme cases (in conditions of low environmental productivity and/or temperature) may last up to 8-years (Power, 1969). Varying numbers of males may mature in freshwater prior to seaward migration; in their first (or subsequent) years. Migration to the sea occurs in the spring following the completion of a well-defined complex of physiological, morphological and behavioural changes known collectively as smoltification (this is explained in greater detail in Section 3.1). The migratory stage (smolt) contrasts with the parr in appearance, and is recognisable by its striking silver colouration. Individuals migrating to the sea after one year in freshwater (age 1+) are called S1 smolts and those migrating in subsequent years are termed S2, S3, S4 smolts accordingly. Little is known about the oceanic phase of the salmon's life history. Some populations remain close to their home rivers, for example Finnish and Swedish stock from the Baltic region are not thought to migrate into the Atlantic, whereas other populations range widely at sea (Figure 1.1), with some individuals making trans-Atlantic migrations of up to 2700-km Vladykov (1963). Stocks from the east coast of North America, Britain and Scandinavia share common feeding grounds off the western coast of Greenland (Haslar, 1971).

Maturing adults may return to freshwater up to 12-months before the commencement of spawning. Some fish return after only 1-year at sea (grilse), but the majority remain at sea from 2-4 years before making the return migration to their natal stream to spawn and complete their life history, which in some instances may be up to 1000-km inland. Both sexes develop a gold-brown spawning colouration. In addition, the males develop pronounced kypes on both jaws. After spawning many adults die, but some (mostly females) return to the sea to recover condition and spawn a second time. A few females may spawn a third time (McDowall, 1988).

Wild Atlantic salmon have probably been an important source of food for man since the end of the last Ice Age. At this time these fish would have been enormously abundant in thousands of rivers throughout their range. Salmon has been exported from the U.K. to the continent since at least the thirteenth century. In recent history the salmon has provided a

staple food on both sides of the Atlantic, eaten salted and later canned and has remained one of the most important commercial fish resources. Today, salmon is perceived as a premium product and is highly regarded by consumers around the World. Commercial netting operations are carried out in rivers, coastal waters and on oceanic feeding grounds, although fishing is now closely regulated by national and international regulatory bodies setting catch quotas and enforcing closed seasons. In Europe an increasing proportion of wild salmon is now reserved for sports fisherman. In Scotland the sport fishery is currently worth in excess of £60m to the tourist industry and local economies (Anon., 1994).

The culture of Atlantic salmon is a recently developed and fast expanding industry, which has changed the supply situation greatly and increased further the commercial significance of this fish over a relatively short period of time. Beginning in Norway in 1965 with the rearing of fish in a partitioned section of sea by Mowi A/S, there are now 14 countries farming this species. Farmed Atlantic salmon has quickly become the market standard, and the Worldwide consumption of fresh and smoked salmon products has increased markedly. Norway remains the leading producer, currently harvesting in excess of 200,000-tonnes annually (Figure 1.3). Salmon culture in Scotland has also shown rapid growth since the mid-1980s and the combined farmed production of Norway and Scotland accounts for approximately 90% of the World nominal catch for this species, with a first hand value of £900m.

The continued expansion of the Atlantic salmon farming industry will require the optimisation of all aspects of culture. This thesis addresses two areas of research which may enable commercial producers to sustain the growth so far achieved:

The farming of the salmon is complicated by the anadromous nature of this species. As a consequence, salmon culture is divided into 2 sections: freshwater smolt production, which includes the incubation of eggs, and the rearing of juvenile stages from hatch to the completion of smoltification (ie the achievement of the migratory state seen in the natural environment); and the seawater on-growing industry, which rears fish from the smolt stage (35-70-g) to market size (2-6-kg). Broodstock management and the production of eggs for hatcheries and smolt units is a specialised extension of the on-growing sector. The temporal

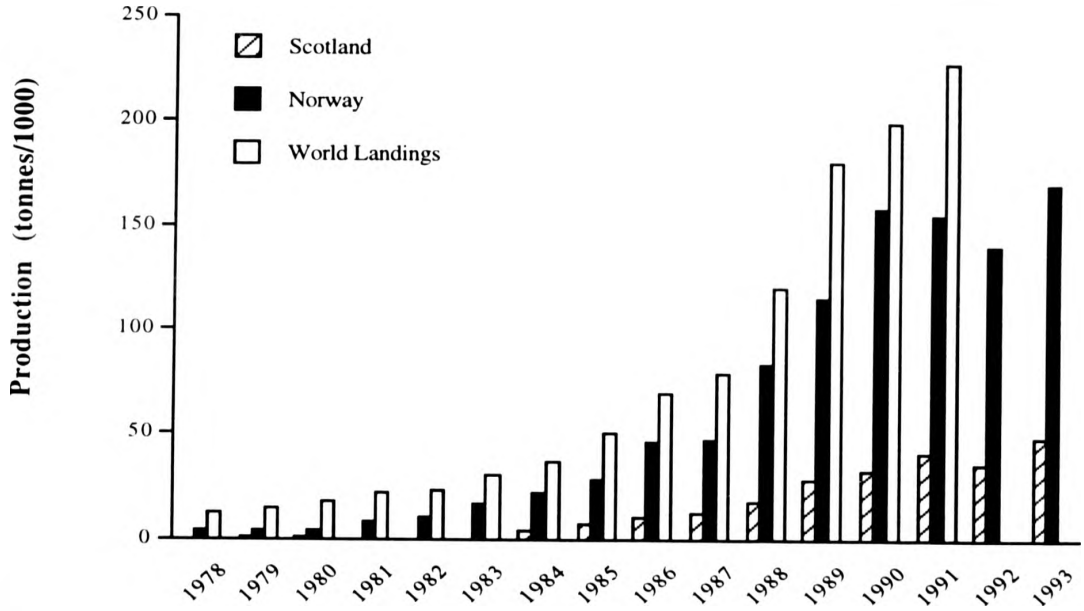


Figure 1.3

The annual production of farmed Atlantic salmon by Norway and Scotland since 1978 and World nominal landings (except recreational). (Heen *et al.*, 1993; FAO,1993)

availability of smolts, has important implications for both the supply and the price of marketable salmon. Figure 1.4 outlines the pattern of production by a large salmon producer over a 12-month period, which is typical for the industry as a whole. This shows a marked seasonal fluctuation in the supply of harvested fish, with the majority of supply concentrated into the last half of the year. There is also an underlying seasonality in the size of fish produced. Ideally the industry would like to guarantee a stable supply of both small (2-3-kg) and larger (3-5-kg) fish for various market outlets throughout the year. However, in addition to there being a general under-supply of salmon in the early part of the year, there is also a shortfall of large fish among those which are available for harvest until the spring. Chapter 3 of this thesis examines the possibility of using environmental control (through photoperiod manipulation) to produce smolts 'out-of-season' at times of the year other than the spring to stabilise both the quantity of salmon being marketed throughout the year, and the size of fish available (ie to standardise the quality of the product).

Another area of salmon farming which requires attention is that of egg production. Maintenance of broodfish is a costly sector of culture, and companies will become increasingly conscious of the need to make broodstock facilities more efficient. However, in the past no assessment has been made of the differences in spawning characteristics or reproductive performance (ie differences in fecundity and eggsize) among the stocks currently maintained by the Scottish salmon farming industry with respect to their suitability for commercial egg production. This would be a worthwhile exercise as subtle differences in the fecundity, for example, of individuals within a brood population will have a marked influence on the overall output of a hatchery. Eggsize is also an important factor in salmon farming. Larger eggs yield larger first-feeding fry and the size advantage awarded to such populations would serve to increase S1 fractions; improving the efficiency of smolt units. The identification of superior qualities belonging to particular stocks would provide valuable information for the incorporation of these traits in future selection programmes. An assessment of the differences in reproductive performance of four broodstocks currently maintained by a large commercial broodstock unit is provided in Chapter 4. This was achieved by making detailed comparisons between total egg number (fecundity), egg size

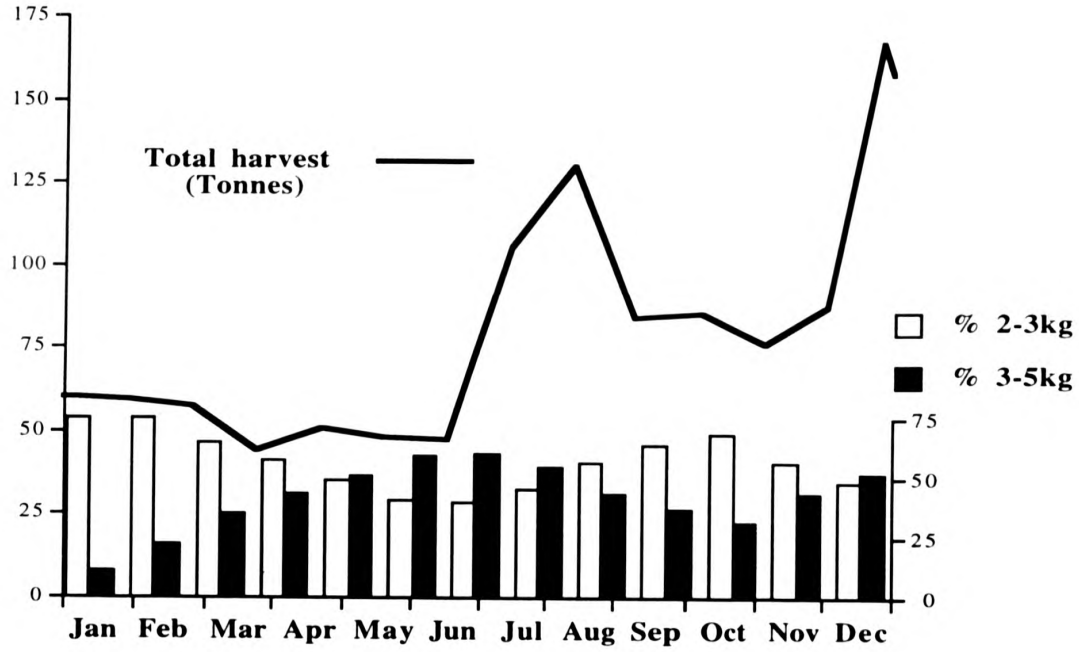


Figure 1.4

The pattern of production by a large salmon farm during 1993, showing the total weight of fish harvested (left axis), and the % of the total weighing 2-3kg and 3-5kg (right axis).

and total egg volume, of the different broodstocks and the relationships of these parameters with fish weight.

Chapter 2

General Materials and Methods

2.1 Fish holding facilities and maintenance

2.1.1 Pre-smolts

A number of different types of holding unit were used to maintain pre-smolts in different trials depending on the scale of the experiment and the site at which the trial was conducted. Experiments 1-6 were conducted at a commercial smolt unit in the south of England (Latitude 52°N). With the exception of Experiment 3, groups were maintained throughout their freshwater residence in 2×2-m square (2,000-l) glass fibre fry tanks supplied with constant temperature (10±0.2°C) spring water at 14-20-l min^{-1}. These tanks were enclosed in individual light-proof plywood boxes with hinged lids to allow access. Standard 60-W pearl tungsten filament bulbs in water-proof bulkhead fittings 0.6-m above the water surface provided an average light intensity of 440-lx (Lightmaster photometer; Evans Electroselenium Ltd., Halstead, Essex, U.K.) at the water surface. Daylengths were controlled by electronic 24-h time clocks (Smith's Industries Ltd., London, U.K.), which were adjusted at weekly intervals. Fish were either fed a commercial dry pellet (BP Nutrition Ltd., Witham, Essex, U.K.) by hand at hourly intervals between 8:00 and 17:00 or by automatic feeders (Skretting) every 15-minutes during the hours of the shortest (current) experimental photophase, at rates recommended by feed manufacturers tables.

In Experiment 3 fish were transferred from fry tanks to 9-m (75-m^3) concrete rearing tanks for final rearing before seawater transfer. These tanks were housed in a wooden framework which was light-proofed with industrial grade black plastic. Cool white fluorescent tubes suspended 3-m above the tanks provided an average light intensity of 25-lx at the water surface.

The freshwater phases of experiments 7 and 8 were conducted at a large smolt unit on the west coast of Scotland (Latitude 56°N) in 4-m glass fibre tanks (12,500-l) under conditions of natural photoperiod and temperature (which ranged from 3 to 13°C over the course of the freshwater phase of the study). Fish were fed a commercial dry pellet (Ewos Ltd., Bathgate, Lothian, U.K.) by automatic feeder.

2.1.2 Post-Smolts

Following the completion of smoltification in the various experimental groups, transfers were made to a number of seawater facilities to assess the survival and growth of post-smolts in a marine environment, and where possible to collect data on the incidence of subsequent maturation:

Smolts from experiments 1, 2 and 6 were transferred to 2×1-m (800-l) rearing tanks at the Hayling Marine Laboratory (Portsmouth University, Hants.) in batches of 50-100. Full strength seawater (35‰) was pumped from Langstone Harbour to a settling tank, to allow partial silt removal, from where it was supplied to the rearing tanks at approximately 20-l min^{-1}. The annual temperature range at this site over the period of study was 2°C (February) to 24°C (July). These fish were hand fed commercial dry pellets to satiation twice daily.

Transfers of smolts from Experiments 4 and 5 were made to a commercial on-grower in the Cleddau Estuary, south Wales (latitude 53°N). These fish were reared in 5-m cages in groups of 850-1,000 and fed to satiation 2-4 times daily. The annual temperature range was 4-19°C. Salinity varied between 28 and 34‰ over the period of study.

Seawater transfers in Experiments 7 and 8 were made to 2-m (1,800-l) glass fibre tanks at the same site. Filtered, oxygenated seawater was supplied at 25-l min^{-1}, and smolts were fed by clockwork automatic feeders (C & H Plastics Ltd., Loanhead, Midlothian, U.K.) at a rate of 1% body weight day^{-1}. Temperature and salinity ranges over the period of study (May to June) were 10-19°C and 30-33‰ respectively.

2.1.3 Broodstock

All broodstocks used in Chapter 4 of this thesis were hatched and reared at a large broodstock facility on the west coast of Scotland (Latitude 56°N). Four discrete stocks, based on eyed eggs obtained from various Norwegian and Scottish sources, were reared through to maturity as 2- and 3-sea winter spawners. Grilse (fish maturing after only 1-sea winter) were graded out and harvested as is normal in the production cycle.

All stocks were maintained in 12-m (225-m^3) concrete tanks at a land based pump-

ashore facility in oxygenated seawater prior to maturation in conditions of ambient temperature and photoperiod. Feed was supplied at rates recommended by manufacturers tables until late summer. After this time it is usual for maturing salmon to stop feeding. The salinity in broodstock tanks was gradually reduced from the beginning of October to achieve freshwater before the expected commencement of spawning.

2.2 Smolt Transport

Smolts were transported from their freshwater rearing facilities to marine sites in a 1200-l insulated transport tank (C&H Plastics Ltd.) at densities of not more than 50-kg tonne^{-1}. Air was supplied to the tank at a rate of approximately 30-l min^{-1} from a 12-V compressor (Charles Austin Pumps Ltd., Weybridge, Surrey, U.K.). Small numbers of smolts were transported short distances in double strength black plastic bags containing *ca* 30-l freshwater (at densities not exceeding 80g-l^{-1}, for up to 2-hours) which were aerated during transit.

2.3 Fish identification

Smolts from all treatments reared in Experiment 1 were transferred to a single seawater on-growing tank. Group identification was achieved by subcutaneously marking the ventral surface of fish (in different positions) with a fine jet of alcian blue dye (1% w/v in water; Sigma Chemical Co. Ltd.) fired from a panjet (F.H. Wright, Dental MFG Ltd., Dundee, U.K.) as described by Johnstone (1981). These markings remained visible for at least 2-months.

2.4 Anaesthesia

To allow ease of handling and to minimise stress and injury, fish were anaesthetised prior to all manipulative procedures. Fish were starved for 24-h prior to handling where possible. Immersion in a solution of 2-phenoxyethanol (Sigma Chemicals Company Ltd., Poole, Dorset, U.K.) in water (1:1,000 v/v) induced full anaesthesia within 1-minute. Subsequent transfer to aerated water resulted in full recovery within 5-minutes.

Phenoxyethanol anaesthetic solutions were made up at the same concentration using 28‰ synthetic seawater for use with challenged pre-smolts or full strength seawater for use with post-smolts maintained at marine facilities.

Broodstock were anaesthetised using a mixture of stock benzocaine (BDH Chemicals Company Ltd., Poole, Dorest, U.K.) in ethanol (100-g l^{-1} w/v) and either fresh or seawater (1:1,000 v/v). Full anaesthesia occurred within 5-minutes of exposure to the anaesthetic solution and subsequent recovery 10-15-minutes following the return to aerated fresh or seawater.

2.5 Fish measurement

During smolt trials (described in trials 1-8, Chapter 3) samples of approximately 100 fish were netted from each group at regular intervals for the assessment of weight, length and condition factor. Fish were anaesthetised and weights determined to 0.1-g (Sartorius L12000S digital balance; Sartorius Instruments Ltd., Epsom, Surrey, U.K.). Lengths were measured to 0.1-cm using a perspex measuring board. Individual condition factors were then determined as:

$$\text{condition factor (K)} = \frac{[\text{weight(g)} \times 100]}{\text{length(cm)}^3}$$

The degree of body silvering, and the occurrence of running (spermiating) males were also recorded. In Experiment 7 (Chapter 3), the specific growth rates (SGR) of post-smolts in successive sampling intervals, as percent increase in body weight per day, were calculated as follows:

$$\text{SGR} = \frac{\ln(\frac{w_1}{w_0})}{t} \times 100$$

where w_0 = mean weight (g) at time_0

w_1 = mean weight at time_1

t = time interval (days)

2.6 Seawater challenge tests

In order to assess the ability of pre-smolts to osmoregulate in a marine environment, batches of fish were subjected to periodic seawater challenge tests; adapted from those described by Blackburn and Clarke (1987). A salinity of 28‰ was chosen in accordance with earlier studies (Clarke and Nagahama, 1977; Clarke *et al.*, 1978;1985; Brauer, 1982).

1. Artificial seawater was made up at a strength of 28‰ in 0.5-m plastic tanks by dissolving 1,440-g Instant ocean (Animal House, Bartlet, W.Yorks, U.K.) in 50-l of freshwater.
2. Challenge tanks were then placed into a 2×2m glass fibre fry tank containing running constant temperature (10°C) spring water to act as a thermal buffer. In Experiments 7 and 8 the challenge medium was circulated through a chiller and heater (Grant Instruments Ltd., Cambridge, U.K.) set to 10°C.
3 The tanks were aerated overnight to ensure that all salt was completely dissolved and that the seawater had equilibrated to the test temperature.
4. The salinity of the test medium was checked using a salinity refractometer (model S/Mill; Atago Company Ltd., Japan) and adjusted if necessary.
5. Fish to be tested were introduced to the test medium in batches of 12-15 per tank.
6. Groups were removed and blood samples taken immediately after 24-h exposure.

2.7 Blood Sampling

Blood samples were drawn from the dorsal aorta of anaesthetised fish using a sterile 1-ml syringe (Terumo Europe N.V., Leuven Belgium) fitted with a 21-gauge, 40-mm needle (Gillette Ltd., Middlesex U.K.). for serum, whole blood was transferred to a micro centrifuge reaction vial (LIP Equipment and Services Ltd., Shipley, W. Yorks, U.K.) and refrigerated for at least 1- hour, to allow clotting, before centrifugation at 14,000-g for 5-minutes (Micofuge B; Beckman, Palo Alto, California, U.S.A). For plasma, syringes were flushed with ammonium heparin (4-mg ml^{-1}; Sigma Chemical Company Ltd.) prior to blood sampling. Heparinised blood was transferred to capped polystyrene tubes (LP3; Luckhams

Ltd., Burgess Hill, U.K.) and centrifuged immediately (MSE Super-Minor centrifuge; Fisons Scientific Equipment Ltd., Loughborough, U.K.) at 2500 rpm for 15-minutes. Serum or plasma was then transferred to new reaction vials or polystyrene tubes and stored at -20°C or -70°C respectively, until analysis.

2.8 Gonadosomatic Index

In some instances the state of maturity of post-smolts was assessed by the calculation of a gonadosomatic index (GSI), expressing the gonad weight as a percentage of somatic weight:

$$GSI = \frac{\text{gonad weight (g)}}{\text{body weight (g)}} \times 100$$

2.9 Determination of serum sodium

Total serum sodium was determined using an atomic absorption spectrophotometer (model 2280; Perkin-Elmer, Beaconsfield, Bucks, U.K.)

2.9.1 Serum Dilution

The spectrophotometer has a maximum detection for sodium of 0.8-mg l^{-1}. As the resting concentration of serum sodium is approximately 155-mmol l^{-1} (3.50g l^{-1}) for salmon parr and smolts acclimated to freshwater; and that for parr challenged for 24-h at 28‰ can reach 225- mmol l^{-1} (5.24g l^{-1}), dilution of serum by a factor of approximately 6600 was adequate to place all samples within an optimum detection range of 0.5-0.7-mg l^{-1}. This was achieved as follows:

1. Add 2.5-ml 0.1% nitric acid (Spectrosol grade; BDH Chemicals Ltd.) in deionised water (v/v) to a 15-ml polystyrene test tube (Bibby-Sterilin Ltd., Stone, Staffs, U.K.) (tube 1).
2. Add 25-µl of serum and vortex mix.
3. Add 6.5-ml 0.1% nitric acid to a second 15-ml test tube (tube 2).

4. Transfer 100-µl from tube 1 to tube 2 (to provide an overall serum dilution factor of 6666) and vortex mix.

2.9.2 Preparation of sodium standards

1. Add 40-µl of sodium nitrate standard (Spectrosol, c(Na^+)=100-mg l^{-1}; BDH Chemicals Ltd.) to a 100-ml volumetric flask.

2. Make up to exactly 100-ml with 0.1% nitric acid to provide a 0.40-mg l^{-1} standard solution.

3. Repeat with 80-µl of sodium nitrate standard to provide a 0.80-mg l^{-1} standard solution.

2.9.3 Sodium analysis.

1. Fit sodium bulb (if not already fitted).
2. Check that GAIN and LAMP1 are turned to zero.
3. Switch on machine and leave for 15-minutes to warm up.
4. Adjust settings: BGKD to AA (Atomic absorption)
   SLIT to 0.7 (normal)
   WAVELENGTH to 589.2-nm
   SIGNAL to lamp1
   MODE to CONT
5. Adjust lamp/energy to 20 (continuous running for Na^+ bulb) with LAMP1
6. Set SIGNAL to ABS
7. Adjust lamp/energy to 50 with GAIN
8. Maximize lamp/energy by moving the bulb within the bulb mounting and by adjusting the two position knobs.
9. Set lamp/energy to 75
10. Position burner head
11. Ignite acetylene flame and run for 10-minutes with sample tube in deionised water
12. Place the sample tube in the 0.80-mg l^{-1} (highest) standard and optimise the flame position and aspiration rate.

13. Calibrate the machine by aspirating the standards (lowest first) and setting the standard curve.

14. Set MODE to CONT (continuous)

15. Check each standard

16. Measure diluted serum samples and record output (mg l^{-1})

17. Make sure lamp/energy remains at 75 (adjust with GAIN) and keep checking standards (Re-calibrate as necessary)

Values recorded from the spectrophotometer were converted to serum sodium concentration as mmol l^{-1} with the following formula:

$$Y = \frac{X \times \text{dilution factor}}{\text{atomic mass of } Na^{+}} \quad \text{ie} \quad Y = \frac{X \times 6666}{23.3} \qquad \text{where} \qquad X = \text{mg } l^{-1}$$

$$Y = \text{mmol } l^{-1}$$

2.10 Determination of plasma melatonin

Salmon plasma samples were analysed for melatonin by direct radioimmunoassay described by Fraser *et al.* (1983), for the measurement of melatonin in human plasma, as modified by Randall (1992), using sheep anti-melatonin antiserum (Guilday Antisera Ltd./Stockgrand Ltd., Guildford, Surrey, U.K.) (used at an initial dilution of 1:2,000) and [0-methyl-^{3}H]melatonin radiolabel (specific activity 70-85-Ci $mmol^{-1}$; Amersham International Ltd.) in a working concentration of approximately 4000-dpm 100-μl^{-1}. Standards were prepared using melatonin (N-actyl-5-methoxytrptamine; Sigma Chemical company Ltd.) and melatonin-free plasma (obtained by charcoal stripped salmon plasma collected during the photophase). Samples taken for melatonin determination in Experiment 6 (Chapter 3) were kindly analysed by C.F. Randall.

2.11 Broodstock spawning and egg measurements

Females approaching maturity show a darkening in colour and a swelling of the abdomen, as well as a protrusion of the anal-urogenital papilla, and may be separated from non-maturing fish up to 2-months before spawning commences. Maturing males turn a deep brown colour, develop a characteristic kype and generally begin to spermiate a few weeks in advance of ovulation in the females (seminal fluid may be expelled from a running male by gently squeezing the ventral abdomen close to the anal-urogenital papilla). An ovulated female has a very soft abdomen and may be identified by gentle hand pressure to the ventral surface. Although females ovulate in captivity, they fail to oviposit and have to be manually 'stripped'. As fish approached maturity, tanks were checked on a regular basis to identify ovulated females and hence the commencement of spawning. Once spawning had started, fish were examined and sorted at approximately 5-day intervals.

Ripe females were weighed to the nearest 100-g (pre-strip weight) using a 50-kg spring balance (Sartorius Instruments Ltd.) and then stripped into clean, dry individually numbered 12-l bowls. Post-strip weights and lengths (measured to the nearest 1-cm) were also recorded.

Milt was collected from up to 20 males, and checked individually under a light microscope for motility when activated with ovarian fluid. Good quality milt was pooled, extended (1:1) with modified Cortland's solution (Truscot *et al*, 1968), and stored on ice. Extended milt was added to the eggs at a ratio of approximately 1-ml l^{-1}, and gently mixed by hand for 1-minute to allow fertilisation. The eggs were rinsed to remove excess milt and debris and then left to stand for a minimum of 2-hours in at least 2-volumes of freshwater to ensure complete hardening before egg diameter and fecundity measurements were taken.

2.11.1 Egg Diameter

The mean egg diameter of each fish was estimated by aligning a sample of water hardened eggs along a 250-mm grooved measuring gauge. These were counted to the nearest 0.5-egg, and the mean diameter calculated as shown overleaf:

$$\text{Mean egg diameter} \quad (\text{OD}) = \frac{250}{\text{No. eggs along gauge}}$$

The accuracy of this method has been validated by Springate (1985), who found no significant difference in egg diameters estimated in this way compared to those calculated by measuring individual eggs with calipers.

2.11.2 Total Egg Volume

Eggs were drained of water with a plastic sieve, and their volume determined by counting 'dry' litres to the nearest 50-ml using a graduated 1-l plastic jug.

2.11.3 Total Fecundity

The total fecundity for each female was calculated using the mean egg diameter (OD) and total egg volume as follows:

$$w = -0.283x + 5.41$$

where $w = \log_{10}$ (number of eggs per litre)

x = egg diameter (OD) (mm)

$$\text{Total Fecundity (TF)} = y\left(10^{w}\right)$$

where y = total egg volume (l)

2.11.4 Relative Fecundity

Relative fecundity, ie the number off eggs per kg for each female was calculated as shown below:

$$\text{Relative Fecundity (RF)} = \frac{\text{TF}}{v}$$

where v = Post-strip weight (kg)

These equations were derived from those of Von Bayer (1950; cited by Leitritz and Lewis, 1976); these were shown to provide accurate estimates of total fecundity for rainbow

trout (Springate, 1985) with a highly significant correlation between estimated and actual (counted) values (r^2=0.99, P<0.001).

2.12 Statistical Analyses

Unless otherwise stated, the statistical techniques used in this thesis are comprehensively described in either Sokal and Rohlf (1981) or Snedecor and Cochrane (1980). All calculations were performed using Minitab release 7.2 (HP-UX version; Minitab Inc., State College, Pennsylvania, USA) on a Hewlett-Packard (HP-9000, series 845) mainframe computer. Where necessary, additional software was written and executed within Minitab.

2.12.1 Estimation of a population mean

The arithmetic mean ($\overline{X}$) provides an estimate of the population mean (μ) which may be derived from a random sample. Throughout this thesis $\overline{X}$ has been used together with the standard error of the mean (sem) as an indicator of sample distribution and written as the arithmetic mean ± one standard error of the mean ($\overline{X} \pm 1$sem).

$$\text{Arithmetic mean } (\overline{X}) = \frac{\sum X}{n} \qquad \text{where: } \sum X = \text{sum of observed sample}$$

$$n = \text{number of observations}$$

$$\text{Standard error of the mean (sem)} = \frac{s}{\sqrt{n}}$$

$$\text{where } s = \text{the sample standard deviation} = \sqrt{\frac{\sum X^2 - \frac{\left(\sum X\right)^2}{n}}{n-1}}$$

2.12.2 Testing assumptions of parametric statistics

Parametric statistical techniques, including t-test and analysis of variance, require that

sample observations are made at random and that test variances are independent. In addition, strict assumptions are made about the distribution of data/observations: the data must fit a normal distribution and sample variances (s^2, the square of the sample standard deviation) must be homogenous (homoscedastic). Violation of the distribution assumptions reduces the power of parametric testing, and in extreme cases a test may be invalidated. For this reason, all data were tested for normality and homogeneity prior to analysis.

2.12.2.1 Normality of distribution

To assess the normality of sample distributions, the normal scores (or ranked normal deviates) of the sample values were calculated. The sample values were then correlated with their normal scores. This essentially provides a Shapiro-Wilk test for normality (Shapiro and Wilk, 1965) and the resulting correlation coefficient (r) was compared with critical tabulated values extrapolated from those supplied by Ryan *et al.* (1981). Data sets with r equal to or greater than the tabulated value for r at P=0.01 were taken to be significantly departed from normality.

2.12.2.2 Comparison of two variances

The F-test was used to compare the variances of two samples, which assesses the departure of the variance ratio from unity.

$$F_s = \frac{s_1^2}{s_2^2}$$ where s_1^2 and s_2^2 are the greater and lesser variances respectively

Degrees of freedom $v_1, v_2 = n_1\text{-}1, n_2\text{-}1$

If the calculated value for F_s was less than the tabulated value at P=0.05 the variances were concluded to be homogenous. Variances with F_s equal to or greater than the tabulated value for F at P=0.05 were taken to be heterogeneous.

2.12.2.3 Multiple comparisons of variance

Bartlett's test was used to test the homogeneity of more than two variances. This test provides an estimation of chi square (χ^2) which may be compared to a tabulated critical value. For a number of variances where f_i represents the degrees of freedom of the ith variance:

$$M = (2.3026)\left[\left(\sum f_i\right)\log \bar{s}^2 - \sum f_i \log \bar{s}_i^2\right] \qquad \text{where: } \bar{s}^2 = \frac{\sum f_i s_i^2}{\sum f_i}$$

$$C = 1 + \frac{1}{3(a-1)}\left[\sum \frac{1}{f_i} - \frac{1}{\sum f_i}\right]$$

$$\chi^2 = \frac{M}{C} \qquad \text{with } (a-1) \text{ degrees of freedom}$$

If the calculated value for χ^2 was less than the tabulated value at P=0.05 the variances were concluded to be homogenous. Variances with χ^2 equal to or greater than the tabulated value at P=0.05 were concluded to be heterogeneous.

2.12.3 Comparison of two samples

If the sample variances were found to be normally distributed and homogenous the means were compared using Student's t-test with a pooled estimate of the variance. For normally distributed samples with heterogeneous variances ($0.05>P>0.01$) means were compared using Student's t-test incorporating a separate estimate for each variance. This effectively reduces the degrees of freedom of the critical t, making the test more conservative thus reducing the probability of a Type-1 error. If one or both of the samples departed significantly from normality, and/or the sample variances were found to be heterogeneous with a significance of $P \leq 0.01$, the Mann-Whitney U-test was employed. This is a non-parametric technique which compares the medians of two (unmatched) samples, and makes fewer assumptions about the distribution of the data within the samples.

2.12.4 Multiple Comparisons

A one-way analysis of variance (ANOVA) was employed for the preliminary analysis of means of three or more normally distributed samples of homogenous variance. If a significant difference was identified among the means ($P \leq 0.05$) paired means were compared with a multiple range test as follows:

$$t_s = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \quad \text{with} \quad (n_1 + n_2) - 2 \text{ degrees of freedom}$$

where $\bar{X}_1$ and $\bar{X}_2$ = sample means

n_1 and n_2 = number of observations in each sample

s^2 = error mean square, as calculated by the ANOVA

Pairs of means were concluded to be significantly different if their calculated t_s was greater than the tabulated value for t at P=0.05. If sample variances were heterogeneous ($P \leq 0.05$; Section 2.12.2.3) or if one or more of the sample distributions departed significantly from normality ($P \leq 0.01$; Section 2.12.2.1), the Kruskal-Wallis test (equivalent to a non-parametric analysis of variance) was employed as a preliminary analysis of three or more samples. If a significant difference ($P \leq 0.05$) was detected among groups, differences between pairs were assessed using Dunn's multiple comparison procedure as described by Zar (1984):

$$Q_{0.5,k} = \frac{\bar{R}_2 - \bar{R}_1}{SE}$$ where $\bar{R}_1$ and $\bar{R}_2$ = mean ranks of the two samples

(e.g. $\bar{R}_1 = \frac{R_1}{n_1}$)

k = number of groups

SE = standard error = $\sqrt{\left(\frac{N(N+1)}{12} - \frac{\sum(t^3 - t)}{12(N-1)}\right) - \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$

N = total number of observations in all (k) groups

t = number of ties for a given (tied) value

n_1 and n_2 = number of observations in each sample

Pairs of means were concluded to be significantly different if their calculated Q was greater than the tabulated value for Q at P=0.05, for k groups.

2.12.5 Linear regression and comparison of regression lines.

Regressions equations for total fecundity, egg size and total egg volume on post-strip fish weight (Chapter 4) were calculated from logarithms (base 10) of data using the method of least squares which is described in full detail by Snedecor and Cochran (1980). The homogenieties of paired residual variances were tested using F_s (variance ratio, see Section 2.12.2.2). The statistical comparisons of common slope and elevation between different strains were made using software designed to emulate the analysis of covariance (ANCOVA) technique set out in Table 14.6.2 in Snedecor and Cochran (1972). Confidence intervals (95%) were calculated from the standard error of $\hat{Y}$ (estimated Y for given $\overline{X}$) for log fecundity, log diameter and log total egg volume of 2- and 3-sea winter stocks adusted to a common log weight ($\overline{X}$) as follows:

$$S_{\hat{y}} = \sqrt{S_{Y.X}^2 \left[\frac{1}{n} + \frac{(X_i - \overline{X})^2}{\sum x^2} \right]}$$

2.12.6 Comparison of percentages

Percentages were statistically compared by calculating the standard error (SE) of sample proportions (% ÷ 100), as described by Fowler and Cohen (1987), and using these values to infer confidence intervals:

$$SE = \sqrt{\frac{p(1-p)}{n-1}} \quad \text{where} \qquad p = \text{sample proportion}$$

n = number of observations

95% Confidence limits = $SE \times 1.96$

99% Confidence limits = $SE \times 2.58$

If the 95% or 99% confidence intervals of two sample proportions did not overlap it was concluded that they were significantly different at $P \leq 0.05$ or $P \leq 0.01$ respectively.

Chapter 3

The effects of photoperiod on smoltification, and the time of seawater transfer on the growth of Atlantic salmon smolts

3.1 INTRODUCTION

The majority of salmonid species (including the genera *Salmo*, *Salvelinus* and *Oncorhynchus*) show some degree of anadromy in the their life history (Rounsefell, 1958; Hoar, 1976; Thorpe, 1989), ranging from members of the genus *Oncorhynchus* which are normally highly anadromous (for example the pink salmon, *O. gorbuscha*, and the chum salmon, *O.* keta) and undergo seaward migration directly after hatching, to species which are optionally anadromous (including the rainbow (steelhead) trout *O. mykiss* and the brown (sea) trout *Salmo trutta*) which show both migratory and resident life history patterns. The Atlantic salmon (*Salmo salar*) shows great flexibility in its life history pattern. Although non-anadromous and landlocked populations of Atlantic salmon have been reported (Power, 1958; Burton and Idler, 1984; Sutterlin and MacLean, 1984; Birt and Green, 1986; Berg and Gausen, 1988) which complete their whole life cycle entirely in freshwater, and maturation of 1+ male parr in freshwater is a relatively common occurrence (Mitans, 1973; Thorpe, 1977; Glebe *et al.*, 1980; Lunqvist, 1980; Murphy 1981; Saunders *et al.*, 1982; Bagliniere and Maise, 1985; Duston and Saunders, 1992), most individuals in the majority of populations migrate to the sea at some point.

The terminology surrounding the processes which prepare freshwater residents for a marine existence is vague. Generally speaking the term smoltification is synonymous with smolting, and parr-smolt-transformation, implying the seasonal changes in physiology, behaviour and morphology which precede the seaward migration of juveniles during the late spring (see Langdon, 1985; Hoar, 1988 for reviews). However, Thorpe (1986) proposes a model whereby potential migrants make a 'decision' to smolt based on an internal perception of growth or accumulation of energy reserves. In the Atlantic salmon this is believed to occur in the summer prior to migration, and is overtly expressed as a surge in growth among individuals which are going to migrate the following spring (Thorpe, 1977; Bailey *et al.*, 1980; Thorpe *et al.*, 1980; Kristinsson, 1985). This results in the marked bimodality of population length-frequency distribution which becomes more evident throughout the autumn (Thorpe *et al.*, 1980). This 'decision' may be considered as the initiation of

smoltification. In this respect smoltification is analogous to maturation in salmonids, which begin gonadal development up to a year before ovulation and spawning (Scott and Sumpter, 1983; Sumpter *et al.*, 1984; Bromage and Cumaranatunga, 1988). But, whereas maturation clearly culminates in spawning, there is no equivalent term for the completion of smoltification. In this thesis, the term smoltification is taken to include all processes from the decision to undertake seaward migration to the achievement of full smolt status (the completion of smoltification). Smolts from a number of salmonid species, retained in freshwater past the natural time of migration, undergo a loss of some smoltification characteristics, a process commonly referred to as parr-reversion or, sometimes inappropriately, de-smoltification (Zaugg *et al.*, 1972; Eriksson and Lundqvist, 1982; Johnston, 1983; Clarke *et al.*, 1985; Soivio et al., 1988; Duston and Saunders 1990; Kurokawa, 1990).

The most striking morphological change during the course of smoltification is the development of body silvering. This is achieved by the synthesis and sub-dermal deposition of purine crystals, predominantly guanine (Johnston and Eales, 1967, 1970; Vanstone and Market 1968) which obscures the brown-green colouration and darker lateral bars characteristic of parr. In addition the paired and caudal fins develop a well defined black margin and the green pigmentation disappears from the fin tissue and the ventral surface. During the later stages of smoltification pre-smolts undergo an increased catabolism of body lipid (Sheridan, 1986, 1989; Bergström, 1989) and an adjustment of body proportions, in particular an increase in proportional post-anal length (Winans, 1984; Winans and Nishioka, 1987; Wessel, 1990), which results in an overall decrease in weight:length ratio (condition factor) and a more streamlined shape (Hoar, 1939; Farmer *et al.*, 1978; Björnsson *et al.*, 1989)

The most important physiological change during smoltification involves the development of the ability to hypo-osmoregulate in a hyper-osmotic (marine) environment. Seawater tolerance has been shown to develop rapidly towards the completion of smoltification (Komourdjian *et al.*, 1976; Saunders *et al.*, 1985, 1989; Duston and Saunders 1992). Key components in the heightened seawater tolerance include increases in intestinal

water absorption (Collie and Bern, 1982; Veillette *et al.*, 1993), urine production (Holmes and Stainer, 1966; Eddy and Talbot, 1985) and in the specific activity of the Na^+K^+-ATPase enzyme responsible for the excretion of monovalent ions (McCartney, 1976; Lasserre *et al.*, 1978; Saunders and Henderson, 1978; Ewing and Birks, 1982; Boeuf and Prunet, 1985; Boeuf *et al.*, 1985; McCormick *et al.*, 1987; Saunders *et al.*, 1989; Zaugg, 1989). The primary site of this enzyme system in gill and opercular epithelium is the mitochondrion-dense chloride cells which have been identified as having a major salt-secretory function (Foskett and Scheffey; 1982). The increased activity of these cells during smoltification (Chernitsky, 1980) is reflected by an increase in both their size (Langdon and Thorpe, 1984) and number (Loretz *et al.*, 1982; Wickes *et al.*, 1983). Na^+K^+-ATPase function is stimulated to its maximum activity on entry to the marine environment (Langdon and Thorpe, 1984). In addition there is a decreased water permeability of the gills (Evans, 1984; Isaia, 1984) and reduction in urine flow (Holmes and Stainer, 1966).

A number of behavioural adaptations occur to facilitate migration (Thorpe, 1987b). Salmon parr generally defend territories, and show strong, positively rheotactic swimming behaviour. However, in the final stages of smoltification, bottom dwelling behaviour is much reduced as the fish hold stations higher in the water column (Kalleberg, 1958) and show an increased tendency to shoal (Hoar, 1976) in anticipation of seaward migration. The ability to hold station in fast water flows becomes reduced (Thorpe and Morgan 1978a) accounting for a degree of passive migration, as well as active downstream migration (Kalleberg, 1958; Solomon, 1978).

A number of endocrine changes have been observed in salmonids throughout the course of the parr-smolt transformation, including an activation of the thyroid, interrenal and pituitary systems. Hoar (1939b) demonstrated a seasonal hypertrophy of thyroid follicles and suggested a regulatory role of thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), in smoltification. The activation of thyroid function as evidenced by a 1- to 2-month period of elevated T_4 prior to seaward migration is now well documented (Nishikawa *et al*, 1979; Dickhoff *et al.*, 1978; 1982; Lindahl *et al.*, 1983; Specker *et al.*, 1984; Boeuf and Prunet, 1985; Yamamuchi *et al.*, 1985; McCormick *et al.*, 1987; Boeuf, 1989). In addition,

the responsiveness and sensitivity to exogenous thyrotropin increases (Specker and Schreck, 1984; Swanson and Dickhoff, 1987). Folmar and Dickhoff (1981) found T_4 to provide the best indicator for survival of coho salmon transferred to seawater. The administration of thyroid hormones or other thyroactive preparations to salmon parr have been shown to elicit a number of the physiological and morphological changes associated with smoltification; including increased somatic growth (McBride *et al.*, 1982), changes in body composition (Sullivan *et al.*, 1987), development of silver colouration (Piggins, 1962; Ikuta *et al.*, 1985) and the development of salinity tolerance (Higgs *et al.*, 1982; Dickhoff *et al.*, 1985) and preference (Baggerman, 1963). Disfunctions in the thyroid axis identified by inactive appearance of thyroid follicles and low plasma T_3 and T_4 have been implicated in the phenomenon of stunting following transfer to seawater (Folmar *et al.*, 1982; Nishioka *et al.*, 1982). However, other studies found that the increased salinity tolerance provided by exogenous thyroid administration was not permanent and that $Na^+ K^+$-ATPase activity was not stimulated (Miwi and Inui 1985; Omeljaniuk and Eales, 1986). A direct causal role of thyroid hormones triggering smoltification has not been established. Dickhoff and Sullivan (1987) suggested a permissive role or synergistic involvement with other hormones are the more likely functions.

In recent years the pituitary, and its production of growth hormone (GH) and prolactin (PRL), its close structural homolog, has received a considerable amount of interest in relation to possible involvement in the control of smoltification. Activation of the pituitary somatotropes and increased production of growth hormone (GH) occurs during smoltification (Clarke and Nagahama, 1977; Boeuf *et al.*, 1989; Prunet *et al.*, 1989) which is reflected in the fast growth rate of pre-smolts. GH has been implicated in the development of hypo-osmoregulatory ability and several studies have shown that GH improves seawater tolerance (Komourdjian *et al.*, 1976; Clarke *et al.*, 1977; Collie *et al.*, 1989; Madsen, 1990). Parr given GH implants in winter months demonstrated increased $Na^+ K^+$-ATPase activity in freshwater, lower plasma osmolarity after seawater transfer and higher growth rate in seawater (Boeuf *et al.*, 1994), the response of parr was greater than that of smolts. The influence of GH on the development of osmoregulation may be effected directly by

stimulating GH receptors in the gills (Sakamoto and Hirano, 1991), through the enhancement of cortisol production, its thyrotropic activity (Grau and Stetson 1979), and in part through its growth-stimulatory properties (Clarke *et al.*, 1977; Higgs *et al.*, 1978; Down *et al.*, 1988,1989). An increasing photoperiod has also been shown to stimulate GH levels (Björnsson *et al.*, 1989; Okumoto *et al.*, 1989).

PRL plays an important role in the control of osmoregulatory homeostasis in freshwater fish, primarily with respect to sodium conservation and decreased gill water permeability (Clarke and Bern, 1980; Loretz and Bern, 1982; Hirano, 1986). Following transfer to seawater, PRL secretory cells of amago salmon (*Oncorhynchus rhodurus*) have been shown to have decreased activity (Nagahama, 1985) and plasma PRL levels of rainbow trout, coho and Atlantic salmon decline (Prunet and Boeuf, 1985; Prunet *et al.*, 1985; Avella *et al.*, 1990). Although PRL has also been shown to have a thyrotropic activity (Leatherland, 1982), it is not considered to have a central role in controlling the development of smoltification (Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980).

Observations of elevated cortisol in Atlantic salmon (Langhorne and Simpson, 1981; Thorpe *et al.*, 1987) and coho salmon (Specker, 1982; Specker and Schreck, 1982; Patino and Schreck, 1986) during smoltification have provided evidence for the involvement of the interrenal gland in the control of this process. However, although cortisol injection induced body silvering and elevated Na^+ K^+-ATPase activity in catadromous eels (*Anguilla rostrata*) (Epstein *et al.*, 1971; Forrest *et al.*, 1973) similar effects were not achieved in Atlantic salmon (Langdon *et al.*, 1984) and the contribution of cortisol to smoltification in salmon remains unclear.

In the commercial environment constraints of physiology require that seawater on-growing facilities can only be stocked during the spring as smoltification must be completed before hatchery smolts can be transferred to marine facilities. In the past, the untimely transfer of smolts has resulted in poor seawater growth and high mortalities (Clarke and Nagahama, 1977; Folmar and Dickhoff, 1980, 1981; Wedemeyer *et al.*, 1980). The restricted temporal availability of smolts ultimately leads to a seasonality in the supply of marketable salmon.

In recent years a number of companies have addressed this problem by attempting to transfer large 0+ parr or 1+ parr to seawater during the autumn and winter. Results have been variable but most transfers of this nature have incurred high mortalities. Higher survivals have generally been obtained where winter seawater temperatures have remained above 4°C. Duston and Knox (1992) found that Na^+K^+-ATPase activity among 1+ parr in October could be stimulated prior to seawater transfer by either gradual marine acclimation, through increasing salinity over a period of 1-month or by a direct change from an ambient to a long day photoperiod (LD18:6) with a combination of these treatments having an additive or synergistic effect, greatly improving over-winter survival. However, it is evident from these trials that non-smolts are very sensitive to low temperature and that although a reasonable survival is possible it is by no means guaranteed. In addition, these fish undergo no growth, or negative growth, until the following spring when the parr complete smoltification in seawater and proceed to grow normally (Duncan *et al.*, 1994).

The possibility of inducing smoltification in salmonids by treatment with exogenous hormones is also under investigation (Almendras *et al.*, 1993; Boeuf *et al.*, 1994). Intraperitoneal GH implantation during winter months has been shown to mimic smolting in pre-smolts, advancing transfer time by up to 6-months and allowing treated fish to survive and grow in full strength seawater (Boeuf *et al.*, 1994). However, the practicality of implanting or injecting pre-smolts on a commercial scale is questionable.

Clearly the way ahead is to control the timing of smoltification itself. The synchronous onset of smoltification is mediated by environmental cues, predominantly photoperiod (Baggerman, 1960; Clarke, 1989). The timing of smoltification has been significantly advanced (Komourdjian *et al.*, 1976; Saunders and Henderson, 1978; Zaugg and Wagner, 1973 Clarke *et al.*, 1985; McCormick et al., 1987; Thrush and Bromage, 1988; Duston and Saunders, 1990;1992) and delayed (McCormick *et al.*, 1987; Zaugg and Wagner 1973; Thrush and Bromage, 1988) by modified light regimes (ie. seasonally-compressed, expanded or direct 'square wave' changes from long to short or short to long daylengths). Accumulating evidence suggests that the development of smoltification may be controlled by one or more endogenous circannual rhythms which are entrained by the seasonal change in

daylength (Conte and Wagner 1965; Hoar 1965; Saunders and Henderson, 1970; Wagner 1974a, b; Clarke *et al.*, 1978; 1985; Eriksson and Lundqvist 1982, Duston and Saunders, 1990, 1992) which may be the same or analogous to the mechanism which is believed to time salmonid maturation and spawning (Pyle, 1969; Poston and Livingstone, 1971; Whitehead *et al.*, 1978; Bromage *et al.*, 1982; 1984; Bromage and Duston, 1986; Duston and Bromage, 1986a, 1991; Randall *et al.*, 1991a).

The use of photoperiod manipulation in altering the timing of smoltification in farmed populations would potentially have a number of important commercial applications. At present, there is considerable demand within the U.K. salmon farming industry for early S1 smolts, as fish which could be introduced and acclimated to the marine environment 2-3-months ahead of conventionally reared smolts would benefit greatly from an extended summer growing period. S2 production by smolt units is not favoured as these fish have to be maintained in freshwater for a further year. S2s are larger than S1s but this additional biomass provides no financial advantage to the smolt producer. For this reason many producers tend to cull S2 fractions. However, the option to advance the smoltification of S2 grades by 6-months and hence introduce them to the sea during the autumn may make them more commercially attractive. For the smolt producer, employment of photoperiod techniques to produce a number of crops from one year class would enhance output and greatly improve cash flow and the overall usage, and hence efficiency, of freshwater facilities. For the sea-farmer, the ability to stock seawater sites with 'true smolts' at times of the year other than the spring would also improve cash flow, enable restocking following escapes or losses through disease, but most importantly, the introduction of out-of-season smolts would remove seasonality (Figure 1.4) from farmed salmon production and, by producing an all-year -round consistency of product, stabilise market prices.

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), the salmon industry has been reluctant to proceed with this technique. The reasons for this stem from three main areas of concern. Firstly, there is a paucity of information on the effects of photoperiod manipulation on the quality of smolts

with respect to their survival and growth following seawater transfer. Secondly, there is no information available concerning what effects photoperiod manipulation and possible improved growth opportunity awarded by altered transfer time may have on the maturation of salmon as either grilse or even post-smolts. Thirdly, there has been a move towards a policy of 'all-in all-out' whereby stocks of fish from different year classes are transferred to seawater and harvested as separate groups to reduce the possibility of transfer of disease between year classes.

The primary aims of the experiments described in this chapter are to monitor the development of morphological and physiological parameters associated with smoltification of both potential S1 and potential S2 smolts reared under a variety of experimental photoperiod regimes to determine the times of completion of smoltification; assess the quality of advanced and delayed smolt populations by continued rearing in a seawater environment; identify other effects of photoperiod manipulation which may have commercial implications, including changes in smolt fraction and in the incidence of maturation in both freshwater and seawater; and evaluate the suitability of out-of-season smolts to culture and provide information which may contribute to the achievement of a year-round supply of Atlantic salmon smolts.

Experiment 1 included a preliminary investigation into the effects of a number of compressed and extended seasonal light cycles, applied from the winter solstice, on the timing of smoltification of potential S1 smolts the following Spring. The same seasonally-changing photoperiods were used over the extended freshwater residence of potential S2 smolts from the same population (Experiment 2). In both trials batches of smolts were transferred to seawater to assess their fitness, ie survival and growth in a seawater environment. In Experiment 2, larger groups were also transferred to the commercial on-growing site to provide further results on the effects of photoperiod manipulation during freshwater stages on subsequent maturation in a fish farm environment. Seasonally-changing light regimes used in Experiment 1 were substituted with direct changes from a short day to a long day at different times during the winter to advance the completion of smoltification of potential S1 smolts and investigate the effects of photoperiod changes on

smolt fraction (S1:S2 ratio) in Experiment 3.

In order to address the possibility of an increased incidence of grilse or post-smolt maturation resulting from the photoperiod manipulation of pre-smolts, a further trial was run applying results gained in Experiment 2 to provide winter smolts among groups of all-female and all-female triploid potential S2 fish (Experiment 4). These were also transferred to the commercial on-grower to provide data on their seawater growth and maturation, and allow an assessment of their value to photoperiod work and the possibility of exploiting the low maturation qualities of these stocks in the future production of out-of-season smolts.

Greater commercial interest provided an opportunity to increase the number of fish used in Experiment 5, which was undertaken in order to provide a greater advancement in the timing of smoltification with the aim of producing S1 smolts ready for seawater transfer during February. This trial was accordingly started in the summer, rather than at the winter solstice, as in the previous experiments with potential S1 populations. A greater advance in the timing of smoltification of S2 smolts was undertaken in Experiment 6. This trial also included a dual intensity light regime to investigate the effects of low intensity night-time illumination on the control of smoltification, and to assess the possibility of employing dual intensity in association with compressed photocycles to increase feeding opportunity of pre-smolts, and thereby enhance both the mean weight and smolt fraction of photoperiodically advanced populations.

To provide more information on the consequences of untimely seawater transfer of smolts, and to help identify both the time over which the smolts may be successfully transferred to seawater (sometimes referred to as the 'smolting window') and the optimum transfer time (data which would be valuable when transferring ambient and out-of-season smolts alike) batches of farmed smolts from the same population were transferred to seawater at different times during the spring and early summer in two consecutive years (Experiment 7a and b). The survival and growth of these fish was observed until the following autumn in both cases.

The freshwater phases of the photoperiod trials (Experiments 1-6) were conducted at a commercial smolt unit in the south of England supplied by spring and borehole water of

constant temperature (10±0.2°C). Two seawater sites were used to for the seawater transfer of photoperiod smolts. Small tanks at the Hayling Marine Laboratory, which although not a commercial site, provided full-strength seawater (35‰) with a large annual temperature range (2-24°C) and thus provided very harsh conditions which were particularly useful for testing the adaptability of small numbers of fish (100-150 per group) to a marine environment. Longer sea-trials were run with larger groups (up to 1000 fish) at a cage farm in west Wales. Both the freshwater and seawater phases of Experiment 7 were conducted at a large smolt and on-growing farm on the west coast of Scotland.

3.2 EXPERIMENT 1. The effects of 6-, 8- and 10-month compressed and 16-month extended seasonal light cycles on smoltification and subsequent seawater growth of potential S1 smolts.

3.2.1 Protocol

A farmed population of mixed-sex Atlantic salmon were reared from hatch, in January, under constant temperature (10±0.2°C) and natural photoperiod (52°N) until their first winter. On December 22 the entire stock was graded into three fractions. The largest grade consisted entirely of upper-mode (potential S1) fish ranging from 9.4-16.3-g (12.6-g mean weight). This grade was used to stock each of five 2000-l glass fibre tanks with 750 fish. Four tanks were housed in light-proof boxes (Section 2.1.1) with independent light sources (60-W tungsten filament bulbs) providing a mean light intensity of 440-lx at the water surface. These groups were reared under artificial seasonally-changing light cycles 6-, 8-, 10- and 16-months in duration, all commencing with a short daylength (LD7.5:16.5), until smolting the following spring (Figure 3.1). The remaining (control) group received a natural 12-month photoperiod. The longest daylengths (LD16.5:7.5) in the artificial photoperiods occurred 3-, 2- and 1-month in advance, and 2-months delayed compared to the ambient photoperiod respectively. Morphometric measurements from 100 fish were taken at monthly intervals (Section 2.5) and samples of 12-15 individuals were removed for seawater challenge tests from February (Section 2.6).

On June 1, 40 smolts from each photoperiod treatment were randomly selected, panjet marked to allow group identification (Section 2.3), and retained for seawater transfer. All remaining fish were returned to the farm population and transferred to a commercial on-grower. The marked smolts were moved to a small seawater facility at the Hayling Marine Laboratory (Portsmouth University) on June 5. These fish were maintained in a single 800-l tank for 2-months under conditions of ambient photoperiod and temperature and full salinity (35‰), after which a pump failure forced the termination of the trial.

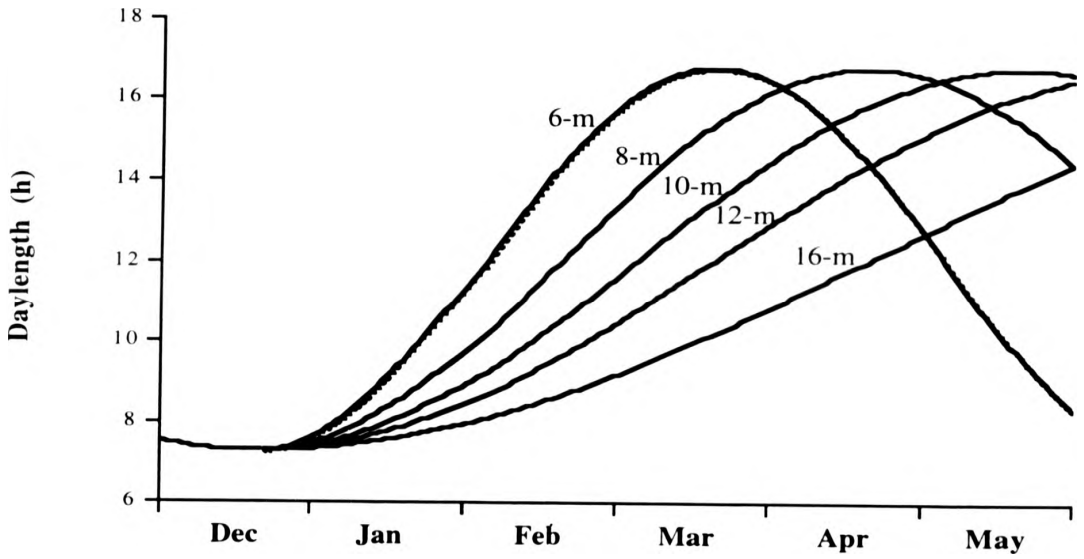


Figure 3.1

6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles used in Experiment 1.

3.2.2 Results

Freshwater

There were no significant differences in weight among treatments between the start of the trial in December and February (Figure 3.2). In March, fish in the control group were significantly heavier than those reared under the 8-month seasonally-compressed photoperiod regime ($P<0.05$). In April, control fish and those reared under the extended photoperiod (16-month) were significantly heavier than fish reared under a 6-month compressed photoperiod ($P<0.05$). However, at the time of seawater transfer, on June 5, there were no significant differences in mean weight between groups. All groups showed a maximal rate of growth between February and March, except for those reared on the extended 16-month photoperiod, where fastest growth occurred between March and April.

The seawater challenge is a test of hypo-osmoregulatory ability in freshwater adapted parr and pre-smolts, which involves the exposure of fish to a hyper-osmotic medium for 24 hours Juvenile salmonids typically respond to being placed directly into a hyper-osmotic environment with a transient rise in plasma ionic concentration (Koch *et al.*, 1959; Houston, 1964; Bath and Eddy, 1979) the duration and extent of this rise is dependent on hypo-osmoregulatory ability and may be readily assessed by subsequent measurement of plasma or serum sodium (Clarke and Blackburn, 1977; Blackburn and Clarke, 1987) or total osmolality (Duston and Saunders, 1990) and has been routinely used as a relatively simple and convenient physiological index of smolt status. Individuals nearing the completion of smoltification should have an improved hypo-osmoregulatory ability and maintain a lower serum sodium concentration than parr, and post-challenge sodium concentrations close to that of unchallenged individuals (*ca* 155-mmol l^{-1}) in conjunction with the development of body silvering, fin darkening and decreases in condition factor were considered to be a good indication that fish have achieved smolt status.

In the 3-months prior to their natural smolting period, the groups of fish reared under compressed photoperiod treatments showed morphological and physiological signs of being at an advanced stage of smoltification compared to those maintained under either control or

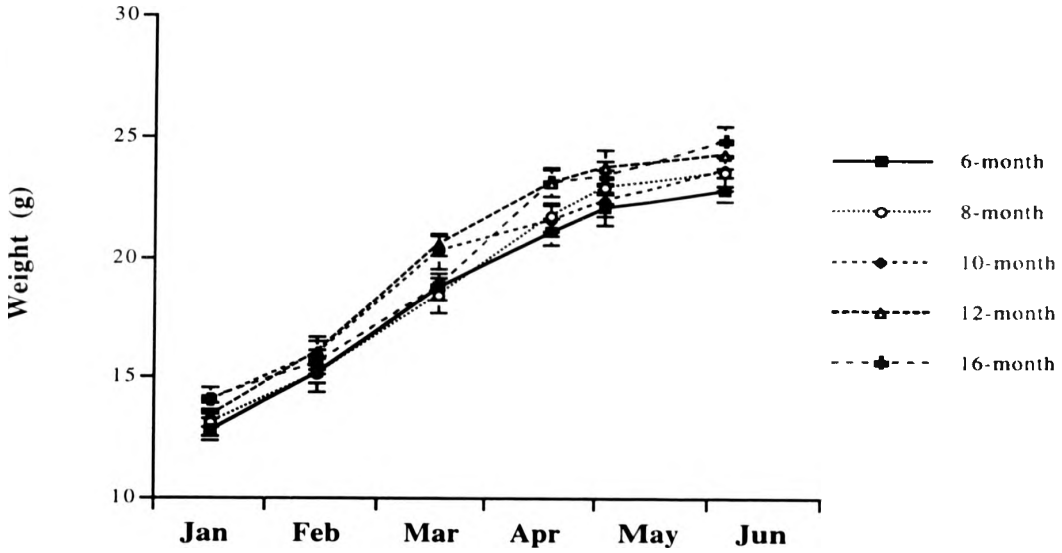


Figure 3.2

Changes in weight of potential S1 smolts reared on 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 1.

extended photoperiods. Before mid-February, fish in all groups retained a strong parr-like appearance. During February some fish in the 6-month and 8-month treatments were beginning to show signs of silvering. By mid-April a majority of the fish in all groups were showing characteristic smolt appearance: with a strong silver colouration and clear fins with well defined black margins.

All groups showed a pattern of decreasing condition factor throughout the freshwater phase of the trial, (Figure 3.3). No significant differences in condition factor were detected until May, when fish reared under the 8-month compressed photoperiod regime had a condition factor significantly lower than all other groups except those reared under a 6-month compressed photoperiod ($P<0.05$).

The first seawater challenge test was performed on March 19, 2-months before the onset of the natural smolting season at this site. At this time the serum sodium concentrations following seawater challenge of groups reared on compressed photoperiods (6-, 8- and 10-month) were significantly lower ($P<0.05$) than those of the control group (12-month) and those reared under the extended (16-month) photoperiod, indicating an advanced development of hypo-osmoregulatory ability, see Figure 3.4. The serum sodium concentration of the 6-month photoperiod group was not significantly different from the unchallenged (control) fish during the April and early-May challenge tests ($P>0.05$). Fish reared under 6- and 10-month photoperiods showed peak osmoregulatory ability on May 5 with post-challenge serum sodium concentrations of 158 and 162-mmol l^{-1} respectively. The rise in serum sodium, following seawater challenge between this point and June is indicative of a loss of osmoregulatory ability associated with smolt reversion. Monthly seawater challenge tests revealed no significant change in osmoregulatory ability in the fish maintained on the 8-month photoperiod between March and June where serum sodium concentration following seawater challenge remained between 164 and 168-mmol l^{-1}. Only the control fish and those reared under the extended (16-month) photoperiod showed a continued development of hypo-osmoregulatory ability between May and June, when samples of fish from all groups were transferred to seawater.

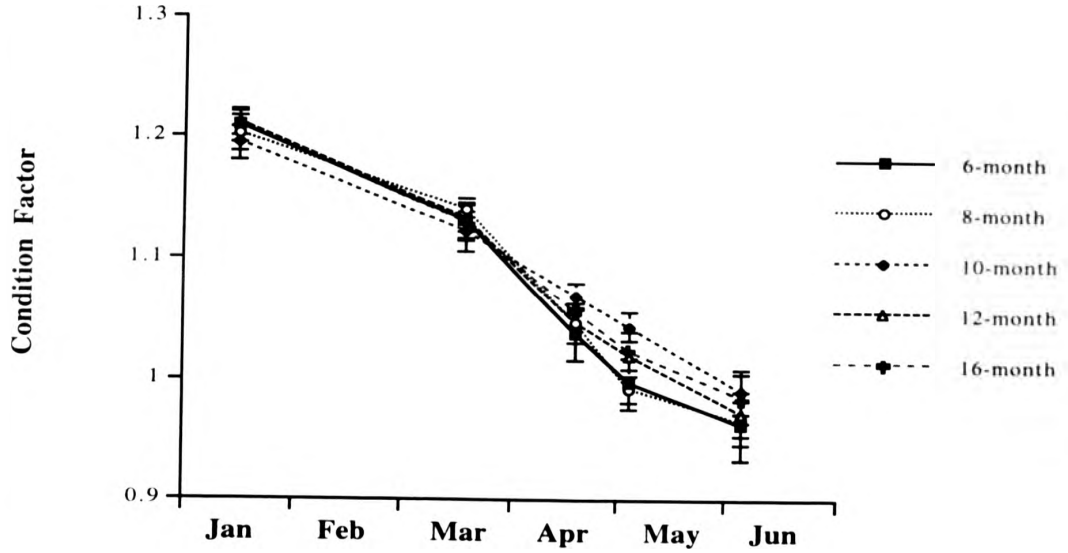


Figure 3.3

Changes in condition factor of potential S1 smolts reared on 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 1.

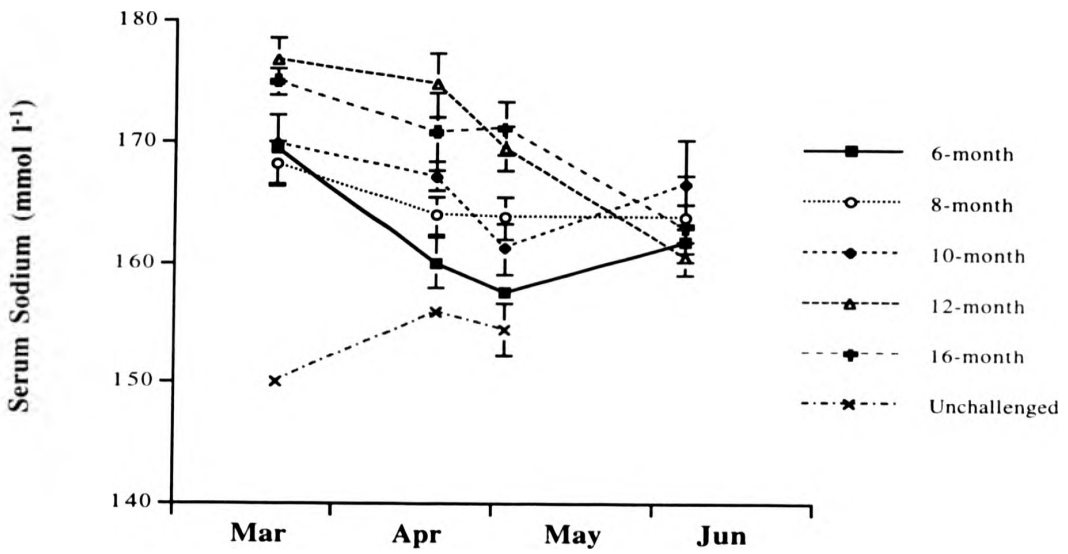


Figure 3.4

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S1 smolts reared on 6-, 8- and 10-month compressed, ambient (12-month), 16-month extended seasonal light cycles and the serum sodium concentration of unchallenged control fish in Experiment 1.

Seawater

Figure 3.5 shows the growth of smolts from each of the 5 photoperiod groups in the first 7-weeks following their transfer to seawater. All groups showed positive growth and negligible mortality over this period. The growth rate among fish held under the compressed photoperiod regimes (6-, 8- and 10-month) prior to seawater transfer was clearly higher than those maintained on either the control or extended (16-month) photoperiods and these advanced groups were significantly heavier ($P<0.001$) at the termination of the experiment in August.

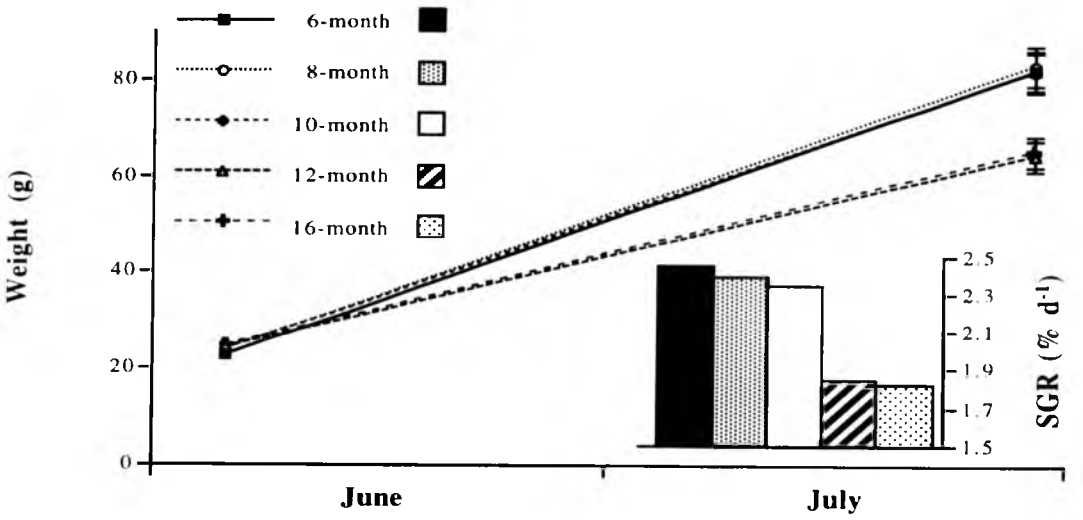


Figure 3.5

Growth following seawater transfer to the Hayling Marine Facility of potential S1 smolts reared under 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 1. The inset shows the specific growth rate (% increase in body weight d^{-1}) for each group over the seawater phase of the trial.

3.3 EXPERIMENT 2. The effects of 6-, 8- and 10-month compressed and 16-month extended seasonal light cycles on smoltification and subsequent seawater growth and maturation of potential S2 smolts.

3.3.1 Protocol

The animals for this experiment were drawn from the smallest grade of the population described in Experiment 1 (Section 3.1.1). All fish in this fraction were lower-mode, potential S2 fish ranging from 1.5-3.9-g with a mean weight of 2.4-g (10-months post-hatch). Five tanks were stocked with 2000 fish on December 22. Identical light regimes to those employed in Experiment 1 were used; ie 6-, 8-, 10- and 16-month period, with a control group reared under natural lighting. Each S2 group was reared along with the S1 groups in Experiment 1, then retained in freshwater for another cycle, after the S1s had been removed to seawater facilities, until the completion of smoltification (Figure 3.6). Successive batches of approximately 50 fish were transferred to the Hayling experimental facility as smoltification was completed in each group. In addition, larger numbers of fish were transferred to a commercial on-growing site from the 6- and 10- photoperiod groups.

3.3.2 Results

Freshwater

There were no significant differences in weight among groups between December and March in the first year (Figure 3.7). Fish maintained on a 6-month photoperiod showed a rapid increase in growth subsequent to the April sample and by June these fish were significantly heavier ($P<0.001$) than those in all other treatments. The stocking densities in the 16-month and control photoperiod groups were reduced by 80% and 40% due to mortalities resulting from interruptions in water supply on June 8 and June 16 respectively. Subsequent growth among surviving fish in these groups increased, and from August their weight remained significantly greater than fish reared under either an 8- or 10-month

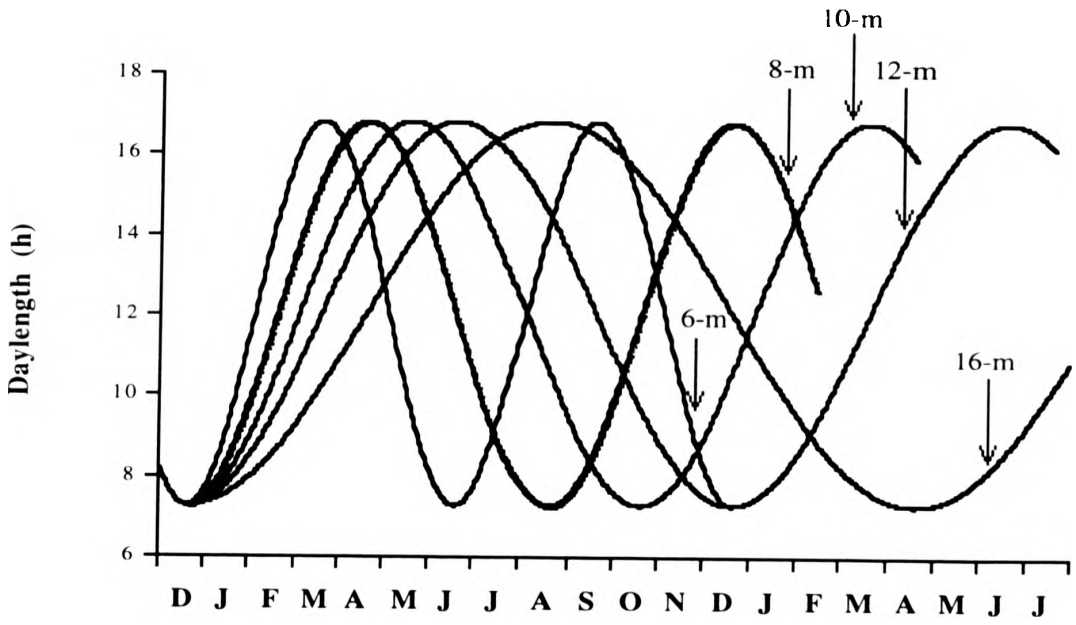


Figure 3.6

6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles used in Experiment 2 and the timing of completion of smoltification of potential S2 smolts reared under these regimes (↓).

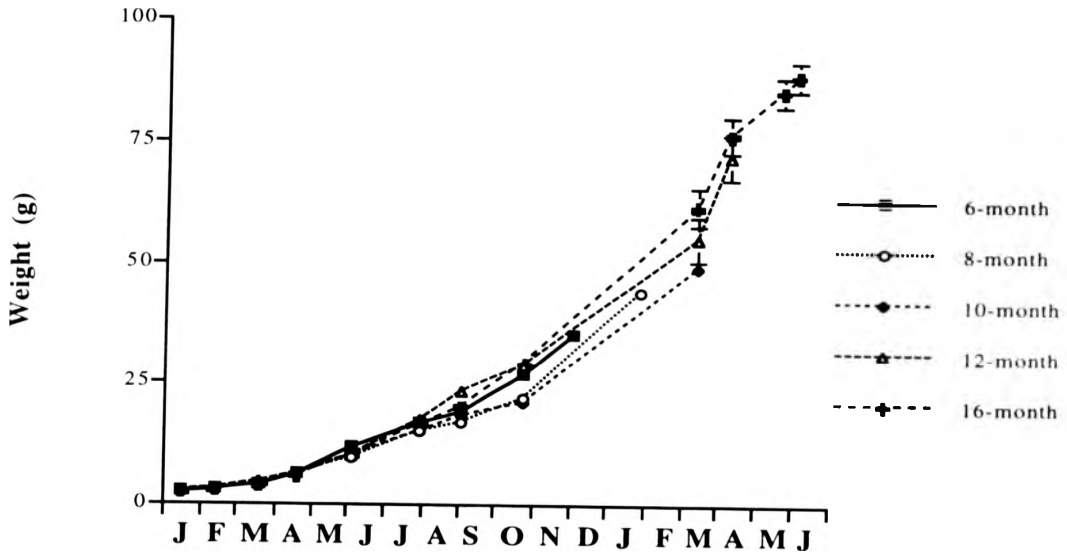


Figure 3.7

Changes in weight of potential S2 smolts reared on 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 2.

photoperiod (P<0.01). In October, fish reared under a 6-month compressed photoperiod were 20% heavier than those in comparably stocked treatments (8- and 10-month compressed photoperiods).

The number of fish completing smoltification decreased with increasing periodicity of experimental light cycles (Table 3.1). Fish in the 6-month photoperiod had a final smolt fraction in excess of 95%, significantly more than all other groups (P<0.01). 73% of fish reared under the 8-month regime smolted, significantly more (P<0.05) than those reared the under 10-, 12- and 16- month seasonal light cycles where 63%, 56% and 55% smolted respectively. Although GSIs were not determined in pre-smolt populations, it was evident that smolt fraction was inversely related to precocious male maturation. In the 6-month group no running (spermiating) males were found during sample weighing, whereas nearly all males reared under the 16-month extended light cycle matured in freshwater (assuming a 50:50 sex ratio).

Fish reared under the 6-month seasonally-compressed photoperiod completed smoltification in late November, 5-months in advance of control fish which completed smoltification in mid-April (Figure 3.6). Fish reared under the 10- and 8-month photoperiods completed smoltification 1 and 3 months earlier than controls, and those reared on a 16-month extended seasonal cycle completed smoltification in early June, 2-months later than the controls.

Pre-smolts in the 6-month group began to show a silver colouration in early-September. Condition factor among these fish peaked in July and decreased sharply before November (Figure 3.8). The first seawater challenge test was performed on July 28, at this time serum sodium following challenge was 198-mmol l^{-1} (Figure 3.9). This had decreased to 169-mmol l^{-1} on October 3 coincident with a decreasing condition factor (Figure 3.8). Fifty smolts, with a mean weight of 35-g were transferred to the Hayling Marine Laboratory on November 23; and 884 smolts were transferred to the commercial on-growing site in west Wales on December 3 and reared in a 5-m cage.

Fish reared under an 8-month seasonally-compressed photoperiod were showing clear signs of silvering in early-December and a full smolt morphology was attained by the third

Group	Smolt Fraction (percent ± 95% CI)	Male GSI (mean ± 1sem)	Female GSI (mean ± 1sem)
6-month	95.6% ± 1.2[a]	0.097 ± 0.036[a]	0.216 ± 0.042[a]
8-month	73% ± 2.6[b]	0.546 ± 0.155[a]	0.232 ± 0.023[ab]
10-month	62.7% ± 3.3[c]	0.401 ± 0.095[a]	0.291 ± 0.020[b]
12-month	56.4% ± 5.4[c]	0.472 ± 0.396[a]	0.273 ± 0.011[b]
16-month	54.6% ± 10.5[c]	-	-

Values in the same column suffixed with a different letter are significantly different ($P<0.05$)

Table 3.1

Smolt fractions of potential S2 smolt populations reared under 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 2, and the gonadosomatic indices (GSI) of male and female post-smolts reared at the Hayling Marine Facility on April 29.

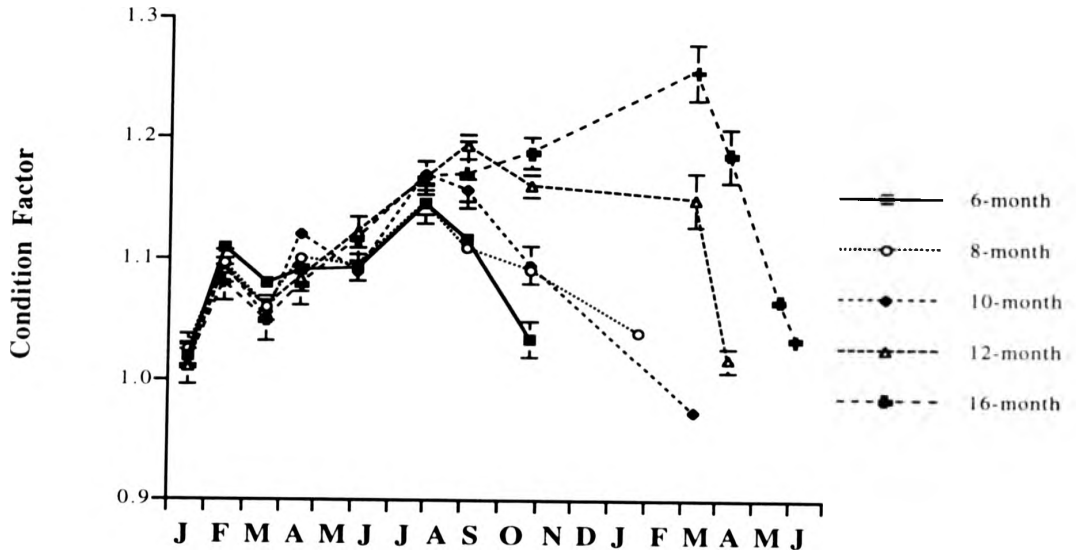


Figure 3.8

Changes in condition factor of potential S2 smolts reared on 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 2.

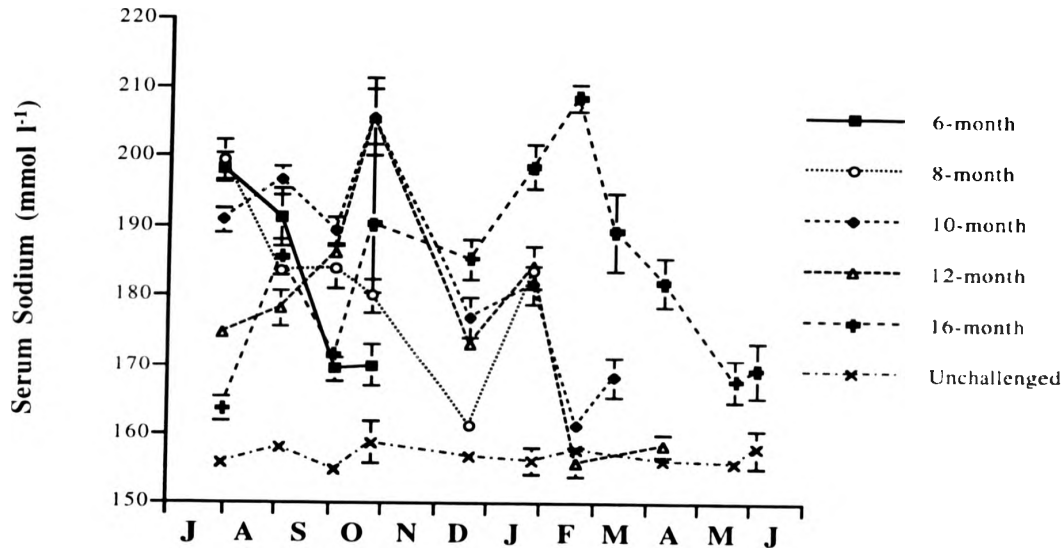


Figure 3.9

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S2 smolts reared on 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles, and the serum sodium concentration of unchallenged control fish in Experiment 2.

week of January. Condition factor peaked in July (Figure 3.8), then decreased rapidly between this point and late-November, in conjunction with an increase in hypo-osmoregulatory ability. Peak hypo-osmoregulatory ability was evident in late-December (fish challenged at this time had a serum sodium concentration 161-mmol l^{-1}; Figure 3.9). However, this had decreased significantly by January 23 ($P<0.01$), immediately prior to seawater transfer. A group of these smolts, with a mean weight of 44-g, were transferred to the Hayling Marine facility on January 25.

Parr maintained on a 10-month photoperiod were showing the first signs of silvering in late-December. Hypo-osmoregulatory ability was at its lowest in late-October (206-mmol l^{-1}) coincident with the shortest day. Hypo-osmoregulatory ability subsequently increased and the mean serum sodium concentration was 161-mmol l^{-1} following seawater challenge in February, 3-weeks before seawater transfer. Condition factor increased between June and July, and decreased during the 7-months prior to smolting (Figure 3.8). Transfers of 50 and 650 smolts with a mean weight of 48-g were made to the Hayling seawater rearing facility and the commercial cage site respectively in the first week of March.

The control group followed a pattern of decreasing hypo-osmoregulatory ability between July and the end of October when peak serum sodium following seawater challenge was 205-mmol l^{-1} (Figure 3.9). Hypo-osmoregulatory ability developed between this point and late-February, when serum sodium following seawater challenge was not significantly different from that of unchallenged fish. At this time the fish were showing clear signs of silvering, but the condition factor, which had shown a peak value in September was still relatively high (Figure 3.8). A strongly developed hypo-osmoregulatory ability persisted for the following 6-weeks, silvering was judged to be complete in early-April and condition factor decreased sharply between March 12 and April 10, when a group of smolts were transferred to the Hayling experimental facility in the same week that production S2 smolts were transferred to commercial rearing sites.

Fish reared under an extended 16-month photoperiod demonstrated a general decreasing hypo-osmoregulatory ability between July and February, coincident with an increasing condition factor. In early-March all fish retained a strong parr-like appearance,

with no signs of silvering and were clearly at a delayed state of smolt development compared to those in either the 10-month or control groups. Condition factor decreased and hypo-osmoregulatory ability developed markedly between March and early-June when body silvering was completed. Fifty smolts were transferred to the Hayling seawater facility on June 5.

Seawater

Batches of smolts moved to the Hayling marine facility from the 6-, 8-, 10- and 12-month photoperiods generally resumed feeding within 2-days of transfer and all incurred mortalities of less than 2% during their first week in seawater. Fish transferred to seawater in November (6-month group) showed very good growth over the winter, their weight increasing from 35-g to 81-g by March (Figure 3.10). Smolts transferred in January showed good seawater growth, almost doubling their weight to 82-g by April 29, when all fish were killed due to a pump failure. Smolts transferred in March (10-month group) and April (controls) showed 10-g and 4-g increases in weight over the 6 and 3-weeks of seawater growth respectively. At this time posts-smolts from the 6-month group were significantly larger than batches transferred from all other photoperiod treatments ($P<0.001$). Gonad samples taken a this time revealed no significant differences in male GSI between these groups (Table 3.1). Females reared under the 6-month photoperiod in freshwater which had been transferred to seawater in November had a significantly lower mean GSI than those reared under 10- or 12-month photoperiods ($P<0.05$). However, males and females in all groups had a mean GSI less than 0.6 and no individuals would have been expected to mature in the next maturation episode.

The final transfer of smolts reared under the 16-month extended photoperiod, was made after the loss of the other groups. Initial growth of these fish following transfer was very poor due to high seawater temperatures, in excess of 22°C, which resulted in a 25% mortality during late-July and early-August. However, surviving fish showed good growth, with a 2-fold increase in mean weight between October and the following January (Figure 3.10). No external signs of maturation were evident during this time.

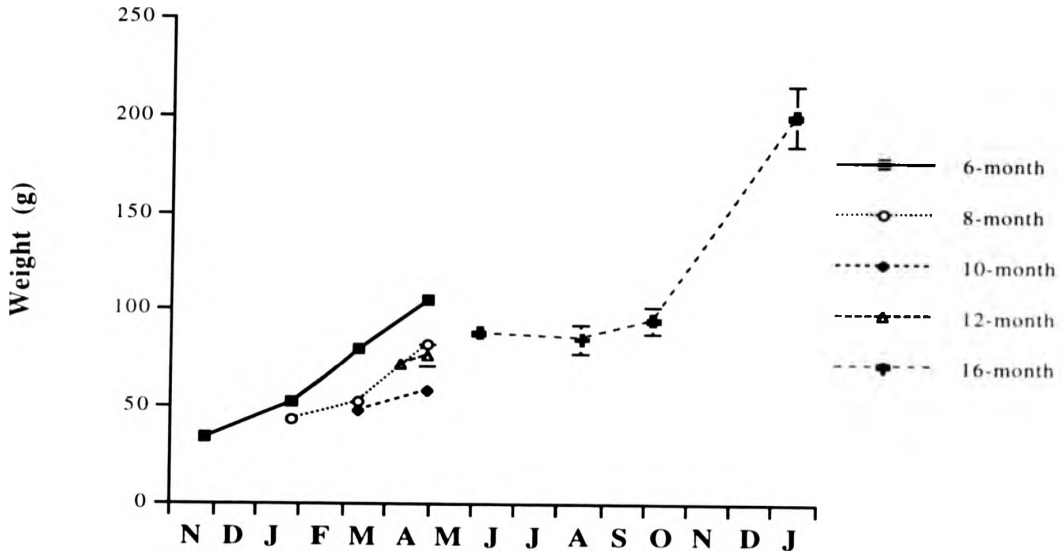


Figure 3.10

Growth following seawater transfer to the Hayling Marine Facility of potential S2 smolts reared under 6-, 8- and 10-month compressed, ambient (12-month) and 16-month extended seasonal light cycles in Experiment 2.

Larger batches of smolts transferred to the commercial cage site from the 6- and 10-month groups showed better growth than those reared at the small scale facility. Fish transferred in November (6-month) showed a 3-fold increase in weight (to 103-g) during their first 3-months in seawater, and their mean weight after 18-months at sea was 2-kg (Figure 3.11). Those transferred in March (10-month) weighed over 1-kg by May of the following year.

Of the smolts transferred to the cage site in November, 6.5% maturated after 1 sea-winter, as determined by secondary sexual characteristics (breeding colouration and kype development among males) which were evident from September (Table 3.2). The following year, 63% of the stock were estimated to be showing signs of maturation in July when the experiment was terminated. Fish transferred to the cage site in March, 1-month early, showed a 24% post-smolt maturation (that is precocious maturation in the same year as seawater transfer).

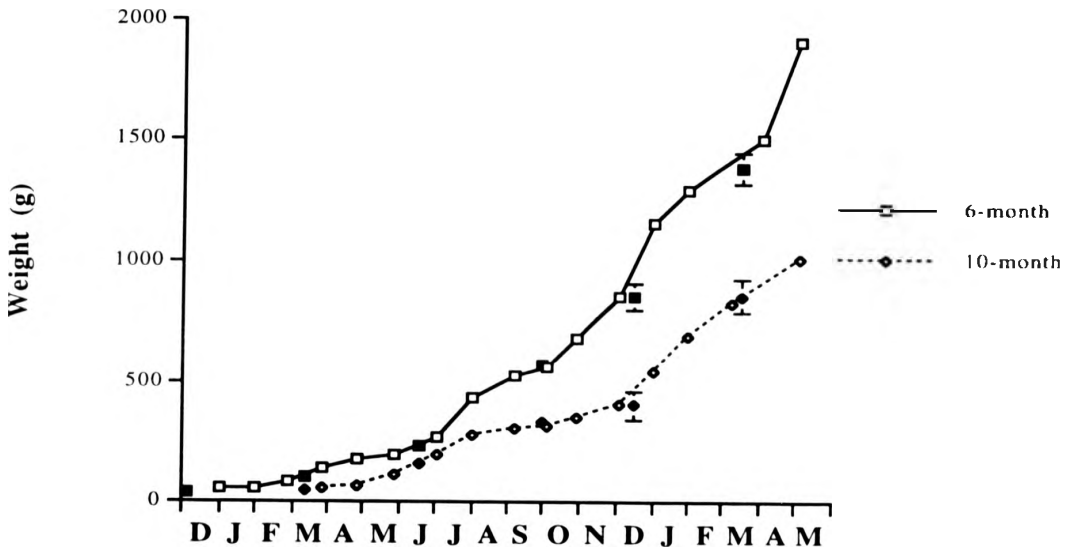


Figure 3.11

Growth following seawater transfer to commercial on-growing facilities of potential S2 smolts reared under 6- and 10-month compressed seasonal light cycles in Experiment 2. Open symbols (without error bars) represent mean weights determined by batch weighing, filled symbols indicate mean weights ± 1sem.

Group	First maturation episode (percent ± 95% CI)	Second maturation episode (percent ± 95% CI)
6-month	6.5% ± 1.8 (1-sw)	63.0% ± 5.5 (2-sw)
10-month	24.2% ± 4.2 (ps)	-

ps post-smolt maturation (fish maturing the same year as seawater transfer)
1-sw maturation after 1-sea winter (grilse)
2-sw maturation after 2-sea winters

Table 3.2

The seawater maturation at a commercial cage site of potential S2 smolts reared in freshwater under 6- and 10-month seasonally compressed light cycles in Experiment 2.

3.4 EXPERIMENT 3. The effects of direct changes from short day to long day on the smoltification of potential S1 smolts.

3.4.1 Protocol

A population of Atlantic salmon parr of mixed stock 2-sea winter parentage were maintained at a commercial smolt unit from hatch under conditions of natural photoperiod (latitude 52°N) and constant temperature (10±0.2°C). On December 14, 1,500 fish were randomly distributed among 3 light proof tanks and held on a short day (LD7.25:16.75). The daylength was changed directly to a long day (LD16.75:7.25) at 30-day intervals on December 22, January 21 and February 20 (Figure 3.12) providing groups A, B and C respectively. On completion of smoltification, 50 fish from each group were transferred to aerated running sea water at the Hayling Marine Laboratory and reared under conditions of ambient temperature and photoperiod. A commercial stock of sibling S1 pre-smolts was monitored as a control for the timing of the completion smoltification.

3.4.2 Results

Freshwater

The S1 fractions in all experimental groups showed a normal development of smoltification as determined by body silvering, fin darkening, decreased condition factor and elevated hypo-osmoregulatory ability. On the basis of the timing of these changes, smolts from groups A-C were transferred to seawater facilities on March 28, April 11 and April 25; 7-, 5- and 3-weeks in advance of smolts reared on an ambient photoperiod respectively (Figure 3.12).

Following the direct change from a short to a long day on December 22 at the commencement of the trial, potential smolts in Group A showed enhanced growth and at the first sampling, on January 22, were significantly heavier than fish in both groups B and C ($P<0.01$; Figure 3.13). This growth advantage over pre-smolts in Group B was lost over

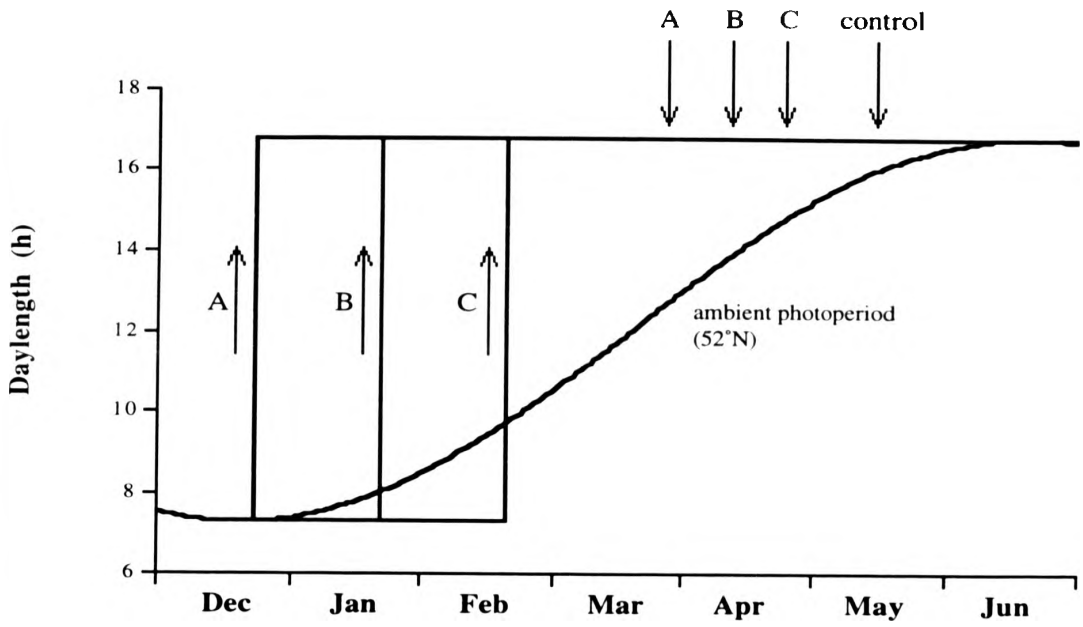


Figure 3.12

Changes from short day (LD7.25:16.75) to a long day (LD16.75:7.25), the ambient (12-month) light cycle and the timing of completion of smoltification (↓) of potential S1 smolts reared under these photoperiod regimes in Experiment 3.

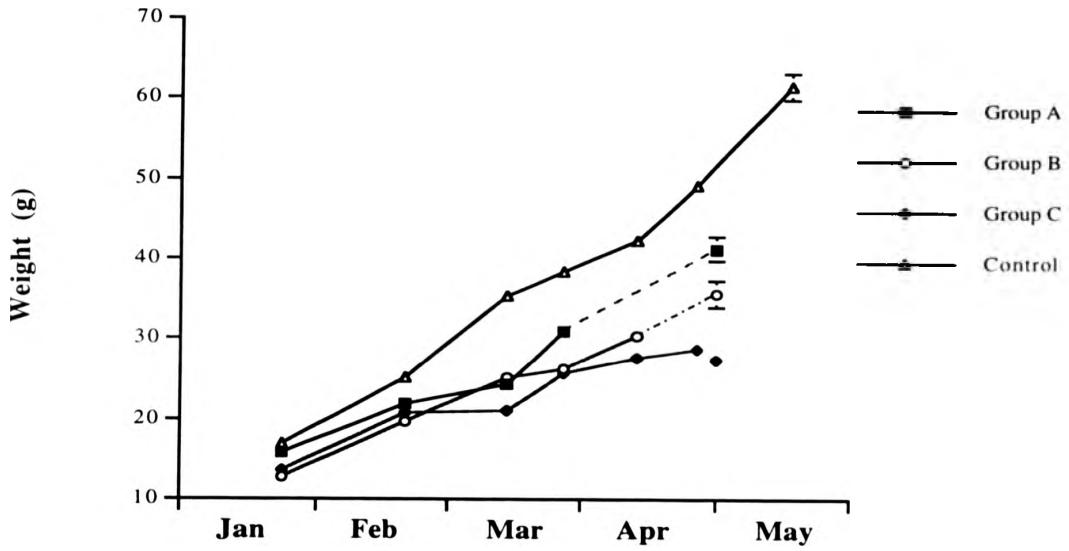


Figure 3.13

Changes in weight of potential S1 smolts transferred from a short day (LD7.25:16.75) to a long day (LD16.75:7.25) on December 22 (Group A), January 21 (Group B), February 20 (Group C) and control fish reared under an ambient (12-month) light cycle in Experiment 3. Dotted lines indicate growth following transfer to seawater.

the next 2-months, which had become marginally heavier than those in Group A on March 13. Pre-smolts in Group C showed comparatively slower growth following transfer to a long day in late-February, and were significantly smaller than those in both groups A ($P<0.05$) and B ($P<0.01$) on March 13. Smolts in Group A showed a growth surge in the last 2-weeks of March, immediately prior to seawater transfer, and were significantly heavier than those in both groups B and C ($P<0.001$) with a mean weight of 31-g on March 26. Smolts in Group B were also significantly heavier than those in Group C when they were transferred to seawater 2-weeks later ($P<0.01$). The mean weights of groups B and C at seawater transfer were 30.4-g and 28.7-g respectively. The production smolts, monitored as a control for the timing of smoltification, which were reared in a large (9-m) tank, grew at a faster rate than all experimental fish, and were significantly heavier than fish in groups A-C from February 20 ($P<0.001$).

Groups A-C showed patterns of decreasing condition factor from January 22, February 20 and March 13 respectively (Figure 3.14). Control fish reared on an ambient photoperiod did not show a decrease in condition factor until April. The mean condition factor of Group A pre-smolts was significantly less than those in either groups B ($P<0.05$) and C ($P<0.01$) on March 13. Pre-smolts in both groups A and B had condition factors significantly less than those in Group C on March 26 ($P<0.001$). Group B showed a slight, but not significant, increase in condition factor prior to seawater transfer on April 11.

All experimental groups showed an advancement in the development of hypo-osmoregulatory ability compared to fish reared on an ambient photoperiod (Figure 3.15). Pre-smolts in groups A and B had significantly lower ($P<0.05$) serum sodium concentrations following seawater challenge on February 20 (170-mmol l^{-1} and 174-mmol l^{-1} respectively) than those of challenged control fish (199-mmol l^{-1}). Pre-smolts in Group A showed a rapid development of hypo-osmoregulatory ability between late-February and March 26 when their serum sodium following seawater challenge, immediately prior to seawater transfer (157-mmol l^{-1}), was not significantly different from that of unchallenged smolts. Groups B and C had post-challenge serum sodium concentrations of 156-mmol l^{-1}

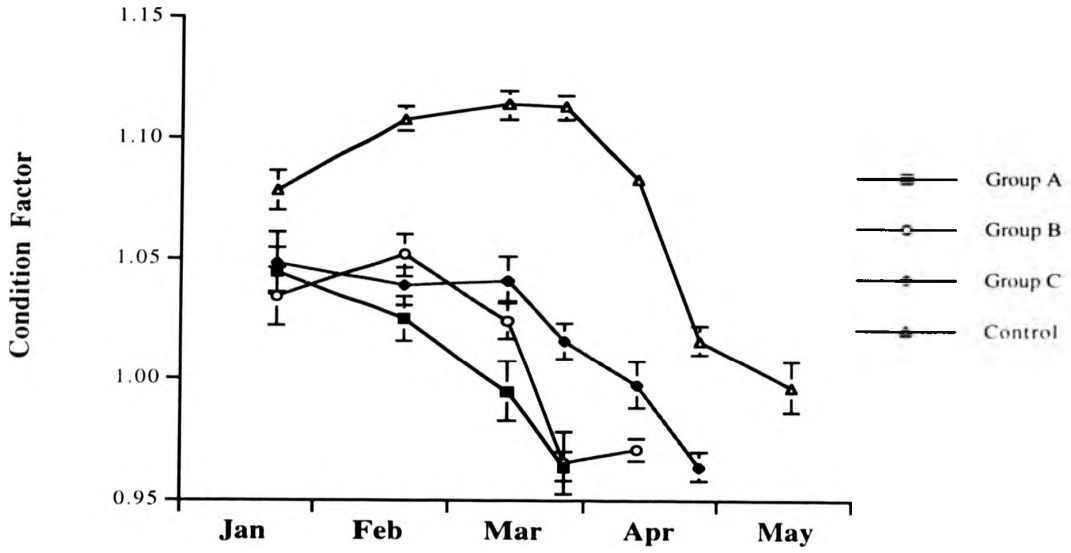


Figure 3.14

Changes in condition factor of potential S1 smolts transferred from a short day (LD7.25:16.75) to a long day (LD16.75:7.25) on December 22 (Group A), January 21 (Group B), February 20 (Group C) and control fish reared under an ambient (12-month) light cycle in Experiment 3.

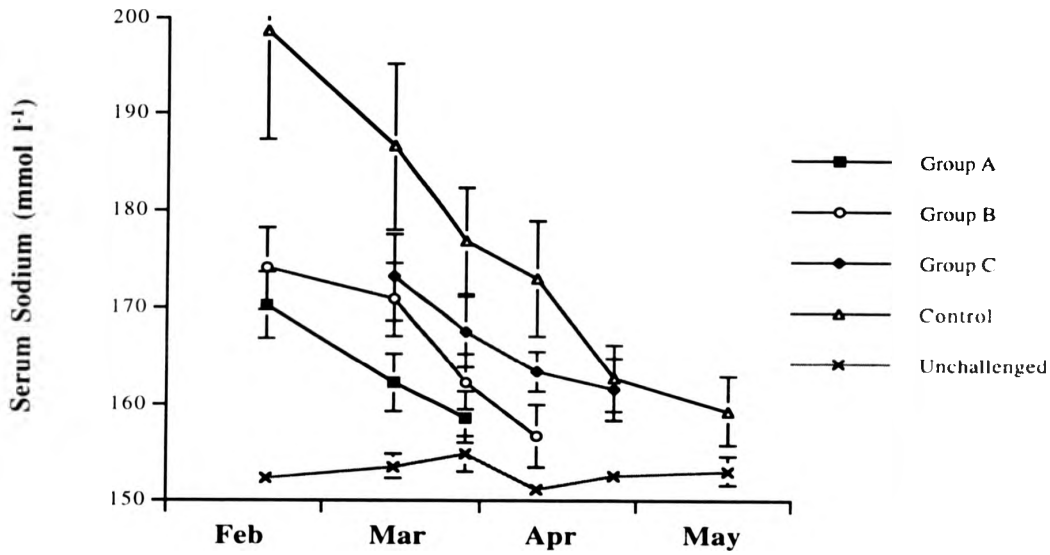


Figure 3.15

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S1 smolts transferred from a short day (LD7.25:16.75) to a long day (LD16.75:7.25) on December 22 (Group A), January 21 (Group B), February 20 (Group C), control fish reared under an ambient (12-month) light cycle, and the serum sodium concentration of unchallenged control fish in Experiment 3.

and 161-mmol l^{-1} when they were transferred to the Hayling Marine facility on April 11 and April 26 respectively.

The final smolt fractions (± 95% confidence intervals), determined by actual counts made at smolt grading prior to seawater transfer, in groups A-C were 23.0% ± 4.2, 28.3% ± 4.4 and 37.8% ± 4.7 respectively. The final S1 fraction on completion of smoltification in Group C, including fish which were transferred to a long day in February, was significantly higher than that of groups A ($P<0.01$) and B ($P<0.05$), which were transferred to a long day in December and January respectively. Figure 3.16 shows length-frequency distributions for all the experimental groups in January, February and March. All groups clearly show the bimodal nature of the populations, with lower modes containing potential S2 fish (parr), while the upper-modes include potential S1 smolts. Similar data were not available for the control population reared on an ambient photoperiod because potential S1 and S2 fish were graded and separated from each other in mid-January. At the end of January all experimental groups had similar length-frequency distributions with approximately 20% of each group contained in the upper (potential S1) modes. In March, the upper modes of groups B and C had increased to 27% and 38% respectively, indicating a migration of individuals from the lower to upper modes in the interval between the February and March samples.

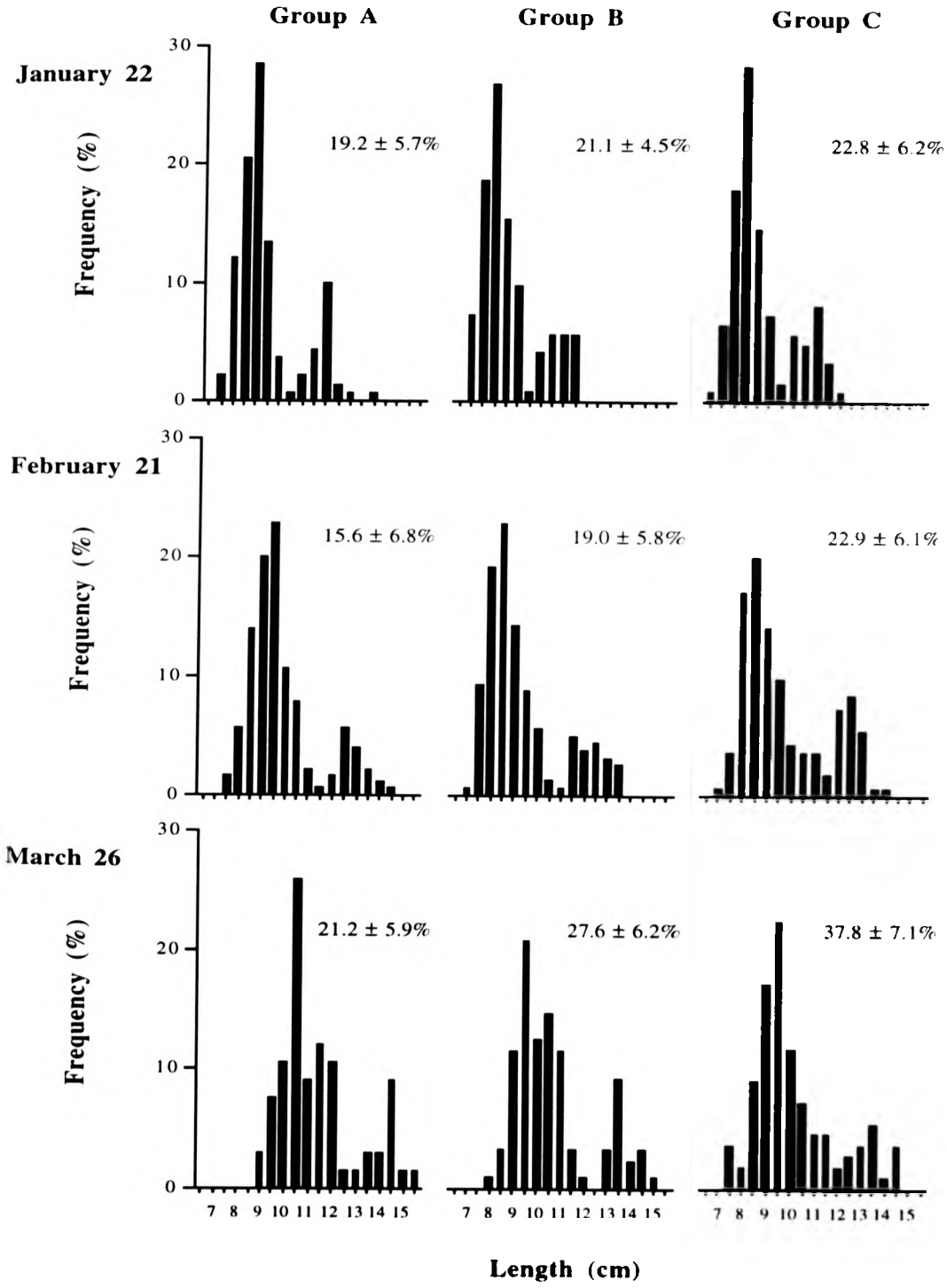
Seawater

Smolts from groups A and B transferred to seawater successfully, with negligible mortality and good initial growth showing 30% and 15% increases in weight after 4 and 2-weeks marine residence respectively (as indicated by the dotted lines in Figure 3.13). All smolts were killed by a pump failure 6-days after the transfer of smolts from Group C. These fish showed a small reduction in weight in this time but had suffered no prior mortalities. Samples taken at the end of the experiment revealed no significant differences in male or female GSI (Table 3.3). Mean GSI for males and females were less than 0.04 and 0.2 respectively and no individuals would have been expected to mature at the next maturation episode (as post-smolts).

Figure 3.16

Length-frequency distributions of 3 populations of 0+ Atlantic salmon transferred from a short day (LD7.25:16.75) to a long day (LD16.75:7.25) on December 22 (Group A), January 21 (Group B), and February 20 (Group C) at 3 different dates prior to the completion of smoltification of S1 smolts, including percentages of fish in the upper-mode ± 95% confidence intervals.

NB. The final smolt fractions determined by actual counts at smolt grading prior to seawater transfer (at the completion of smoltification) were: Group A, 23.0% ± 4.2 (March 28); Group B, 28.3% ±4.4 (April 11); Group C, 37.8% ± 4.7 (April 25).


Group A
Group B
Group C
January 22
February 21
March 26
Frequency (%)
19.2 ± 5.7%
21.1 ± 4.5%
22.8 ± 6.2%
15.6 ± 6.8%
19.0 ± 5.8%
22.9 ± 6.1%
21.2 ± 5.9%
27.6 ± 6.2%
37.8 ± 7.1%
0
10
20
30
7 8 9 10 11 12 13 14 15
Length (cm)

Group	Smolt Fraction (percent ± 95% CI)	Male GSI (mean ± 1sem)	Female GSI (mean ± 1sem)
Group A	23.0% ± 4.2[a]	0.027 ± 0.004[a]	0.163 ± 0.032[a]
Group B	28.3% ± 4.4[a]	0.028 ± 0.006[a]	0.159 ± 0.022[a]
Group C	37.8% ± 4.7[b]	0.038 ± 0.010[a]	0.118 ± 0.019[a]

Values in the same column suffixed with a different letter are significantly different ($P<0.05$)

Table 3.3

Smolt fractions of potential S1 populations transferred from a short day (LD7.25:16.75) to a long day (LD16.75:7.25) on December 22 (Group A), January 21 (Group B) and February 20 (Group C) in Experiment 3, and the gonadosomatic indices (GSI) of male and female post-smolts reared at the Hayling Marine Facility on April 29.

3.5 EXPERIMENT 4. The effects of a compressed light cycle on smoltification and subsequent seawater growth of potential S2 all-female and all-female triploid smolts.

Considering the possibility of early maturation among smolts transferred to seawater at times of the year other than the spring and in the light of the significant proportion of fish reared on a 10-month photoperiod cycle showing post-smolt maturation in Experiment 2, an evaluation of the low precocious maturity provided by all-female triploid and stocks for possible use in the production of out-of-season smolts was considered worthwhile. The following experiment was therefore undertaken in an attempt to provide temporally advanced stocks of diploid and triploid all-female smolts for transfer to a commercial on-growing site for assessment of subsequent seawater growth and maturation.

3.5.1 Protocol

Stocks of diploid and triploid all-female Atlantic salmon parr of mixed Scottish stock origin were reared under ambient temperature and photoperiod (56°N) from hatch at a smolt-unit on the west coast of Scotland. Groups of 600 triploid and all-female potential S2 fish (of 8.9-g and 10.3-g respectively) were transferred (from LD 14:10, 13°C) directly to LD18:6 and a constant temperature water supply (10°C ± 0.2) on April 23 and then subjected to a photoperiod approximating to a 6-month compressed seasonal cycle, which was held at a long day (LD18:6) from October (see Figure 3.17).

A proportion of the triploid stock (approximately 50 individuals) were found to have bad spinal and tail deformities, resulting from water contamination during yolk-sac reabsorption, making them commercially unattractive. These fish were separated from the remainder of the stock and maintained on a natural photoperiod (52°N) to provide a control for the development of hypo-osmoregulatory ability. Weight data was also collected from these fish, but due to the deformities, lengths were not recorded and condition factor not calculated. Mixed-sex potential S2 production stock were monitored from August to provide control information from fish reared under an ambient photoperiod. Advanced

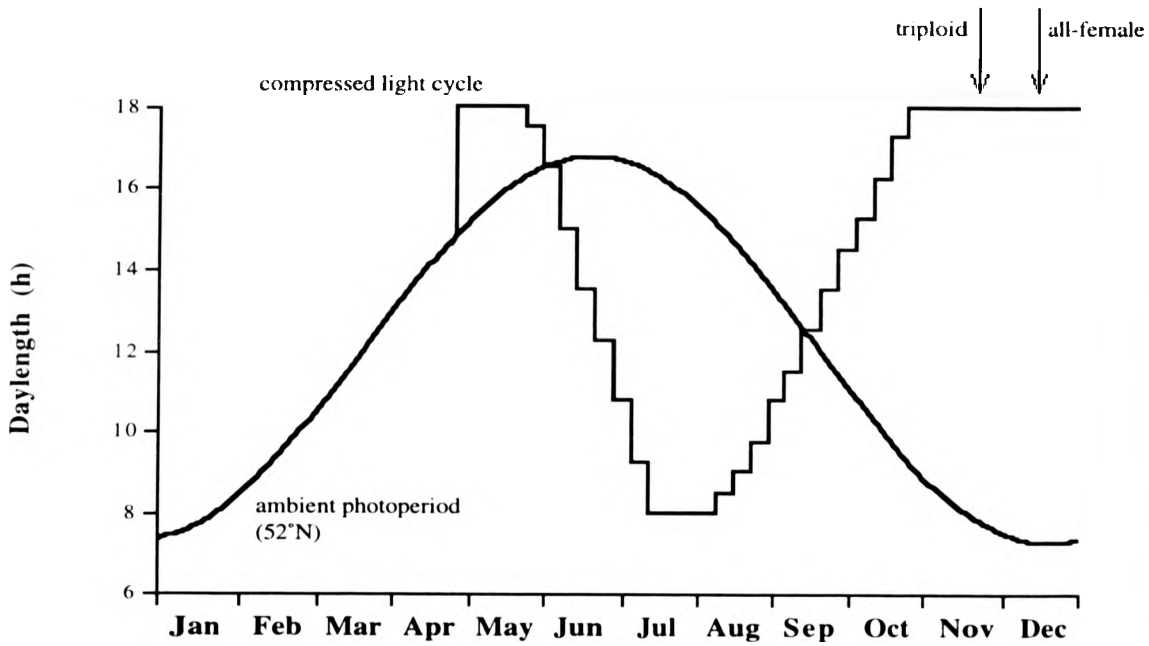


Figure 3.17

Compressed and ambient (12-month) light cycles and the timing of completion of smoltification (↓) of potential S2 all-female and all-female triploid smolts reared under the compressed photoperiod in Experiment 4.

triploid and all-female smolts were transferred to the commercial cage facility in west Wales for on-growing in seawater.

3.5.2 Results

Freshwater

The photoperiod triploid and all-female groups grew at a comparable rate between the start of the trial and the end of October (see Figure 3.18). During this period the all-female stock remained significantly heavier than the triploid group ($P<0.001$). During November the triploids showed particularly good growth, and by November 20 were slightly larger than the photoperiod all-female fish.

The first signs of silvering occurred among the photoperiod triploid fish in late-August, approximately one month in advance of the all-females, and was fully complete by mid-November. The triploids were transferred to seawater on November 21 (mean weight 44-g), but the all-female stock, which had not attained full smolt morphology at this time, was kept in freshwater for a further month and transferred to the cage site on December 15 (mean weight 48-g). The development of morphological smolt characteristics was supported by the seawater challenge tests. Hypo-osmoregulatory ability developed among photoperiod triploids approximately one month in advance of that of the all-females (see Figure 3.19) increasing significantly between October 9 and 22 ($P<0.001$) and providing a serum sodium concentration following seawater challenge of 158-mmol l^{-1} prior to seawater transfer. The first significant increase in hypo-osmoregulatory ability among the photoperiod all-females occurred between October 22 and November 5 ($P<0.01$), and serum sodium following seawater challenge before transfer to seawater was 162-mmol l^{-1}.

The weight per unit length of the all-female fish was much higher than that of the photoperiod triploids throughout the freshwater phase of the trial ($P<0.001$) (Figure 3.20). However, relative changes in condition factor between these two groups remained very similar until early-October. Between October 9 and November 5 the photoperiod triploids showed a significant decrease in condition factor ($P<0.001$). Over the same period the

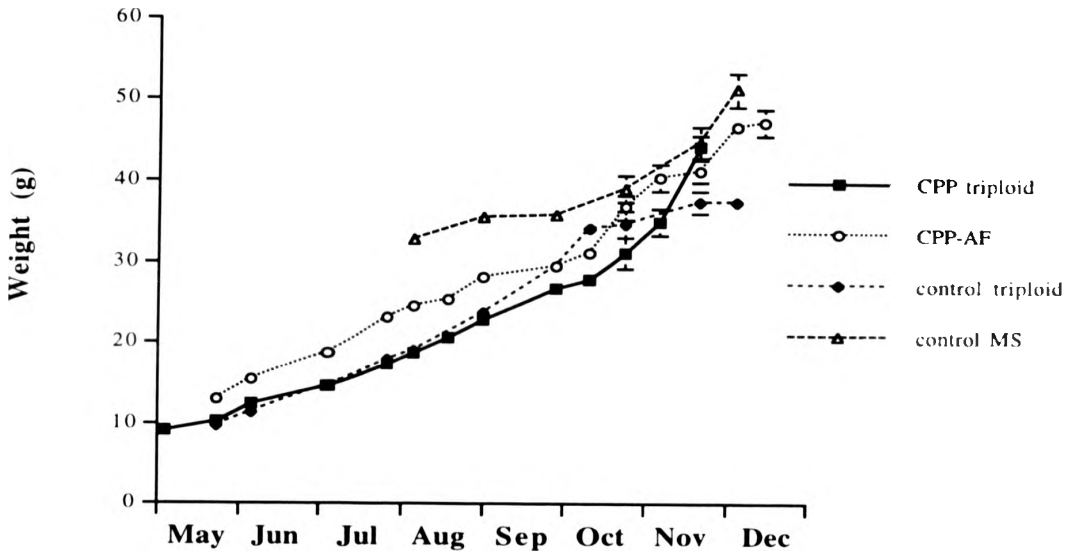


Figure 3.18

Changes in weight of potential S2 all-female smolts (CPP-AF), and potential S2 all-female triploid smolts (CPP triploid) reared under a compressed photoperiod and of mixed sex (control MS), and all-female triploid (control triploid) potential S2 smolts reared under an ambient (12-month) light cycle in Experiment 4.

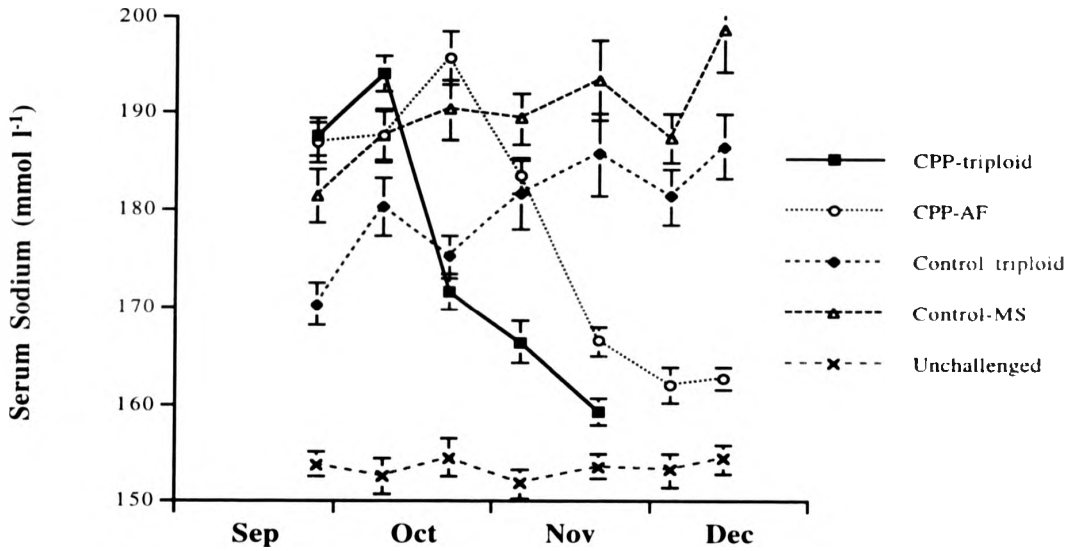


Figure 3.19

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S2 all-female smolts (CPP-AF), and potential S2 all-female triploid smolts (CPP-triploid) reared under a compressed photoperiod and of mixed sex (control-MS), and all-female triploid (control triploid) potential S2 smolts reared under an ambient (12-month) light cycle and the serum sodium concentration of unchallenged control-MS fish in Experiment 4.

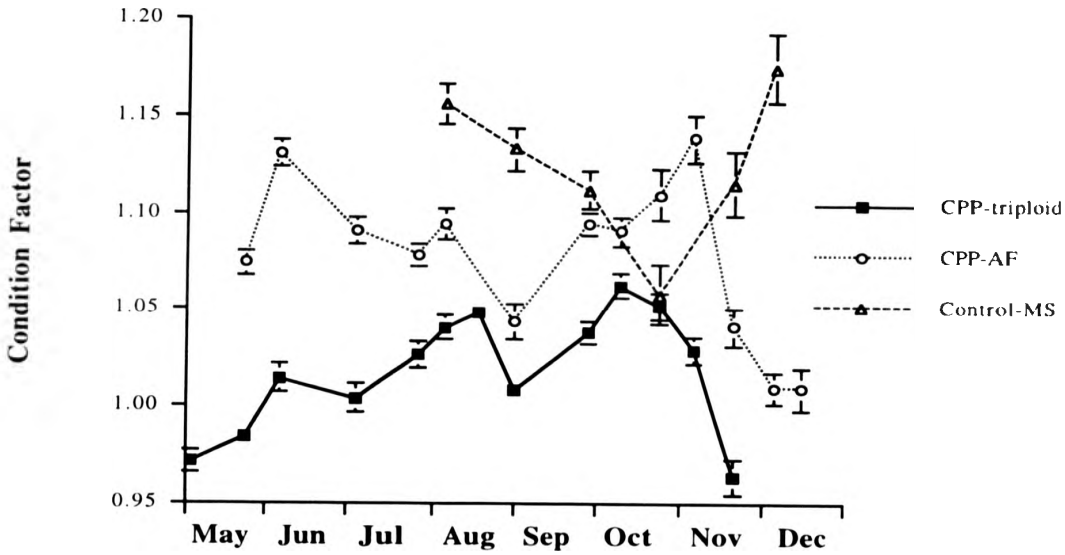


Figure 3.20

Changes in condition factor of potential S2 all-female smolts (CPP-AF), and potential S2 all-female triploid smolts (CPP-triploid) reared under a compressed photoperiod and of mixed sex (control-MS) potential S2 smolts reared under an ambient (12-month) light cycle in Experiment 4.

photoperiod all-females showed a significant increase in condition factor ($P<0.001$), but subsequently showed a sharply decreasing condition factor before transfer to seawater. During the period that the photoperiodically-advanced triploid and all-female groups were transferred to seawater, the control mixed sex fish had a strong parr-like appearance, with a high and increasing condition factor. Throughout November and December both the control mixed sex and triploid fish maintained on an ambient photoperiod had a poorly developed hypo-osmoregulatory ability, with serum sodium concentrations following seawater challenge in excess of 180-mmol l^{-1}.

Seawater

Mortality among advanced triploid and all-female groups was less than 2% in the month following transfer to seawater. Both groups showed good growth over the winter (see Figure 3.21), and the triploids in particular showed exceptional growth from March onwards achieving a mean weight of 300-g by June, when smolts reared under ambient photoperiods in freshwater, averaging 40-50-g, are transferred to seawater. In July, when the trial was terminated, neither of the groups were showing any external sign of maturation.

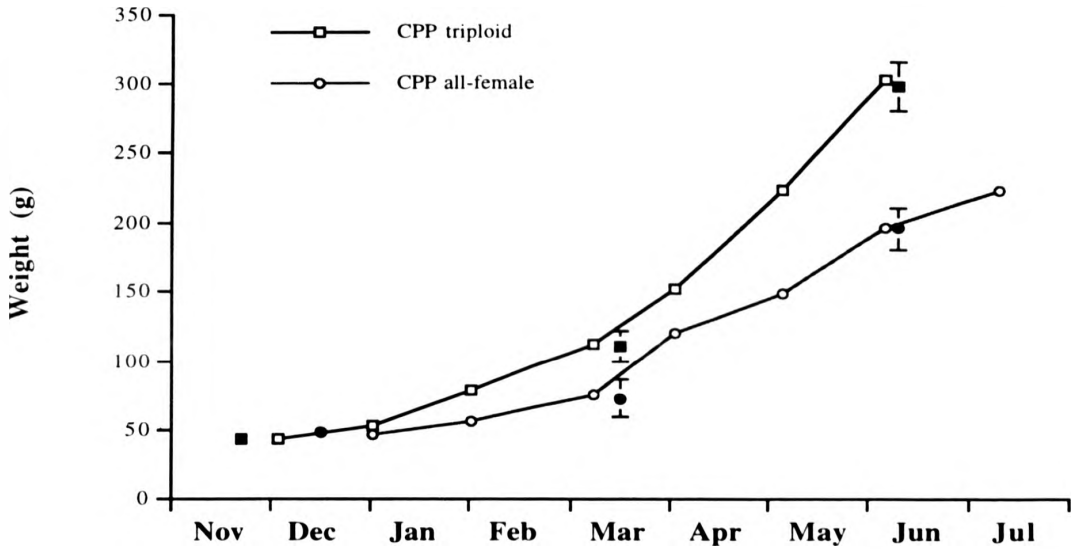


Figure 3.21

Growth following seawater transfer of all-female (CPP all-female) and all-female triploid (CPP triploid) potential S2 smolts reared under a compressed photoperiod in Experiment 4. Open symbols (without error bars) represent mean weights determined by batch weighing, filled symbols indicate mean weights ± 1sem.

3.6 EXPERIMENT 5 The effects of a compressed light cycle on smoltification of potential S1 smolts: a commercial trial.

3.6.1 Protocol

Parr of Sundalsøra stock origin were reared from hatch (in January) on a constant long daylength (LD20:4) in 2000-l tanks until July 14. The fish were then graded into 2 fractions. Those over 1.8-g were placed on a light cycle approximating to a 6-month seasonally-compressed photoperiod until December 12, after which they were held on a constant long day (LD18:6), see Figure 3.22. The smaller grade was returned directly to an ambient photoperiod (52°N). On September 12, the advanced population was moved to a single 9-m concrete rearing tank, illuminated by cool white fluorescent tubes suspended 3-m above the tank providing an average light intensity of 25-lx at the water surface (Section 2.1). On December 22, this population was further graded into 3 fractions and distributed among similar 9-m concrete tanks for continued rearing in freshwater. The final grading of the stock reared on an ambient photoperiod was made on January 25. Morphometric measurements were taken and seawater challenge tests made on the largest grade of advanced fish from December 30. The largest grade of ambient fish was monitored from February 2 as a control. A large group of the advanced stock were kept in freshwater under a constant long day (LD18:6) photoperiod until the control smolts were transferred to seawater in May.

3.6.2 Results

Fish reared on the 6-month seasonally-compressed photoperiod, which were originally drawn from a larger grade, maintained a clear size advantage over those reared on the control photoperiod throughout their freshwater residence (Figure 3.23). The advanced smolts were showing clear signs of body silvering in late-December. These fish were fully silvered in early-February and a transfer was made to seawater on February 18, 3-months in

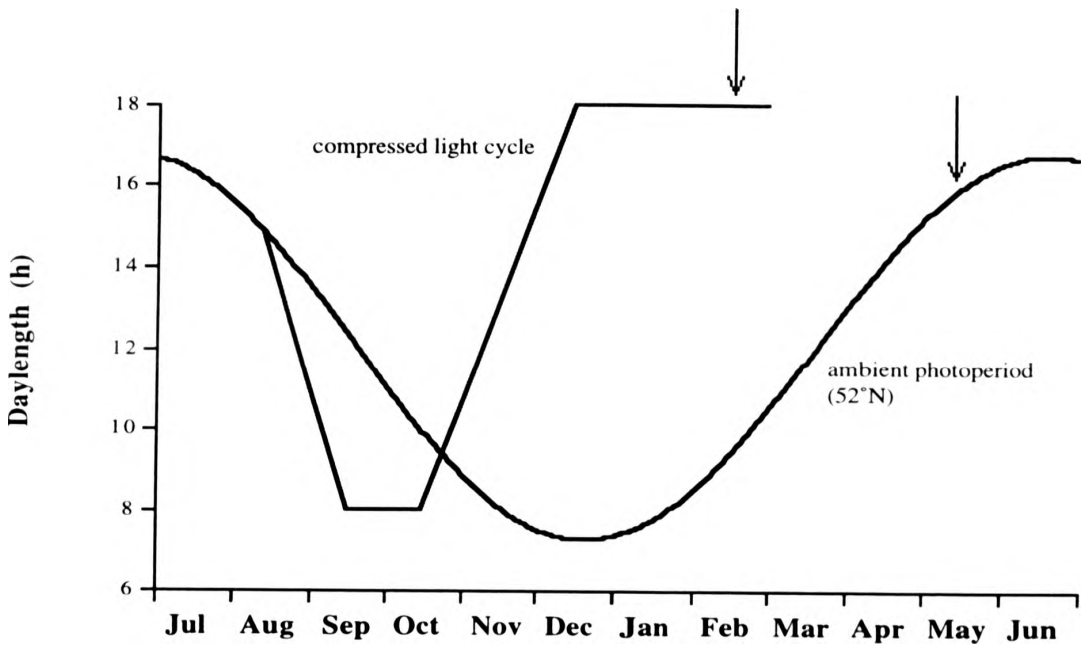


Figure 3.22

Compressed and ambient (12-month) light cycles and the timing of completion of smoltification (↓) of potential S1 smolts reared under these regimes in Experiment 5.

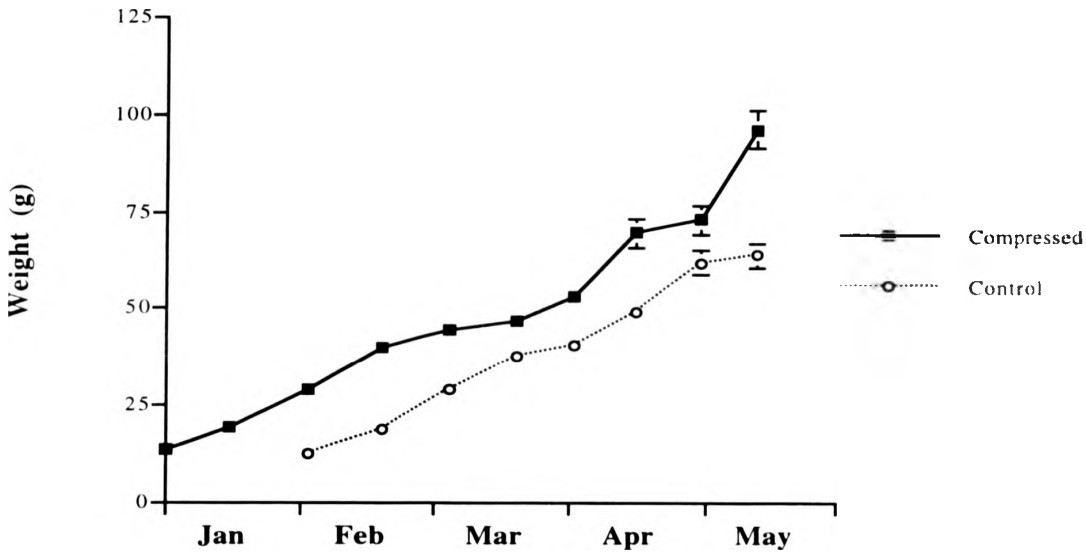


Figure 3.23

Changes in weight of potential S1 smolts reared under ambient (12-month) and compressed light cycles in Experiment 5.

advance of fish reared from July under ambient photoperiod conditions. At this time, the advanced smolts had shown a significantly reduced condition factor ($P<0.01$; Figure 3.24) and an increased hypo-osmoregulatory ability ($P<0.001$; Figure 3.25); serum sodium following seawater challenge in early-February was 159-mmol l^{-1}. High losses (43%) were incurred shortly after transfer to seawater. Although surviving fish were reported to grow well, it was not possible to collect any further growth or maturation data. Advanced smolts remaining in freshwater until May retained a high degree of hypo-osmoregulatory ability, and condition factor continued to decrease.

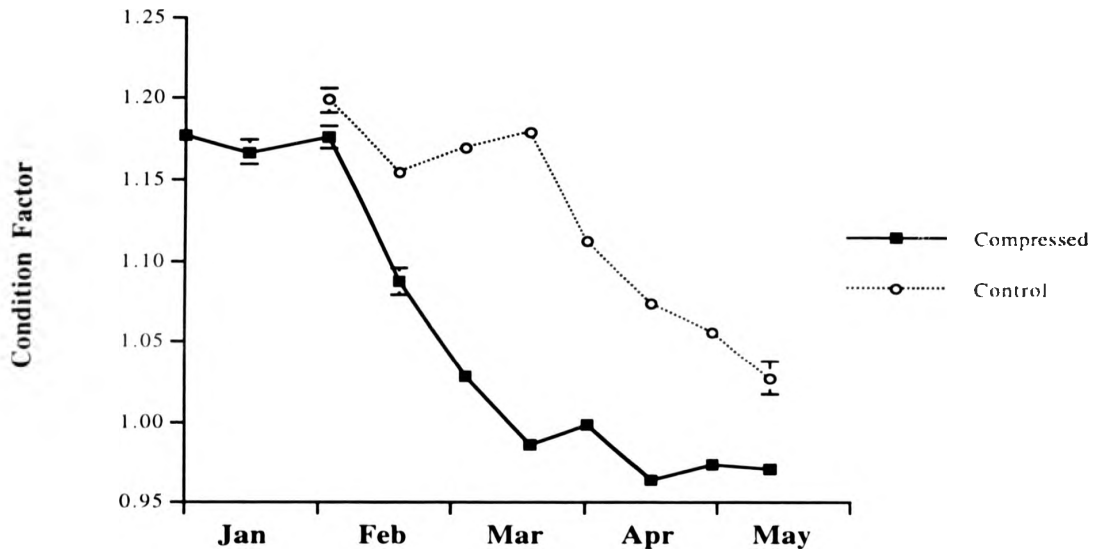


Figure 3.24

Changes in condition factor of potential S1 smolts reared under ambient (12-month) and compressed light cycles in Experiment 5.

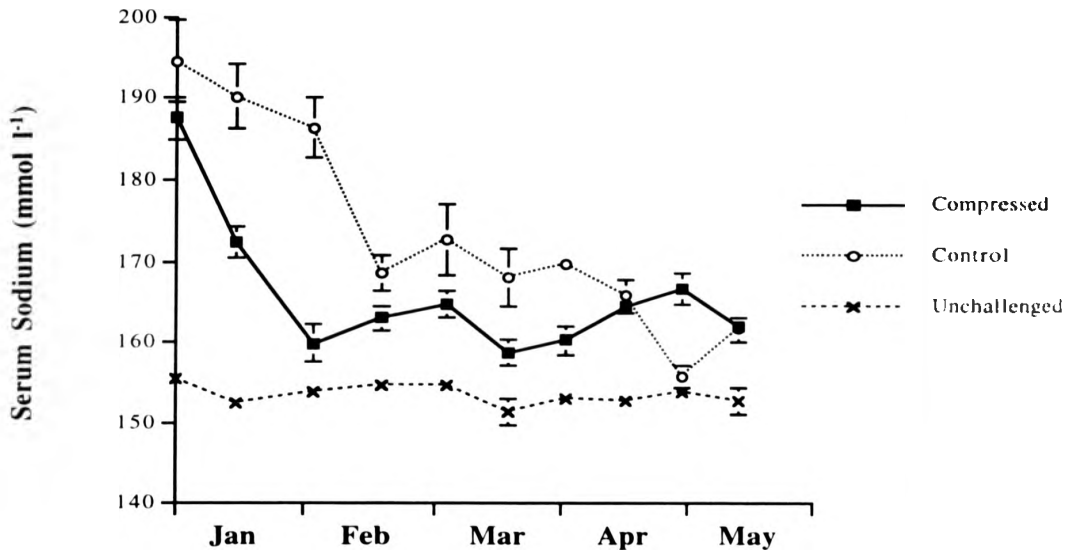


Figure 3.25

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S1 smolts reared under ambient (12-month) and compressed light cycles, and the serum sodium concentration of unchallenged control fish in Experiment 5.

3.7 EXPERIMENT 6. The effects of a compressed light cycle and low intensity night illumination on smoltification of potential S2 smolts.

3.7.1 Protocol

A farm population of underyearling Atlantic salmon (of 2-sea winter Mowi female × 3-sea winter male Sundalsøra stock origin) were reared from hatch under conditions of ambient photoperiod (latitude 52°N) and constant temperature (10±0.2°C). These were graded on November 28 and those in the lowest fraction, less than 1.5-g (potential S2 fish), were maintained on an ambient photoperiod until January 23. Three tanks were then each stocked with 550 fish. Two groups were maintained on a compressed photoperiod approximating to a 5-month seasonal light-cycle (L:D) (Figure 3.26). One of these groups received, in addition, a 24-h low intensity continuous red light illumination (L:R). The third (control) group was reared under a simulated natural (52°N) photoperiod. As in previous experiments, light was supplied using 100-W tungsten filament bulbs (providing 400-lx at the water surface). The continuous low illumination in the L:R group was provided by a 15-W low intensity red tungsten filament bulb. The incidence of direct illumination from this source was reduced by painting out the horizontal surface of the lamp condenser with matt-black enamel paint, reducing the light intensity at the water surface to 4-8-lx. All groups were fed according to manufacturers tables by electronic automatic feeders during the light phase of the shortest photoperiod. Fish in the L:R group were supplied an additional 0.25% body weight day^{-1} during the 'scotophase' by an automatic clockwork feeder to take advantage of the additional feeding opportunity provided by the night-time illumination.

3.7.1 Results

Freshwater

Fish in the L:R group showed an early growth advantage, and were significantly heavier than both the L:D group and the control group ($P<0.001$) from February 18 and

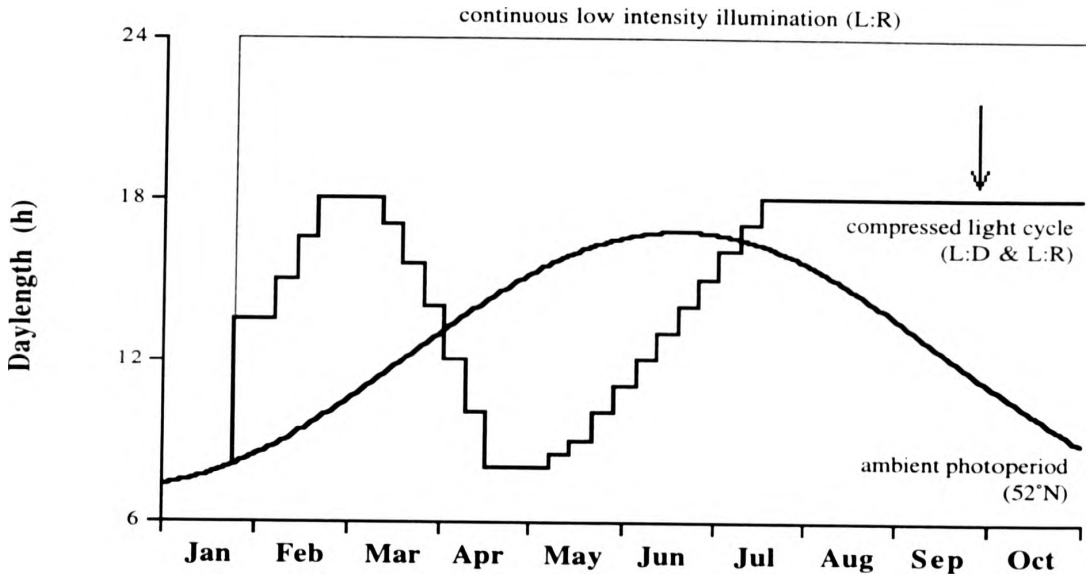


Figure 3.26

Compressed and ambient (12-month) light cycles, the provision of continuous low intensity illumination (L:R) and the timing of completion of smoltification (↓) of potential S2 smolts reared on the compressed light cycle without continuous low intensity illumination (L:D) in Experiment 6.

March 17 respectively (Figure 3.27). At the end of May, the mean weight of the L:R group was 9.8-g; which was 20% higher than that of control fish reared on an ambient photoperiod (8.2-g) and 46% higher than the L:D group (6.7-g).

By late-May the L:D population had developed a clear bimodal distribution, with only approximately one third of the group contained in the upper-mode (potential smolts). No bimodal distributions were apparent in either the L:R or control groups at this time and these populations remained unimodal at the termination of the freshwater phase of the trial in September. On June 6, the mean weights of the parr and potential smolt fractions in the L:D population were 5.6-g and 12.4-g respectively. The mean weight of the L:D potential smolts was not significantly different from the mean weight of the L:R group between June and mid-September. However, on September 29 the L:R group were significantly heavier than the L:D smolts ($P<0.05$).

Smoltification was completed by 34% of the fish reared under the L:D photoperiod regime (Table 3.4). In the first 2-weeks of the trial the L:D population, as a whole, showed a significant decrease in condition factor ($P<0.05$), but subsequent to this, an increasing condition factor was observed between February and April. No significant change in the mean condition factor was evident between the end of March and May 26 (Figure 3.28). Considering the potential smolts (upper-mode fish) and non-smolts (lower-mode fish) in the L:D group separately from June onwards; non-smolts showed a pattern of increasing condition factor, while the pre-smolts showed a steadily decreasing ($P<0.001$) condition factor from July through to October. This was accompanied by a significantly increased hypo-osmoregulatory ability over the same period ($P<0.001$; Figure 3.29). These fish were fully silvered and considered to have achieved smolt status by late-September, 7-months in advance of the natural smolting season, as gauged by normal times of seawater transfer of farm stocks reared on an ambient photoperiod (the experimental control group was not monitored beyond October). A group of 150 smolts were transferred to the Hayling marine facility on September 29.

Fish reared under the L:R regime failed to smolt at the same time as those in the L:D group. The L:R group exhibited a sharp increase in condition factor between the start of the

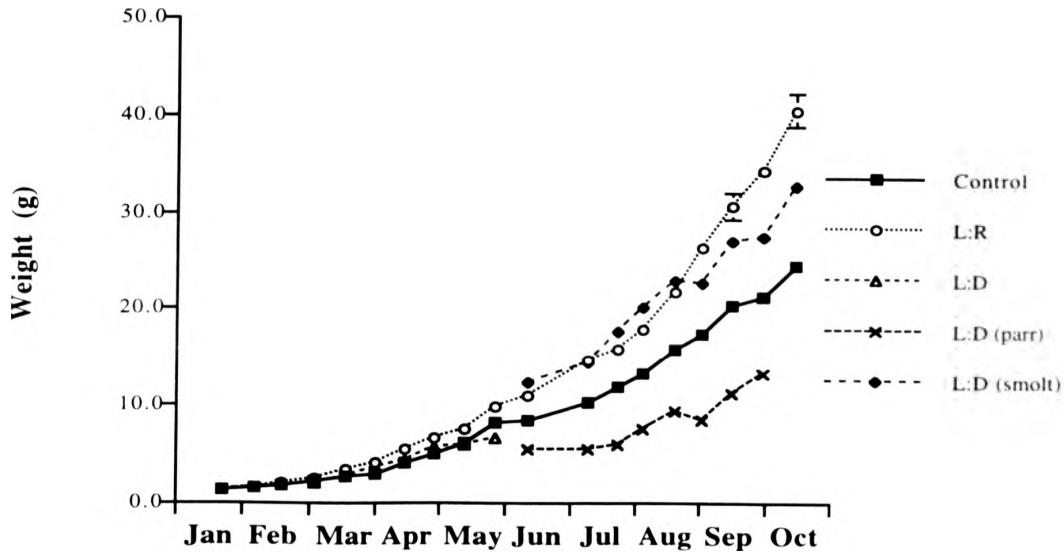


Figure 3.27

Changes in weight of potential S2 smolts reared under a compressed light cycle with (L:R) and without (L:D) continuous low intensity red light illumination, and control fish reared under an ambient (12-month) photoperiod in Experiment 6. (L:D fish are separated into smolts and parr from June 8)

Group	Smolt Fraction (percent ± 95% CI)	Male GSI (mean ± 1sem)	Female GSI (mean ± 1sem)
Control	-	5.25 ± 1.37^{bc}	0.363 ± 0.012^{b}
L:R	0%	1.74 ± 0.84^{ab}	0.280 ± 0.011^{a}
L:D	33.8% ± 4.1	-	-
L:D (smolts)	-	0.06 ± 0.01^{a}	0.345 ± 0.029^{b}
L:D (parr)	-	6.36 ± 0.99^{c}	0.477 ± 0.019^{c}

Values in the same column suffixed with a different letter are significantly different ($P<0.05$)

Table 3.4

Smolt fractions of potential S2 populations reared under a compressed light cycle with (L:R) and without (L:D) continuous low level red light illumination in Experiment 6, and the gonadosomatic indices (GSI) of male and female pre-smolts and parr on September 2.

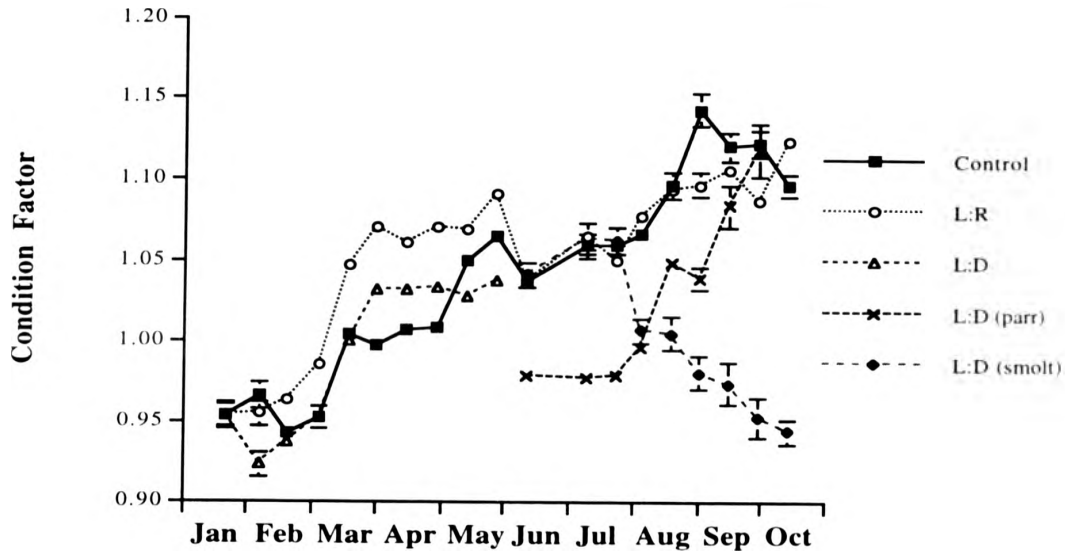


Figure 3.28

Changes in condition factor of potential S2 smolts reared under a compressed light cycle with (L:R) and without (L:D) continuous low intensity red light illumination, and control fish reared under an ambient (12-month) photoperiod in Experiment 6. (L:D fish are separated into smolts and parr from June 8)

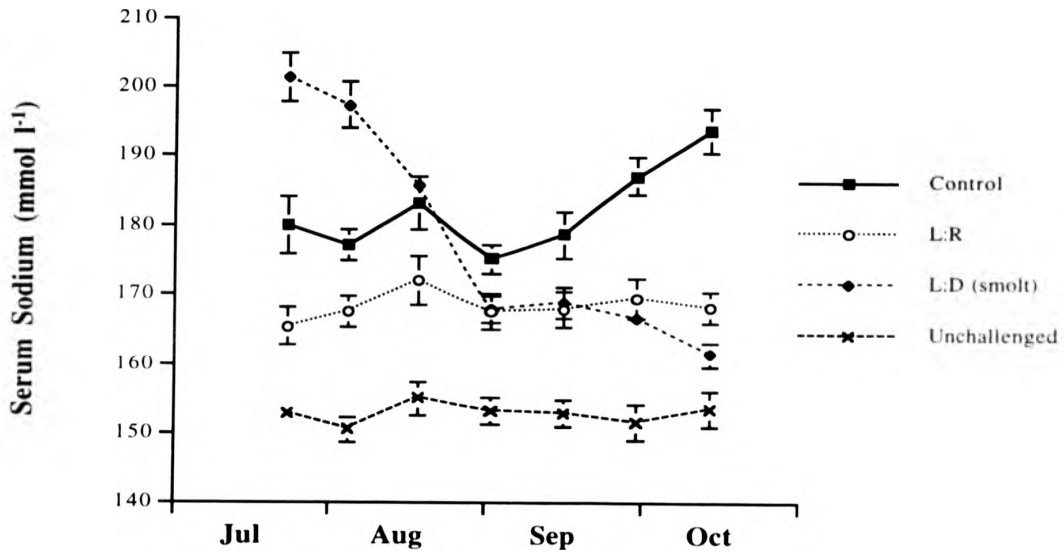


Figure 3.29

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S2 smolts reared under a compressed light cycle with (L:R) and without (L:D) continuous low intensity red light illumination, control fish reared under an ambient (12-month) photoperiod and the serum sodium concentration of unchallenged control fish in Experiment 6.

trial in January and mid-March, from which point it remained high (above 1.05) and relatively stable. The condition factor of the L:R fish was significantly higher than that of the control group and fish reared under the L:D photoperiod regime between February 18 and May 26 ($P<0.05$). Hypo-osmoregulatory ability in the L:R group was elevated compared to control fish from July to October, and was significantly higher than that of both challenged control and L:D pre-smolts fish in July and August ($P<0.05$). However, full hypo-osmoregulatory ability failed to develop. Serum sodium following seawater challenge was relatively stable (between 165 and 172-mmol l^{-1}), remained significantly higher than that of unchallenged controls throughout the testing period ($P<0.05$) and was significantly higher than that of L:D smolts prior to their transfer to seawater ($P<0.01$).

Condition factor in the control fish, reared under an ambient photoperiod, showed an increasing pattern from mid-February, peaking at the end of August, and then decreasing before the end of the freshwater stage of the trial in October. Hypo-osmoreglatory ability among these fish remained poorly developed throughout this period, with serum sodium concentration following seawater challenge remaining above 176-mmol l^{-1}.

Sacrifices were made in mid-September to assess the matuaration status among fish in each of the groups. Female parr in the L:D group had significantly higher GSIs than female smolts in the L:D group and females reared under both the L:R regime and those reared under an ambient photoperiod ($P<0.05$; Table 3.4). Female L:D smolts and control females also had a signicantly higher mean GSIs than those reared under the L:R regime ($P<0.05$). However, all females sampled had a GSI of less than 0.6 and would not have been expected to mature at the next maturation episode. Male parr in the L:D group had a significantly higher mean GSI and a significantly higher percentage of maturing individuals (males which where either running or which had a GSI greater than 10.0) than L:D smolts and males reared under the L:R regime (Table 3.4; Figure 3.30). Control males, reared under an ambient photoperiod, had a significantly higher mean GSI than L:D male smolts, and a significantly higher percentage of maturing individuals than L:R males and L:D male smolts.

L:D smolts moved to the Hayling marine laboratory in late-September transferred to seawater with no mortalities and resumed feeding within a few days. Growth over the

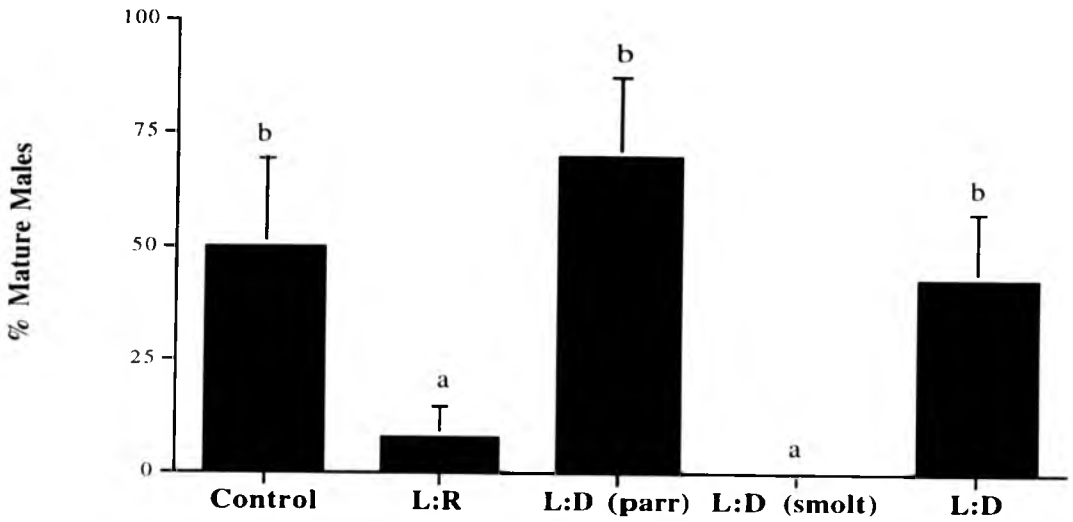


Figure 3.30

Precocious maturation among male parr and smolts reared under a compressed light cycle (L:D; parr, smolts and all fish combined), fish reared under a compressed light cycle with continuous low intensity red light illumination (L:R) and control fish reared under an ambient (12-month) photoperiod in Experiment 6. Percentages ± 95% confidence intervals. (Bars labelled with a different letter are significantly different ($P<0.05$))

winter was good with a 3-fold increase in weight from 27g at transfer to 85-g by March (Figure 3.31). The mean weight of these fish at the termination of the trial the following September was 145-g. There was no evidence of maturation among the post-smolts at this time. No transfers to seawater were made from either the L:D or control groups.

Plasma melatonin was measured in the L:R population over a 24-h sampling period (Figure 3.32). Melatonin increased rapidly after the transition from high intensity white to low intensity red lighting, remained elevated throughout the period of low intensity and returned to 'daytime' levels within 1-h after the return to high intensity. The mean 'photophase' and 'scotophase' levels over the 24-h (L:R18:6) cycle were 85.6-pg ml^{-1} and 155.8-pg ml^{-1} respectively. Changes in melatonin were coincident with changes from low to high and high to low intensity. Melatonin profiles were not determined for either L:D or control fish.

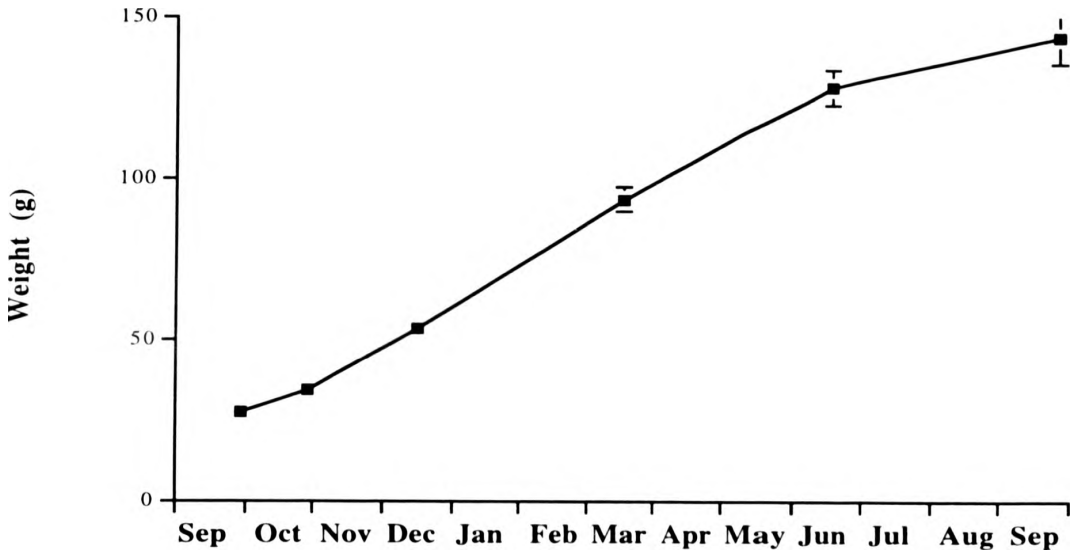


Figure 3.31

Growth following seawater transfer to the Hayling marine facility of potential S2 smolts reared under a compressed photoperiod (L:D) in Experiment 6

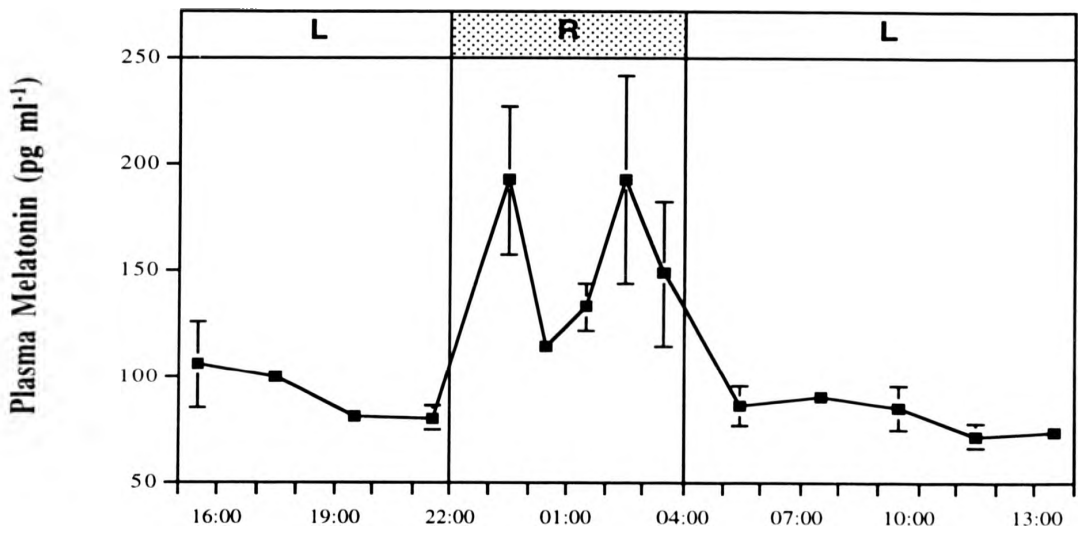


Figure 3.32

Changes in plasma melatonin over a 24-h period among potential S2 smolts maintained under a dual light intensity photoperiod (LR18:6); intensities of the 'photophase' (L) and 'scotophase' (low intensity red light illumination; R) were 440-lx and 4-lx respectively.

3.8 EXPERIMENT 7. The effects of time of seawater transfer on the subsequent growth of S1 post-smolts.

The purpose of this experiment was to investigate the effects of the timing of seawater transfer on the subsequent survival and growth of Atlantic salmon smolts. In two successive years, the development of smoltification characteristics of a group of production pre-smolts was monitored in freshwater from February until the completion of smoltification. Transfers of sub-populations to seawater were made at regular intervals between early-April and late-May; commencing prior to and ending after the period during which farmed smolts were transferred to seawater on-growing sites. The subsequent growth of experimental groups was followed until September.

3.8.1 Protocol

Experiment 7A

A population of *ca*5000 medium grade potential S1 pre-smolts (Sundalsøra strain, mean weight 41.4-g) were reared in a 4-m (12,500-l) glass-fibre tank under conditions of natural photoperiod (56°N) and temperature at a commercial hatchery from mid-January. Morphometric measurements from 100 fish were taken at 2-weekly intervals from February 24, and samples of 12-15 individuals were removed for sea water challenge tests. Groups of 150 fish were moved to 2-m (1800-l) glass fibre tanks supplied with running oxygenated full strength seawater on April 8, April 22, May 6. The test population was moved to an on-growing site on May 8, but sufficient fish were retained in freshwater to allow a final seawater transfer on May 20. The experimental groups were fed a commercial dry pellet daily to excess using automatic feeders. Assessments of growth were made at 2-weekly intervals until September 23 when the trial was terminated.

Experiment 7B

This trial was repeated the following season, again using medium grade smolts (of Mowi strain, mean weight 63.0-g). Transfers to seawater were made on April 7, April 21, May 5 and May 19. The test population was moved to a commercial on-grower on April 24. Experimental groups were fed 2.5% body weight day-1 by automatic (clockwork) a feeders during daylight hours. Morphometric measurements of the groups moved to seawater were continued at 2-weekly intervals until September 9.

3.8.2 Results

Experiment A

Pre-smolts grew steadily throughout the freshwater phase of the experiment (Figure 3.33). Condition factor increased significantly between the start of the trial in late-February and March 25 ($P<0.001$) (Figure 3.34). On April 8, when the first transfer to seawater was made (Group 1), condition factor was still high and hypo-osmoregulatory ability was not very well developed. Although the fish were generally silvery in appearance, faint parr marks were still visible on most fish, and significant amounts of green pigment remained in their ventral surfaces and pectoral and pelvic fins. Condition factor decreased to a minimum on May 6. Hypo-osmoregulatory ability developed steadily from late-March reaching a maximum on May 6, when the serum osmolarity of challenged smolts was not significantly different from that of unchallenged fish (159-mmol l^{-1} Figure 3.35). At this time, silver colouration in the population was considered to be complete, no parr marks were visible and the ventral surfaces were white. The tank population was moved to a commercial on-growing site. A significant increase in condition factor was observed among the fish retained in freshwater after May 6 for late transfer to seawater on May 20 ($P<0.01$), but hypo-osmoregulatory ability remained elevated.

All groups transferred to seawater well and there were no post-transfer mortalities, although some fish in all groups were lost immediately after sampling (these failed to recover from anaesthesia after morphometric measurements were made). Changes in weight

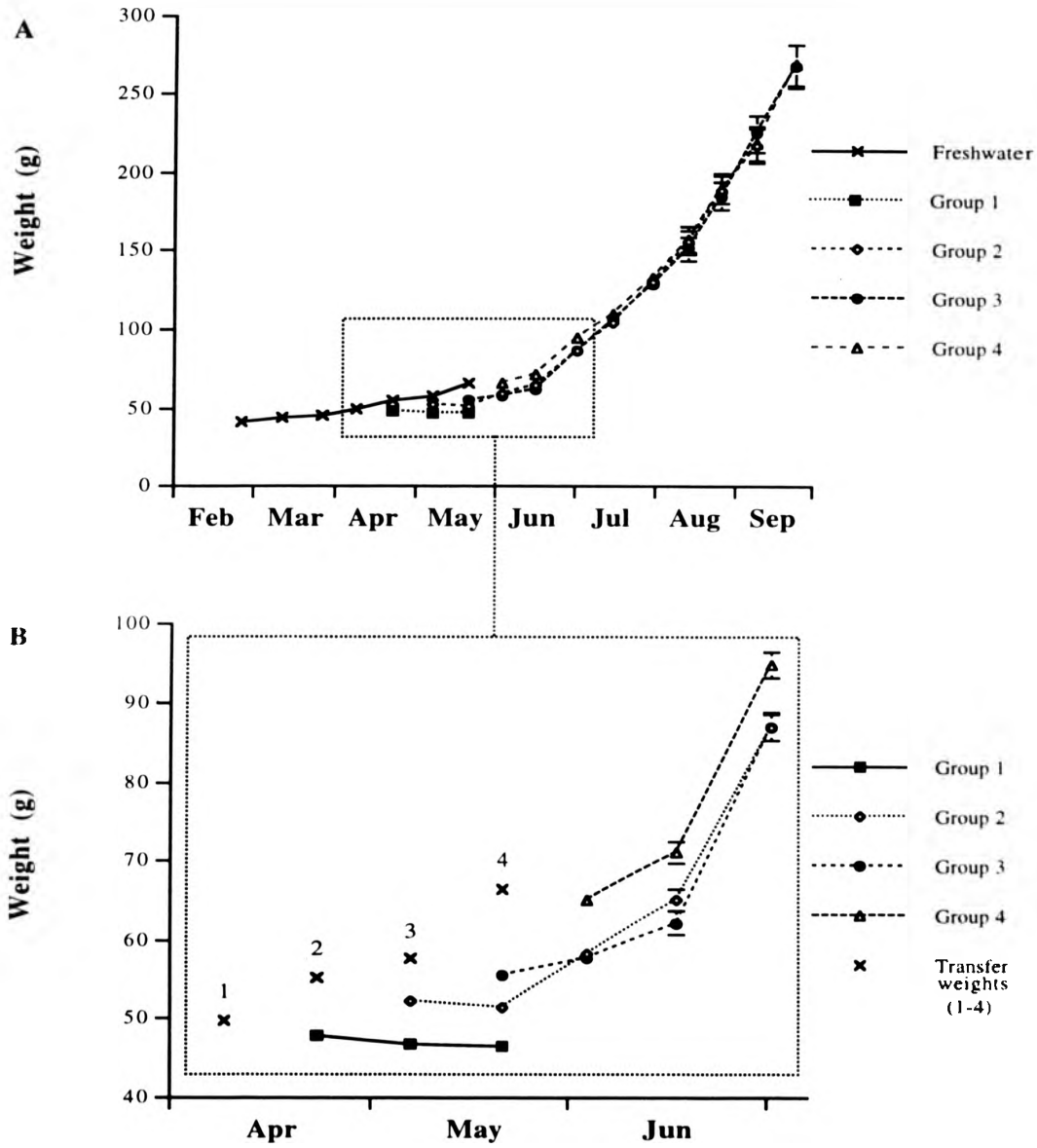


Figure 3.33

A: Changes in weight of potential S1 smolts in freshwater and following transfers made to seawater at different times in Experiment 7a. B: Expanded detail of weight changes immediately after seawater transfer.

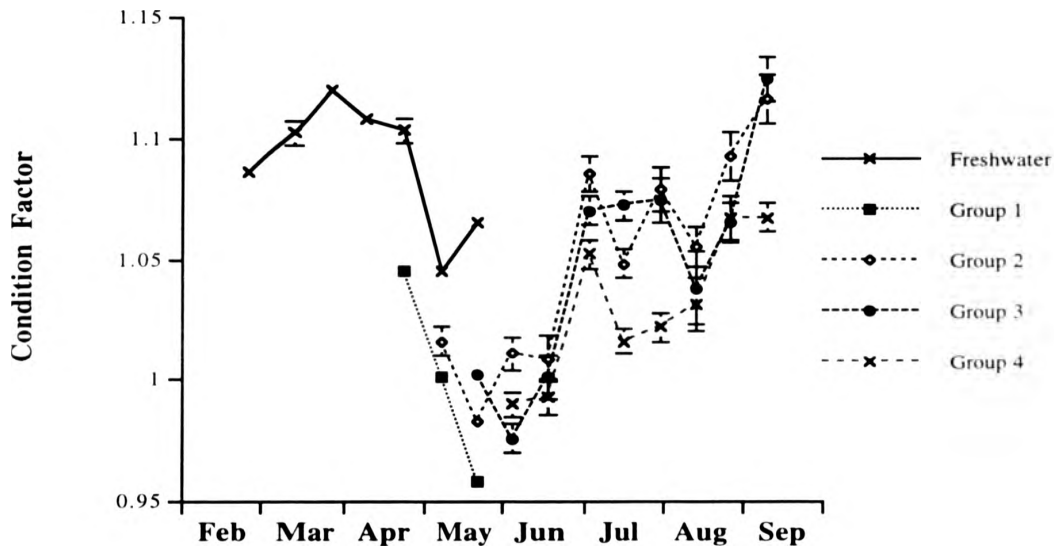


Figure 3.34

Changes in condition factor of potential S1 smolts in freshwater and following transfers made to seawater at different times in Experiment 7a.

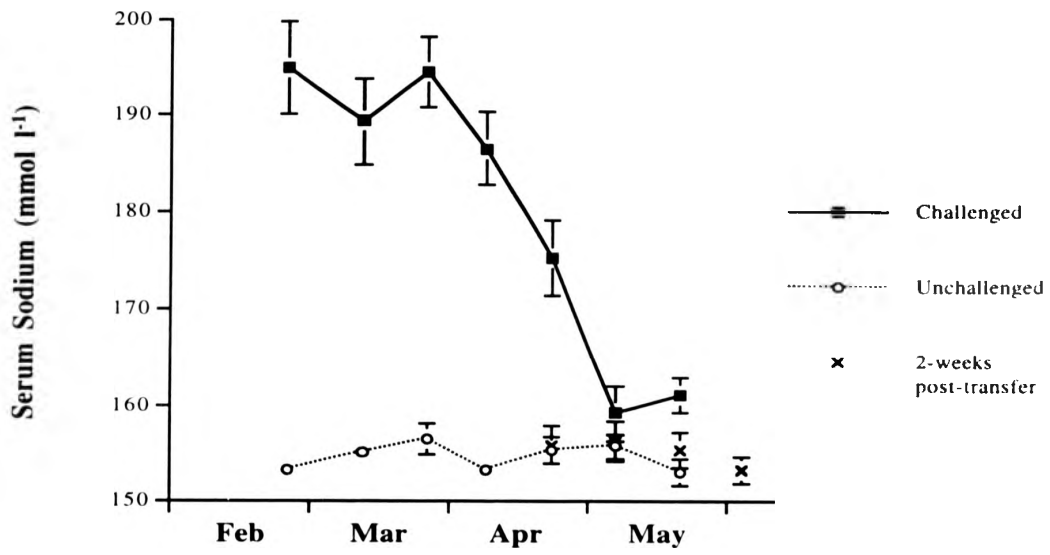


Figure 3.35

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S1 smolts, serum sodium concentrations of unchallenged fish in freshwater and of smolts two weeks after successive transfers to seawater in Experiment 7a.

following the transfer of groups to seawater are shown in Figure 3.33, specific growth rates of fish between successive sampling times are presented in Figure 3.36. Smolts in all groups moved to seawater suffered an initial loss in weight. Group 1 (transferred on April 8, 4-weeks early) showed a pattern of decreasing weight for 6-weeks after transfer. This group was subsequently lost due to an interruption in water supply. Fish transferred to seawater on April 22, 2-weeks early (Group 2) continued to loose weight for 4-weeks. Positive growth was resumed between 4 and 6-weeks post-transfer ($P.<0.001$) but the population only became significantly heavier than their weight at transfer after 10-weeks in seawater ($P<0.01$). Smolts in Group 3 were transferred to seawater on May 6, when the remainder of the tank population and the majority of the production stocks were moved to commercial on-growing sites. At this time both condition factor and serum sodium following seawater challenge were at their lowest values and the population was considered to be at the peak of smoltification. This group showed a decrease in weight during the first 2-weeks in seawater, after which positive growth was restored. A significant increase on the transfer weight was only achieved after 8-weeks in the sea. Group 4 was transferred to seawater on May 20 (late transfer) these fish showed minimal loss in weight, had exceeded their transfer weight after 4-weeks and were significantly heavier than their transfer weight at 6-weeks ($P<0.001$). Weight loss as a percentage of weight at transfer for groups 2-4 were 7.0%, 3.8% and 1.9% respectively (Group 1 showed a 6.8% reduction in weight at their final assessment on May 20).

As a result of the arrest in growth seen in all groups following seawater transfer, on May 20 ,when the final group was moved to seawater, the mean weights of the 4 groups were all significantly different from each other and ranked in order of transfer time: ranging from 46.7-g (Group 1) to 66.4-g (Group 4). Fish in Group 4 remained significantly heavier than those in groups 2 and 3 until July 1 but from this time until the termination of the trial on September 29 trial there were no significant differences in the mean weights of the 3 surviving groups.

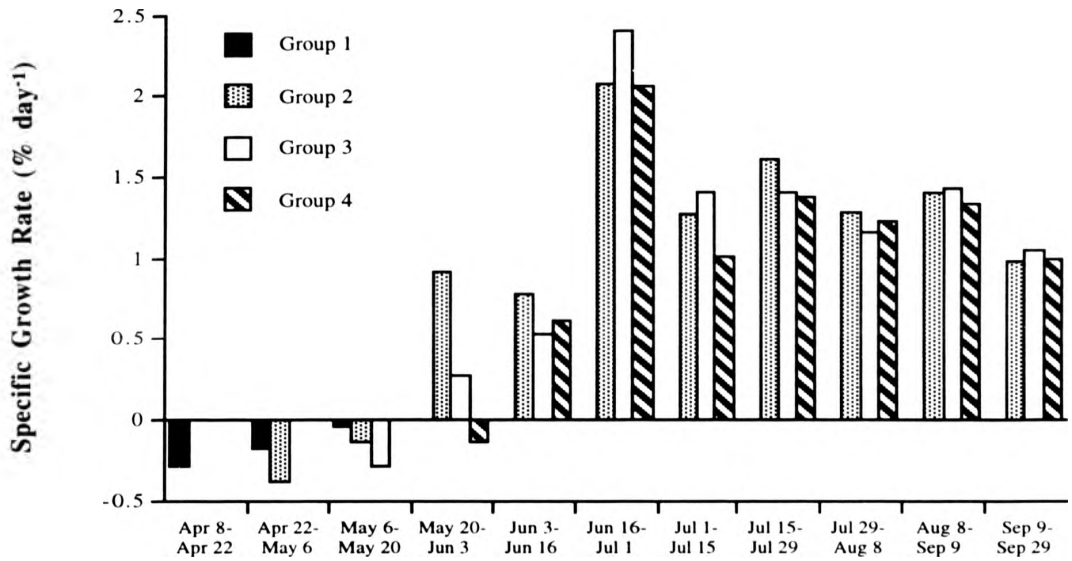


Figure 3.36

Changes in specific growth rate of S1 post-smolts following transfers to seawater at different times in Experiment 7a. (Groups 1-4 were transferred to seawater on April 8, April 22, May 6 and May 20 respectively)

Experiment B

Due to a higher average winter freshwater temperature, the medium grade pre-smolts selected for Experiment B were larger than those used the previous year (see Figure 3.37). At the start of the trial, in late-February, these fish had a mean weight of 63.0-g compared to 41.4-g in Experiment A. At the first seawater transfer in early-April, smolts in Experiment B weighed 78.8-g, whereas in Experiment A the mean weight at first transfer was 49.8-g. The larger fish in Experiment B showed a completion of smolt colouration in advance of the (correspondingly) smaller fish one year previously.

On April 8, when the first transfer to seawater was made, fish in Experiment B were very silvery, no parr marks were visible and a small amount green pigment persisted in the caudal and paired fins. As in Experiment A, condition factor increased significantly in the first month of the trial ($P<0.001$), but decreased more rapidly from late-March through to May 19 when the last transfer to seawater was made (Figure 3.38).

Hypo-osmoregulatory ability appeared to be late in developing (Figure 3.39), with serum sodium after seawater challenge remaining above 180-mmol l^{-1} until the end of April, but developed rapidly between this point and May 6 when the Group 3 was transferred to seawater. Hypo-osmoregulatory ability did not change significantly between May 6 and May 20 when the last group was transferred to seawater.

In this trial, weight loss following transfer to seawater was less severe and return to positive growth was faster than for groups transferred to seawater than in Experiment A (Figure 3.33). Groups 1, 2 and 3 (transferred on April 7, 21 and May 5 respectively) weighed slightly less after 2-weeks in a marine environment. In all cases an increase in weight had occurred at 4-weeks post-transfer and mean weights were significantly greater than the transfer weights 6-weeks post-transfer for groups 1 and 2; and at 8-weeks for Group 3. Weight loss was not evident among fish in Group 4 which was transferred on May 19, 2-weeks after transfer to seawater (see Figures 3.33 and 3.40), and showed a significant increase on transfer weight after 6-weeks. There were no significant differences in weight between any of the groups from the time of the final transfer in mid-May until the end of July. From mid-August until the end of the trial on September 8, post-smolts which

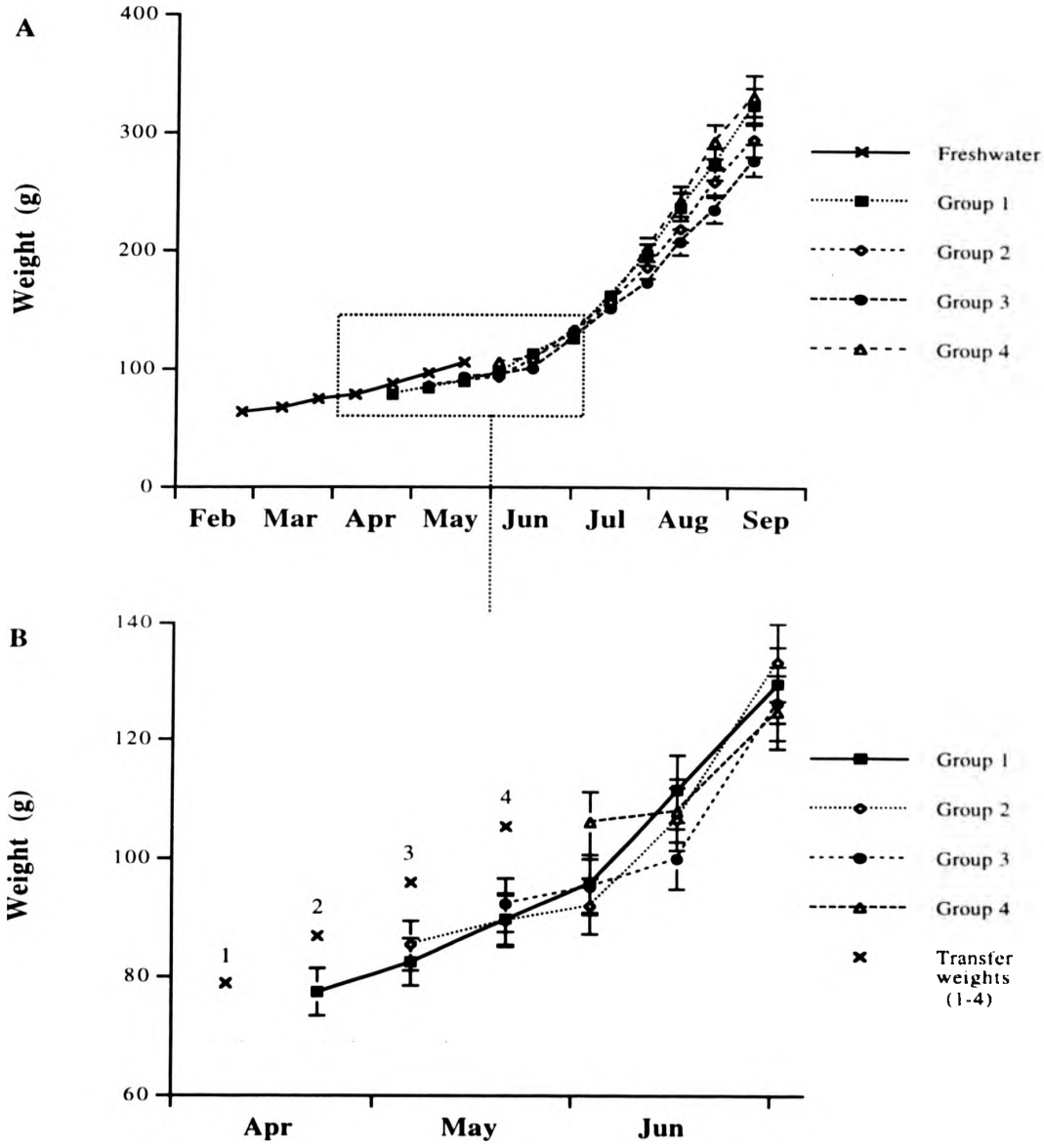


Figure 3.37

A: Changes in weight of potential S1 smolts in freshwater and following transfers made to seawater at different times in Experiment 7b. B: Expanded detail of weight changes immediately after seawater transfer.

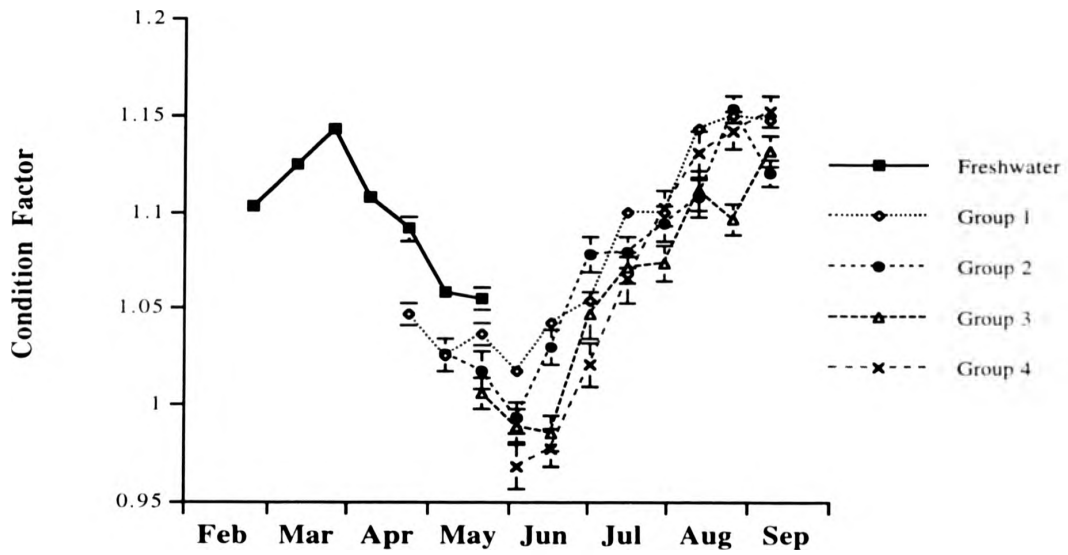


Figure 3.38

Changes in condition factor of potential S1 smolts in freshwater and following transfers made to seawater at different times in Experiment 7b.

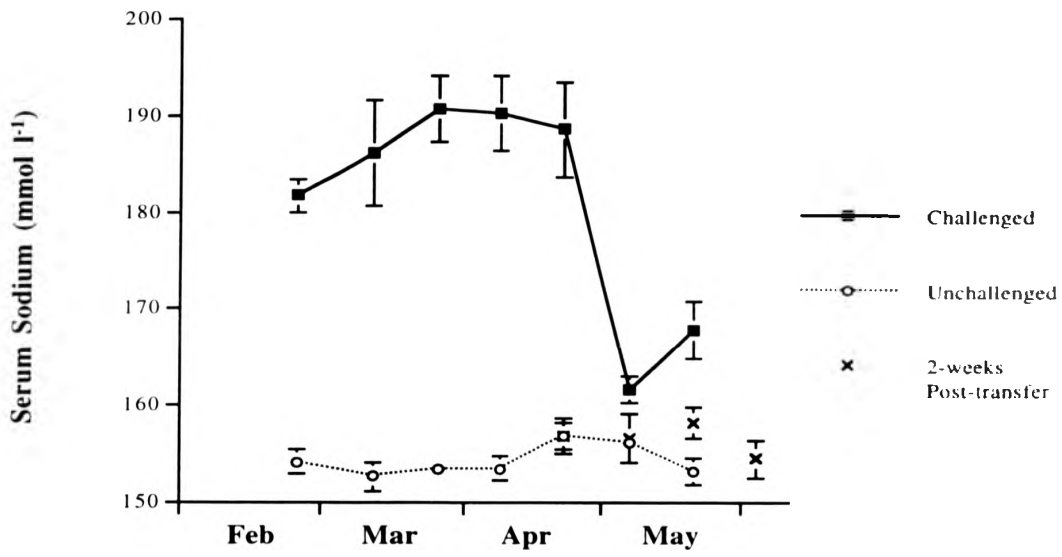


Figure 3.39

Changes in serum sodium following 24-h, 28‰ seawater challenge of potential S1 smolts, serum sodium concentrations of unchallenged fish in freshwater and of smolts two weeks after successive transfers to seawater in Experiment 7b.

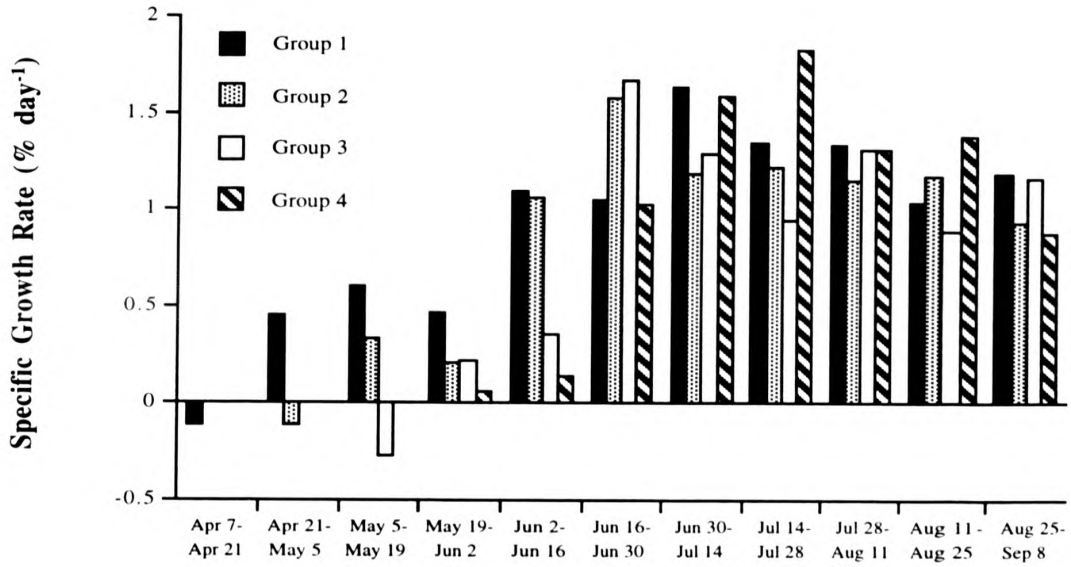


Figure 3.40

Changes in specific growth rate of S1 post-smolts following transfers to seawater at different times in Experiment 7b. (Groups 1-4 were transferred to seawater on April 7, April 21, May 5 and May 19 respectively)

had been transferred on April 7 and May 19 (groups 1 and 4) were significantly heavier than those transferred on either April 21 or May 5 (groups 2 and 3).

3.9 Discussion

The present work shows clearly that the timing of smoltification can be altered by exposing either potential S1 or S2 smolts (under-yearling and 1+ year-old parr respectively) to modified (seasonal) light cycles. The manipulation of photoperiod during freshwater residence has previously been employed in a number of salmonid species to enhance smolt size; (Komourdjian *et al.*, 1976; Knutsson and Grav, 1976; Brauer, 1982; Saunders *et al.*, 1985; 1989) and to both advance or delay the timing of smoltification (Zaugg and Wagner, 1973; Komourdjian *et al.*, 1976; Saunders and Henderson, 1978; Clarke *et al.*, 1985; McCormick *et al.*, 1987).

In the present work, potential S1 smolts were advanced up to a maximum of 3-months compared to fish reared under conditions of natural or simulated natural photoperiod, and advances of up to 7-months were achieved with potential S2 smolts. Smolts in all trials exhibited significantly increased hypo-osmoregulatory abilities and significantly decreased condition factors in response to increases in daylength, in agreement with previous works showing that exposure to long or increasing daylengths subsequent to a period of short 'winter' daylength are stimulatory to the completion of smoltification (Wagner, 1974a; Komourdjian *et al.*, 1976; Farmer *et al.*, 1978; Clarke *et al.*, 1978; 1985; Zaugg, 1981; McCormick *et al.*, 1987; Okumoto *et al.*, 1989; Sigholt *et al.*, 1989; Duston and Saunders, 1990; 1992)

In Experiment 1 accelerated seasonal light cycles of 6-, 8- or 10-months duration, applied from December to June yielded advances of up to one month in the completion of smoltification as assessed by changes in body silvering and the development of hypo-osmoregulatory ability. These observations are in agreement with Clarke *et al.* (1985) who, by using a seasonally-accelerated light cycle (approximately 7-months in period length, commencing in early February) induced an earlier silvering and a 2-3 week advancement in hypo-osmoregulatory ability of potential S1 smolts. In Experiment 1 fish in the 6- and 8-month groups also showed signs of an early loss of smolt characteristics evidenced by a significant loss of hypo-osmoregulatory ability (6-month fish only) and a return of yellow

pigment to the body by the end of the freshwater phase of the trial (before the control fish had achieved smolt status), this occurrence was also noted by Clarke *et al.*(1985). The maximum advancement in the completion of smoltification of S1 smolts in the present work was achieved in Experiment 5 using an approximation to a 6-month accelerated seasonal photoperiod commencing in mid-July. This regime served to advance the completion of smoltification by 3-months, to the first week of February, compared to fish reared under a control photoperiod.

In Experiment 3, the use of squarewave photoperiods, ie direct change from a short day to a long day, during the winter proved more stimulatory to the advancement of smoltification in potential S1 fish than those achieved with seasonally compressed light cycles starting in December (Experiment 1). Direct change from short day (LD7.25:15.75) to a long day (LD16.75:7.25) on December 22, January 21 and February 20 advanced the completion of smoltification by 7, 5 and 3-weeks respectively compared to that of control fish which completed smoltification in mid-May. Duston and Saunders, (1990) made 4 direct transfers from LD:8.25:15.75 to LD16:8 between December 31 and March 31 all of which provided advancements in the completion of smoltification, assessed by seawater tolerance, from late February to mid-April, compared to controls which completed smoltification in late May. Sigholt *et al.* (1989), working with potential S2 smolts, achieved a comparable advancement of 6-weeks by abruptly increasing the daylength from ambient to a constant LD12:12 on December 15.

Seasonally-compressed photoperiods were again used to alter the timing of smoltification of potential S2 fish in Experiment 2; this was a continuation of the light regimes tested on S1 smolts in Experiment 1, (after the S1s had been transferred to sea). Six-, 8-, or 10-month seasonal photoperiods were shown to advance the completion of smoltification by 5, 3 and 1-month respectively. Similar advances were achieved in Experiments 4 and 6 using photoperiods approximately 5-months in period length, commencing in April and January; these provided advances of 5 and 7-months and supplied early S2 smolts in November/December and late September respectively.

Results provided by these studies concerning the photoperiodic advancement and delay

of the timing of smoltification are consistent with the hypothesis that changes in daylength entrain an endogenous circannual timing mechanism which controls the development and completion of smoltification (Conte and Wagner, 1965; Hoar, 1965, 1988; Wagner, 1974a; 1974b; Clarke *et al.*, 1978; 1985; Eriksson and Lundqvist, 1982, Duston and Saunders, 1990; 1992). The seasonal nature of smoltification, as well as growth patterns and gonadal development in salmonids is well established (Baggerman, 1960; Conte and Wagner, 1965; Eriksson and Lundqvist, 1980; Duston and Saunders, 1992). The presence of an underlying timing mechanism (an endogenous oscillator) controlling the development of such rhythmic processes among animals inhabiting seasonally-changing environments ensures that they proceed at times of the year which are optimal to survival (Gwinner, 1981; 1989). True endogenous rhythms should 'free-run' (exhibit their natural frequency) in the absence of environmental cues (constant conditions) with a periodicity approximating to, but significantly different from, one year (Gwinner, 1981). Such circannual mechanisms have been identified in more than 40 species representing a variety of taxonomic groups (reviewed by Gwinner, 1986).

Evidence that there was an endogenous component to some of the seasonal processes seen in salmonid fish was first provided by Brown (1946), who demonstrated free-running growth rhythms among brown trout maintained under constant conditions. Duston and Bromage (1986a) demonstrated circannual rhythms of maturation in rainbow trout maintained on constant temperature and constant short days (LD6:18), long days (LD18:6) and continuous light (LL) over 3 spawning cycles. Eriksson and Lundqvist (1982) demonstrated approximate 10-month cyclic variations in specific growth rate, condition factor and skin colouration in Atlantic salmon over a 14-month study period, under constant photoperiod and temperature regime.

Under ambient photoperiod conditions, it is suggested that the endogenous mechanism(s) controlling the various aspects of smoltification are entrained by information provided by decreasing and increasing daylengths. Advances in the timing of smoltification under the compressed-seasonal photoperiods in the course of the present study may be interpreted as overt expressions of corrective adjustments (phase-advances) in the

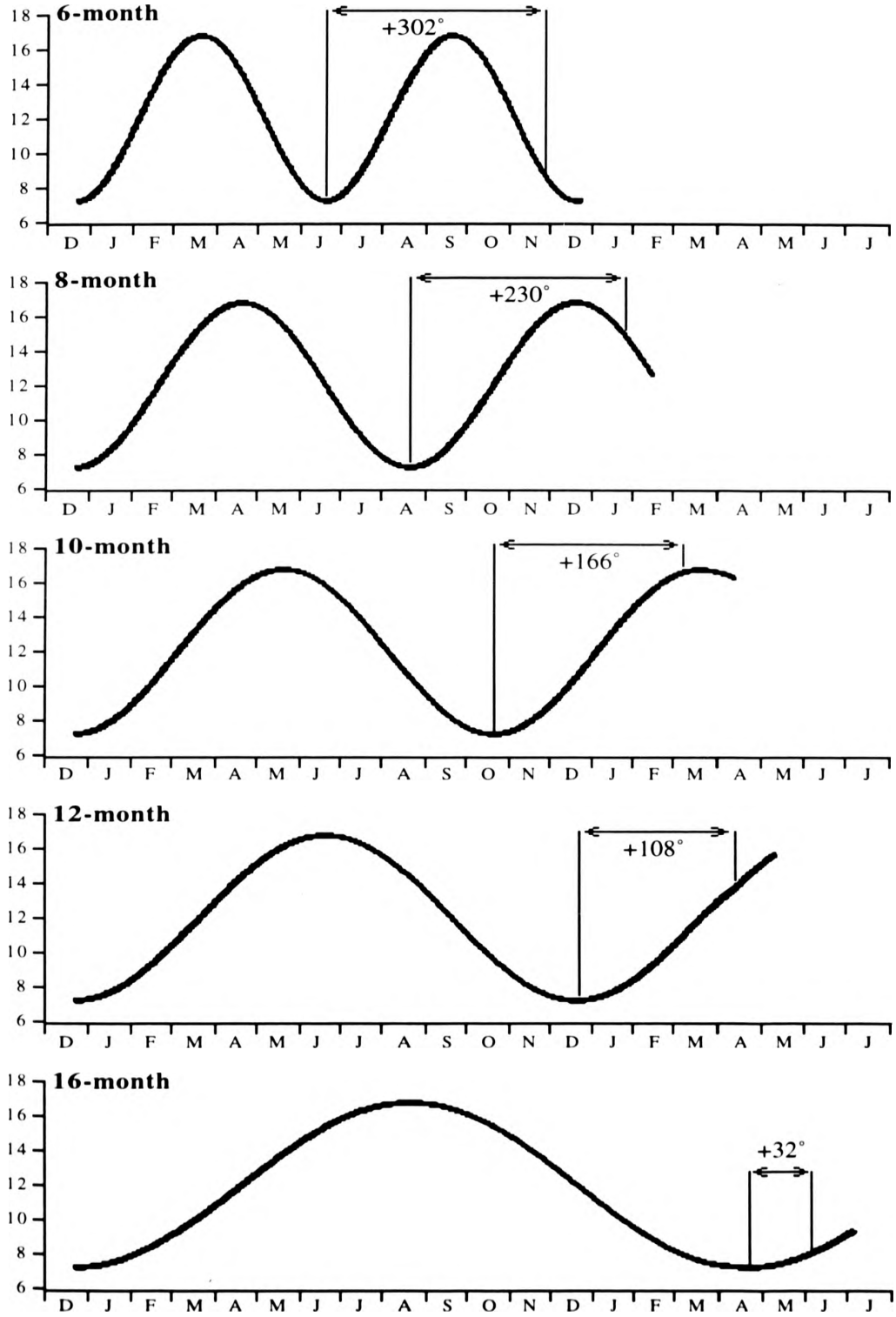
endogenous oscillator which is seen to be running 'behind time' with respect to a photoperiodic zeitgeber which is running at an increased frequency. Similarly, the later smoltification seen in Experiment 2, among fish reared under the 16-month photoperiod, may be described as the result of a corrective phase-delay.

In this study, all groups which were exposed to compressed photoperiod cycles demonstrated a completion of smoltification at a point in the experimental photoperiodic cycle later the than would be expected under an ambient photoperiod; similar effects were reported by Clarke *et al.* (1985) and Duston and Saunders (1992). This was particularly evident among the potential S2 smolts reared under the 6-, 8- and 10-month seasonally compressed photoperiods in Experiment 2. Control fish reared under an ambient 12-month photoperiod completed smoltification, as expected, on an increasing photoperiod of 12-14-h daylength (Figure 3.6). Smoltification under the 10- and 8-month photoperiods was completed just before and just after the respective solstices, whereas with the most forcing zeitgeber ie the 6-month photoperiod, smoltification was completed after a significant period of decreasing daylength. Fish reared on a light cycle with a period greater than 12-months (16-months) completed smoltification earlier than would have been expected, on a short daylength of 9-h, soon after the winter solstice. This is clarified in Figure 3.41, which shows relative phase angle differences between actual and expected times of completion of smoltification of fish reared under the compressed and extended seasonally-changing photoperiods in Experiment 2. These phase angle differences were calculated relative to the winter solstice in each light-cycle. Smoltification is completed at a progressively later phase of the photocycle as the period of the photocycle is decreased. With an increased zeitgeber period (16-month) a decreased phase angle difference is apparent

This response to seasonal photoperiods of varying frequency is characteristic of a processes controlled by an endogenous mechanism, and provides evidence that the completion of smoltification is not a result of direct induction by photoperiodic stimuli. The advances in the timing of smoltification among S1 fish subjected to direct (square-wave) changes from short to long daylengths during the winter in Experiment 3 may also be explained in terms of corrective phase-shifts made to an endogenous oscillator. From

Figure 3.41

Relative phase angle differences between the time of completion of smoltification and the winter solstice for potential S2 salmon reared under 6-, 8-, and 10-month compressed, 12-month (ambient) and 16-month extended seasonal photoperiods.


6-month
+302°
8-month
+230°
10-month
+166°
12-month
+108°
16-month
+32°
18
16
14
12
10
8
6
D J F M A M J J A S O N D J F M A M J J

December 14 the photoperiod was maintained on a solstice hold (LD 7.25:16.75) providing constant environmental conditions, of photoperiod and temperature, under which the endogenous mechanism(s) controlling smoltification would be expected to free-run (Eriksson and Lunqvist, 1982). Group A experienced a single abrupt change from a short day to a long day (LD16.75:7.25) on December 22, during the 'subjective winter' of the circannual oscillation. This change would be perceived by the clock as running 'behind time' which responded with a compensatory phase advance that was expressed overtly as a 7-week advance in the timing of the completion of smoltification. The clocks in groups B and C would have been advancing (free-running) in the absence of environmental cues, such that subsequent daylength changes made on January 21 and February 20 occurred successively later, closer to the 'subjective spring' in the circannual cycle, which prompted the oscillator to make smaller corrective phase-advances. This will have resulted in respectively earlier completions of smoltification of 5 and 3-weeks compared to control smolts. Duston and Saunders (1990) demonstrated that in the absence of a change to a long day, the development of salinity tolerance and condition factor occurred at approximately the same time as control fish reared under an ambient photoperiod.

In the past, questions have been raised regarding the ability of Atlantic salmon smolts produced under unnatural photoperiods to survive and continue grow well when removed to a marine environment. A number of studies have yielded smolts from alternative photoperiod regimes which were not subsequently transferred to seawater, as a result their true 'smolt quality' could not be properly assessed. Saunders *et al.* (1985) reared potential S1 smolts under continuous light from late November, and although these fish grew at an enhanced rate in freshwater, compared to that of control fish reared under a natural photoperiod, and developed morphological smolt characteristics, their growth rate was much reduced following transfer to seawater. It was concluded from this study that these fish had not physiologically smolted. In a later study (McCormick *et al.*, 1987), fish reared under continuous light from first feeding failed to develop physiological smolt characteristics and grew at a decreased rate following seawater transfer compared to fish reared under an ambient photoperiod. Also in this study, parr reared from first feeding on continuous light

but returned to an ambient photoperiod in December showed an impaired development of hypo-osmoregulatory ability. Post-smolts from this treatment showed reduced seawater performance. Saunders and Henderson (1970) subjected potential smolts to a photoperiod which was 6-months out of phase with that of the natural light cycle (reciprocal photoperiod). These fish grew well in freshwater, developed a silver colouration but had an increased rather than a decreased condition factor at sea water transfer. Following sea water transfer, this group grew more slowly than fish reared under an ambient photoperiod. These authors suggested that the reciprocal photoperiod had disturbed the smoltification process. Komourdjian *et al.* (1976) placed potential S1 parr under a reciprocal photoperiod in December. These fish showed morphological signs of smoltification and an increased survival in 40 ‰ seawater in February, but had lost some smoltification characteristics by June when the control (natural photoperiod) fish smolted. Again, these advanced smolts were not transferred to seawater, so their ability to grow in a marine environment and hence their commercial value was not known.

An important finding of the present work was that, with the exception of fish reared in Experiment 5, all smolts reared under experimental photoperiods in freshwater showed good survival and growth following transfer to seawater. Potential S1 smolts introduced up to 7 weeks early (Experiment 3), benefited from their extended period in seawater and were growing well by the time conventionally-reared control smolts were tranferred. Potential S2 smolts transferred to seawater in late-autumn or early-winter (Experiments 2, 4 and 6) showed better growth in seawater than control fish remaining in freshwater, generally tripling their weight over the winter before the introduction of smolts reared under ambient photoperiod conditions. Smolts reared at the cage site grew faster than those reared at the small scale facility; due to improved culture conditions, including better water quality and a more favourable annual temperaure range. High mortalities occurred following the transfer of advanced potential S1 smolts, which were produced on a greater scale for a larger cage site, in February (Experiment 5). Although the reason for this remains unclear, there are a number of factors which may have contributed to the mortality. These fish were reared in larger tanks (9-m) at higher rearing densities. The final stocking density prior to the

seawater transfer of these smolts was 57-kg m^{-3}, compared to maximum rearing densities in Experiments 1-4 and 6 of 20-30-kg m^{-3}. High rearing density has been shown to reduce Na^+K^+-ATPase activity in coho salmon (Sower and Fawcett, 1991), increase serum cortisol in coho salmon and rainbow trout (Patino *et al.*, 1986; Leatherland and Cho, 1985) and to reduce growth following seawater transfer in Atlantic salmon. Fluorescent tubes were used to provide the lighting in the freshwater phase of this experiment which provided a low light intensity, with maximum a of 20-lx at the water surface, compared to 400-lx supplied by tungsten filament lamps in the other photoperiod trials. Considering that the natural lighting under light cloud cover is approximately 30,000-lx, the smolts transferred in Experiment 5 will have received a considerable light shock on transfer to seawater; this is now recognised as a particularly severe form of stress for smolts. In addition, the transport of these fish between the smolt unit and the ongrowing farm was particularly stressful; involving both a longer road journey and additional handling at the seawater site in transferring the smolts to the sea cages. Increased circulating cortisol during smoltification (Specker and Schreck, 1982; Patino and Schreck, 1986) makes smolts particularly susceptible to stress, so any factors causing stress at this time may accumulate and result in poor seawater performance following transfer.

Potential S2 parr reared on a seasonally accelerated (5-month) photoperiod from January with no additional night-time illumination (L:D) smolted in late-September, 7-months in advance of control smolts (farmed stocks) reared on an ambient light:dark cycle. However, fish reared under the same accelerated photoperiod with a supplementary continuous low level red lighting providing a night-time illumination of 4-lx (L:R) had not smolted by the end of the trial the following January. L:R fish maintained a high condition factor and did not develop a silvery appearance or black fin margins. Hypo-osmoregulatory ability was elevated from the commencement of testing in August but not at the same level as fish which had completed smoltification. This is in agreement with the results of Thorarensen *et al.* (1989), who demonstrated that low intensity night-time illumination resulted in incomplete silvering and poor seawater adaptability in coho salmon juveniles. These authors suggested that the threshold level over which night-time light levels may

interfere with the completion of smoltification was close to 10^{-4}-lx.

The L:R regime was successful in increasing growth rate and, after 4-months, the L:R group were 46% heavier than the L:D fish. However, these fish were provided with a supplementary 0.25% body weight feed during the 'scotophase' and, in the absence of an L:R group which was not fed during the course the low intensity phase, it is not possible to determine whether this increased growth was a result of the additional light, the additional feed or a combination of the two factors. It was considered possible that the growth rate among the L:R population was high enough to allow a majority of the fish to mature in freshwater rather than proceed with smoltification (Thorpe, 1986). However, when groups of fish were sacrificed in September, no maturing females were found and the incidence of maturing or running males was less than that of both L:D non-smolts and control fish reared on an ambient light-dark cycle.

A 24-h blood sampling profile was undertaken in order to investigate the effect of the L:R (high-low intensity) light regime on circulating melatonin. This was conducted in January, after the population had been held on a 'long day' (LR18:6) for 6-months. It is well documented that the pineal gland, through the release of melatonin, plays a central role in photoperiodism in some mammals and the removal of this organ renders them unable to respond to changes in photoperiod (for reviews see Underwood and Goldman, 1987; Bartness and Goldman, 1989; Bartness *et al.*, 1993; Reiter, 1993). The translation of temporal information provided by light:dark cycles into daily rhythms of melatonin secretion is believed to control a number of seasonal events including reproduction, in both higher vertebrates (Bartness *et al.*, 1993) and fish (Zackmann *et al.*, 1992a), and its involvement in smoltification has also been suggested (Randall *et al.*, 1994). Changes in daylength modify both the duration and the amplitude of the nocturnal elevation in melatonin, and in a number of species it is the duration of increased circulating melatonin levels which has been implicated in the mediation of neuroendocrine responses to photoperiod (Bartness *et al.*, 1993). The first evidence to suggest that fish possessed a similar mechanism was provided by Fenwick (1970) who demonstrated that melatonin was synthesised by the chinook salmon pineal. Since then diel rhythms of circulating melatonin release have been reported

in Atlantic salmon (Lindahl, 1986; Randall *et al.*, 1989, 1991a), rainbow trout (Gern *et al.*, 1978a; Owens *et al.*, 1978; Duston and Bromage, 1986b; Randall *et al.*, 1991a,b), brook trout (Zachmann *et al.*, 1992b), pike, *Esox lucius* (Falcon *et al.*, 1989), common carp, *Cyprinus carpio* (Kezuka *et al.*, 1988), goldfish, *Carassius auratus* (Kezuka *et al.*, 1992) dace, *Leuciscus leuciscus* (Brook, 1989) and tilapia, *Oreochromis niloticus* (Randall, 1992).

In Experiment 6 circulating melatonin measured over a 24-h period among fish held on a light regime alternating between a high intensity (400-lx) 'photophase' and a low intensity (4-lx red light illumination) 'scotophase' (LR18:6) exhibited a diel cycle with elevated levels coincident with the period of low intensity illumination. Melatonin concentrations increased rapidly with the transition from high to low intensity, with levels remaining elevated throughout the course of the low intensity period and returning to 'daytime' levels within 1-h of the change to high intensity lighting. This profile is comparable with the type III pattern of melatonin secretion described by Reiter (1988) for several higher vertebrates, and similar to that observed in rainbow trout (Randall *et al.*, 1991a,b) and pike (Falcon *et al.*, 1989). Mean plasma melatonin concentrations during the high intensity phase (86-pg ml^{-1}) were comparable with those found in the photophase for salmonids entrained to light-dark cycles (Gern *et al.*, 1978a; Owens *et al.*, 1978; Duston and Bromage, 1986b; Randall *et al.*, 1989, 1991a,b; Alvariño *et al.*, 1993). However, the mean concentration of melatonin during the low intensity phase (156-pg ml^{-1}) was lower than levels previously reported for the scotophase in fish (mentioned above) entrained to light-dark cycles. The amplitude of the nocturnal increase in melatonin has been shown to decrease with increased size (Gern *et al.*, 1978b; Randall, 1992) and to increase with increasing temperature (Falcon *et al.*, 1989). Juvenile rainbow trout of approximately 300-g maintained under either LD16:8 or LD8:16 with ambient temperatures of 12-15°C had night-time melatonin concentrations in the order of 500-600-pg ml^{-1} (Randall *et al.*, 1991a). The closest comparison may be made with Atlantic salmon of a similar age and size (potential S2) reared at the same smolt unit (at constant 10°C) entrained to an ambient photoperiod (latitude 52°N), which had nocturnal melatonin levels of 200-300-pg ml^{-1}. (Randall *et al.*, 1989; see Figure 3.42)

So although the fish in the present study showed a clear diel rhythm in melatonin levels

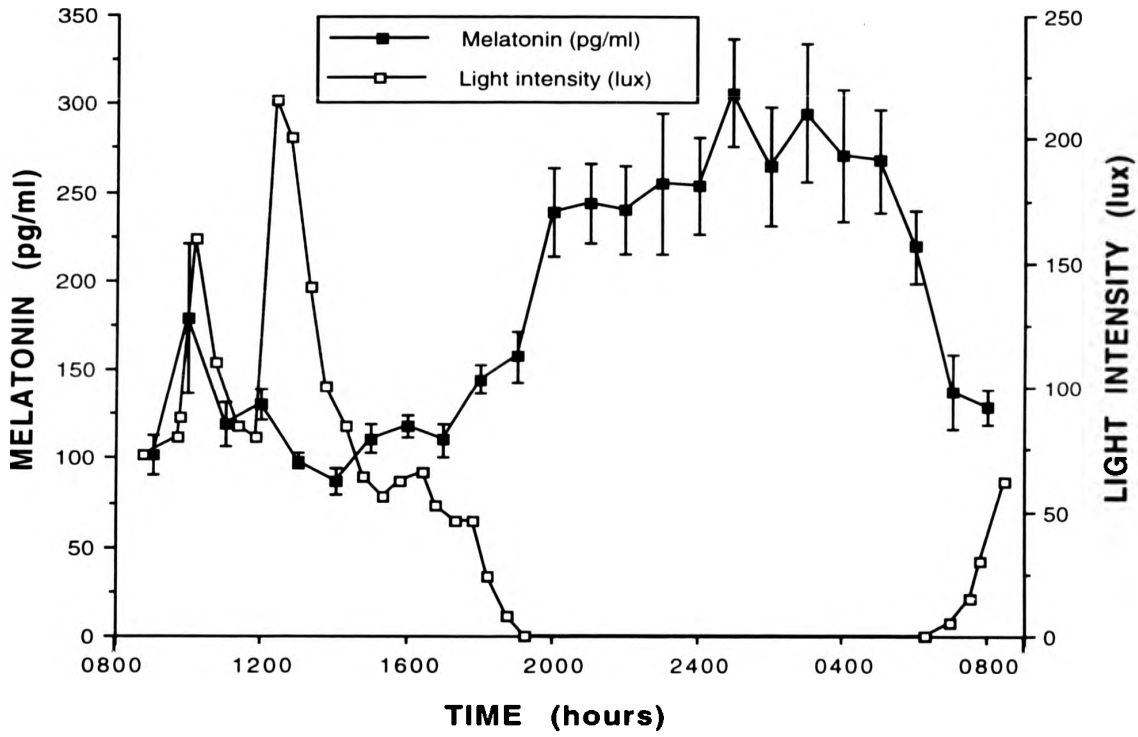


Figure 3.42

Diurnal changes in serum melatonin concentration in Atlantic salmon parr maintained under a natural daylength of approximately 12.5L:11.5D. Between 0900 and 1700 hours each point represents the mean (± 1SEM) of 4-5 pooled samples containing equal volumes of serum from 2 fish. Between 1800 and 0800 hours each point represents the mean (± 1SEM) of 8-10 individual fish (from Randall *et al.*, 1989).

and appeared to be entrained to the LR18:6 photocycle, the 4-lx low intensity night-time red illumination appears to have depressed the night time rise of this hormone. This is in agreement with the observation that Atlantic salmon post smolts accidentally exposed to a night-time illumination of 1-2-lx showed a nocturnal increase in melatonin only one-third of that of fish sampled in complete darkness (Randall and Bromage, unpublished) and other observations that increasing light intensities were increasingly effective at decreasing melatonin in dark acclimated trout (Zachmann *et al.*, 1992b; Alvariño *et al.*, 1993).

It is clear that smoltification can be entrained to a 5- or 6-month accelerated seasonal light-dark cycle (L:D group, Experiment 6; Experiment 2; Experiment 4). The absence of smoltification in September among the L:R fish suggests that night-time illumination either interferes with the smoltification process directly or that the timing mechanism(s) underlying the smoltifiction process was not entrained to the accelerating information provided by the changing high-intensity fraction of the photoperiod regime. The latter argument would question the involvement of melatonin rhythms in the entrainment of smoltification, as the nocturnal rise in circulating melatonin was not abolished. Alternatively, the melatonin rhythm may not have been of sufficient amplitude for the fish to perceive the daily change in photoperiod provided by the compressed photocycle.

It is possible that the endogenous component of the timing mechanism in these fish was free running in the absence of complete photic information. If this were the case, these fish would have been expected to complete smoltification close to the time when fish reared under an ambient photoperiod were transferred to seawater, ie between May and June. Unfortunately the experiment was terminated in January. However, Stefansson *et al.* (1991) reared pre-smolts under a dual light intensity regime simulating a natural (12-month) seasonal-photoperiod. These fish completed smoltification at the normal time and were transferred successfully to a marine environment.

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 early signs of post-smolt maturation, although maturation at the post-smolt episode is a

relatively uncommon occurrence among S1 smolts reared under ambient photoperiod conditions (Herbinger, 1987; Herbinger and Newkirk, 1990). S1 smolts transferred 2-months early in a commercial scale trial (Thrush *et al.*, 1994), also showed no post-smolt maturation. Grilse maturation (maturation after 1-sea winter) among these fish was 19%, this was slightly higher than sibling stocks reared at other cage sites which had been transferred to seawater at the normal time.

Potential S2 smolts transferred to the Hayling Marine Facility in November, January and early-March, which had been reared on compressed seasonal cycles in Experiment 2, were showing no signs of maturation at the end of April when GSIs were assessed at the end of the experiment. None of the smolts transferred to seawater in June, which had been reared on an extended photoperiod, matured over the following 7-months. Similar experiments where potential S2 smolts have been transferred to seawater between November and December have shown rates of maturation after 1-sea winter acceptable to the farming industry (Duncan and Bromage, unpublished). However, smolts from Experiment 2 transferred to the commercial cage site in west Wales in March showed a post-smolt maturation of 24%, and 7% of those transferred to the cage site in November matured after 1-seawinter. Inter-site differences in maturation were also reported by Thrush *et al.* (1994), where grilsing among potential S2 smolts transferred in early November to two sea farms was 0% and 12%. In both cases the sea site where post-smolts had shown greater initial growth reported the higher incidence of maturation in the first autumn, 1-year following transfer.

These results are in agreement with Thorpe's model of the development of life history patterns in Atlantic salmon (Thorpe, 1986). Whereby fish accumulating energy reserves above a genetically determined threshold during a 'decision sensitive period' for maturation mature at the next maturation episode. Post-smolts maintained on a restricted diets during late winter and early-spring, when this decision is thought to be made, showed reduced rates of grilse maturation (Thorpe, 1989). Therefore, smolts which gain a growth advantage from early seawater transfer and acclimation which coincides with the period of decision may be expected to show increased rates of maturation.

The problem of maturation could potentially be solved by taking advantage of the low maturing qualities of all-female and all-female triploid stocks in the production of out-of-season smolts. Experiment 4 provided encouraging results for their use. Potential S2 all-female and all-female triploid groups transferred to the cage site in Wales during November and December grew very well throughout the seawater phase of this trial. Although data was not collected for the second maturation episode following seawater transfer, none of the post-smolts among these groups showed signs of maturing after one winter at sea.

The development of length-frequency distributions among populations of Atlantic salmon prior to smoltification is well established (Thorpe, 1977; Thorpe and Morgan, 1978b; Bailey *et al.*, 1980; Thorpe *et al.*, 1980; 1982; Saunders *et al.*, 1982; Kristinsson *et al.*, 1985; Duston and Saunders, 1992). With detailed studies of individually-marked fish, Kristinsson *et al.* (1985) showed that fish which ultimately appeared in the upper-mode and smolted as S1s had shown an initial growth equivalent to that of lower mode fish, but subsequent to a growth surge exceeding that of the lower-mode (potential S2) fish became permanently segregated. Thorpe *et al.* (1980) found that under ambient photoperiod conditions, divergence between groups became evident in late summer or early autumn. Fish in the upper-mode have a higher motivation to feed (Metcalfe *et al.*, 1988) and sustain a higher relative food intake compared to lower mode fish (Higgins and Talbot, 1985) from July onwards. The initiation of smoltification is a cause or an effect of this increased growth and the mechanism which mediates it remains unclear. Elson (1957) suggested that parr attaining a critical length of 10-cm in the autumn would smolt the following spring. The decreasing phase of the photoperiod has been implicated as having an influence on the establishment of the two size modes (Villarreal *et al.*, 1988; Thorpe *et al.*, 1989; Saunders *et al.*, 1989; Duston and Saunders 1992). Thorpe (1986) postulated that each fish monitors it's growth performance through the rate of acquisition of surplus energy, possibly via a pathway of hormone kinetics associated with energy storage: so that if the rate of acquisition is above a genetically determined threshold during 'a sensitive' period for a decision to smolt; appetite remains high and the fish continue to feed and grow and complete smoltification the following spring. The 'sensitive period' for the decision to smolt was

suggested to be close to mid-summer.

Groups A-C in Experiment 3 were transferred directly from a short day (LD7.25:16.75) to a long day (LD16.75:7.25) on December 22, January 21 and February 20 respectively. In late January the length-frequency distributions of groups showed similar patterns of bimodality with approximately 20% of the population included in the upper-mode. These modal proportions remained stable for a further month and were not significantly different at the end of February. However at the end of March the proportion of fish in the upper-mode of Group C had increased from 22.9% (in February) to 37.0%. The final smolt fractions at seawater transfer for groups A-C were 23.0%, 28.3% and 30.7% respectively. This is in contradiction to Bailey *et al.*, (1980) and Thorpe *et al.*, (1980) who both state that once established the proportion of fish in each mode remained unchanged (ie that there was no further migration between modal populations). However, Saunders *et* al. (1989) and Skilbrei (1991) demonstrated that a long day photoperiod which is continued into the autumn or winter extends the period over which fish may be recruited into the upper-mode. Kristinsson *et al.* (1985) showed that the entrance to the faster growing phase continued from late August until January or possibly February, providing the water temperature remained above 10°C. Fish reared on an extended 18-month photoperiod showed a delay in the establishment of bimodality and contained almost twice as many potential smolts in the upper-mode to fish reared on a 12-month photoperiod (Duston and Saunders, 1992) It would appear that from the results provided here and the observations made in other studies, that the duration of the decision period proposed by Thorpe (1986), to be in the order of 1-month, commencing in mid-summer, is actually much longer and extends into the winter. The decision period is possibly terminated by the increasing photoperiod, which entrains the completion of smoltification, as suggested by Duston and Saunders (1992).

An extended decision period, terminated by an increasing daylength would explain the relatively similar S1 smolt fractions achieved by two smolt farms growing sibling stocks under different temperature regimes obsereved during this study. On one farm, supplied with constant temperature spring water (10°C), eggs develop more quickly and hatch earlier.

As a result, the fry are feeding and growing in advance of the sensitive period for maturation proposed by Thorpe (1986), although growth at this stage is not sufficient to allow (0+) maturation to proceed. Eggs over-wintered on an ambient temperature (below 10°C) regime develop, hatch and become first feeding fry later than those reared on constant temperature. As the water temperatures rise during the spring (above 10°C), growth rate among fish reared on an ambient temperature regime increases, so that by the onset of the summer sensitive period for smoltification these fish are larger and growing faster than those reared on constant temperature. However, the S1 smolt fraction and smolt size of the same genetic stocks reared under the two regimes were similar over a number of seasons. If the decision period was confined to mid-summer, the smolt fraction for populations reared on constant temperature should have been much smaller, as it would if the attainment of a critical length in the autumn was necessary for smoltification to proceed. If the decision period for smoltification was extended beyond this time, any fish subsequently achieving the threshold growth-rate or rate of accumulation of surplus energy would be able to proceed into the upper-mode. According to Kristinsson *et al* (1985) migration into the upper-mode among fish reared at 10°C will continue throughout the winter (see also Kazakov *et al.*, 1988; Nicieza *et al.*, 1991). The presence of inter-mode fish may delay the development of a clearly segregated groups during this period (Skilbrei, 1991). There is a delay between the initiation of the growth surge and the time at which it becomes evident, so assuming the increasing phase of the photoperiod terminates the option to smolt; final entrance to the upper mode will be seen a few weeks after a change in daylength has been perceived. In populations reared on ambient temperature, faster growth in summer should result in greater numbers of fish committing themselves to smolt earlier in the year, but the falling temperature in the autumn will prevent later recruitment to the upper-mode, making clearer and earlier modal segregations, which having become evident, would be unlikely to lead to significant changes in their relative proportions. This possibly explains observations made by Thorpe *et al.* (1980) and Bailey *et al.* (1980) that, once established, the modes do not change in proportion, as winter temperatures in both studies decreased well below 10°C. In Experiment 3 then, it would have been expected that groups B and C which were held on an

extended short day past the winter solstice for successively longer periods (ie until late-January and late-February respectively) compared to Group A (changed to a long day in late-December) at a 'high' winter growing temperature would have progressively larger upper-modes. Final smolt fractions for groups A-C were 23%, 28% and 38% respectively.

Chapter 4

Relationships between fecundity egg size, egg volume and fish weight in four stocks of farmed Atlantic salmon

4.1 INTRODUCTION

Aquaculture in general remains far behind other areas of livestock husbandry with respect to the realisation of biological potential. Relatively little attention has been paid to the role of selective breeding and, after a century of rearing, only a few strains of rainbow trout and carp may be considered as domesticated. Efforts in genetic selection centred on improving size or growth have been undertaken in a number of commercially important species including: chinook salmon, *Oncorhynchus tshawytscha* (Donaldson and Menasveta, 1961), rainbow trout, *Oncorhynchus mykiss* (Kincaid *et al.*, 1977; Bergot *et al.*, 1981), channel catfish, *Ictalurus punctatus* (Eknath and Doyle, 1985; Bondari, 1983; Dunham and Smitherman, 1983), common carp, *Cyprinus carpio* (Moav and Wohlfarth, 1976; Kirpichnikov and Faktorovich; 1969, 1972) and tilapia, *Oreochromis niloticus* (Bondari *et al.*, 1983; Teichert-Coddington, 1983). Selective breeding for disease resistance has been addressed (Wolf, 1953; Ehlinger, 1964; Kirpichnicov *et al.*, 1979). In addition, it is probable that autumn spawning in various strains of rainbow trout (compared to spring spawners) is a characteristic developed by selective breeding (Behnke, 1979 cited by Bromage and Cumaranatunga, 1988)

Stocks of Atlantic salmon currently maintained by the Scottish farming industry remain just a few generations from the wild and commercial interest in breeding programmes has been overshadowed by continuing efforts to optimise husbandry techniques, disease control and diet formulations. However, in Norway a considerable effort is being made to identify and improve commercially desirable traits within farmed stocks with the establishment of the Norwegian selection programme for Atlantic salmon (see Gjerde, 1993 for review). This programme, directed by the Breeding Council (set up jointly by the Norwegian Fish Farmers, Sales and Smolt Producers Associations), incorporates two breeding centres and a number of marine testing and marine multiplier stations. The breeding centres run parallel family and within-family selection programmes. About 350 families are reared each year in separate units from which samples of 150 tagged individuals are transferred to the marine testing stations. Traits monitored currently include survival and growth rate in fresh water,

early sexual maturity and body weight at harvest. In total, 150 males selected from the highest 10-15 ranking families; and 500 females from the highest 20-30 ranking families at each breeding centre are reared through to spawning. A nested mating design is employed whereby each male is used to fertilise eggs from 2-6 females. As it is impossible for only two breeding centres to supply the whole Norwegian industry with eggs, smolts are transferred from the breeding centres to a number of multiplier stations along the coast which are reared as broodstock and used to supply eggs for distribution to smolt producers.

The comprehensive data recording and hierarchical design of the programme makes transfer of 'genetic gain' to the industry is very effective. The system also allows for the estimation of other genetic parameters so that new traits can be easily included in the selection programme. Criteria for flesh quality traits for possible inclusion in the breeding programme are currently under review.

Ideally, all aspects of performance should be considered when assessing stock suitability or superiority for culture. In the future, hatchery managers will be under increased pressure to produce the maximum number of high quality eggs from available broodstocks. Subtle differences in the relationships between fecundity, egg size and fish size significantly affect the number of eggs which can be produced per fish or per tonne of broodstock (Springate and Bromage, 1984; Bromage *et al.*, 1992), which ultimately has profound implications for the efficiency of broodstock facilities, particularly as 5-year-old (12-20-kg) 3-sea winter broodstock salmon are very expensive to produce and maintain. Hence, the quantification of various aspects of reproductive performance among stocks already held by the industry should provide important additional criteria for future selection programmes.

In the natural environment, both salmonid and non-salmonid fish species have been shown to have considerable intra-specific differences in reproductive performance. Assessments of either fecundity or egg size revealing significant population or race differences have been described for Atlantic salmon, *Salmo salar* (Randall, 1989), brown trout, *Salmo trutta* (Nicholls, 1958; McFadden *et al.*, 1965), steelhead trout, , (Bulkley, 1967; DuBois *et al.*, 1989), chum salmon, *Oncorhynchus keta* (Rounsefell, 1957; Beacham,

1982), chinook salmon (Godfrey, 1968; Healey and Heard, 1984), coho salmon, *Oncorhynchus kisutch* (Zorbidi *et al.*, 1990; Flemming and Gross, 1990; Beacham, 1982), sockeye salmon, *Oncorhynchus nerka* (Rounesfell, 1957; Murray *et al.*, 1989; Manzer and Miki, 1986) and Arctic char, *Salvelinus alpinus* (Rounesfell, 1957). For example, Manzer and Miki (1986), in a study of 14 sockeye salmon stocks across British Columbia, showed an 18% difference in the range of fecundity; and Bulkley (1967) found differences of up to 51% in the fecundity of female steelhead trout from neighbouring Californian streams. Comparable studies made on other commercially valuable species have shown similar results for cod, *Gadus morhua* (May, 1967), herring, *Clupea harengus* (Kandler and Dutt, 1958) and plaice, *Pleuronectes platessa* (Bagenal, 1973).

Considering differences in the reproductive performance of cultured salmonid populations: results from a number of studies made over several years (Gall 1970, 1974, 1975; Gall and Gross 1978a, 1978b) showed several stocks of domesticated rainbow trout to have significant differences in reproductive performance, and it was suggested that selection of broodstock lines could be made on the basis of egg size. Bromage *et al.*, (1990) assessed fecundity, egg size and total egg volume differences in 12 stocks of farmed rainbow trout from a number of hatcheries around the U.K. They found that some stocks produced almost twice as many eggs as less fecund stocks, significant differences in egg size and inter-stock variations in total egg volume of 55%.

It would appear then that there are genetic differences in various aspects of reproductive performance among stocks of cultured rainbow trout. This chapter examines the relationships between total egg number (fecundity), egg size and total egg volume with fish weight data collected from 2- and 3-sea winter spawning females from four Atlantic salmon stocks currently maintained by a commercial broodstock unit in order to ascertain whether a similar reproductive variability exists which may be of value to future commercial selection programmes. As in a majority of the above cited works, analysis of covariance (ANCOVA) has been employed to partition the influence of fish size (and other variables which may exert a bias, through their effect on fish size, eg. continually improving husbandry techniques and different environmental temperatures between years) on the variates used to

assess reproductive performance. Post-strip fish weight was used to measure fish size as this is most commonly used by fish farms. Pre-strip (total) weight, however, was purposefully not used in order to avoid the autocorrelatory effect of egg weight in the various regressions.

4.2 MATERIALS AND METHODS

Four broodstock lines (Stocks A-D), of Scottish and Norwegian ancestry, originating from intakes, over 4 successive years, of eyed ova were maintained separately under normal production conditions. Fish maturing as grilse (after 1-sea winter) were removed from the populations. These fish are not normally stripped as their eggs are not commercially saleable. The grilse fraction was less than 5% for all of the stocks over the period of study. Data were collected from females maturing after 2- and 3-sea winters from individuals randomly selected throughout the course (October to December) of 5 successive spawning seasons. Section 2.11 includes a detailed account of broodstock handling and spawning techniques. Progeny from the spawning of 2-sea winter females in Stock A were also assessed when they matured at 2-sea winters in the next production cycle (ie Stock A-f1) Details of egg handling and the calculation of egg diameter, total fecundity, total egg volume and relative fecundity are provided in Section 2.11 (1-3).

Regression equations for total fecundity, egg size and total egg volume were calculated from the common (base 10) logarithms of all variates (see Section 2.16.2). Log-transformation served to improve regression coefficients of determination and reduced heterogeneity between residual variances. Assessment of goodness-of-fit of the regressions were provided by calculation of correlation coefficients (r), coefficients of determination (r^2) and F-test of regression slopes. The homogeneities of paired residual variances were compared by F-test (see Section 2.12.2.2). Statistical comparison of slope and elevation of regressions between different stocks and between individual stocks and common regressions were made using analysis of covariance (see Section 2.16.2). Confidence intervals (95%) were calculated from the standard error of $\hat{Y}$ (the estimated Y for given $\overline{X}$) for log fecundity, log egg diameter and log total egg volume of 2- and 3-sea winter stocks adjusted to a common log weight for each year class as described by Sokal and Rohlf (1981).

4.3 RESULTS

4.3.1 Total Fecundity

All four stocks showed total fecundity increases with increasing post-strip weight, in both 2-sea winter (Figure 4.1) and 3-sea winter spawning females (Figure 4.2). Coefficients of determination (the proportion of variation in log total fecundity which may be attributed to its linear regression on post-strip weight, r^2) ranged from 12.7% (Stock C) to 62.3% (Stock D); and 23.2% (Stock A) to 57.4% (Stock B) in 2- and 3-sea winter fish respectively. All regressions were highly significant ($P<0.001$), the details of these are shown in tables 4.1 and 4.2.

The r^2 value for all data (2 and 3-sea winter stocks combined) was 56.7% ($P<0.001$, Table 4.3). This was higher than for data pooled for either 2 or 3-sea winter stocks where r^2 was 44.3% ($P<0.001$) and 43.8% ($P<0.001$) respectively. Analysis of covariance showed the slope for combined 3-sea winter stocks to be significantly steeper than that for combined 2-sea winter stocks, (Table 4.4, Figure 4.3) that is to say a unit increase in log post-strip weight produced a significantly greater increase in log total fecundity among 3-sea winter stocks than 2-sea winter stocks. Individual comparisons of 2 and 3-sea winter females within the same stock revealed significant differences in the slopes of fecundity on weight regressions between year classes in stocks B and C (Table 4.4) with probabilities of $P<0.005$ and $P<0.001$ respectively (see Figure 4.4, b and c).

As the slopes for the different year classes of stocks A and D were not significantly different, the elevations were compared. Stock D showed no significant difference between elevations of fecundity on weight for the 2 and 3-sea winter year classes. Stock A however, showed a significant difference in elevation ($P<0.05$) between age groups such that, adjusted to a common log weight, 3-sea winter spawning females were 10% more fecund than 2-sea winter fish, producing 1000 more eggs.

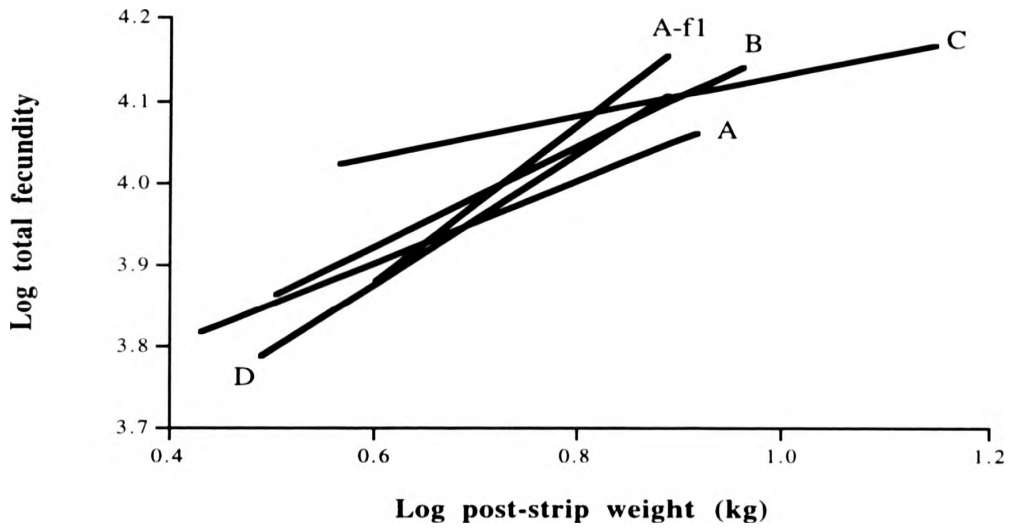


Figure 4.1

Regressions of log total fecundity on log post-strip weight for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of stock A).

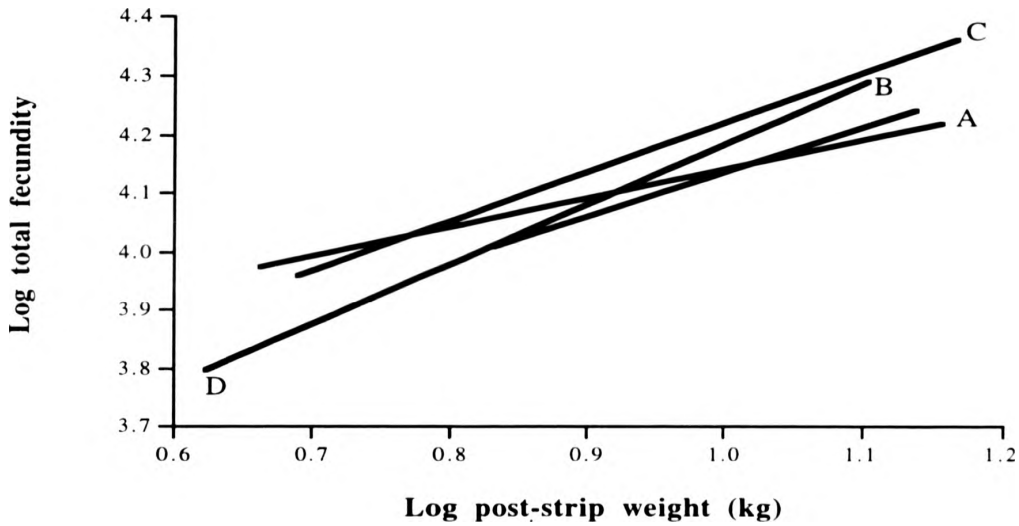


Figure 4.2

Regressions of log total fecundity on log post-strip weight for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
All 2-SW stocks	LTF = 3.55 + 0.607 LWt	0.666	44.3	0.0066	281.18	354	< 0.001
All stocks less A	LTF = 3.59 + 0.565 LWt	0.639	40.8	0.0062	189.46	275	< 0.001
All stocks less B	LTF = 3.55 + 0.608 LWt	0.677	45.8	0.0070	227.25	269	< 0.001
All stocks less C	LTF = 3.49 + 0.671 LWt	0.670	44.9	0.0060	191.83	235	< 0.001
All stocks less D	LTF = 3.58 + 0.570 LWt	0.635	40.3	0.0069	205.26	304	< 0.001
All stocks less A(f1)	LTF = 3.56 + 0.599 LWt	0.664	44.1	0.0068	261.15	331	< 0.001
A	LTF = 3.60 + 0.500 LWt	0.518	26.8	0.0071	28.18	77	< 0.001
B	LTF = 3.56 + 0.600 LWt	0.610	37.2	0.0058	49.2	83	< 0.001
C	LTF = 3.88 + 0.249 LWt	0.356	12.7	0.0053	16.99	117	< 0.001
D	LTF = 3.40 + 0.790 LWt	0.789	62.3	0.0043	79.36	48	< 0.001
A(f1)	LTF = 3.31 + 0.944 LWt	0.725	52.6	0.0048	23.35	21	< 0.001

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.1

Regression equations and analyses of log total fecundity (LTF) on log post-strip fish weight (LWt) for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of Stock A).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
All 3-SW stocks	LTF = 3.38 + 0.803 LWt	0.662	43.8	0.0081	235.63	302	< 0.001
All stocks less A	LTF = 3.31 + 0.883 LWt	0.703	49.4	0.0077	226.13	232	< 0.001
All stocks less B	LTF = 3.48 + 0.705 LWt	0.605	36.6	0.0076	139.78	242	< 0.001
All stocks less C	LTF = 3.44 + 0.713 LWt	0.622	38.7	0.0089	106.58	169	< 0.001
All stocks less D	LTF = 3.34 + 0.858 LWt	0.698	48.7	0.0076	248.27	261	< 0.001
A	LTF = 3.65 + 0.489 LWt	0.482	23.2	0.0080	20.54	68	< 0.001
B	LTF = 3.16 + 1.020 LWt	0.758	57.4	0.0089	78.10	58	< 0.001
C	LTF = 3.38 + 0.838 LWt	0.739	54.6	0.0047	157.34	131	< 0.001
D	LTF = 3.37 + 0.763 LWt	0.575	33.1	0.0081	19.31	39	< 0.001

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.2

Regression equations and analyses of log total fecundity (LTF) on log post-strip fish weight (LWt) for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

Stock(s)	Intercept + slope	r	r^2 (%)	RV	*F*	df	*P*
Total Data	LTF = 3.49 + 0.69 LWt	0.753	56.7	0.0074	860.8	1, 658	< 0.001
All 2-SW stocks	LTF = 0.61 + 3.55 LWt	0.666	44.3	0.0066	281.2	1, 354	< 0.001
All 3-SW stocks	LTF = 0.80 + 3.38 LWt	0.662	43.8	0.0081	235.6	1, 302	< 0.001

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.3

Regression equations and analyses of log total fecundity (LTF) on log post-strip fish weight (LWt) for all 2-sea winter stocks, all 3-sea winter stocks, and all data combined.

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All 2-SW vs All 3-SW	1.217	302, 354	< 0.05	9.897	1, 656	< 0.002	0.117	1, 657	-
A (2-SW vs 3-SW)	1.133	68, 77	ns	0.006	1, 145	ns	4.733	1, 146	< 0.05
B (2-SW vs 3-SW)	1.154	58, 83	< 0.05	8.957	1, 141	< 0.005	5.146	1, 142	-
C (2-SW vs 3-SW)	1.142	117, 131	ns	42.439	1, 248	< 0.001	15.247	1, 249	-
D (2-SW vs 3-SW)	1.889	39, 48	< 0.025	0.022	1, 87	ns	3.896	1, 88	ns

ns (not significant) $P > 0.05$

Table 4.4

Analyses of covariance of regressions of log total fecundity on log post-strip weight for different year classes (2- and 3-sea winter) for individual Atlantic salmon stocks (A-D) and all stocks combined.

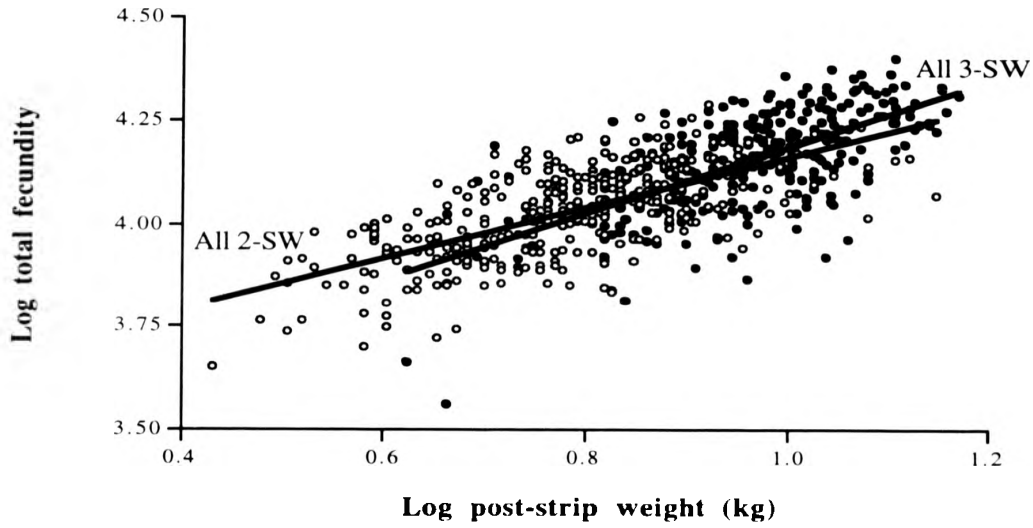
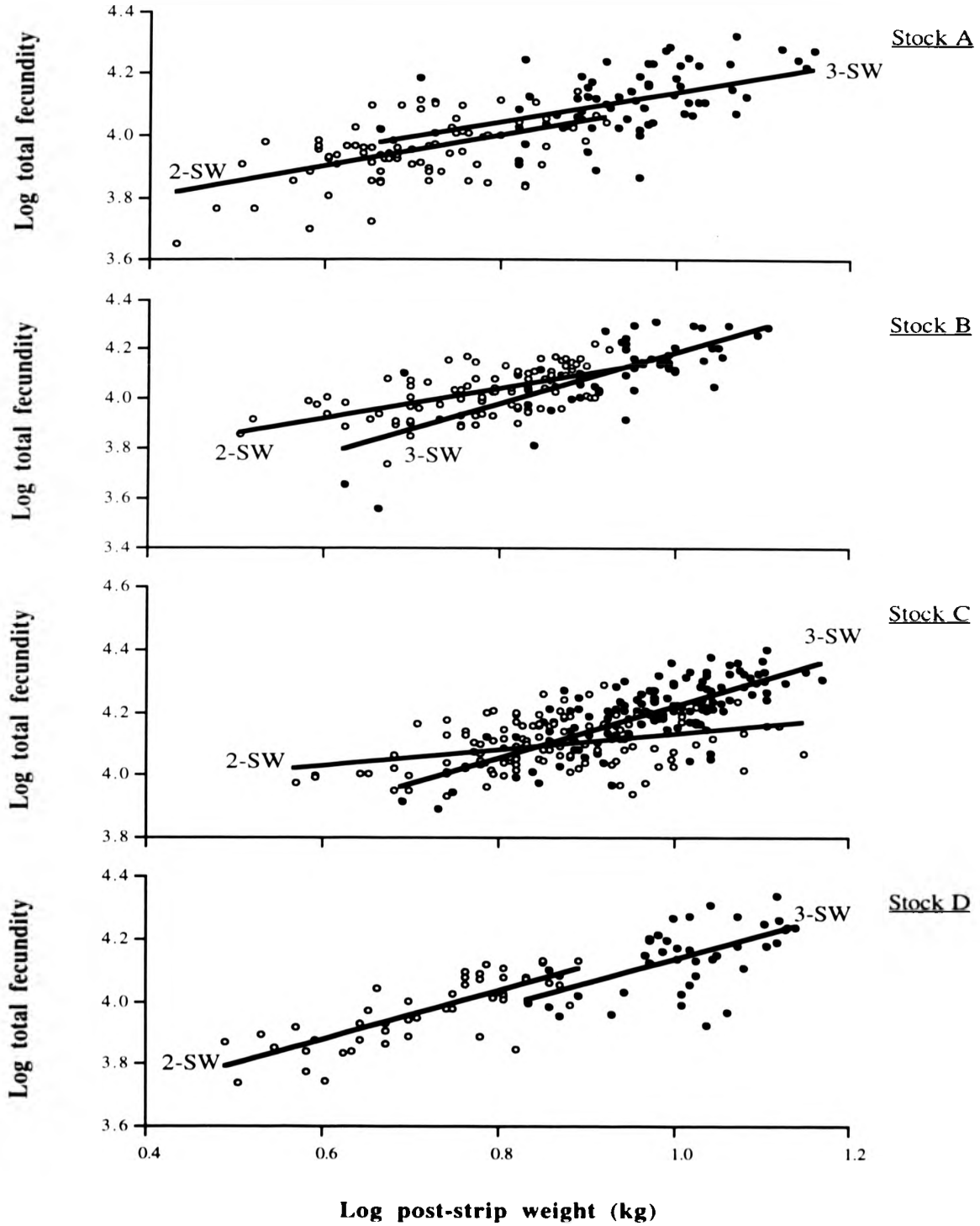


Figure 4.3

Regressions of log total fecundity on log post-strip weight for all 2-sea winter stocks combined (Stocks A-D and A-f1; open circles) and all 3-sea winter stocks combined (Stocks A-D; closed circles).

Figure 4.4

Regressions of log total fecundity on log post-strip weight; year class comparisons within stocks. (Stocks A-D; open circles represent 2-sea winter fish and closed circles represent 3-sea winter fish).


Stock A
Stock B
Stock C
Stock D
2-SW
3-SW
Log total fecundity
Log post-strip weight (kg)
4.4
4.2
4.0
3.8
3.6
3.4
4.6
0.4
0.6
0.8
1.0
1.2

Stock comparisons between 2-sea winter fish

Covariance analysis of the individual regressions of fecundity on weight among stocks of 2-sea winter fish revealed the slope of the regression for Stock C to be significantly different to all other stocks of the same age, (Table 4.5, Figure 4.1). This was confirmed by the comparison of Stock C with all other 2-sea winter stocks combined, which showed a significant difference between the slopes of the regressions ($P<0.001$). The slope of the fecundity on weight regression for Stock D was steeper than that of Stock A, but this difference was invalidated at $P<0.05$ as the variances were found to be heterogeneous by the F-test ($P<0.05$). Further covariance testing showed the elevation of the slope for Stock B to be significantly higher than that of Stock A ($P<0.01$). When adjusted to a common log of weight, Stock B produced 880 (9%) more eggs than Stock A (Figure 4.5). The regression of fecundity on weight for the f1 generation of Stock A at 2-sea winter age showed no significant differences in homogeneity, slope or elevation to their parents.

Stock comparisons between 3-sea winter fish

Covariance analysis of the individual 3-sea winter regressions identified Stock C to be significantly heterogeneous to each of the other groups of the same age. This was also seen in the comparison of Stock C with all other stocks combined ($P<0.001$, Table 4.6). The slope of the fecundity on weight regression for Stock C was steeper than that of Stock A, this result was considered significant at $P<0.005$. The slope of the fecundity on weight regression for Stock B was also significantly steeper than that of Stock A ($P<0.002$, Table 4.6), showing this stock to have a lower relative rate of increase in fecundity per unit increase in weight than either of stocks B or C (Figure 4.2).

Valid comparisons among the elevations of fecundity on weight regressions were made between groups that were found to be homogeneous and of common slope. Significant differences among 3-sea winter groups were revealed by the paired comparison of stocks. Analysis of covariance comparisons of the elevations of the regressions of fecundity on weight which had been not shown to have significantly different slopes revealed differences between stocks B and C ($P<0.001$), stocks B and D ($P<0.05$) and Stocks C and D

Table 4.5

Analyses of covariance of regressions of log total fecundity on log post-strip weight for different 2-sea winter stocks (Stocks A-D, and the f1 progeny of Stock A).

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All stocks less A vs A	1.142	77, 275	ns	0.440	1, 352	ns	16.391	1, 353	< 0.001
All stocks less B vs B	1.198	269, 83	ns	0.007	1, 352	ns	0.065	1, 353	ns
All stocks less C vs C	1.133	235, 117	ns	28.586	1, 352	< 0.001	23.853	1, 353	-
All stocks less D vs D	1.613	304, 48	< 0.025	3.585	1, 352	ns	3.461	1, 353	ns
All stocks - Af1 vs Af1	1.401	331, 21	ns	2.213	1, 352	ns	0.115	1, 353	ns
A vs B	1.218	77, 83	ns	0.620	1, 160	ns	7.347	1, 161	< 0.01
A vs C	1.393	77, 117	ns	5.379	1, 194	< 0.025	48.613	1, 195	-
A vs D	1.649	77, 48	< 0.05	4.548	1, 125	< 0.05	0.521	1, 126	-
A vs A (f1)	1.465	77, 21	ns	3.273	1, 98	ns	3.661	1, 99	ns
B vs C	1.092	83, 117	ns	11.432	1, 200	< 0.001	12.480	1, 201	-
B vs D	1.355	83, 48	ns	2.231	1, 131	ns	1.635	1, 132	ns
B vs A (f1)	1.203	83, 21	ns	2.310	1, 104	ns	0.089	1, 105	ns
C vs D	1.241	117, 48	ns	23.111	1, 165	< 0.001	25.088	1, 166	-
C vs A (f1)	1.102	117, 21	ns	10.713	1, 138	< 0.002	8.893	1, 137	-
D vs A (f1)	1.126	21, 48	ns	0.549	1, 69	ns	1.678	1, 70	ns

ns (not significant) $P > 0.05$

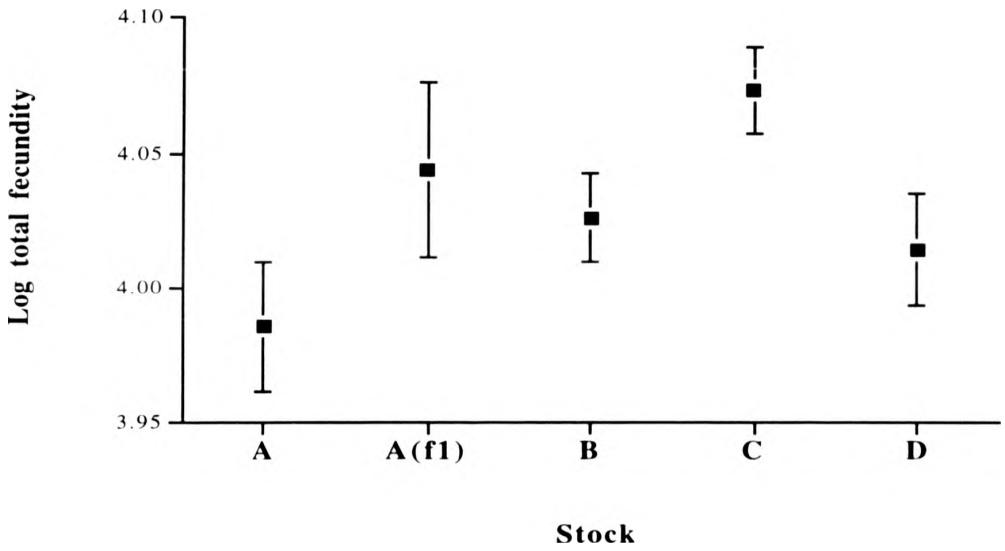


Figure 4.5

Log total fecundity of 2-sea winter Atlantic salmon broodstocks adjusted to a common (overall mean) log weight ± 95% confidence intervals (Stocks A-D, and the f1 progeny of stock A).

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All stocks less A vs A	1.046	68, 232	ns	10.566	1, 300	< 0.002	5.026	1, 301	-
All stocks less B vs B	1.173	58, 242	ns	6.340	1, 300	< 0.025	4.163	1, 301	-
All stocks less C vs C	1.902	169, 131	< 0.001	1.501	1, 300	ns	46.404	1, 301	< 0.001
All stocks less D vs D	1.066	39, 261	ns	0.288	1, 300	ns	18.596	1, 301	< 0.001
A vs B	1.115	58, 68	ns	11.287	1, 126	< 0.002	0.003	1, 127	-
A vs C	1.720	68, 131	< 0.005	8.756	1, 199	< 0.005	27.776	1, 200	-
A vs D	1.011	39, 68	ns	1.807	1, 107	ns	0.684	1, 108	ns
B vs C	1.918	58, 131	< 0.002	2.185	1, 189	ns	16.752	1, 190	< 0.001
B vs D	1.103	58, 39	ns	1.446	1, 97	ns	4.916	1, 98	< 0.05
C vs D	1.739	39, 131	< 0.025	0.226	1, 170	ns	46.613	1, 171	< 0.001

ns (not significant) $P > 0.05$

Table 4.6

Analyses of covariance of regressions of log total fecundity on log post-strip weight for different 3-sea winter stocks (Stocks A-D).

($P<0.001$, Table 4.6). Adjusted to a common log of weight, 3-sea winter females in Stock C produced 1500 (11%) more eggs than those in Stock B; which in turn were 9% more fecund, producing an additional 1100 eggs compared to females in Stock D (Figure 4.6).

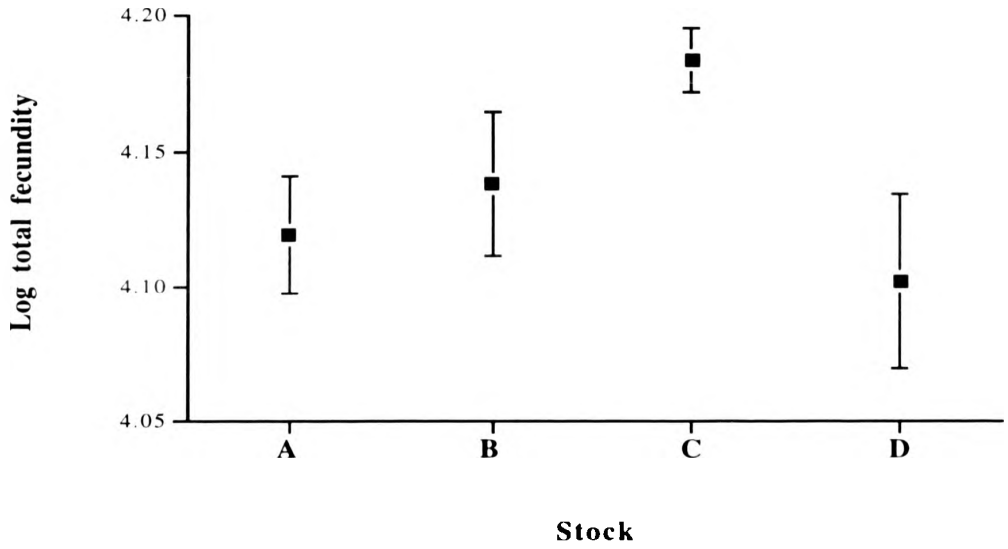


Figure 4.6

Log total fecundity of 3-sea winter Atlantic salmon broodstocks adjusted to a common (overall mean) log weight ± 95% confidence intervals (Stocks A-D).

4.3.2 Ova Diameter

Figures 4.7 and 4.8 show linear regression lines of best fit for log ova diameter on log post-strip weight for 2-sea winter and 3-sea winter females respectively. Figures 4.9 and 4.10 show the regression lines for combined year class and for year class comparisons within stocks. However, ova diameter was found to be poorly related to female post-strip weight. Tables 4.7 and 4.8 show details of individual regressions of the 2 and 3-seawinter stocks among the four stocks. In 2-sea winter fish, regression of ova diameter on post-strip weight yielded r^2 values of 15% or less. Stock D and Stock A-f1 showed no significant correlation (Table 4.7). The 3-sea winter ova diameter on weight regressions showed r^2 values of less than 7%. Only Stock B showed a significant correlation ($P<0.05$, Table 4.8). Stock D showed an inverse relationship between body weight and egg size (Figure 4.8). The regression of all data from the four stocks of the two ages combined provided a coefficient of determination of only 18.2% ($P<0.001$, Table 4.9) For regressions of pooled 2 and 3-sea winter data, r^2 values were 9.6% ($P<0.001$) and 2.8% ($P<0.005$) respectively. Adjusted to a common log mean weight, the differences between largest and smallest egg diameters for 2 and 3-sea winter stocks were only 260-µm (Figure 4.11) and 160-µm (Figure 4.12) respectively. However, there was a considerable degree individual varition within stock (Figure 4.10), with differences in egg diameter in excess of 20%. Due to the poor nature of the relationships between ova diameter and fish weight, the results obtained from covariance analyses are not discussed in further detail, but for completeness are presented Table 4.10 (comparisons between years), Table 4.11 and Table 4.12 (comparisons between 2- and 3-sea winter stocks respectively).

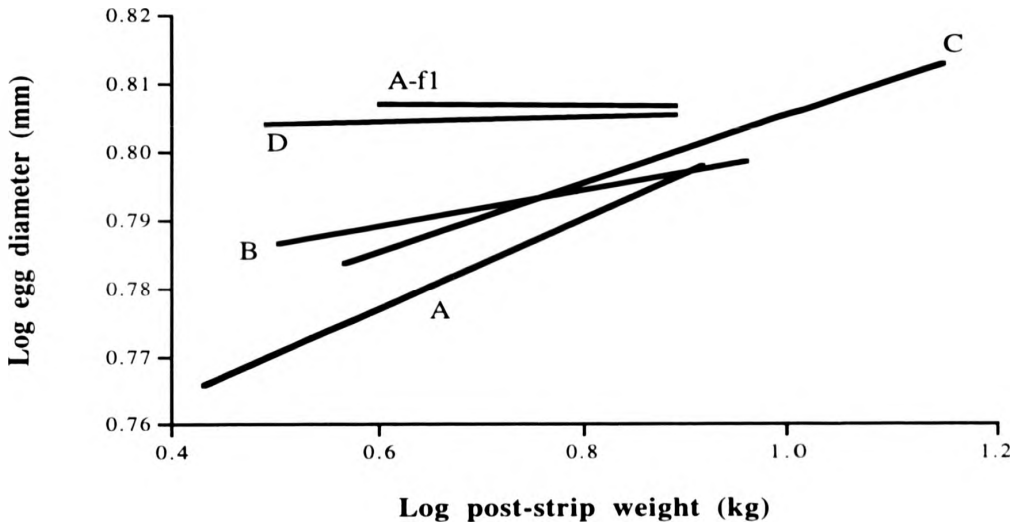


Figure 4.7

Regressions of log egg diameter on log post-strip weight for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of stock A).

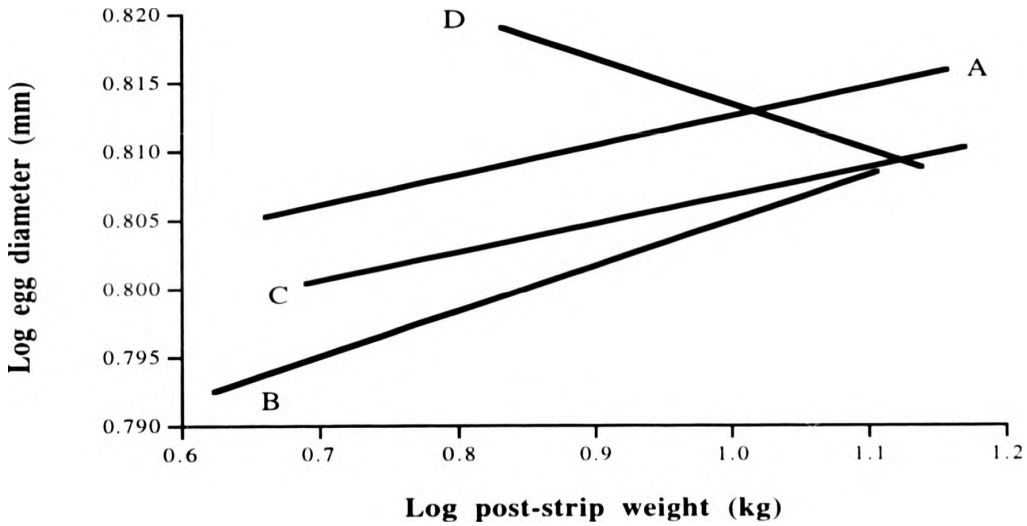


Figure 4.8

Regressions of log egg diameter on log post-strip weight for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

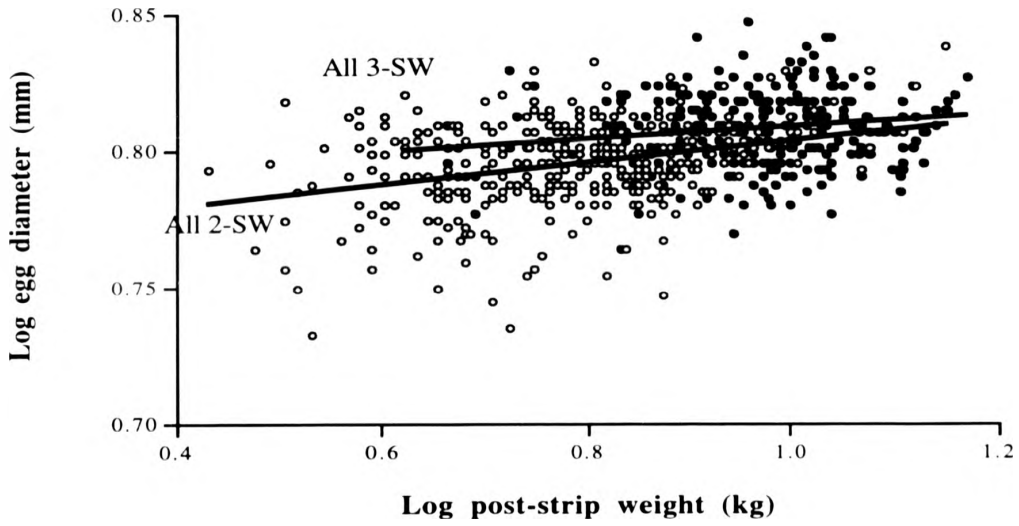
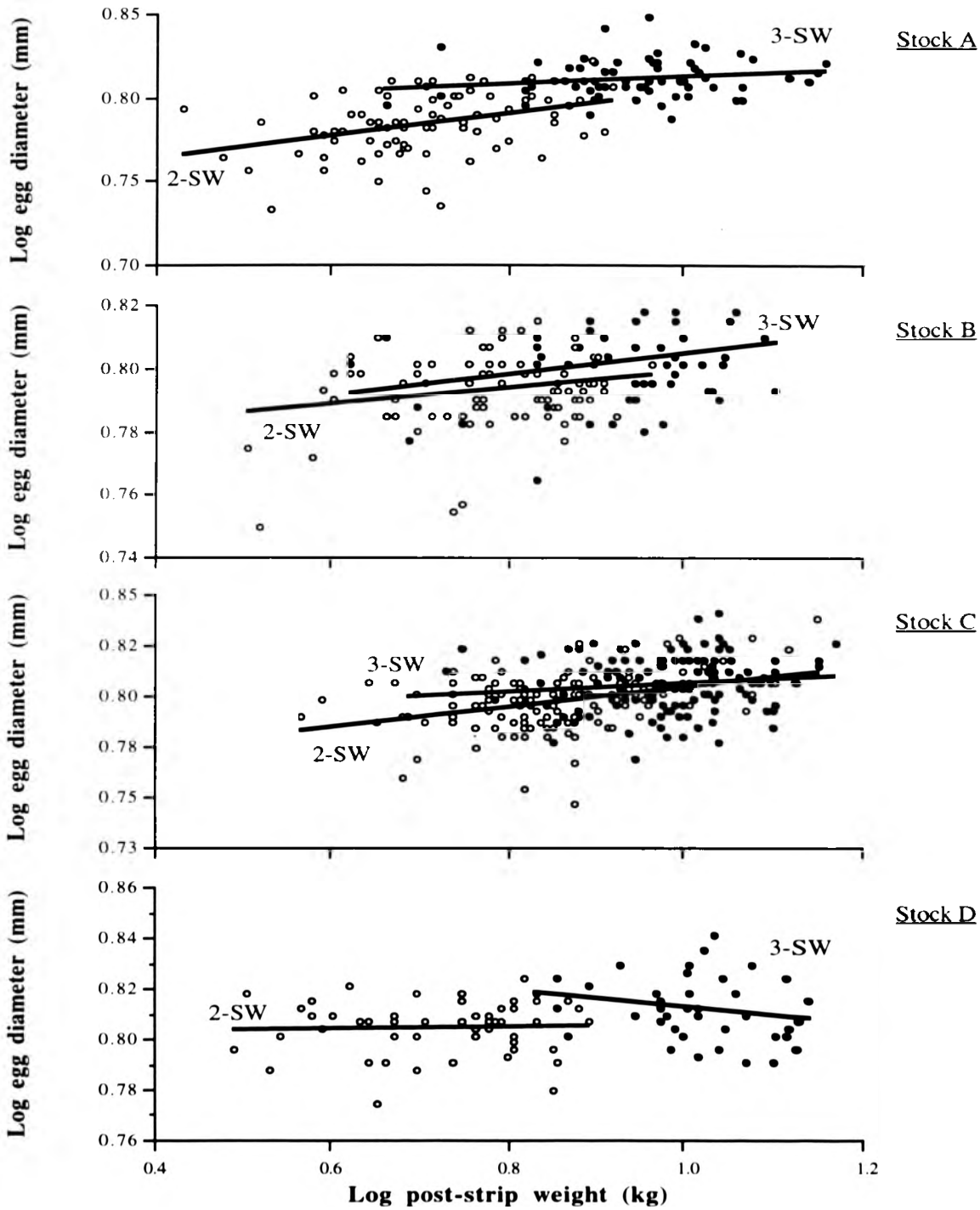


Figure 4.9

Regressions of log egg diameter on log post-strip weight for all 2-sea winter stocks combined (Stocks A-D and A-f1; open circles) and all 3-sea winter stocks combined (Stocks A-D; closed circles).

Figure 4.10

Regressions of log egg diameter on log post-strip weight; year class comparisons within stocks. (Stocks A-D; open circles represent 2-sea winter fish and closed circles represent 3-sea winter fish).


Stock A
Stock B
Stock C
Stock D
3-SW
2-SW
Log egg diameter (mm)
Log post-strip weight (kg)
0.85
0.80
0.75
0.70
0.82
0.78
0.76
0.74
0.73
0.86
0.84
0.4
0.6
0.8
1.0
1.2

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
All 2-SW stocks	LED = 0.763 + 0.041 LWt	0.310	9.6	0.00023	38.64	1363	< 0.001
All stocks less A	LED = 0.784 + 0.018 LWt	0.152	2.3	0.00018	6.78	284	< 0.025
All stocks less B	LED = 0.762 + 0.044 LWt	0.327	10.7	0.00026	32.93	274	< 0.001
All stocks less C	LED = 0.766 + 0.038 LWt	0.243	5.9	0.00025	15.07	240	< 0.001
All stocks less D	LED = 0.751 + 0.054 LWt	0.404	16.3	0.00021	60.56	312	< 0.001
All stocks less A (f1)	LED = 0.760 + 0.045 LWt	0.345	11.9	0.00022	45.97	340	< 0.001
A	LED = 0.737 + 0.066 LWt	0.374	14.0	0.00028	12.51	77	< 0.001
B	LED = 0.773 + 0.027 LWt	0.224	5.0	0.00013	4.57	87	< 0.05
C	LED = 0.755 + 0.050 LWt	0.387	15.0	0.00018	21.42	121	< 0.001
D	LED = 0.802 + 0.004 LWt	0.010	0.01	0.00011	0.09	49	ns
A (f1)	LED = 0.807 - 0.001 LWt	0.000	0.0	0.00016	0.00	21	ns

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.7

Regression equations and analyses of log egg diameter (LED) on log post-strip fish weight (LWt) for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of Stock A).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
All 3-SW stocks	LED = 0.786 + 0.023 LWt	0.167	2.8	0.000175	8.75	305	< 0.005
All stocks less A	LED = 0.780 + 0.027 LWt	0.192	3.7	0.000182	8.95	234	< 0.005
All stocks less B	LED = 0.796 + 0.013 LWt	0.089	0.8	0.000167	2.08	245	ns
All stocks less C	LED = 0.783 + 0.027 LWt	0.207	4.3	0.000175	7.57	170	< 0.01
All stocks less D	LED = 0.783 + 0.025 LWt	0.182	3.3	0.000172	9.03	264	< 0.005
A	LED = 0.791 + 0.021 LWt	0.187	3.5	0.000129	2.47	69	ns
B	LED = 0.772 + 0.033 LWt	0.255	6.5	0.000182	4.01	58	< 0.05
C	LED = 0.786 + 0.021 LWt	0.138	1.9	0.000173	2.61	133	ns
D	LED = 0.846 - 0.033 LWt	0.207	4.3	0.000162	1.76	39	ns

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.8

Regression equations and analyses of log egg diameter (LED) on log post-strip fish weight (LWt) for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
Total data	LED = 0.760 + 0.048 LWt	0.427	18.2	0.00021	149.08	670	< 0.001
All 2-SW stocks	LED = 0.763 + 0.041 LWt	0.310	9.6	0.00023	38.64	363	< 0.001
All 3-SW stocks	LED = 0.786 + 0.023 LWt	0.167	2.8	0.00017	8.75	305	< 0.005

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.9

Regression equations and analyses of log egg diameter (LED) on log post-strip fish weight (LWt) for all 2-sea winter stocks, all 3-sea winter stocks, and all data combined.

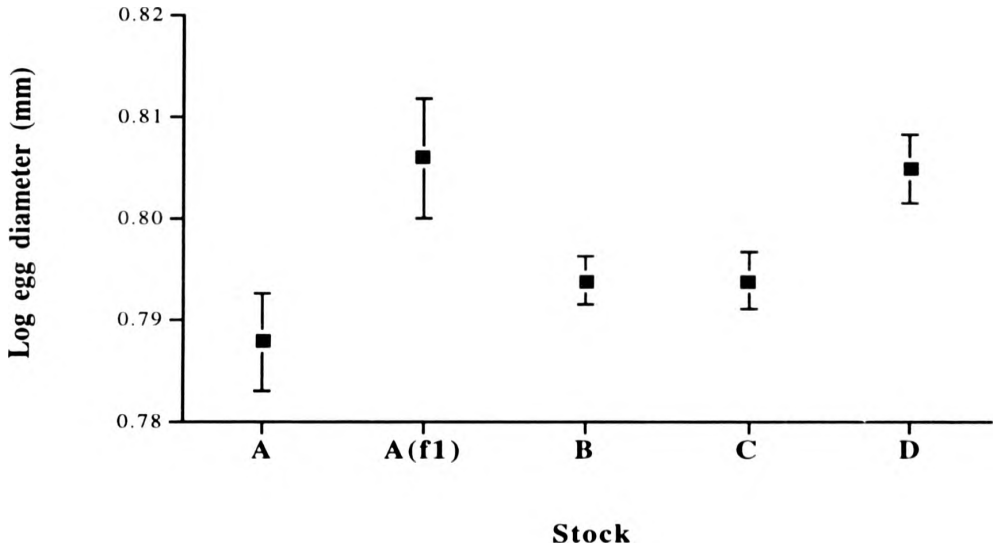


Figure 4.11

Log egg diameter of 2-sea winter Atlantic salmon broodstocks adjusted to a common (overall mean) log weight ± 95% confidence intervals (Stocks A-D, and the f1 progeny of stock A).

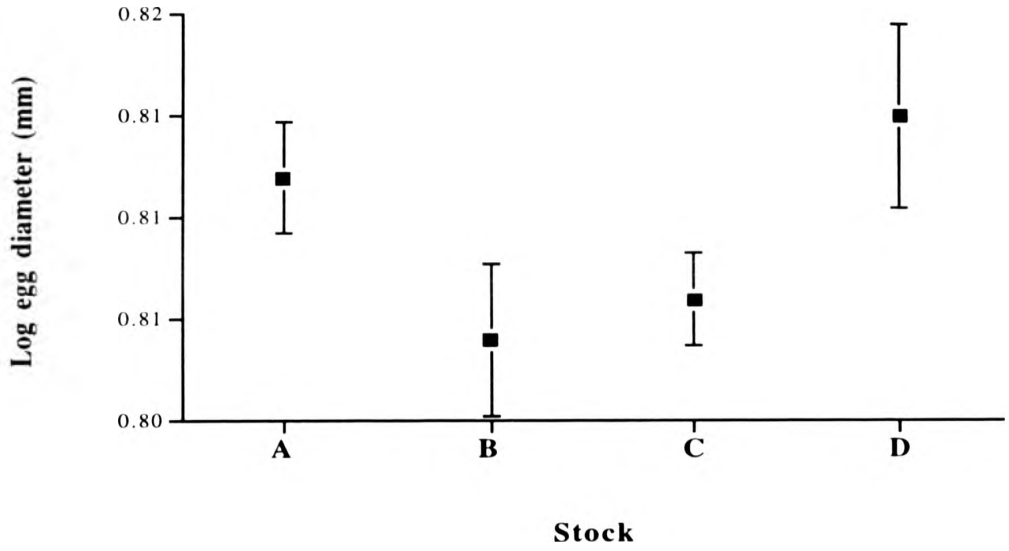


Figure 4.12

Log egg diameter of 3-sea winter Atlantic salmon broodstocks adjusted to a common (overall mean) log weight ± 95% confidence intervals (Stocks A-D).

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All 2-SW vs All 3-SW	1.290	363, 305	< 0.05	3.148	1, 668	ns	17.156	1, 669	< 0.001
A (2-SW vs 3-SW)	2.171	77, 69	< 0.001	3.577	1, 146	ns	20.981	1, 147	< 0.001
B (2-SW vs 3-SW)	1.424	58, 87	ns	0.101	1, 145	ns	3.231	1, 146	ns
C (2-SW vs 3-SW)	1.015	121, 133	ns	3.072	1, 254	ns	3.808	1, 255	ns
D (2-SW vs 3-SW)	1.442	39, 49	ns	1.764	1, 88	ns	5.491	1, 89	< 0.025

ns (not significant) $P > 0.05$

Table 4.10

Analyses of covariance of regressions of log egg diameter on log post-strip weight for different year classes (2- and 3-sea winter) for individual Atlantic salmon stocks (A-D) and all stocks combined.

Table 4.11

Analyses of covariance of regressions of log egg diameter on log post-strip weight for different 2-sea winter stocks (Stocks A-D, and the f1 progeny of Stock A).

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All stocks less A vs A	1.571	77, 284	< 0.005	7.682	1, 361	< 0.01	41.124	1, 362	-
All stocks less B vs B	2.005	274, 87	< 0.001	0.959	1, 361	ns	2.072	1, 362	ns
All stocks less C vs C	1.434	240, 121	< 0.025	0.591	1, 361	ns	0.202	1, 362	ns
All stocks less D vs D	1.903	312, 49	< 0.005	6.129	1, 361	< 0.025	41.448	1, 362	-
All stocks - Af1 vs Af1	1.327	340, 21	ns	1.175	1, 361	ns	20.859	1, 362	< 0.001
A vs B	2.189	77, 87	< 0.001	3.221	1, 164	ns	6.725	1, 165	< 0.025
A vs C	1.590	77, 121	< 0.025	0.621	1, 198	ns	5.115	1, 199	< 0.025
A vs D	2.484	77, 49	< 0.001	5.898	1, 126	< 0.025	56.413	1, 127	-
A vs A (f1)	1.709	77, 21	ns	1.905	1, 98	ns	30.682	1, 99	< 0.001
B vs C	1.377	121, 87	ns	1.916	1, 208	ns	0.670	1, 209	ns
B vs D	1.135	87, 49	ns	1.402	1, 136	ns	37.523	1, 137	< 0.001
B vs A (f1)	1.281	21, 87	ns	0.594	1, 108	ns	28.328	1, 109	< 0.001
C vs D	1.562	121, 49	< 0.05	5.475	1, 170	< 0.025	23.708	1, 171	-
C vs A (f1)	1.074	121, 21	ns	1.718	1, 142	ns	20.066	1, 143	< 0.001
D vs A (f1)	1.454	21, 49	ns	0.014	1, 70	ns	0.656	1, 71	ns

ns (not significant) $P > 0.05$

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All stocks less A vs A	1.417	234, 69	< 0.05	0.099	1, 303	ns	10.444	1, 304	< 0.002
All stocks less B vs B	1.090	58, 245	ns	1.236	1, 303	ns	10.090	1, 304	< 0.002
All stocks less C vs C	1.011	170, 133	ns	0.137	1, 303	ns	3.074	1, 304	ns
All stocks less D vs D	1.060	264, 39	ns	4.657	1, 303	< 0.05	5.006	1, 304	-
A vs B	1.413	58, 69	ns	0.295	1, 127	ns	16.922	1, 128	< 0.001
A vs C	1.346	133, 69	ns	0.001	1, 202	ns	9.478	1, 203	< 0.005
A vs D	1.260	39, 69	ns	4.011	1, 108	< 0.05	0.168	1, 109	-
B vs C	1.050	58, 133	ns	0.346	1, 191	ns	2.045	1, 192	ns
B vs D	1.121	58, 39	ns	4.736	1, 97	< 0.05	10.269	1, 98	-
C vs D	1.068	133, 39	ns	3.565	1, 172	ns	6.604	1, 173	< 0.025

ns (not significant) $P > 0.05$

Table 4.12

Analyses of covariance of regressions of log egg diameter on log post-strip weight for different 3-sea winter stocks (Stocks A-D).

4.3.3 Total Egg Volume

The most significant measure of egg productivity was the regression of total egg volume on post-strip weight. As with total fecundity, regression analysis showed volume to increase with increasing fish size for all stocks (Figures 4.13 and 4.14). The different year classes of each stock showed significant regressions of total egg volume on fish weight ($P<0.001$, tables 4.13 and 4.14). Coefficients of determination ranged, for 2-sea winter fish of stocks C and A(f1) respectively, from 36.2% to 77.0%; and for 3-sea winter fish of stocks A and C respectively, from 31.1% and 72.9%. Values of r^2 for combined 2-sea winter and 3-sea winter groups were 58.7% ($P<0.001$) and 55.7% ($P<0.001$) respectively. The coefficient of determination for the regression of total egg volume on post-strip weight for all data combined was 71.2% (Table 4.15).

Analysis of covariance of the regressions for combined 2 and 3-sea winter data revealed significant differences in slope ($P<0.025$, Table 4.16) with 3-sea winter fish showing a significantly faster increase in total egg volume for a unit increase in post-strip weight than 2-sea winter fish (Figure 4.15). Comparisons of the slopes of the regressions between year classes within individual stocks showed that only stocks B and C were different (Table 4.16). Stock A showed a significant difference ($P<0.001$) in elevation for the regression of total egg volume on fish weight between year classes, such that, adjusted to a common log of weight, 3-sea winter females yielded a greater volume of eggs than 2-sea winter females. (Table 4.16, Figure 4.16)

Stock comparisons between 2-sea winter fish

Analyses of covariance of total egg volume on post-strip weight regressions among the 2-sea winter stocks showed the rate of increase in volume per unit increase in weight for females in Stocks C to be significantly less than females in each of the other groups. No significant differences were found between the slopes of the regressions of total egg volume on post-strip weight among any of the other stocks (Table 4.17).

Further covariance tests found that, among the groups of common slope, there were a

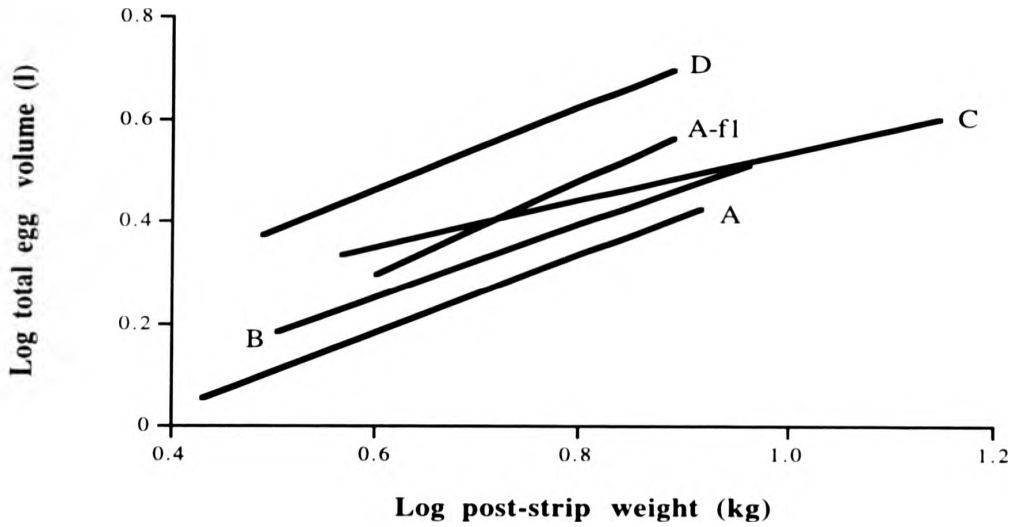


Figure 4.13

Regressions of log total egg volume on log post-strip weight for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of stock A).

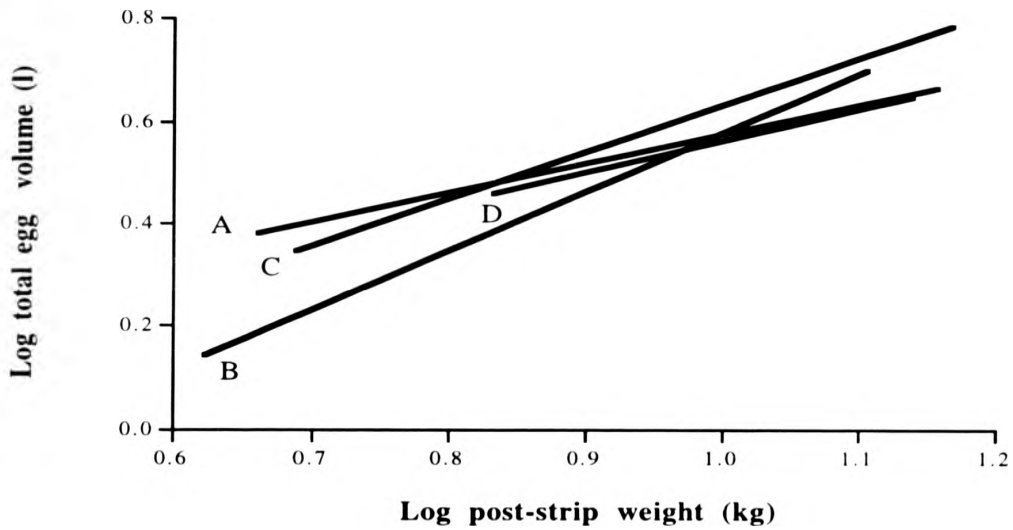


Figure 4.14

Regressions of log total egg volume on log post-strip weight for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
All 2-SW stocks	LTV = -0.219 + 0.774 LWt	0.767	58.8	0.0060	507.26	356	< 0.001
All stocks less A	LTV = -0.092 + 0.638 LWt	0.731	53.5	0.0047	318.97	277	< 0.001
All stocks less B	LTV = -0.227 + 0.787 LWt	0.785	61.6	0.0061	433.90	271	< 0.001
All stocks less C	LTV = -0.268 + 0.825 LWt	0.738	54.4	0.0062	283.16	237	< 0.001
All stocks less D	LTV = -0.235 + 0.788 LWt	0.764	58.3	0.0063	428.52	306	< 0.001
All stocks less A (f1)	LTV = -0.231 + 0.783 LWt	0.778	60.6	0.0059	508.25	331	< 0.001
A	LTV = -0.275 + 0.762 LWt	0.758	57.5	0.0045	104.07	77	< 0.001
B	LTV = -0.175 + 0.709 LWt	0.677	45.9	0.0057	70.54	83	< 0.001
C	LTV = +0.066 + 0.466 LWt	0.650	42.2	0.0037	85.29	117	< 0.001
D	LTV = -0.214 + 0.802 LWt	0.834	69.5	0.0032	109.59	48	< 0.001
A (f1)	LTV = -0.260 + 0.920 LWt	0.840	70.6	0.0020	55.16	23	< 0.001

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.13

Regression equations and analyses of log total egg volume (LTV) on log post-strip fish weight (LWt) for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of Stock A).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
All 3-SW stocks	LTV = -0.300 + 0.895 LWt	0.756	57.2	0.0058	404.37	302	< 0.001
All stocks less A	LTV = -0.393 + 0.992 LWt	0.803	64.5	0.0052	421.36	232	< 0.001
All stocks less B	LTV = -0.152 + 0.753 LWt	0.713	50.9	0.0048	250.68	242	< 0.001
All stocks less C	LTV = -0.255 + 0.822 LWt	0.711	50.5	0.0073	172.75	169	< 0.001
All stocks less D	LTV = -0.354 + 0.957 LWt	0.784	61.4	0.0057	414.34	261	< 0.001
A	LTV = -0.000 + 0.573 LWt	0.571	32.6	0.0069	32.85	68	< 0.001
B	LTV = -0.576 + 1.150 LWt	0.837	70.0	0.0066	135.07	58	< 0.001
C	LTV = -0.292 + 0.919 LWt	0.863	74.5	0.0023	383.25	131	< 0.001
D	LTV = -0.066 + 0.627 LWt	0.593	35.2	0.0050	21.18	39	< 0.001

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.14

Regression equations and analyses of log total egg volume (LTV) on log post-strip fish weight (LWt) for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

Stock(s)	Intercept + Slope	r	r^2 (%)	RV	*F*	df	*P*
Total Data	LTV = -0.293 + 0.879 LWt	0.851	72.4	0.0060	1729.2	660	< 0.001
All 2-SW stocks	LTV = -0.219 + 0.774 LWt	0.767	58.8	0.0060	507.26	356	< 0.001
All 3-SW stocks	LTV = -0.300 + 0.895 LWt	0.756	57.2	0.0058	404.37	302	< 0.001

r = correlation coefficient r^2 = coefficient of determination RV = residual variance (for homogeneity tests)

Table 4.15

Regression equations and analyses of log total egg volume (LTV) on log post-strip fish weight (LWt) for all 2-sea winter stocks, all 3-sea winter stocks, and all data combined.

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All 2-SW vs All 3-SW	1.040	356, 302	ns	12.103	1, 658	< 0.001	20.052	1, 659	-
A (2-SW vs 3-SW)	1.414	68, 77	ns	0.311	1, 145	ns	69.558	1, 146	< 0.001
B (2-SW vs 3-SW)	1.167	58, 83	ns	11.742	1, 141	< 0.001	1.908	1, 142	-
C (2-SW vs 3-SW)	1.616	117, 131	< 0.005	41.972	1, 248	< 0.001	47.585	1, 249	-
D (2-SW vs 3-SW)	1.559	39, 48	ns	1.379	1, 87	ns	0.270	1, 88	ns

ns (not significant) $P > 0.05$

Table 4.16

Analyses of covariance of regressions of log total egg volume on log post-strip weight for different year classes (2- and 3-sea winter) for individual Atlantic salmon stocks (A-D) and all stocks combined.

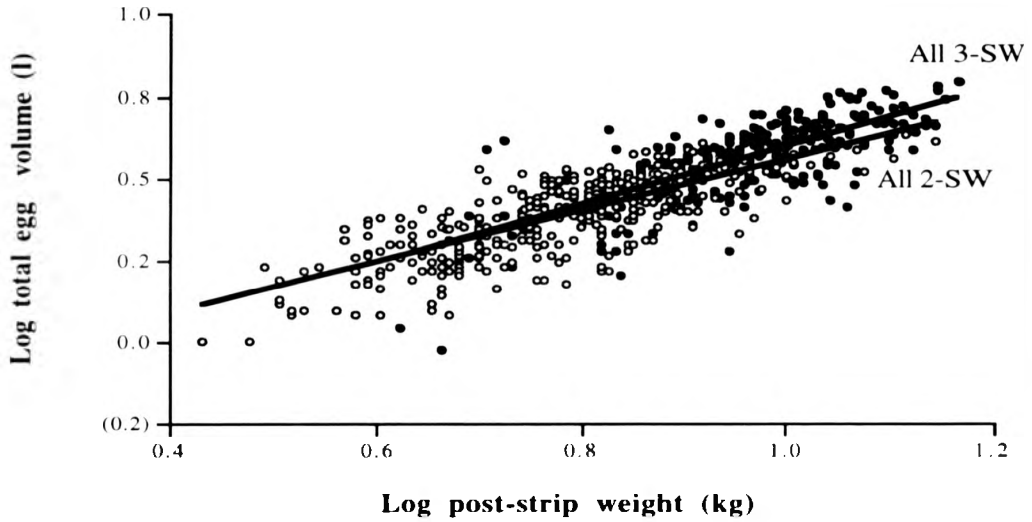


Figure 4.15

Regressions of log total egg volume on log post-strip weight for all 2-sea winter stocks combined (Stocks A-D and A-f1; open circles) and all 3-sea winter stocks combined (Stocks A-D; closed circles).

Figure 4.16

Regressions of log total egg volume on log post-strip weight; year class comparisons within stocks. (Stocks A-D; open circles represent 2-sea winter fish and closed circles represent 3-sea winter fish).

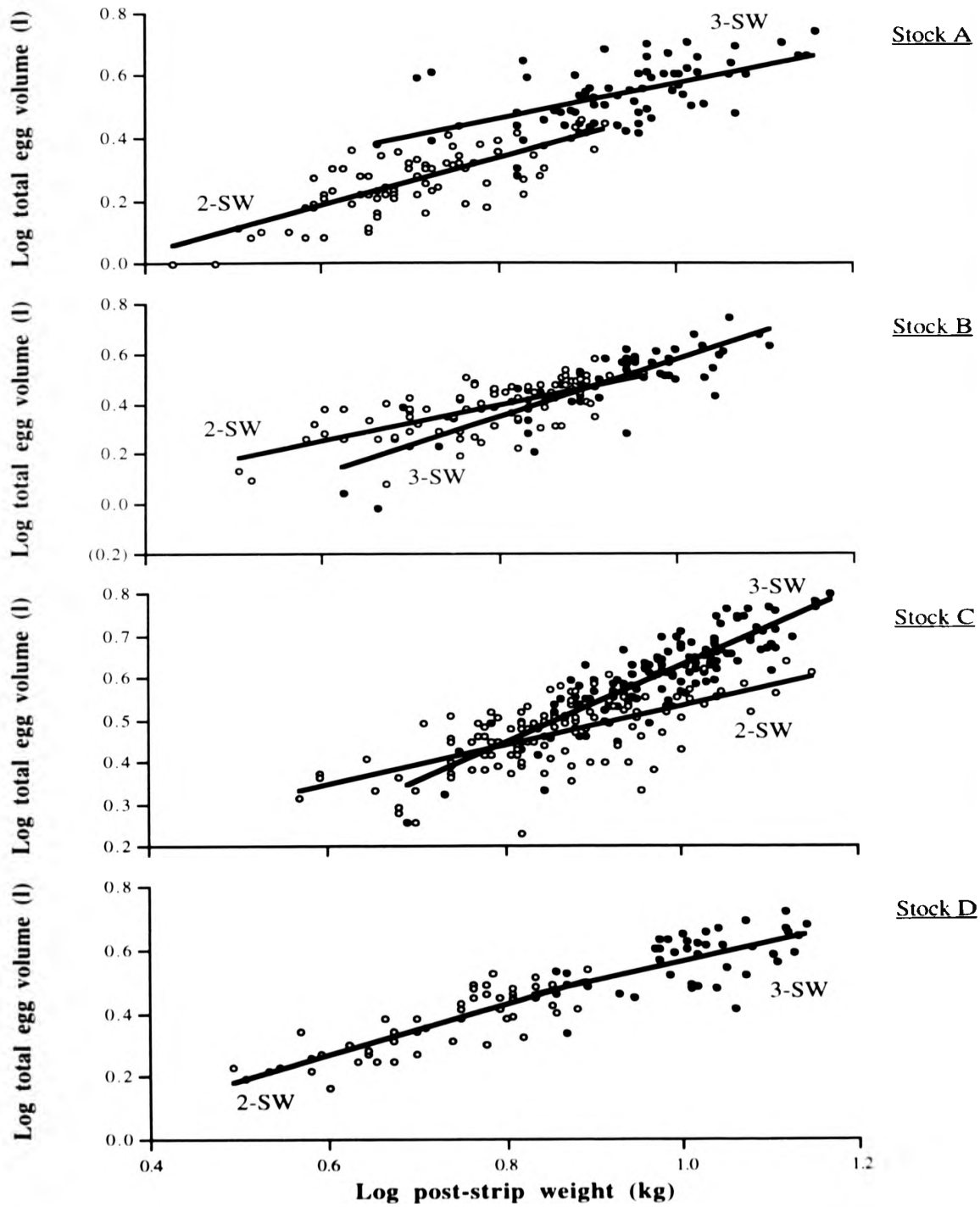

Stock A
Stock B
Stock C
Stock D
3-SW
2-SW
Log total egg volume (l)
Log post-strip weight (kg)
0.8
0.6
0.4
0.2
0.0
(0.2)
0.7
0.5
0.3
0.4
0.6
0.8
1.0
1.2

Table 4.17

Analyses of covariance of regressions of log total egg volume on log post-strip weight for different 2-sea winter stocks (Stocks A-D, and the f1 progeny of Stock A).

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All stocks less A vs A	1.037	77, 277	ns	3.820	1, 354	ns	99.393	1, 355	< 0.001
All stocks less B vs B	1.099	271, 83	ns	0.002	1, 354	ns	1.207	1, 355	ns
All stocks less C vs C	1.735	237, 117	< 0.001	9.769	1, 354	< 0.002	27.005	1, 355	-
All stocks less D vs D	2.024	306, 48	< 0.002	0.774	1, 354	ns	4.117	1, 355	< 0.05
All stocks - Af1 vs Af1	2.988	331, 23	< 0.002	0.989	1, 354	ns	18.495	1, 355	< 0.001
A vs B	1.165	83, 77	ns	3.969	1, 160	< 0.05	30.589	1, 161	-
A vs C	1.312	77, 117	ns	0.381	1, 194	ns	127.357	1, 195	< 0.001
A vs D	1.519	77, 48	ns	8.192	1, 125	< 0.005	52.939	1, 126	-
A vs A (f1)	2.398	77, 23	< 0.025	4.839	1, 100	< 0.05	77.414	1, 101	-
B vs C	1.528	83, 117	< 0.025	6.721	1, 200	< 0.025	19.727	1, 201	-
B vs D	1.770	83, 48	< 0.025	0.587	1, 131	ns	5.917	1, 132	< 0.025
B vs A (f1)	2.793	83, 23	< 0.005	1.026	1, 106	ns	20.118	1, 107	< 0.001
C vs D	1.158	117, 48	ns	12.584	1, 165	< 0.001	4.481	1, 166	-
C vs A (f1)	1.828	117, 23	< 0.05	7.262	1, 140	< 0.01	0.937	1, 141	-
D vs A (f1)	1.578	48, 23	ns	0.517	1, 71	ns	9.727	1, 72	< 0.005

ns (not significant) $P > 0.05$

number of significant differences in elevation (Table 4.17). Adjusted to a common log weight, stocks B and A(f1) produced total egg volumes 15% and 23% greater than Stock A respectively (Figure 4.17). Stock D produced a total egg volume 18% greater than Stock B (P<0.005) and 35% (690-ml) greater than Stock A

Stock comparisons between 3-sea winter fish

Analysis of covariance between total egg volume on weight regressions for the 3-sea winter stocks identified Stock C to be significantly heterogeneous to all other stocks combined (P<0.001, Table 4.18). Individual paired tests between stocks showed Stock C to be heterogeneous with each of the other 3-sea winter stocks.

The rate of increase in total egg volume per unit increase in weight was found to be greater in Stock C than in Stock A (P<0.001). In addition, the slope of total egg volume for on post-strip weight for Stock B was significantly steeper than those for either Stock A (P<0.001) or Stock D (P<0.025, Table 4.18). Adjusted to a common log weight, females in Stock C produced a total egg volume which was 530-ml (15%) greater than those in Stock D (Figure 4.18).

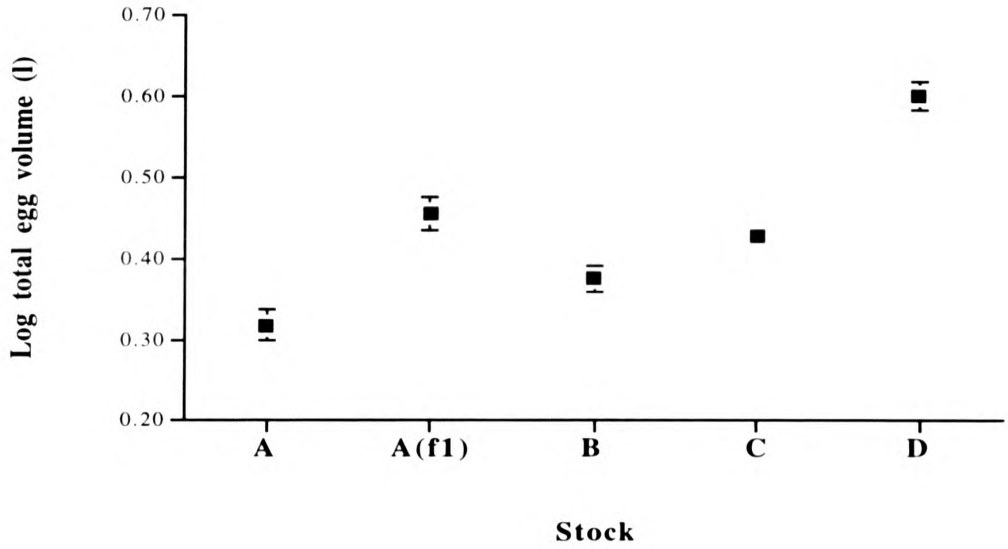


Figure 4.17

Log total egg volume of 2-sea winter Atlantic salmon broodstocks adjusted to a common (overall mean) log weight ± 95% confidence intervals (Stocks A-D, and the f1 progeny of stock A).

Stocks	Homogeneity			Slope			Elevation		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All stocks less A vs A	1.324	68, 232	ns	16.628	1, 300	< 0.001	0.116	1, 301	-
All stocks less B vs B	1.368	58, 242	ns	15.706	1, 300	< 0.001	24.019	1, 301	-
All stocks less C vs C	3.162	169, 131	< 0.001	1.239	1, 300	ns	45.726	1, 301	< 0.001
All stocks less D vs D	1.135	261, 39	ns	4.741	1, 300	< 0.05	11.681	1, 301	-
A vs B	1.040	68, 58	ns	16.881	1, 126	< 0.001	6.345	1, 127	-
A vs C	2.997	68, 131	< 0.001	12.874	1, 199	< 0.001	15.624	1, 200	-
A vs D	1.377	68, 39	ns	0.094	1, 107	ns	0.051	1, 108	ns
B vs C	2.882	58, 131	< 0.001	6.104	1, 189	< 0.025	43.685	1, 190	-
B vs D	1.325	58, 39	ns	8.840	1, 97	< 0.005	0.103	1, 98	-
C vs D	2.176	39, 131	< 0.001	6.226	1, 170	< 0.025	44.616	1, 171	-

ns (not significant) $P > 0.05$

Table 4.18

Analyses of covariance of regressions of log total egg volume on log post-strip weight for different 3-sea winter stocks (Stocks A-D).

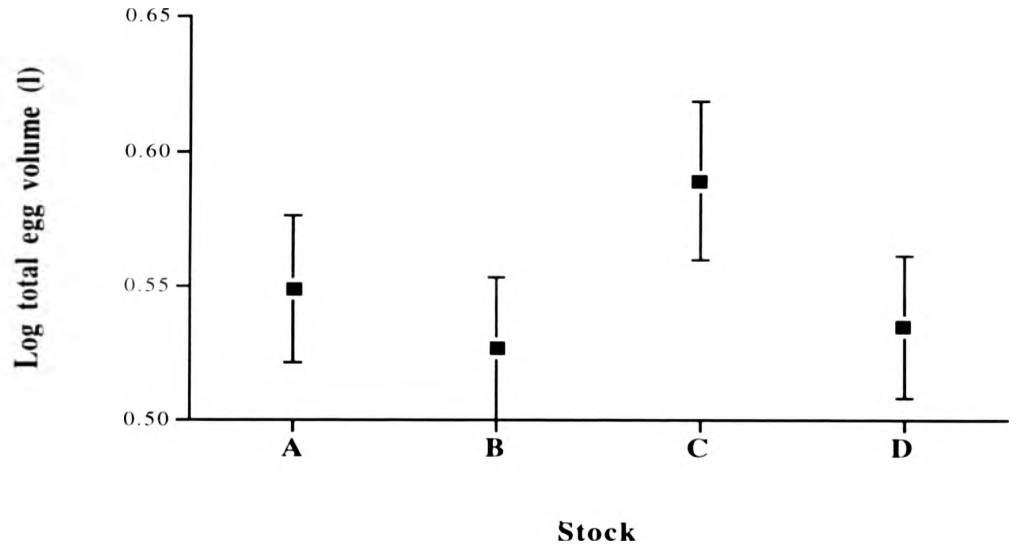


Figure 4.18

Log total egg volume of 3-sea winter Atlantic salmon broodstocks adjusted to a common (overall mean) log weight ± 95% confidence intervals (Stocks A-D).

4.3.4 Relative fecundity

Figure 4.19 and Figure 4.20 show the regressions of log relative fecundity on log post-strip weight (ie the number of eggs per kg of post-stripped body weight) for 2- and 3-sea winter stocks respectively. All stocks showed an inverse relationship between relative fecundity and post-strip weight, such that with increasing size, female Atlantic salmon produced fewer eggs per unit weight. Relative fecundities are frequently used as a measure of stock productivity by rainbow trout farmers. However, regression of eggs per kg on weight (kg) is subject to autocorrelation and slopes produced by regression analysis are only the inverse of those obtained by regressing fecundity on weight directly. For this reason the relationships between relative fecundity and fish weight in the salmon stocks was not subjected to further detailed analysis.

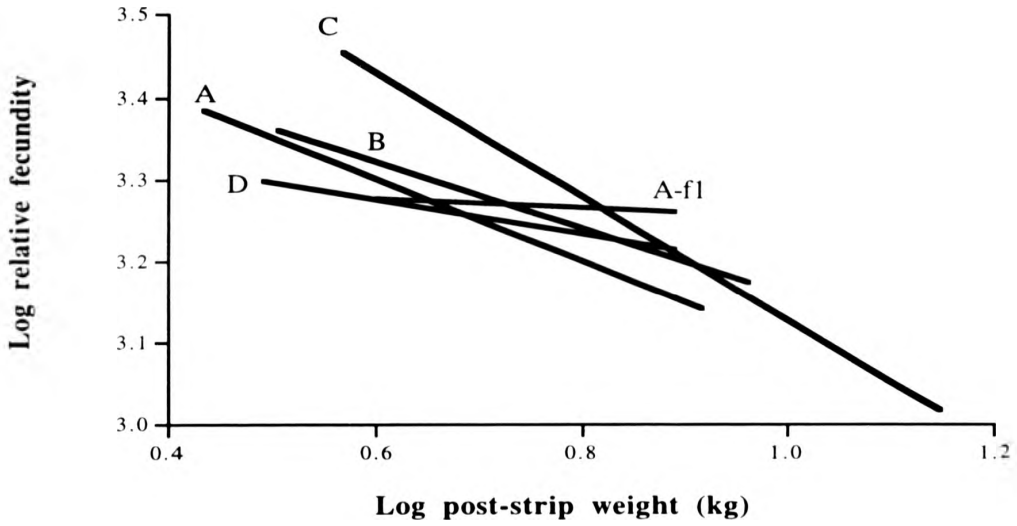


Figure 4.19

Regressions of log relative fecundity on log post-strip weight for 4 stocks of 2-sea winter Atlantic salmon (Stocks A-D, and the f1 progeny of stock A).

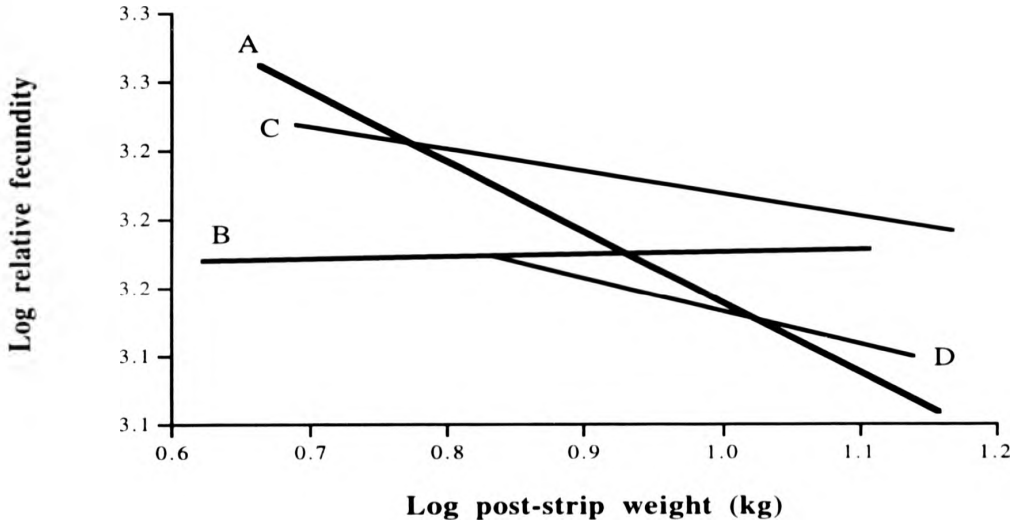


Figure 4.20

Regressions of log relative fecundity on log post-strip weight for 4 stocks of 3-sea winter Atlantic salmon (Stocks A-D).

4.4 DISCUSSION.

The results clearly show fecundity to increase linearly with post-strip weight among the four stocks of farmed Atlantic salmon investigated. Fecundity has been repeatedly shown to increase with fish size among salmonids (Rounesfell, 1957; Pope *et al.*, 1961; Nomura, 1963; McFadden *et al.*, 1965; Bagenal, 1969; Gall and Gross, 1978a,b; Boreman, 1981; Springate and Bromage, 1984; Manzer and Miki, 1986; Bromage and Cumaranatunga, 1988). Within populations, coefficients of determination in excess of 65% have been reported for the linear regression of fecundity on (various expressions of) length (Nicolls, 1958; Beacham, 1982; Healey and Heard, 1984; Thorpe *et al.*, 1984; DuBois *et al.*, 1989; Randall, 1989; Iwaszhiw and Padin, 1990) and on somatic (post-strip) weight (Bulkley, 1967; Baum and Meister, 1971; Gibson *et al.*, 1976). Few studies compare both length and somatic weight on fecundity (Rounesfell, 1957; Bulkley, 1967; Gibson *et al.*, 1976) and neither measure consistently appears to be a better predictor of fecundity. Bromage *et al.* (1990) showed length and weight to be highly significantly correlated, with a coefficient of determination (r^2) greater than 90%. However, it may be argued that the weight of female anadromous salmon is influenced by the loss in condition factor which occurs from the time the fish cease feeding; this occurs up to 10-months before spawning in wild migratory fish (Hoar, 1976) and up to 3-months before spawning in cultured salmon. This would introduce more variation into weight measurements, whereas length data remains unaffected.

Few departures from linearity for regressions of fecundity on fish size have been established: Blackett (1973) reported a curvilinear relationship between fecundity and body length for Alaskan populations of dolly varden, *Salvelinus malma*, although regressions for egg number on body weight remained linear; Nicholls (1958) showed a curvilinear relationship for egg number on length for a Tasmanian population of brown trout (*Salmo trutta*), although he admitted that some of the females included in the study may have commenced spawning before capture. The fecundity on weight data for these fish was still best fitted by a straight line. Partial oviposition was also suggested as a possible cause for the curvilinear fecundity on length relationship described for brook trout (*Salvelinus*

fontinalis) by Titcombe (1897) (quoted by Rounesfell, 1957).

In the present study significant differences in the slope of the regression of fecundity on post-strip weight were detected between sea-age within stocks and between stocks of the same age. Two stocks showed a greater rate of increase in fecundity per unit increase in weight in 3-sea winter fish than 2-sea winter fish, as did combined 2 and 3-sea winter data for all stocks. A similar relationship was seen by Thorpe *et al.* (1984), who reported the fecundity of 2-sea winter females to increase significantly more rapidly than 1-sea winter females. Generally, reports of slope differences among fecundity on fish size relationships between stocks within species are not common, and Bromage *et al.* (1990) suggested that the rate of increase of fecundity with size was a closely defined species-specific characteristic. However, significant slope differences were detected by analysis of covariance among 4 out of 14 stocks of chinook salmon taken throughout their range in an exhaustive study by Healey and Heard (1984). Nicholls (1958), again using analysis of covariance, showed the rate of increase of fecundity to be significantly different between 2 out of 3 stocks of brook trout from different Tasmanian rivers when regressed on body weight, and significantly different between all 3 stocks when regressed on fish length. Manzer and Miki (1986) observed clear slope differences between stocks of sockeye salmon when correlating fecundity with length but quoted no significances.

Among groups with common slope, further covariance tests indicated highly significant differences in elevation. Adjusted to a common weight, the 3-sea winter females in Stock A produced significantly more eggs than the 2-sea winter females from the same stock. Although age has been suggested as an important determinant of fecundity (Rounesfell, 1957; Thorpe *et al.*, 1984) variation in fecundity attributable to age has been shown to be minimal once variation due to fish size has been accounted for (Beacham, 1982; Healey and Heard, 1984; Manzer and Miki, 1986; DuBois *et al.*, 1989). The data for 2 and 3-sea winter year classes for each stock were collected in consecutive spawning seasons. Fecundity in wild salmonid populations have been shown to vary between seasons (Godfrey, 1968; Manzer and Miki, 1986). Such variation has been attributed to changes in population density (Healey, 1978) and changes in mean water temperature (Rounesfell,

1957), possibly through its effect on food availability. Changes in food availability (or ration in cultured stocks) have been shown to have a marked effect on fecundity in salmonids (Fry, 1949; Scott, 1962; Bagenal, 1969, 1978; Martin, 1970; Springate and Bromage, 1985; Springate *et al.*, 1985). The stocks used in this study were maintained on the same farm at comparable stocking densities, with no change in feeding regime over the study period. Although changes in temperature between years may have resulted in minor alterations in feed conversion and growth rate, it is unlikely that relationships between fecundity, or other parameters of reproductive performance measured, and post-strip weight were altered beyond that which could be accounted for by differences in size by analysis of covariance (Healey and Heard, 1984).

After 2 sea winters, Stock A had a significantly higher fecundity than Stock B, and a number of significant differences were apparent between 3-sea winter stocks adjusted to a common weight. Using the same statistical techniques, Pope *et al.* (1961) discovered significant 18% and 22% differences in elevation of fecundity on length among 6 wild Scottish stocks; and Randall (1989) found a significant difference between 2 stocks taken from rivers in New Brunswick. Healey and Heard (1984) found a number of significant elevation differences among 10 populations of chinook salmon (*Oncorhynchus tshawytscha*) of common slope, with almost 2-fold differences in fecundity of fish adjusted to common length. Bromage *et al.* (1990) found comparable fecundity differences among 12 populations of farmed rainbow trout, *Oncorhynchus mykiss*. Further significant intra-specific differences in fecundity determined by analysis of covariance have been reported for coho, *Oncorhynchus kisutch*, and chum, *Oncorhynchus keta* (Beacham, 1982), sockeye salmon, *Oncorhynchus nerka* (Manzer and Miki, 1986), Steelhead trout, *Oncorhynchus mykiss* (DuBois *et al.*, 1989), and brown trout (McFadden *et al.*, 1965). Blackett (1973) employed analysis of covariance to show the significant difference between anadromous and resident dolly varden adjusted to a common length. A number of studies, although not employing analysis of covariance as a means of accounting for parent size differences between groups, provide more examples of stock specific fecundity. Nomura (1963) presented parallel fecundity on length regressions for two hatchery stocks of rainbow trout,

a clear difference in elevation was apparent, but no statistical significance was quoted. Bulkley (1967) comparing his data with that of Shapovalov and Taft (1954), found steelhead trout of 22.5-cm from the Alsea river, Oregon to have 32% and 51% fewer eggs than fish of the same size from 2 Californian rivers (as calculated from individual population regression equations for fecundity on length). A similar approach was used by Rounesfell (1957) to demonstrate varying stock fecundities in chinook and sockeye salmon and char using information provided by McGreggor (1922, 1923), Aro and Broadhead (1950) and Smith (1947) respectively. Additional information for salmonid species of equal size yielding different numbers of eggs were reported by Galbreath and Ridenhour (1964), Murray *et al.* (1989), Zorbidi (1990), and Flemming and Gross (1990).

Although log egg size increased with log post-strip weight in most instances, the relationship provided very low coefficients of determination, with at best, fish weight accounting for only 15% of the variation in egg size. In 5 out of 9 cases there was no significant correlation. In one group egg size decreased with increasing body weight. Egg size has been shown to increase with increasing fish size in individual populations of Atlantic salmon (Pope *et al.*, 1961; Aulstad and Gjedrem, 1973; Thorpe *et al.*, 1984), coho salmon (Flemming and Gross, 1990), pink salmon (Kaev and Kaeva, 1987), rainbow trout (Nomura, 1963; Springate and Bromage, 1984; DuBois *et al.*, 1989; Bromage *et al.*, 1990), brown trout (McFadden *et al.*, 1965) and brook trout (Gibson *et al.*, 1976). Bulkley (1967) reported 98% of the variation in egg size (measured as the mean individual egg volume) to be attributable to fish length in a stock of steelhead trout. However, there are also reports of poor, non-significant and negative correlations between egg size and fish size (Galinka, 1970; Gall and Gross, 1978; Flemming and Gross, 1990). Compared with the present study, Bromage *et al.* (1990) found a better relationship between ova diameter and post-strip weight in rainbow trout (the coefficient of determination for one group was 89%), however, correlations for ova diameter and weight were poorer than those for fecundity and weight (with 7 stocks showing r^2 values less than 15%). Gibson *et al.* (1976), Thorpe *et al.* (1984) and Kaev and Kaeva (1987) also found the relationship between fish size and egg size to be poorer and more variable than fish size and fecundity.

The poor and non-significant correlations between egg size and body size among the 4 stocks in the present study make comparisons of slope for the regressions of little value. The only comparable study using covariance to identify significant differences in the rate of change of egg size with increasing fish size was that of Bromage *et al.* (1990), who reported considerable variations between stocks of rainbow trout from one which exhibited large changes in egg size with modest increments in fish weight to another showing virtually no change in egg size over its entire weight range. These authors went on to find that within the stocks having ova diameter on fish weight regressions of common slope, there were also significant elevation differences. Other workers who have established significant differences in elevation in egg size on body size regression for salmonid populations with common slope, using analysis of covariance include: Pope *et al.* (1961) who identified a maximum inter-stock difference in egg size of 15% for Atlantic salmon; and McFadden *et al.* (1965) working with brown trout. Aulstad and Gjedrem (1973) reported a significant difference in egg size among 16 Norwegian populations using a multivariate regression analysis. Other reports of significant differences in egg size between stocks or strains of the same species using statistical methods other than covariance are provided by Rounesfell (1957), Glebe *et al.* (1979), DuBois *et al.* (1989) and Murray *et al.* (1989).

In this study, total egg volume was clearly seen to be the most reliable measure of reproductive performance. Each group showed post-strip weight to be more closely related to egg volume than either total fecundity or egg size. All regressions were highly significant, with at least 50% of the variation in egg volume being accounted for by body weight in most cases. These results are in good agreement with those of Bromage *et al.* (1990), who found total egg volume to be more strongly correlated with fish size than either fecundity or egg size in 8 out of 12 individual stocks of rainbow trout. This observation was also made, again with rainbow trout, by Gall and Gross (1978).

Significant differences were found in the rate of increase of total egg volume per unit increase in fish weight between different stocks of the same age and within stocks of different age. Bromage *et al.* (1990) found all stocks investigated to have a common slope, but did find significant differences in elevation such that, adjusted to a common weight,

there was a between stock variation of up to 55% in the volume off eggs produced.

Fecundity and egg size (or individual egg volume) may be regarded as integral components of total egg volume. The strong correlation of total egg volume with fish size restricts the independent variation of either fecundity or egg size and gives rise to the 'trade-off' phenomenon described by Springate and Bromage (1984) and Bromage *et al.* (1990), whereby fish of a common weight can either mature a large number of small eggs or a reduced number of large eggs. The inverse relationship between these two parameters is evident when comparing individuals within species (Rounesfell, 1957; Nicholls, 1958; Nomura, 1963; Gall and Gross, 1978; Healey and Heard, 1984; Murray *et al.*, 1989; Flemming and Gross, 1990) and also when making comparisons between species (Wootton, 1984).

As separate stocks spawn in discrete ecological situations, it is likely that this trade-off has been adjusted in response to 'local' selection pressures to favour either increased or decreased fecundity or egg size. The geographical separation of resident stocks; and the accurate homing of migratory stocks to natal streams (Hoar, 1976) restricts gene flow between populations (Quinn, 1984). This genetic isolation of stocks may provide the opportunity for population-specific differences in egg size and fecundity characteristics to become established and maintained (Flemming and Gross, 1989). Manzer and Miki (1986) showed that, after adjustment for fish size, sockeye salmon spawning in coastal regions were 18% more fecund than interior spawning stocks, and suggested that the difference represented adaptive mechanisms of stocks utilising markedly different environments. Rounesfell (1957) attempted to establish a geographical trend in fecundity among *Oncorhynchus* species, hypothesising that egg number in these species increased from north to south; and that the reason for this trend was attributable to the increased growth rates achieved by stocks at southern latitudes. His data though were limited and unconvincing. Since this work a number of contradictory studies have shown fecundity to increase with increasing latitude (Crone and Bond, 1976; Beacham, 1982; Healey and Heard, 1984). It is unclear from these studies whether the changes in fecundity were a result of fecundity/egg size trade-off or whether there was a clinal pattern of investment into

gonadal material. Flemming and Gross (1990) observed total egg biomass (a parameter comparable to total egg volume) to decline with latitude, so that increasing egg number with latitude could not be explained by an increased total investment in eggs. These authors also showed egg size to decrease with latitude and suggested that the latitudinal patterns in egg number were a consequence of selection pressure on egg size.

The trade-off between fecundity and egg size complicates efforts to make inter-population comparisons of either of these parameters individually. Total egg volume combines these parameters and remains, to a certain extent, independent from the relationship between them. The total egg volume also provides an indication of total energetic investment in reproductive material. These facts allied with the greatly improved correlations seen between egg volume and fish size in this, and other studies present the measurement of total egg volume as a better indicator of reproductive performance.

The data presented here clearly illustrate substantial differences in fecundity and egg volume; and the relationships between these two parameters and fish weight among the 4 stocks of farmed Atlantic salmon investigated. The identification of such inter-stock differences in reproductive productivity is of direct economic importance to aquaculture, as clearly the choice of stock maintained by an egg producer will have implications on the efficiency and productivity of his broodstock unit. These criteria should be taken into account with other measures of (stock) performance such as growth, survival, feed-conversion and precocious maturation, and should be available to hatchery managers.

The consideration of egg size, however, may be of more significant to smolt producers, and the salmon industry as a whole: larger eggs yield larger alevins which result in larger first-feeding fry. A 10% increase in egg diameter increases individual egg volume by 33% and has a comparable effect on the weight of emerging fry. Fish from larger eggs will get off to a better start in the hatchery and this will ultimately have a favourable effect on the S1:S2 ratio of the resulting population (see Section 3.9 for discussion on the effects of early growth on recruitment to the upper-mode and the decision to smolt). This is a phenomenon peculiar to anadromous species which makes egg size of particular importance to the salmon farming industry.

Although analyses of the data gathered from the four stocks in this study showed that egg size was poorly related to fish size (compared to either fecundity and total egg volume) and clear inter-stock differences in egg size were not established by covariance techniques, a considerable amount of intra-stock variation in egg size was evident, with differences in the egg diameter of individuals in excess of 20%. This advocates family selection procedures as the most appropriate approach to taking commercial advantage of the variability in this trait in accordance with the selection programme already in operation within the Norwegian salmon industry.

Chapter 5

General conclusions and suggestions for future work

The results in Chapter 3 clearly show that the timing of the completion of smoltification in Atlantic salmon can be significantly altered by employing novel light regimes. Seasonally-compressed photoperiods served to advance the completion of smoltification by up to 3- and 7-months in potential S1 and S2 smolts respectively. Advanced smolts consistently showed all the characteristics associated with the completion of smoltification (body silvering, increased hypo-osmoregulatory ability and reduced condition factor), which occurred in a synchronous fashion, and transfers to seawater were, with the exception of Experiment 5, successful. Mortalities following the seawater transfer of photoperiod smolts at both the Hayling Marine Facility and the commercial ongrowing site were very low and their subsequent growth indicated these fish to be of good quality.

Temporally advanced S1 smolts benefited from an early transfer to seawater and significant growth advantages were established with even modest advances in transfer time. Potential S2 smolts transferred to seawater between late-September and early-December showed good growth over the winter (or at least better growth than control fish remaining in freshwater). Fish transferred at this time generally showed a 3-fold increase in weight prior to the transfer of smolts reared under ambient conditions. The knowledge that smolts can be successfully transferred to seawater out-of-season should encourage the use of these techniques in the salmon farming industry. Clearly, experience in the scaling up these techniques to produce commercial numbers of out-of-season smolts is required, and work to this effect is already proceeding at a number of large smolt units in the U.K. Unfortunately, the efforts to increase the scale of production in this project resulted in a high mortality on transfer of S1 smolts in February. However, the problematic transfer of these fish was, in part, attributed to stress. Losses of this scale amongst smolts produced by conventional methods are not uncommon within the industry, and this experience highlights the importance of an awareness of accumulating stress factors close to the time of smolt transport.

It was evident that the increased growth opportunity provided by advanced seawater transfer increased both post-smolt and grilse maturation. Due to the comparatively small number of fish transferred to the ongrowing phases of these trials (ie less than 1,000) the

data collected in this area are far from complete. Again, larger scale sea trials with photoperiod smolts are required to determine fully the effects of various photoperiod regimes on the subsequent maturation of smolts transferred to sea at different times. The low maturing qualities of all-female or all-female triploid stocks make them particularly attractive in the continued development of photoperiod techniques.

Use of light regimes incorporating direct change from a short day to long day in the winter was also found to be conducive to the completion of smoltification. In addition these photoperiods were found to more stimulatory to the advancement of smoltification than seasonally-changing regimes. Continued research into the use of 'square wave' light regimes with a view to replacing seasonally-changing photoperiods would be worthwhile as these are easier to implement and do not require constant readjustment of time clocks controlling artificial photoperiod lighting.

Incorporation of the photoperiod regimes described in these experiments into smolt production could allow the supply of S1 smolts at any time between February and May and of S2 smolts between September and May; although restricting the introduction of S2s to the autumn would to be most beneficial to smolt producers. To maximise the output of a smolt unit three clear crops of smolts from each intake of eggs could be produced: Fry hatching in the spring could be graded in mid-summer and the top fraction committed to a compressed photoperiod with the aim of providing an early S1 smolt crop in February. Potential S2 smolts in the remaining population would be evident by October or November and could be graded out and reared under a compressed light regime advancing them to the following autumn.

The employment of photoperiod techniques in the production of out-of-season salmon eggs would further widen scope for smolt production, particularly with regard to the earlier supply of S1 smolts. It may be more convenient to achieve temporal advances in spawning, and rear resulting progeny from hatch on an 'expected' simulated natural 12-month photoperiod, out of phase with the ambient light cycle. This would obviate the need to employ 'forcing' photoperiods to produce winter S1s. The use of heated water to accelerate

the development of eggs, to advance the time of first feeding and enhance early growth would also make a contribution to the achievement of a "year-round" supply of smolts.

All the photoperiod work in this project was conducted using a constant temperature water supply. However, most smolt hatcheries operate with water supplies of ambient temperature. Previous work has shown that temperature has a regulatory effect on the timing of the completion of smoltification in steelhead trout and coho salmon (Adams *et al.*, 1973; Wagner, 1974; Zaugg and McLain, 1976), and recent studies on Atlantic salmon have indicated that temperature controls the rate of loss of smoltification characteristics (McCormick, *pers com.*). For this reason, similar photoperiod trials should be run in association with seasonally-changing temperatures to ascertain the effects of temperature on the timing of completion of smoltification in Atlantic salmon, or whether high summer or low winter water temperatures interfere with the development of smoltification characteristics, in particular the development of hypo-osmoregulatory ability.

The results contributed to accumulating evidence that photoperiod entrains an endogenous circannual rhythm which controls the development of smoltification. However, to prove conclusively that smoltification is controlled by a true endogenous circannual clock, it must be demonstrated to free run over 2 or more complete cycles under conditions of constant temperature and unchanging daylength. To avoid the confusing influence of maturation among post-smolt males retained in freshwater, this kind of experiment would be best conducted using all-female triploid stocks. The results were also in agreement with the hypothesis that potential smolts make a decision to smolt in the year preceding seaward migration. However, it would appear that the decision period is not confined to a 1 or 2-month interval, and fish may be recruited into the smolt fraction at any time preceding the spring increase in day length.

Assessments of four stocks of cultured salmon identified total egg volume as being the most reliable measure of reproductive performance. There were clear differences in egg productivity (measured as either egg volume or fecundity) between stocks, and also between ages within stocks, once the influence of fish size had been removed by covariance techniques. However, egg size may be considered a more important reproductive

characteristic in the farming of salmon than in the culture of other species due to its influence on S1:S2 ratio. Although improvements in husbandry and diet over the past few years have significantly increased the growth of juvenile salmon in the hatchery/freshwater phase of culture; and improved the industry S1 yield considerably, this parameter once again becomes of increased importance with the exploitation of photoperiod techniques and the production of increasing earlier S1 or even S1/2 (0+) smolts. With the time to smolting being much reduced, the decision to smolt will be taken earlier, in response to accelerated photoperiods, at a smaller size resulting in a reduced smolt fraction. This makes the effect of egg size on the size and early growth of emerging fry more critical and suggests that the use of larger eggs would be appropriate for this kind of work. In addition, the photoperiod advancement of spawning in rainbow trout has been shown to significantly reduce egg size (Bromage *et al.*, 1984; Duston and Bromage, 1988; Randall, 1992). Therefore work directed towards increasing egg size within broodstocks would be of great value for the continued develpoment and commercial application of photoperiod techniques and the achievement of year-round supplies of smolts and eggs in the salmon farming industry. This study illustrated considerable within stock rather than between stock variation in egg size, suggesting that a programme of family selection for increased egg size may be the best method of achieving this objective with Atlantic salmon.

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Appendix: Research Publications

Work presented in this thesis has been published in the following refereed articles:

Thrush, M.A., and Bromage, N. R. (1988). Photoperiodic effects on smoltification in Atlantic salmon (*Salmo salar*). J. Int. Disp. Cycle Res., **19**: 213. (abstr.)

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.

Thrush, M.A., and Bromage, N.R. (1991). Relationships between fecundity, egg size, egg volume, and fish weight in four stocks of farmed Atlantic salmon (*Salmo salar*). In: Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish. Scott, A.P., Sumpter, J.P., Kime, D.E. and Rolfe, M. (Eds.), p. 291. Sheffield: FishSymp 91.