COMPENSATORY GROWTH, LIFE-HISTORY DECISIONS AND WELFARE OF FARMED ATLANTIC SALMON (*SALMO SALAR* L.) PARR

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This thesis is submitted for the degree of Doctor of Philosophy,
Division of Environmental and Evolutionary Biology,
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University of Glasgow,
July 1999

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Declaration

I declare that the work described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It is entirely of my own composition and has not, in whole or in part, been submitted for any other degree.

Amanda MacLean

July 1999

For Mum and Dad

Acknowledgements

First and foremost I would like to thank my supervisor at Glasgow, Neil Metcalfe, for giving guidance and direction to the project and particularly for his help in the final stages.

Thanks are also due to David Mitchell, my CASE supervisor from Marine Harvest McConnell (MHM). Mitch and David Cahill together co-ordinated tank space at MHM sites and organised fish transport. The project was funded by a grant from the Natural Environment Research Council.

My thanks go to all the people who looked after the fish and made sure that enough survived to produce a decent set of results: Alistair McInnes and the team at Mingarry Hatchery, Jim Raffell and Andy MacLean at Kinlochmoidart, Gordon Beaton, Kathy, Norm, Tony and Shona at Invergarry, John Laurie and Roddy MacDonald in the Glasgow University Aquaria, and Vivien Cameron at the Field Station.

Many thanks are also due to: Anders Alänära for designing and setting up the tanks used in Chapter 3; Mikael Sjöberg, who wrote the programme for data analysis used in Chapter 3; Duncan Perrin and the MHM lab at Lochailort for their valuable assistance with the seawater challenge. Thanks also to Duncan Perrin, Carol Cox, Shona Robertson and Jill Ashburner for useful discussions on the subject of opercular erosion. The fin damage scoring system was developed by Jimmy Turnbull, who also gave help and advice regarding opercular erosion.

Lots and lots of thanks too to all the people who sacrificed their time and sanity to assist me with data collection, chasing fish around tanks and getting soaked (not just in the pub), whether being alternately midge-bitten and frozen in the shed at Kinlochmoidart or in relative comfort at Mingarry and Invergarry: Alexandra Hay, Mel Fletcher (gold star for multiple attendances), Colin Bull, Ally Walsh, Mat

O'Hare, Mat Cottam, Mark Cole, Murray Roberts, Dave Nairn, Dave Stevens, Kat Jones, and Nick Rogers (triple gold star, see below!). Thanks, too, to Shona and Aileen McCauley for their hospitality when it was needed.

There are lots of other people – too many to mention here – who had input one way or another, giving advice with statistics, references, techniques, etc. So thank you, all of you, for your time and patience. Plenty of people helped too in other ways, by listening, encouraging or just having a laugh. This is the tricky bit because I don't want to miss anyone out, so thanks to all you DEEB staff, post-grads and post-docs, and all my other pals, but special mention has to go to Colin Bull, Dave Fraser, Svenni Valdimarsson, Kate Greaves, Andrea Fidgett, Shona McCauley and Anne Whiteford who took more than their fair share of ear-bending.

Very special thanks go to my family for their support and encouragement throughout this project: to my brother, Calum MacLean, and his partner (now wife) Carol; and a double mention goes to my dad, Malcolm MacLean, for putting up with it all (and also because I forgot to mention him in the acknowledgements for my M.Sc. thesis, even though he helped me collect bivalves in Millport. Recognition at last!).

Finally, heartfelt thanks go to my partner Nick Rogers, who has done more than anyone and gave more than he received. We must both be equally delighted that this is finished!

Abstract

In nature, growth rates are rarely maximised but are instead optimised by natural selection. Compensatory growth, a common phenomenon in the animal kingdom, is evidence of this: when growth conditions improve after periods of poorer than normal growth, many organisms will grow at faster than normal rates. This allows them to achieve a desired body size despite unexpected setbacks in growth. This thesis investigates compensatory growth in the context of social interactions and life-history decisions in Atlantic salmon *Salmo salar*. The fish in the experiments were 0+ parr on an accelerated smolt regime, producing out-of-season smolts ca. 6 months in advance of the natural cycle.

In the experiment described in Chapter 2, six groups of individually marked fish were subjected to periods of unseasonably low temperature during successive periods of three or six weeks in the spring. Their growth rates were compared to that of a control group that was not exposed to low temperature. Four out of the six experimental groups showed clear compensatory growth spurts when they were returned to warm water. The mean size of fish in the two groups that did not exhibit compensatory growth was close or equal to that of fish that had already compensated. Thus, fish only initiated a period of compensatory growth if they were below a target size threshold for the time of year.

Chapter 3 examines the behavioural mechanisms behind compensatory growth responses. By automatically registering movements of fish that were individually identified with passive integrated transponder (PIT) tags, the exact feeding and activity patterns of individual fish within groups were recorded over a period of days. While on average growth-compensating fish did not spend more time feeding than controls, they were more aggressive; as a result dominant fish within each group gained more exclusive access to the feeding area during periods of compensatory growth. The extent to which compensatory growth could be achieved

was therefore dependent on both the social status of the individual and the dominants' ability to monopolise the food patch.

Sexual maturation is an increasing problem in aquaculture due to the use of accelerated smolt regimes, probably because rapid growth rates trigger maturation during a crucial decision period in the spring prior to spawning. The experiment described in Chapter 4 used three groups of parr, exposed to successive three-week periods of low temperature between April and June, in an attempt to reduce the incidence of sexual maturation. There was some evidence that a growth setback caused a reduced and delayed investment in gonads. However, contrary to expectations, the incidence of maturation did not differ between the experimental groups and a control group. Periods of compensatory growth after the experimental groups were returned to warm water may have negated the effects of the low temperature treatment on the decision to mature. In addition, the absence of seasonal cues due to the constant photoperiod may have resulted in a less strictly defined decision window.

Sexual maturation and smolting are often considered to be mutually inhibitory processes. However, some mature parr make the decision to smolt and do so with varying degrees of success. The question of whether the fish could undergo the processes of maturation and smolting at the same time was investigated in chapter 5 by following the development of smolt characteristics (smolt coloration and sea-water adaptability) in sexually mature and immature parr.. Smolt characteristics were more developed in immature fish, but nevertheless the mature males did show signs of smolting, and larger mature males could adapt to sea water. I suggest that the inhibition of smolting by sexual maturation is a result of two processes: firstly, that mature parr often do not fulfil the necessary requirements to make the smolt decision; secondly, that in mature parr that do decide to smolt, androgens inhibit or delay the development of smolt characteristics, but do not entirely prevent smolting due to the delay between spawning and emigration.

The development of dorsal fin damage, which is primarily caused by aggression, was followed in Chapter 6 in four groups of parr of different mean length. The probability of having fin damage was strongly related to relative body size within each group: the largest fish in a tank were up to six times more likely to have damaged fins than the smallest fish. Studies of small groups of salmonids have demonstrated that subordinates are the main recipients of fin damage, but the present study indicates that the reverse is true in larger groups. This may be because dominant fish compete aggressively amongst themselves and incur fin damage, while less aggressive individuals adopt alternative feeding strategies that reduce the risk of injury.

Erosion of the operculae is often seen in cultured fish, but little is known about the causes of the condition. While vitamin deficiency has been implicated in some cases, fish that show none of the other symptoms of vitamin deficiency may still develop opercular erosion. Chapter 7 describes how the development of opercular erosion was recorded in four groups of parr of different mean length. Erosion of the operculae developed during the early summer but healed completely during the late summer and autumn. The larger fish in a tank were generally more likely than smaller fish to have eroded operculae. I suggest that the condition resulted from physical injury caused by aggressive interactions between fish, and that a shift in behaviour with increasing body size reduced the rate of attack on the operculae later in the year, allowing them to recover from injury.

The final chapter brings together the findings and concepts of the previous chapters. The implications of the findings for aquaculture are also discussed.

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Chapter 1: General Introduction

1.1 Growth Rates

It is self-evident that, all other things being equal, rapidly growing individuals will attain a large body size sooner than slower growing individuals. In organisms where large body size is associated with fitness benefits such as increased survival, social dominance or greater fecundity, we might naïvely expect that growth would proceed at the maximum possible rate at all times. However, there is a considerable body of evidence that growth frequently proceeds at sub-maximal rates (Calow, 1982; Arendt, 1997).

Since natural selection applies to all aspects of an organism's biology, it will of course affect patterns of growth. While rapid growth may be advantageous to some organisms in some environments, it does not necessarily follow that it will always be so, even if large size is in itself advantageous. In plants, rapid growth may be highly advantageous when competition is severe, or when mortality is high and early reproduction is a prerequisite of success, but not in nutrient-poor environments if it leads to the exhaustion of nutrients prior to reproduction (reviewed by Arendt, 1997). A large body size can be produced either by short periods of rapid growth or by long periods of slower growth. A requirement for rapid growth may put organisms at a disadvantage if they are subject to periods of starvation or food shortage (Arendt, 1997), even though larger bodies can usually carry more storage tissues and can thus improve fasting endurance (Millar & Hickling, 1990). In the case of altricial birds (those that give parental care to their young in the nest), slower juvenile growth rates may allow parents to raise more young for the same rate of food provision, preventing exhaustion of the parents and loss of the whole group (Case, 1978). Optimal foraging theory predicts that predators should prefer those prey items that provide the greatest gain per unit handling time, and since the faster-growing individuals in a cohort will be larger, and so often the most profitable to a predator,

selection may favour slower growth (Calow, 1982). In animals that exhibit determinate growth, i.e. those where structural growth does not continue beyond maturation, there may be some benefit in growing rapidly in order to reach the adult size as soon as possible. In indeterminately growing species, there is more reason for regulation of growth below the maximum rate.

In addition to these types of fitness trade-offs, there is evidence that fast growth can have more direct costs. Some possible costs, summarised by Arendt (1997) and drawn from studies in a wide variety of taxa, include developmental errors (e.g. greater levels of fluctuating asymmetry, skeletal deformities or changes in body proportions); reduced protein turnover leading to accumulation of proteins with errors; genetic damage caused by repeated reading of DNA; and immune suppression. In many circumstances, the costs of an increased growth rate may outweigh its benefits, hence resulting in sub-maximal growth rates.

There is a difference, however, between growth rates that are expressed at a species or population level and those of individual organisms. Related species, or populations of the same species from different habitats, could differ in their growth rates while still growing at physiologically maximal rates, if natural selection alters the physiological maximum to suit the environment. However, there is evidence that individual organisms often do not grow at maximal rates, but rather maintain growth at lower rates. Growth rates in many species can be increased by administering naturally-occurring hormones, indicating that the endocrine system works actively to control growth rates (see Calow, 1982). Further evidence that growth is maintained as lower than maximum rates comes from the existence of compensatory growth (also known as catch-up or recovery growth). When growth conditions improve after periods of poorer than normal growth, many organisms will grow at faster than normal rates. Compensatory growth is a widespread phenomenon and has been reported in birds, mammals, fish, invertebrates and plants (e.g. Wilson & Osbourn, 1960; Miglavs & Jobling, 1989; Bejda at al., 1992; Russell & Wootton, 1992; Wairimu et al., 1992; Carlstein, 1993; Jobling et al., 1993; Mortensen & Damsgård, 1993; Reimers et al., 1993; Dieterich & Anderson, 1995; Kapkowska, 1995;

Leichter, 1995; Jaremo et al., 1996; Hayward et al., 1997; Lawrence & Fowler, 1997; Nicieza & Metcalfe, 1997; Speare & Arsenault, 1997; Chmilevskii, 1998; Maxwell et al., 1998; Le Francois et al., 1999). This is clear evidence that normal growth rates are often slower than is physiologically possible, and suggests that growth is regulated with respect to required growth trajectories (Calow, 1982).

Because organisms regulate their growth with respect to internal targets, growth rates are not simply the automatic product of environmental factors such as temperature and the abundance of food, although these both have a strong influence. At least in animals, the effect of behaviour must also be considered. Food acquisition often incurs risks, which will vary with the season and the habitat. The growth rate attained by an individual will depend in part on the extent to which it is willing to run those risks. Predation is one of the chief risks associated with finding food, but another risk is competition from conspecifics. Socially subordinate individuals that are unable or unwilling to compete successfully in the presence of social dominants will obtain less food and grow at slower rates. Consequently, when studying growth rates in groups of animals it is essential to consider the effects of individual behaviour, rather than treating the group as a homogeneous whole.

It is plain that many factors (environment, behaviour, season, desired growth trajectory, stage of the life-cycle and nutritional state) combine to determine growth rates. Growth rates in their turn can influence some of these factors. In particular, there is a strong interaction between growth rates and life-history stages. Fast juvenile growth and the rapid accumulation of fat reserves often lead to early sexual maturation, while in indeterminate growers the diversion of resources to the maturation process itself leads to a reduction of somatic growth rates (Stearns, 1992).

1.2 The Atlantic Salmon

As an indeterminate grower that exhibits remarkable life-history plasticity, the Atlantic salmon *Salmo salar* is an excellent species in which to study the effects of deviations from expected growth trajectories on growth rates and the choice of

life-history strategies. All aspects of the biology of Atlantic salmon have been subject to intense scrutiny, because of its importance for conservation and its commercial value. Both are important areas: a recent estimate claimed that nearly 95% of all the Atlantic salmon alive today are in aquaculture (Gross, 1998). The protection of wild stocks is essential, while salmon-farming is clearly of great economic importance. An understanding of the interaction of growth rates and life-history decisions is vital for the management of Atlantic salmon both in aquaculture and in the wild. For instance, growth rates during the first year after hatching have a major effect on the absolute numbers and the proportions of fish that mature or migrate to sea in the first year (Thorpe et al., 1989; Rowe et al., 1991; Saunders et al., 1994a; Berglund, 1995; Friedland, 1998; Hutchings & Jones, 1998). This in turn has an effect on the ecology of rivers and oceans, and on the efficiency of salmon-farming.

The Atlantic salmon is an anadromous species, migrating from the marine environment to spawn in freshwater rivers and lakes. Its natural marine range is the north Atlantic ocean, while the freshwater stages inhabit streams and rivers on both sides of the Atlantic, from Iceland and Greenland in the north to Portugal and Connecticut in the south (Jones, 1959). Its range is extended in aquaculture to include parts of the southern hemisphere and the Pacific Ocean (Gross, 1998).

Like all salmonids, Atlantic salmon are thought to be descended from a marine ancestor that buried its eggs along shorelines (as capelin and grunions still do today) (Thorpe, 1987, 1994a). During the course of evolutionary history, the diverging species of salmonid fish began to spawn in estuaries and gradually moved farther upstream. Today, the eggs are laid in fresh water rivers and streams, and in some cases in lakes. The females excavate pockets known as redds in gravel beds, where the eggs are laid, fertilised by the male and then buried. Spawning occurs in the autumn and winter and the eggs hatch in early spring, producing alevins that remain in the gravel until they have absorbed their yolk-sac (Jones, 1959). Once the yolk-sac has been absorbed the young fish emerge from the gravel and establish feeding territories where they feed on invertebrates (small crustaceans and the larvae

and pupae of insects) that are carried in the water current. Between the time of first feeding and seaward migration the fish are referred to as parr.

In the wild, the parr spend at least a year in fresh water before undergoing the physiological, morphological and behavioural changes (termed smolting or smoltifying) that prepare them for seaward migration. At this time they lose their characteristic brown markings and become silvery in colour, with darkened fin margins and a more streamlined appearance (Hoar, 1976). Sexual maturation may occur either before or after the seaward migration. Maturation as parr, prior to migration, is at a small size (5-20 cm in length) and is almost exclusively confined to males, although some mature female parr have been reported. (Such fish are commonly referred to as "precocious parr", but I have avoided this terminology here in favour of the less judgmental term "sexually mature parr" or simply "mature parr". The term "precocious parr" is surely inappropriate when 80% or more of males in some populations will mature before migrating to sea (Myers, 1984; Fleming, 1998), and maturation as parr is therefore the norm rather than the exception.) Females rarely mature in fresh water, as they cannot meet the greater energetic requirements of egg production. After seaward migration, the fish spend a year or more in the sea, and then return to their natal rivers to spawn. Large anadromous females spawn with anadromous males and also with sexually mature male parr. Although many adults die after spawning, the survivors return to sea and may repeat the spawning migration in following years.

The decisions to become sexually mature and to smolt appear to be based on a comparison of the current physiological state of the fish (a combination of size and growth rate or physiological correlates of these factors) with a genetically-determined threshold during seasonally-determined decision periods (Thorpe et al., 1998). If a fish exceeds the threshold level of state at the time of the decision period, it will proceed with maturation or with smolting, whereas if its state is below the threshold then the relevant process will be "switched off". In the case of smolting, the decision is normally taken around midsummer (Wright et al., 1990) while for maturation there appear to be two decision periods, one in November a year prior to spawning and the

other in early summer (Thorpe et al., 1990; Rowe et al., 1991). In the autumn, some individuals put on a brief growth spurt and will form the upper modal group (UMG) (Kristinsson et al., 1985; Metcalfe et al., 1988), smolting and migrating to sea the following spring, while the remainder that belong to the lower modal group (LMG) reduce their food intake and growth and will remain in fresh water for at least a further year. This leads to the development of a bimodal size distribution by the end of the first growing season (Thorpe, 1977).

There are enormous differences between individual fish in the life-history adopted. While males may adopt either or both of the mating strategies described above, there are also large variations in the age and size of maturity and smolting. In the wild, sexual maturity may first be reached at ages ranging from 0+ (under one year of age) to 10 years of age, while size at maturity is also highly variable, ranging from just over 5 mm to over 1 metre in length (summarised by Hutchings & Jones, 1998). The age at which seaward migration occurs can range from one to six or more years. Further plasticity is evident in female fecundity, which varies five-hundred-fold, and egg size, which varies up to three-fold (Hutchings & Jones, 1998). Finally, in addition to differences between individuals, there are large differences between populations and between years in the percentage of fish maturing or migrating at each age or size.

1.3 Accelerated Smolt Regimes in Aquaculture

The fish used in much of this thesis were raised on an accelerated smolt regime which is becoming increasingly common in aquaculture. The process is described here using details of the system used by Marine Harvest McConnell (MHM), the CASE partner in this Ph.D. project.

Traditional aquaculture techniques for Atlantic salmon have used natural photoperiod and ambient temperatures. Thus, smolting has occurred in spring as it does in nature. However, this makes it difficult to provide a year-round supply of fish within the size range required commercially. By manipulating photoperiod and

temperature regimes, smolting can be induced approximately six months in advance of the natural cycle, producing a more even supply of commercially acceptable fish through the year (Duston & Saunders, 1995).

Successful transfer to sea water depends on the completion of the smolting process, which in turn is dependent on the size of the fish (e.g. Berglund 1995; Thorpe & Metcalfe, 1998). Consequently, fish are raised at elevated water temperatures to ensure a large size by the mid-summer or early autumn after hatching. Constant long days (typically 22L:2D) also contribute to rapid growth. Once the fish have reached a minimum size of 10g, they are subjected to a "photoperiod winter" of short days (10L:14D) for eight weeks, and are then returned to long days. These changes in daylength (from long to short days and back again) are the signal for the completion of smolting, since photoperiod acts as the natural synchroniser, so "tricking" the fish into behaving as if it is really spring. This has the effect that the fish are ready to be transferred to sea water in the autumn, 6-7 months in advance of smolting under normal conditions.

1.4 Outline of Thesis

The degree of life-history plasticity in an indeterminately growing species of commercial value and importance for conservation makes the Atlantic salmon *Salmo salar* an excellent species for studies of deviations from expected growth trajectories and the choice of life-history strategies. The research presented in this thesis has been conducted with this in mind. By using periods of unseasonably cold temperatures, I demonstrate that 0+ parr will compensate for a deviation from their expected growth trajectory, but only when they have fallen sufficiently behind schedule (Chapter 2). As little is known of the behavioural basis of compensatory growth, I investigated this aspect in Chapter 3. The effects of manipulations of growth rates on the maturation decision are investigated in Chapter 4, and how this decision affects the process of smolting is discussed in Chapter 5. Since social status has considerable influence on the ability of fish to obtain food, I used dorsal fin damage (which is principally caused by aggression) as a tool to investigate social interactions during

the experiments (Chapter 6). Finally, I investigated the occurrence of opercular erosion, which may be also be an indicator of aggression in groups of Atlantic salmon in culture (Chapter 7).

Chapter 2: Compensatory growth and developmental targets in juvenile Atlantic salmon

2.1 Introduction

Body size, like any other phenotypic trait, is subject to natural selection. Natural selection should favour individuals that possess the ability to achieve the body size that provides optimal benefits in terms of fitness. Therefore, since environmental conditions are variable and only partially predictable, natural selection should favour individuals that are able to achieve the "right" body size despite fluctuations in environmental conditions. Hence, we would expect natural selection to favour adaptations that allow organisms to not only exploit opportunities for growth when they are available, but also to compensate for periods of slow growth when conditions improve. Compensatory growth (also known as "catch-up" or "recovery" growth) is exactly such an adaptation, as it allows organisms to increase growth rates over and above normal rates in response to a growth setback when conditions improve. The importance of achieving the "right" body size is demonstrated by the existence of compensatory growth in a wide array of taxa and in response to a variety of types of growth inhibition.

Many studies have demonstrated compensatory growth in organisms that have suffered periods of starvation or food restriction. When food is restored to normal levels, compensatory growth occurs in species as diverse as stoneflies *Soyedina interrupta* (Dieterich & Anderson, 1995), wapiti stags *Cervus elaphus* (Wairimu et al., 1992), several domesticated mammals and birds (Wilson & Osbourn, 1960) and numerous species of fish (e.g. Russell & Wootton, 1992; Jobling et al., 1993; Reimers et al., 1993; Hayward et al., 1997). Compensatory growth has been observed in children after renal transplantation (Maxwell et al., 1998), in brook charr *Salvelinus fontinalis* after exposure to ionising radiation (Le Francois et al., 1999), in rainbow trout *Oncorhynchus mykiss* during recovery from repeated exposures to hydrogen peroxide (Speare & Arsenault, 1997) and after birth in rats whose mothers have been exposed to cigarette smoke (Leichter, 1995). More natural

types of growth setback can also result in compensatory growth when conditions are restored to normal. Thus, compensatory growth has also been reported after water restriction in hens (Kapkowska, 1995), exposure to low levels of dissolved oxygen in the winter flounder *Pseudopleuronectes americanus* (Bejda at al., 1992) and after periods of unseasonably low temperatures in tilapia *Oreochromis mossambicus* (Chmilevskii, 1998), Atlantic salmon *Salmo salar* and Arctic charr *Salvelinus alpinus* (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997). Those studies that have demonstrated compensatory growth in response to growth setbacks other than starvation or food restriction are of particular interest, as they confirm the view that compensatory growth occurs as a response not simply to hunger or a nutritional deficiency but to the deviation from a desired growth trajectory.

Atlantic salmon are particularly suitable for studies of compensatory growth, as they face a major life-history decision affected by size and growth rates during their first year in fresh water. During their first autumn after hatching, populations of Atlantic salmon juveniles develop a bimodal length-frequency distribution comprising an Upper Modal Group (UMG) and a Lower Modal Group (LMG) with an anti-mode around 7-10 cm fork length (reviewed by Saunders et al., 1994a). The UMG consists of individuals that will maintain growth rates and migrate to sea the following spring, while the LMG comprises smaller individuals that become anorexic and arrest growth until the spring, remaining in fresh water for at least another year. The decision to smolt is taken during the summer months, and appears to depend on a future projection of size at the time of smolting, based on a combination of size and growth rates at the time of the smolt decision (Thorpe et al., 1998). It is thought that if the projected body size is below a genetically-determined threshold length, smolting is delayed for a further year. Since overwinter survival and seawater survival are both largely dependent on body size (Bilton et al., 1982; Holtby et al., 1990; Lundqvist et al., 1994; Smith & Griffith, 1994; Meyer & Griffith, 1997), and since smolting itself occurs during a time-limited window of opportunity in the spring (Lundqvist et al., 1994), there is considerable selection pressure for fish to attain an ideal smolt size, for which there is mounting evidence (Nicieza & Braña, 1993). This produces the ideal candidate conditions under which we should expect to see compensatory growth responses if fish are caused to deviate from their expected

growth trajectory.

Although numerous studies have demonstrated the existence of compensatory growth in salmonids and other species, little attention has been paid to the importance of the seasonal timing of the growth setback and to the degree to which growth is reduced. The extent to which Atlantic salmon parr exhibit compensatory growth may vary according to both the time of year and the size already attained by the fish. By subjecting groups of Atlantic salmon parr to periods in cold water of different durations and at different times of year, I was able to compare the compensatory growth response of groups that varied in timing and the degree of growth setback.

2.2 Materials and Methods

The experiment involved a population of farmed Atlantic salmon parr of pooled hatchery stock belonging to Marine Harvest McConnell Ltd. It started approximately six weeks after first-feeding, on 21-27 April 1996, when 1050 fish were selected from a stock population of 4,000 (± 10%) and individually marked with combinations of fin marks and body marks using alcian blue dye and red and purple acrylic paints (Herbinger et al., 1990; Hill & Grossman, 1987). The marked fish were kept together in a stock tank from which fish were randomly selected for use in experimental groups. In order to manipulate growth rates, group A, the control, remained in warm water (heated water with a mean temperature of 15.7°C ± 0.04 until 1 June; ambient temperatures thereafter, see Figure 2.1a), while groups B to G successively spent either three weeks (B, D, E and G) or six weeks (C and F) in colder water (mean of 7.3°C ± 0.1), according to the schedule shown in Table 2.1a.

Each experimental group comprised either 150 (groups B and C), 116 (group D), or 140 marked fish (groups A, E, F, and G), selected at random from the stock tank. (The numbers of marked fish varied because of deaths caused by oxygen pump failures. Additional fish were marked on 4-5 June to make up the deficit in groups A, E, F and G). Groups B to G included an additional 150 unmarked fish, taken from the unmarked stock tank, of which 50 unmarked fish were removed on 18-19 September

for use in other experiments.

Further measurements of all marked fish were taken on 14-20 May, 31 May-7 June, 28 June-4 July, 22-25 July, 19-22 August, 17-20 September and 12-15 November. There were thus seven growth periods between measurement dates as shown in Table 2.1b.

The fish were anaesthetised in an aerated water bath using Benzocaine in 95% alcohol, and then measurements were made of fork length (to $\pm$ 1 mm) and weight (to 0.01 g, after blotting with damp tissue paper to remove excess water). Fading fin marks or body marks were renewed when necessary. On 12-15 November, the marked fish were measured and all the fish (marked and unmarked) were assessed for sexual maturity by squeezing the body of the fish gently between thumb and forefinger and looking for the expression of milt. Specific Growth Rate in length (% increase per day) between measurement periods (SGRL) was calculated as:

$$SGRL = 100 \times [\ln (FL_{t2}) - \ln (FL_{t1})] / (t_2 - t_1)$$

where t_1 = first sampling day; t_2 = second sampling day, and FL = fork length. Specific Growth Rate in weight (SGRW) was calculated using the equivalent formula for weight.

Four locations were used during the course of the experiment. For the first six weeks of the experiment, the control group and those fish that had not yet undergone cold temperature treatment were kept at Mingarry Hatchery, South Uist. On 1 June, they were transferred to Kinlochmoidart Hatchery, Morvern, where they remained at ambient water temperatures until the end of the experiment (except when undergoing cold temperature treatment). Cold temperature treatment occurred at Glasgow University's Aquaria. The fish were transferred to Kinlochmoidart immediately after the end of low temperature treatment, with the exception of Group B, which was kept in similarly warm water (mean of 14.7°C $\pm$ 0.04) at the University Field Station, Rowardennan, from 17 May to 3 June and then transferred to Kinlochmoidart. In all locations the fish were kept in fibre-glass tanks (diameter 0.6-2 m, water depth 0.25-

0.40 m).

The fish were exposed to the natural photoperiod (except for a 24 day period where photoperiod was kept constant) (Figure 2.1b). At Mingarry and Kinlochmoidart, the fish experienced natural daylight, while at the other sites the natural photoperiod was simulated using automatic timers and overhead fluorescent striplights (240 lux). At night, floodlighting was used at Kinlochmoidart and fluorescent strip-lights (40-64 lux) at the other sites, to allow the fish to feed 24 hours per day.

The fish were fed to excess throughout the experiment on a pelleted diet of an appropriate size according to manufacturer's tables. Food was available 24 hours per day until the point when the photoperiod was briefly held constant, after which feeding only occurred during daylight hours. Food was dispensed from clockwork belt feeders at Mingarry and Kinlochmoidart, and from automated hoppers at Glasgow and Rowardennan.

2.3 Results

Effects of treatment on life-history strategy

Three marked and two unmarked male fish were sexually mature at the end of the experiment. Since the growth patterns of maturing fish differ from those of immature fish, the mature fish have been excluded from further analysis.

By the end of the experiment, there was a clear bimodal split in the length-frequency distribution of all seven groups of fish, with fish falling either into a Lower Modal Group (LMG) with fork length less than 100 mm, or an Upper Modal Group (UMG) with fork length greater than 100 mm. There were significant differences between groups in the proportion of fish belonging to the LMG ($\chi^2 = 38.1$, 6 d.f., $p < 0.001$) (Table 2.2). Paired comparisons using the sequential Bonferroni criterion (Rice, 1989) to test for significance revealed that there were significantly greater

proportions of fish in the LMG in groups C (χ^2 = 12.0, 1 d.f., $p < 0.001$) and F (χ^2 = 8.1, 1 d.f., $p < 0.005$) than in the control. Both of these groups had spent six-week periods in the cold.

Relationship between growth rate and size

Marking did not have any effect on the final size of fish, as marked and unmarked fish (in groups B to G only, as there were no unmarked fish in group A) did not differ significantly in final fork length (three-way ANOVA of final fork length, effects of: marking $F_{1, 918} = 0.6$, n.s.; life-history strategy (UMG or LMG) $F_{1, 918} = 826.9$, $p < 0.001$; treatment group $F_{5, 918} = 1.7$, n.s.; all interactions n.s.).

The nature of the relationship between growth rate and body size changed throughout the course of the experiment. This is exemplified by data from the control group (A), as follows. At first, SGRL during a given growth period was not related to fork length at the start of the growth period (period 1 $r^2 = 0.00$, 114 d.f., n.s.; period 2 $r^2 = 0.00$, 93 d.f., n.s.). By the third growth period, however, SGRL was positively related to fork length ($r^2 = 0.14$, 101 d.f., $p < 0.001$). During the fourth and fifth growth periods, the relationship between SGRL and fork length at the start of each growth period was curvilinear and best described by a regression equation including a quadratic term (period 4: $r^2 = 0.29$, 58 d.f., $p < 0.001$; period 5: $r^2 = 0.29$, 58 d.f., $p < 0.001$). The asymptotic value of FL was between 88 mm and 89 mm: thus, growth rate increased with body size in fish with an initial length at the start of the growth period up to and including 88 mm, but decreased with body size in fish with an initial length of 89 mm or greater (Figure 2.2). It should be noted that there were no fish with an initial fork length > 88 mm before growth period 4. During growth period 6, growth rate in the control group was negatively related to initial size at the start of the growth period (period 6 $r^2 = 0.50$, 59 d.f., $p < 0.001$), while during period 7 the same tendency was evident but was not statistically significant ($r^2 = 0.05$, 59 d.f., $p < 0.10$).

In all groups, the growth patterns of LMG and UMG fish began to diverge during growth period 4 and continued to do so until the end of the experiment

(Figure 2.2), although a small number of UMG fish showed similar patterns of growth and size as the LMG fish (i.e. small size and slow growth) until the final growth period. The mean final fork length of marked LMG fish at the end of the experiment did not differ between groups (one-way ANOVA, effect of group $F_{2, 37}$ = 0.8, n.s; this analysis includes only groups C, D and F, as there were not enough LMG fish in the other three groups for meaningful statistical analysis). Thus, the temperature manipulation affected the number but not the final size of fish in the LMG. Unfortunately, as only a single marked fish in group A belonged to the LMG, the effect of temperature manipulation on growth patterns in LMG fish could not be meaningfully compared to the control.

Compensatory growth

Unless otherwise stated, only marked UMG fish for which there was a complete growth history (i.e. measurements of length and weight taken on all measurement dates) from the third measurement date (28 June-4 July) onwards are used in further analyses. For all comparisons, the control group includes UMG fish in group A plus fish from the other groups prior to temperature manipulation (since these were kept in the same tanks as the controls up until the manipulation periods began). Growth curves for the UMG fish in each group show that, while growth continued in cold water, the treatment represented a setback in growth from which the fish never fully recovered (Figure 2.3), so that the UMG fish in the control group (A) were still significantly larger than those in the other groups at the end of the experiment (one-way ANOVA of final fork length, $F_{6, 336} = 25.2$, $p < 0.001$; one-way ANOVA of final weight, $F_{6, 336} = 27.4$, $p < 0.001$; comparisons of pairs of groups using Tukey's pairwise comparisons with a family error rate of 0.05). However, the final mean size of UMG fish in the experimental groups (B to G) did not differ, except in the case of group F, which was significantly smaller than all groups but C.

Although the experimental groups did not catch up in size with the control group, there was evidence of compensatory growth. For each growth period, I used analysis of covariance (ANCOVA) to compare the growth rates of experimental groups with those of the control during each growth period, with group as a fixed factor and

initial size (length or weight) at the start of the relevant growth period as the covariate (Table 2.3, Figure 2.4). I only present data on SGRL, as the analyses for SGRW showed identical trends. Because of the curvilinear nature of the relationship between SGRL and fork length during growth periods 4 and 5 (see earlier), data from fish with an initial fork length of $\leq$ 88 mm and > 88 mm were analysed separately for these periods. For growth period 6, there was only one control fish (FL = 88 mm) in the smaller size category, but as the inversion in the relationship between SGRL and FL was still evident in the other groups (Figure 2.2c), I excluded all fish $\leq$ 88 mm from analysis for this growth period. However, dividing the population into two sections in this manner reduced the numbers of fish in each group. Thus, only groups A, B, D and G had sufficient numbers of fish with initial length > 88 mm for period 4. Only six fish in control group A were $\leq$ 88 mm in length at the start of period 5, but they have been included in the analysis as it was necessary to compare the experimental groups to the control.

Except in growth period 7, there was no significant interaction between group and initial length. Thus, the relationships between SGRL and FL described for the control group (above) were also evident amongst the treatment groups. However, during the final growth period, there was an interaction between initial length and treatment group. While the control regression line was flatter than the regression line for the entire data set for that period, other groups had steeper regression lines. Because the interactions during the first six growth periods were not significant, the ANCOVA results could be used to compare the growth rates of the experimental groups to those of the control group during each of these growth periods. As expected, all groups of fish grew more slowly than the control when in cold water. Immediately after return to warm water, four groups (B, C, E and F) showed compensatory growth, growing faster than controls of the same size (Figure 2.4). Smaller fish in group E also continued to grow faster than the control during the second growth period after return to warm water, although the effect was not evident in fish with an initial length > 88 mm.

Except when in cold water, fish in groups E and F never grew more slowly than controls of the same size. However, the other groups had growth rates

significantly slower than the control during some of the periods after return to warm water. During period 4, all fish in group B grew more slowly than the control, as did large fish (i.e. those with an initial length > 88 mm) in group D. The same was true of large fish in groups B, C, D and G during period 5, and of groups B and D during period 6.

Compensatory growth and body size

Groups D and G did not show compensatory growth spurts on return to warm water. The growth setback experienced by these groups was significantly less than that experienced by the groups (C and F, respectively) that were returned to warm water at the same time, and that did show compensatory growth. The mean length of UMG fish in groups that showed compensatory growth was significantly smaller, at the time of return to warm water, than that of the groups that had been returned to warm water at an earlier date and had already compensated (Figure 2.3. One-way ANOVA of length: at end of period 2, $F_{3, 339} = 59.3$, $p < 0.001$; at end of period 3, $F_{5, 337} = 62.3$, $p < 0.001$; at end of period 4, $F_{6, 336} = 54.3$, $p < 0.001$; comparisons of pairs of groups using Tukey's pairwise comparisons with a family error rate of 0.05). In contrast, the mean length of UMG fish in the two groups (D and G) that did not exhibit compensatory growth did not differ significantly from that of UMG fish in the groups that had already compensated. (It should be noted, however, that in the case of group D, logistical requirements dictated that group B, the only group that had already compensated, had to be measured several days before the other groups. Thus, although the mean fork length of group B measured at the end of period 2 was not significantly different from that of group D, group B would have been larger by the time groups C and D were measured.)

2.4 Discussion

As expected, the low temperature treatment resulted in a reduction in growth rates, with the result that the control group was always larger than the groups that had spent three or six weeks in cold water. Longer periods of low temperature treatment increased the proportion of fish in the LMG. The same effect of cold water

treatment has been found before in Atlantic salmon (Mortensen & Damsgård, 1993), and the results agree with theoretical predictions that the smolt decision is based on a future projection of size based on a comparison of state (size and energy reserves) and rate of change of state (growth rate) in the summer months (around August) (Thorpe et al, 1998). Therefore, fish that experienced longer periods in the cold were less likely to exceed the predetermined threshold and made the decision to remain in fresh water for a further year.

In upper modal group fish, the return to warmer water was followed in four out of six experimental groups by a period of rapid growth over and above the growth rates of control fish of the same size. This growth spurt occurred during the first growth period after return to warm water, in accordance with previous findings in both Arctic charr and Atlantic salmon (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997).

Metcalfe et al. (submitted) predicted that the extent of compensatory growth would depend on the discrepancy between current state and the expected state for the time of year, and the relative cost of reducing that discrepancy. The fact that the rapid growth rates seen during spells of compensatory growth are not observed under normal conditions suggests that they incur a cost in terms of fitness. Rapid growth in salmonids is known to be associated with increased tendencies towards muscle damage (Christensen et al., 1992) and coronary arterial lesions (Saunders et al., 1992). If such costs are indeed associated with compensatory growth, then it should only occur when a growth restriction has been sufficiently disadvantageous for the benefits in terms of the size attained to outweigh the costs of compensatory growth. My results confirm this view. When comparisons could be made, groups that did not show compensatory growth were larger in size than the groups that were returned to warm water at the same time and that did compensate. More importantly, one of the groups that did not show compensatory growth was already the same size as those that had already compensated. If these fish had exhibited compensatory growth, they might have attained the same size as the control. The fact that they did not, suggests that the costs of compensatory growth would have outweighed its benefits, and that they had already reached an appropriate size for the time of year, despite the setback

in growth. My results suggest, then, that fish only initiated a period of compensatory growth if their size at the time of return to warm water was below a target size threshold for the time of year.

The fact that the temperature-manipulated fish did not compensate fully (so as to catch up completely with the size of the controls) should not be considered surprising, as the average size attained by the control fish need not be interpreted as the absolute target. While there is a positive relationship between fitness and body size at smolting, the costs of increasing the rate of growth (so as to achieve a larger smolt size) are likely to increase at an accelerating (non-linear) rate. Therefore the optimal final smolt size will vary between fish according to this trade off between the benefits of a given final size and the costs of attempting to achieve it, given the fish's current size and the time available. The conditions for growth were very good throughout the experiment: food was available in excess at all times of the day and night, while the temperature from April to September (other than during the periods of cold temperature manipulation) was close to the optimum for salmon identified by Elliot & Hurley (1997). Since the conditions for growth experienced by wild salmon are generally less favourable than this, the lower end of the genetically-determined optimal size range should be expected to be below the size attained by the controls, and therefore some of the fish in the experimental groups may have emerged from the cold at a size already within the optimal range. However, the target size range of fish that have been farmed for several generations may differ from those of wild fish, due to culling of the smallest, slowest-growing fish, thus artificially selecting for fish with larger target sizes.

The final size reached by fish in this experiment was not, of course, the size that the fish would have reached by the time of the smolt migration, as several months more would have elapsed before the smolt migration. In all but the smallest UMG fish, growth was negligible during October and November, as temperatures had fallen and food was available for a maximum of ten hours per day. Had the experiment continued, further growth would be expected prior to the smolt migration in the spring. It is therefore possible that further compensatory growth might have been observed during the spring months. Nicieza & Braña (1993) demonstrated that

the growth increment in the spring prior to smolting is inversely proportional to size in the previous autumn. Thus, larger fish that had already reached an (apparently optimal) smolt size showed little spring growth, while fish that had not reached that size grew more. Indeed, I found a similar phenomenon during the final growth period. Growth rate at this time was unrelated to length in the group with the largest mean length (the control), but in the smaller groups the relationship was strongly negative (Figure 2.4g). This can be interpreted as a compensatory response that was limited to the smallest of the UMG fish. Had the experiment continued into the spring, I predict that the same effect would have continued, allowing smaller fish to catch up in size with the larger fish, and resulting in a narrowing of the gap in size between the experimental and control groups.

However, during periods of compensation that occurred immediately after return to warm water, fish of all sizes showed compensatory growth to the same extent (indicated by non-significant interactions between group and the covariate, fork length). This indicates that fish of all sizes had fallen behind their desired growth trajectories to a similar extent (as expected, as the effects of low temperature on physiology should not be related to size), and that all fish were equally able to recoup the loss in growth. Little is known of the physiological aspects of compensatory growth, but it may be that adaptations to metabolism in the cold (such as an increase in the concentration of RNA (Foster et al., 1992) or other factors that increase the rate of protein synthesis (McCarthy & Houlihan, 1996)) are retained for some time after return to warm water, and allow growth to continue at faster than normal rates. Since such adaptations should be shared by all members of the population, they would allow all fish to show compensatory growth, even if social interactions acted to suppress growth. As I argue below, I did find some evidence for the social suppression of growth rates in smaller fish. Since these fish nevertheless showed compensatory growth, the possibility remains that physiological adaptations allowed them to grow at faster than normal rates.

Although food was supplied in abundance throughout the experiment, I nevertheless did find evidence for the competitive suppression of growth rates. In the absence of direct behavioural observations, information regarding social interactions

can be obtained by investigating the relationship between growth rates and fish size. At the start of the experiment, growth rate was unrelated to initial size. This makes sense, as social dominance in salmon parr soon after first feeding is not related to body size but to aggression and metabolic rate (Huntingford et al., 1990; Titus & Mosegaard, 1991; Metcalfe et al., 1992, 1995). However, the higher food intake and growth rates associated with social dominance soon result in a size advantage that in turn reinforces social rank. Thus, the relationship between growth rate and size soon became positive (in accordance with Jobling's (1985) assertion that a positive relationship between growth rate and size indicates that the food intake and growth of social subordinates is being suppressed by competitively superior, dominant fish). This leads to growth depensation, where small initial differences in size are reinforced, causing the variation in the size-frequency distribution to increase over time. In the present experiment, since food was supplied in excess, we would expect that most fish would be able to achieve good rates of food intake, and that social suppression of growth rates would only affect a minority of fish. We will assume, then, that most fish in the present experiment were growing at close to optimal rates, while the growth rates of a small number of fish in each tank (primarily those in the LMG) were socially suppressed. Within a species, the maximum potential growth rates of smaller fish are usually greater than those of larger fish, resulting in a negative correlation between growth rate and size (Jobling, 1985). Therefore, when there is large variation in the size-frequency distribution of a population, we would expect the maximum potential growth rate of the larger fish to be considerably lower than that of the smaller fish. During growth period 3, the size range in each tank was still relatively small, and thus the maximum potential growth rates of the largest (and presumably dominant) fish would have been similar to those of the smallest (subordinate) fish. However, as the difference in size between the largest and the smallest fish increased over time, the maximum potential growth rate of the larger fish would have declined, producing the curvilinear regression of growth rate on fork length during growth periods 4 and 5.

While the growth rates of some of the smaller fish remained suppressed and they entered the LMG, others eventually achieved growth rates more typical of their size and joined the UMG. My results are consistent with the findings of Kristinsson

et al. (1985), who found that Atlantic salmon parr were continually recruited to the UMG over the course of the autumn while temperatures remain above 10°C. However, in the present study the majority of the fish that joined the UMG did not show the growth surge typical of the smolt decision. This may have been because they were already on course for a large smolt size and thus did not need to put on a growth spurt once they had decided to join the UMG. In a model predicting the life-history decisions of Atlantic salmon, Thorpe et al. (1998) claimed that the smolt decision may sometimes appear to be based on growth rates, and sometimes on size. The present study reinforces this case. Where most fish are growing at or near their full physiological potential, the smolt decision may appear to be based on size, rather than growth rates, as the values of SGR obtained for the larger fish are similar to those obtained from the smallest fish. However, in studies where the growth rates of most fish are suppressed, and the size differential between the smallest and largest fish is relatively small, the decision should appear to be based on growth rate as the values of SGR of the larger fish might be greater than those of the smaller fish. In the latter case, final size would be better predicted by growth rate than by current body size. Clearly, growth rate, body size and social factors should not be studied in isolation.

Table 2.1: (a) Periods spent in cold water by six groups of juvenile Atlantic salmon. **(b)** Growth periods between measurement dates

(a)

	Cold water period:	
Group	Start date	End date
B	25 April	17 May
C	25 April	7 June
D	16 May	7 June
E	7 June	28 June
F	7 June	23 July
G	29 June	23 July

(b)

	Measurement Dates at:	
Growth Period	Start of growth period	End of growth period
1	21-27 April	14-20 May
2	14-20 May	31 May - 7 June
3	31 May - 7 June	28 June - 4 July
4	28 June - 4 July	22-25 July
5	22-25 July	19-22 August
6	19-22 August	17-20 September
7	17-20 September	12-15 November

Table 2.2: Percentage of fish belonging to the Lower Modal Group (LMG) (i.e. with a fork length of 100 mm or less) in seven groups of juvenile Atlantic salmon. Groups B, D, E and G spent three weeks, and groups C and F spent six weeks, at atypically cold temperatures. Group A is the control. Data include all marked and unmarked fish that survived to the end of the experiment.

Group	n	Percentage of fish in LMG
A	83	1.5%
B	135	2.2%
C	147	15.5%
D	132	7.0%
E	187	4.6%
F	145	11.6%
G	124	3.1%

Table 2.3: Analyses of covariance of Specific Growth Rate in length (SGRL) against initial length at the start of seven growth periods for seven treatment groups of juvenile Atlantic salmon. The ANCOVA was performed first including an interaction term between group and SGR (to test for homogeneity of regression slopes) and, if the interaction term was not significant, was repeated without it to test for differences in elevations. Groups that differed significantly in elevation from the control are identified in Figure 2.4. Statistically significant p-values are given in bold type. Separate ANCOVA's were performed for fish ≤ 88 mm and > 88 mm in initial length for periods 4, 5 and 6 (data not presented for fish < 88 mm in period 6 as they were too few in number). See text for explanation.

Growth Period	Effects	With Interaction Term			Without Interaction Term		
		df	F	p	df	F	p
1	Group	2	1.1	0.345	2	234.4	**<0.001**
	Fork length	1	0.3	0.570	1	0.4	0.512
	Interaction	2	0.1	0.945			
	Error	190			192		
2	Group	3	1.7	0.165	3	126.1	**<0.001**
	Fork length	1	0.2	0.668	1	1.0	0.314
	Interaction	3	0.7	0.563			
	Error	188			191		
3	Group	5	2.0	0.081	5	433.3	**<0.001**
	Fork length	1	23.9	**<0.001**	1	42.5	**<0.001**
	Interaction	5	1.4	0.222			
	Error	331			336		
4	Group	6	2.0	0.067	6	135.0	**<0.001**
(≤ 88 mm)	Fork length	1	62.7	**<0.001**	1	61.2	**<0.001**
	Interaction	6	1.9	0.088			
	Error	254			260		
4	Group	3	1.7	0.184	3	136.6	**<0.001**
(> 88 mm)	Fork length	1	0.6	0.435	1	76.5	**<0.001**
	Interaction	3	0.9	0.426			
	Error	64			67		
5	Group	6	1.8	0.103	6	15.2	**<0.001**
(≤ 88 mm)	Fork length	1	32.8	**<0.001**	1	42.0	**<0.001**
	Interaction	6	1.3	0.251			
	Error	122			128		
5	Group	6	1.5	0.187	6	17.1	**<0.001**
(> 88 mm)	Fork length	1	15.1	**<0.001**	1	119.7	**<0.001**
	Interaction	6	1.0	0.445			
	Error	193			199		
6	Group	6	2.5	**0.023**	6	11.1	**<0.001**
(> 88 mm)	Fork length	1	247.1	**<0.001**	1	301.6	**<0.001**
	Interaction	6	2.0	0.062			
	Error	305			311		
7	Group	6	7.6	**<0.001**			
	Fork length	1	237.9	**<0.001**			
	Interaction	6	8.1	**<0.001**			
	Error	329					

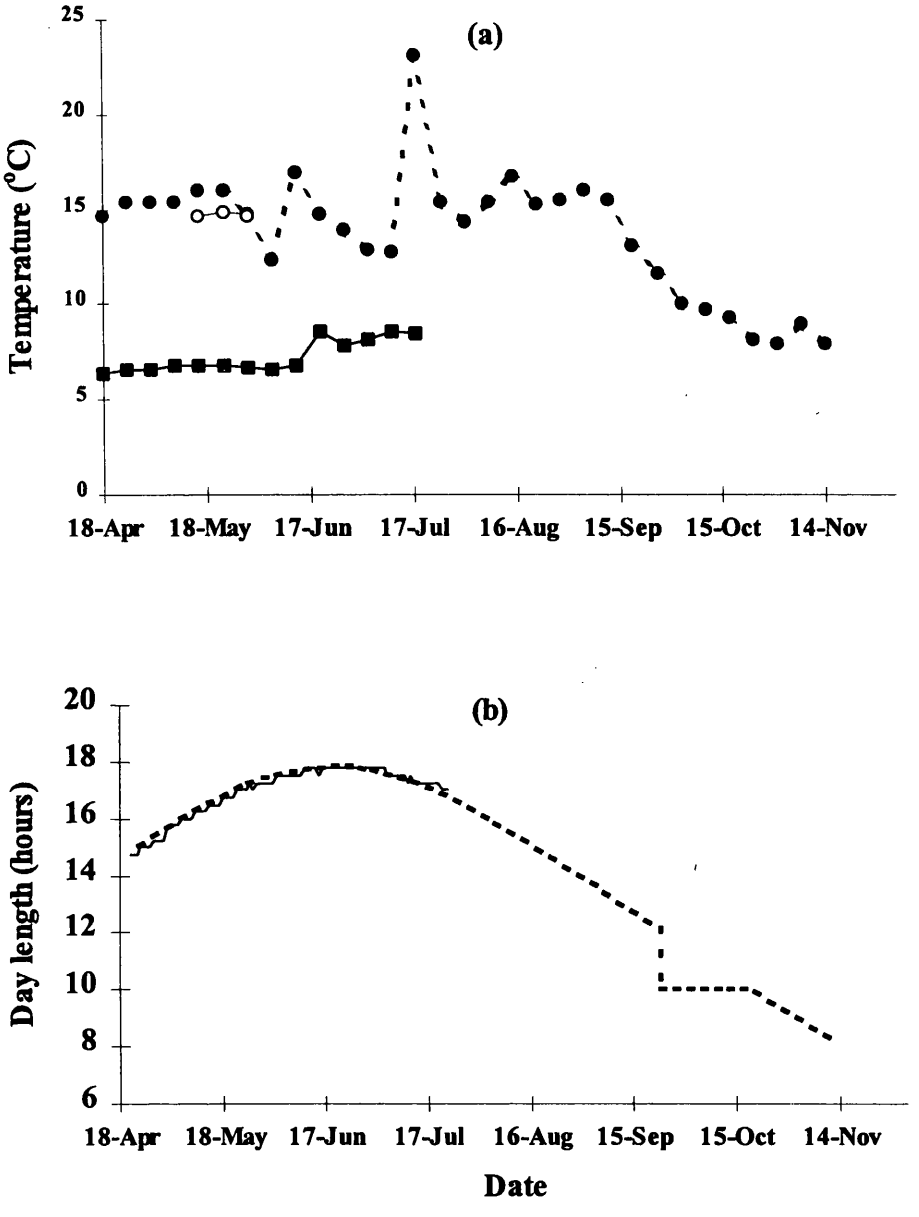

Figure 2.1
(a) Mean weekly daytime temperatures and **(b)** photoperiod during the course of the experiment. Filled circles and dashed lines indicate conditions experienced by the control throughout and by groups A-C except when subjected to the cold water manipulation (squares and solid lines). Open circles indicate the conditions experienced by group B while at the University Field Station, Rowardennan.

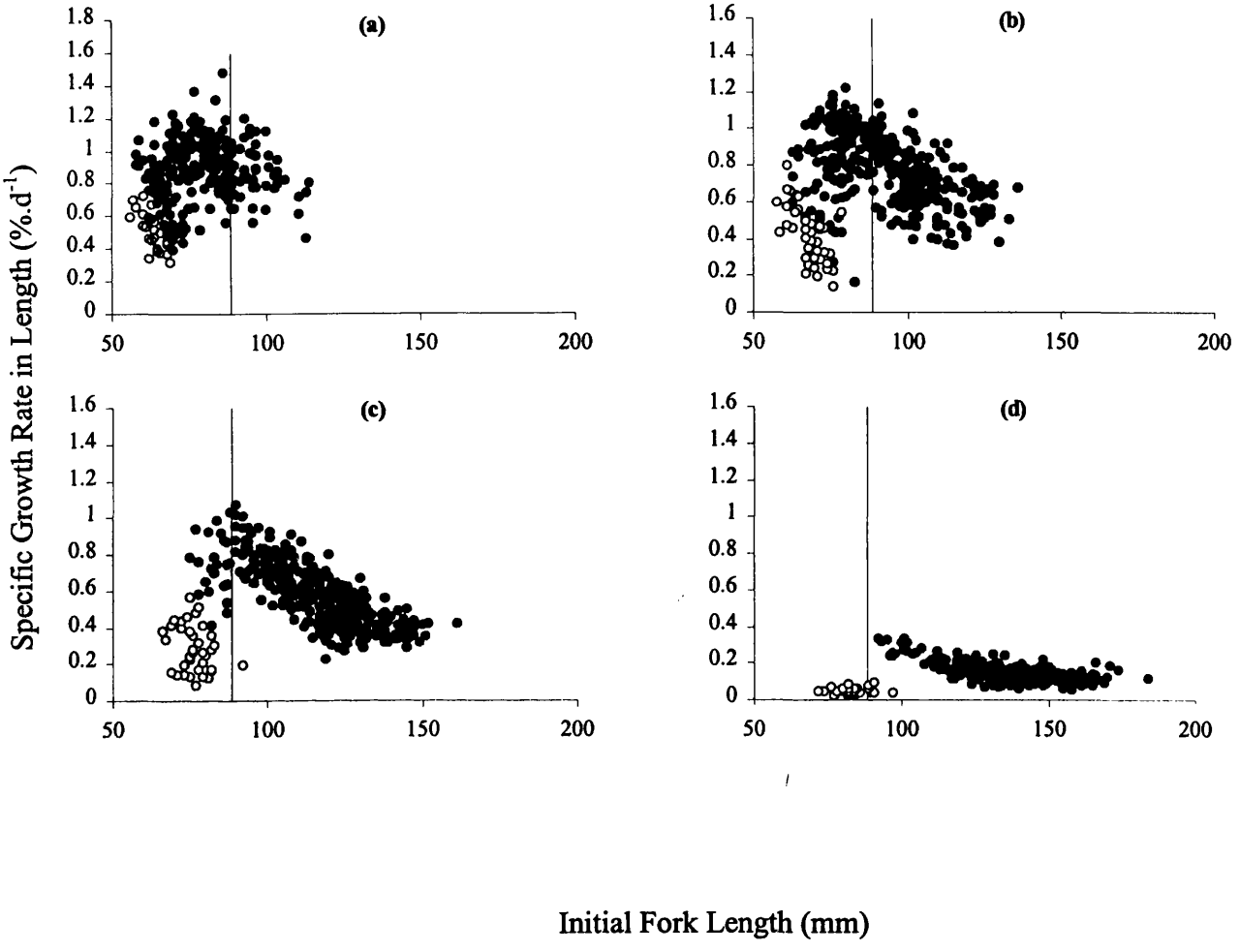

Figure 2.2
Plots of SGRL against initial fork length (FL) at the start of a given growth period, for growth periods **(a)** 4 **(b)** 5 **(c)** 6 and **(d)** 7. Open circles represent fish belonging to the LMG (final length < 100 mm). Closed circles represent fish belonging to the UMG (final length > 100 mm). Groups F and G are not represented in **(a)** as they were in cold water at the time. The vertical line at FL = 88.5 mm indicates the asymptotic value of FL derived from the regression equation of control group SGRL on FL and FL^2 in periods 4 and 5.

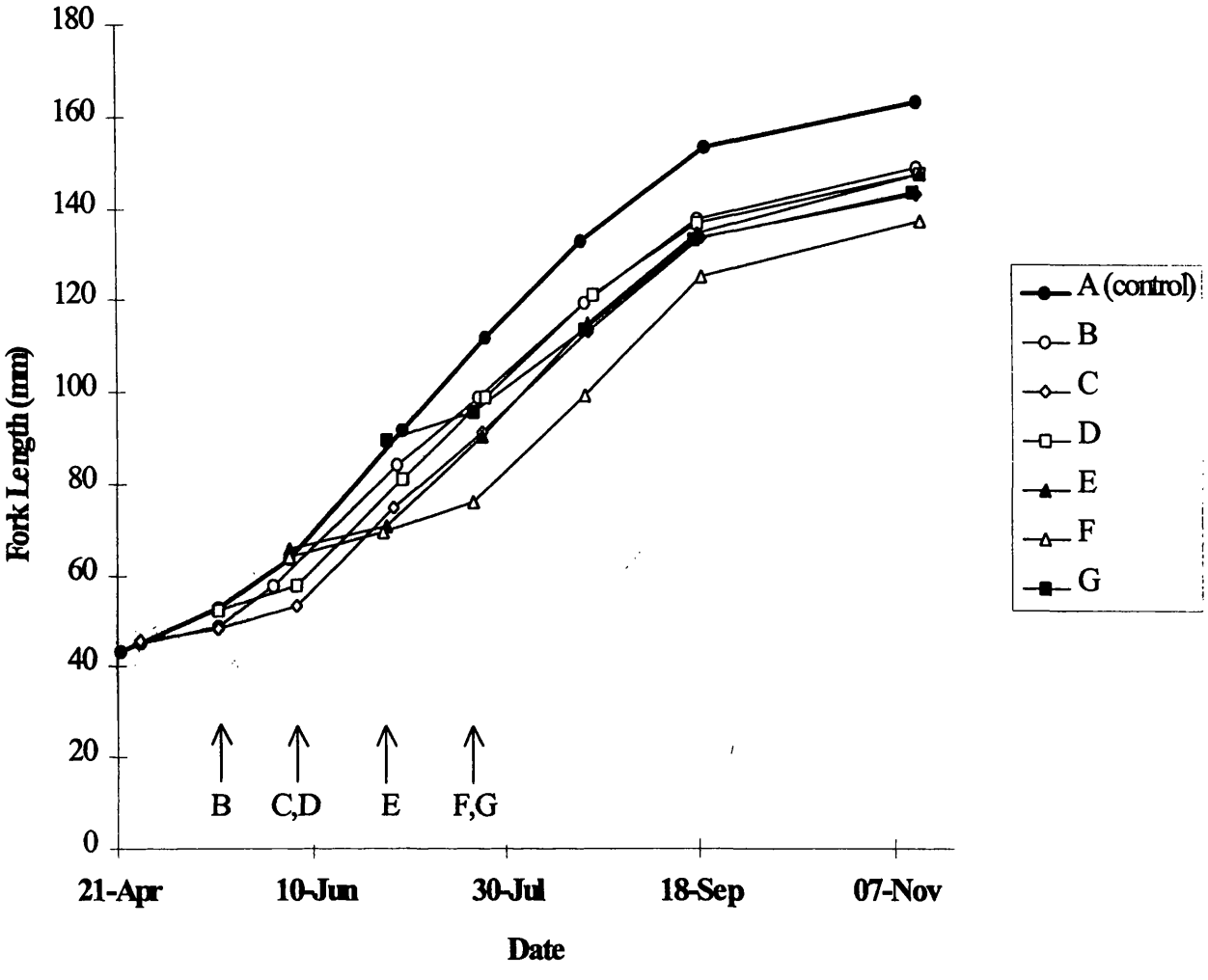

Figure 2.3
Mean fork length (mm) of UMG fish in seven groups of farmed Atlantic salmon parr during the experiment. Arrows indicate the time of return to warm water of the group indicated by the accompanying letter. Standard errors have been omitted to improve clarity, but varied between 0.25 and 2.23.

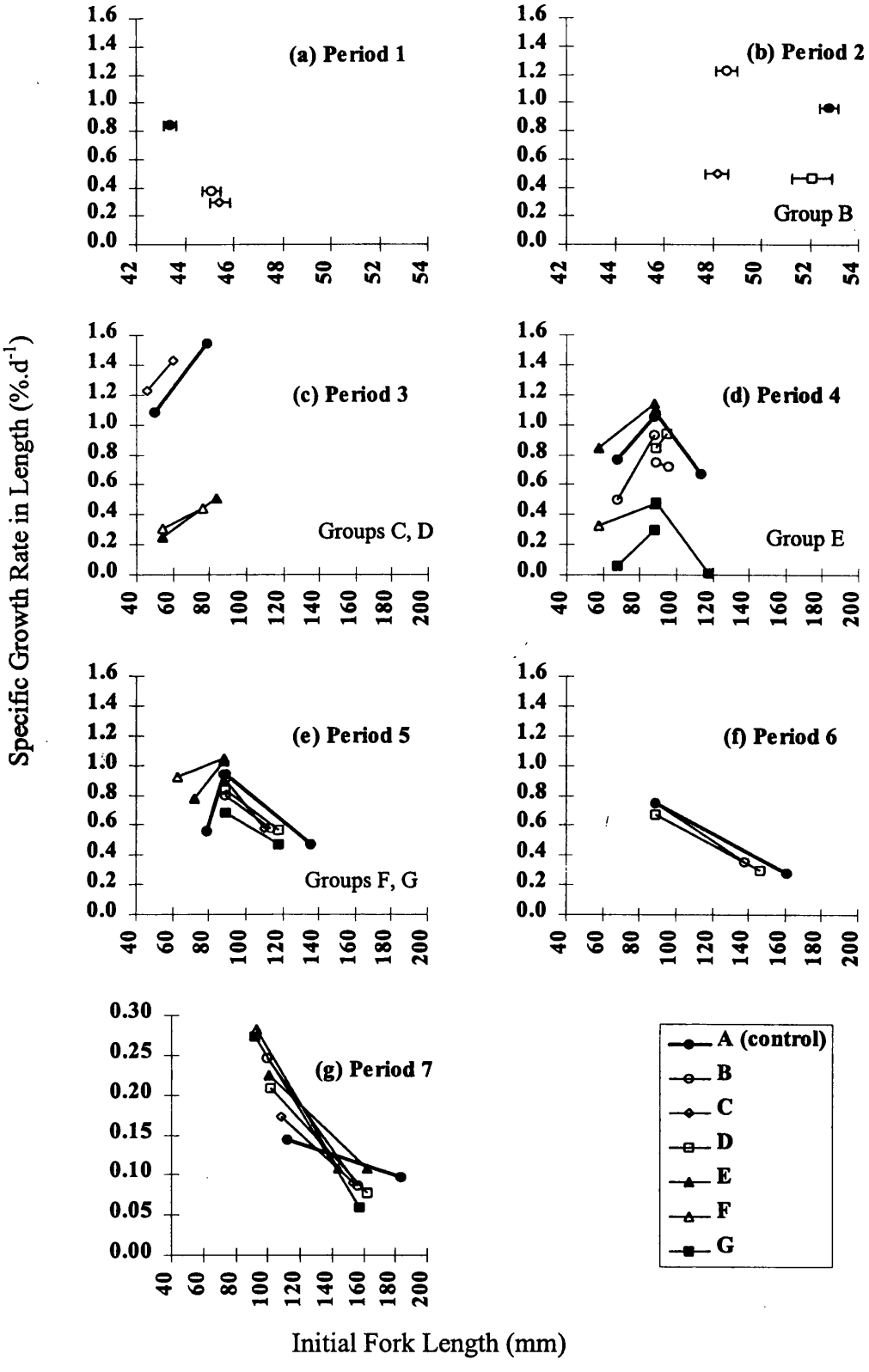

Figure 2.4

Comparison over seven time periods of growth rates (SGRL) of Atlantic salmon parr exposed to seven treatments. Data are only shown for the control group and for those treatment groups whose growth rates differed significantly from the control using the sequential Bonferroni criterion (Rice, 1989) (see Table 2.3 for statistical analyses). **(a)** and **(b)** show the mean initial fork length (±SE) and mean SGRL of each group, as statistical analysis showed no effect of the covariate (fork length). Standard errors for SGRL are not shown due to their small size, but varied from 0.02-0.04. **(c)** to **(g)** show the regression lines of SGRL on initial fork length for each group, plotted over the range of initial fork lengths found in that group. Data for fish with fork length ≤ 88 mm and > 88 mm are treated separately (see text for details). Note that the axes scales of **(a)**, **(b)** and **(g)** differ from those of **(c)** to **(f)**. The letters in the bottom right corners of (b) to (e) indicate the groups that were returned to warm water at the start of the relevant growth period.

Chapter 3: Social status, access to food, and the behavioural basis for compensatory growth in juvenile Atlantic salmon

3.1 Introduction

There is considerable evidence that many animals have ideal growth schedules or growth trajectories that they seek to maintain, and that they adjust growth rates to compensate for any deviation from these trajectories (Arendt, 1997). Much evidence for this phenomenon comes from studies of compensatory growth, in which animals that have experienced starvation or reduced food rations will put on a growth spurt over and above normal growth rates when food levels are restored. Compensatory growth is a wide-spread phenomenon in the animal kingdom, and has been investigated in several vertebrate taxa, including mammals and birds (reviewed by Wilson & Osbourn (1960) and Lawrence & Fowler (1997)). Most work regarding compensatory growth has focused on farm animals with a view to reducing the cost of food and increasing production efficiency (e.g. Greeff et al., 1986; Kindschi, 1988), with the result that the ecological and evolutionary implications of compensatory growth have been largely ignored. Nevertheless, compensatory growth does pose interesting questions in this regard.

Compensatory growth has been demonstrated at various stages of the life-cycle in salmonid fish such as rainbow trout *Oncorhynchus mykiss* (Weatherley & Gill, 1981; Dobson & Holmes, 1984; Kindschi, 1988; Quinton & Blake, 1990), Arctic charr *Salvelinus alpinus* (Miglavs & Jobling, 1989; Jobling et al., 1993; Mortensen & Damsgård, 1993), sockeye salmon *Oncorhynchus nerka* (Bilton & Robins, 1973) and Atlantic salmon *Salmo salar* (Mortensen & Damsgård, 1993; Reimers et al., 1993). This comes as no surprise given that in salmonids, life-history pathways are not fixed and life-history decisions depend on growth rates and/or size during decision windows several months prior to the transformation itself. For instance, the decision to become sexually mature as parr (the freshwater stage of

salmonids) is linked to growth rates or fat deposition rates in the spring, although the maturation process itself is not initiated until later in the year (Rowe & Thorpe, 1990a; Rowe et al., 1991; Berglund, 1992; Silverstein et al., 1997). Similarly, the decision to smolt (the process of physiological, behavioural and morphological changes that equip the fish for migration to, and life in, the sea) appears to be taken by midsummer in the year preceding the spring migration and is based on growth rates around that time (Wright et al., 1990). The faster-growing fish will continue growing during the autumn and winter and will smolt the following spring, while slower-growing fish will arrest growth until the spring and will remain in fresh water for at least another year (reviewed by Saunders et al., 1994a). Once the decision to smolt has been taken it is not reversible (Thorpe & Metcalfe, 1998) and, since larger fish have higher survival rates in sea water (Holtby et al., 1990; Lundqvist et al., 1994) and successful transfer from fresh to sea water is limited to a few weeks in the spring (Lundqvist et al., 1994), there should be strong selection pressure to reach a large size by the time of the smolt migration. The ability to compensate for set-backs in growth is an important adaptation that allows the fish to remain on target in a fluctuating and unpredictable environment.

The mechanisms that mediate compensatory growth are not well understood. Unless all the adjustments that allow compensatory growth are physiological, behavioural adjustments must be involved as high growth rates require an increase in food intake. Alterations in the behaviour of salmonids during compensatory growth after food-restriction or starvation involve increased risk-taking (Damsgård & Dill, 1998), an increase in aggression (Nicieza & Metcalfe, 1997) and hyperphagia (Jobling & Koskela, 1996; Miglavs & Jobling, 1989). Hyperphagia has also been reported in minnows *Phoxinus phoxinus* (Russell & Wootton, 1992) and hybrid sunfish *Lepomis spp.* (Hayward et al., 1997). Presumably in these cases the fish respond to feelings of hunger and seek to increase food intake directly in response to their nutritional requirements. Indeed, they appear to regulate both their physiology and feeding behaviour according to the ratio of fat stores to structural tissue (Broekhuizen et al., 1994; Jobling & Miglavs, 1993).

However, growth compensation after a period of abnormally low temperature without food restriction should not be regulated by the same mechanism, since fish that have been fed to satiation throughout the low temperature period should have grown at a slower rate than normal but should not have depleted fat reserves and may not feel hungry as such. Nevertheless, compensatory growth has been demonstrated after a period of water temperatures atypically cold for the time of year in Arctic charr (Mortensen & Damsgård, 1993) and Atlantic salmon (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997). While fish have little influence over the temperature of their immediate environment, their appetite may be affected by previous growth history. Therefore part of the compensatory response after medium-term (several days or weeks) reductions in temperature may involve alterations in feeding behaviour that lead to increased food intake. The behavioural option used may depend on social status, the nature of the food supply (reliability and abundance) and the complexity of the environment. Nicieza & Metcalfe (1997) found that fish previously exposed to colder than normal temperatures did not subsequently become more aggressive than controls, and appeared instead to spend longer feeding per day. However, this was in the presence of larger fish, previously given other experimental treatments, and so most of the temperature-manipulated fish were probably socially subordinate. Other behavioural responses may be involved where all fish in a group are growth-compensating after temperature manipulation, and the fish involved cover the whole range of the social hierarchy.

In this chapter, I use automated monitoring systems to examine how fish of differing social status altered their behaviour to achieve compensation after a period of cold temperatures that suppressed growth. I show that fish attempting to compensate become more aggressive and that, in a situation where food can be defended, this results in a greater polarisation of the dominance hierarchy.

3.2 Materials and Methods

The experiment was conducted between 30 May and 23 December 1997. The fish were farmed Atlantic salmon parr from pooled hatchery stock and originated

from Marine Harvest McConnell's (MHM) freshwater site at Invergarry, NW Scotland. There were two groups of fish, control and low-temperature, (LT), each of which initially consisted of 1,150 (±7%) fish. On 30 May the LT stock group was transferred to Glasgow University's aquaria where they spent three weeks in cooled water (8.4°C ± 0.03), while the control group remained at Invergarry at ambient water temperature (mean of 16.4°C ± 0.03). On 19-20 June, random samples of 135 (± 5) fish per group were retained for use in the present experiment (the rest of the fish being used in the experiments reported in Chapters 4-7). On 20 June, the control group was transferred to an adjacent tank in the aquaria in Glasgow, and the water temperature was adjusted to an intermediate level for both groups (13.8°C ± 0.1) for the remainder of the experiment. Throughout the experiment the tanks were lit by overhead fluorescent strip-lights; the photoperiod regime was that used commercially to produce accelerated "S½" smolts, with long days (22L:2D) followed by a photoperiod "winter" (10L:14D) in the (real) autumn.

The fish were kept in small, circular tanks (diameter 0.6 m, water depth 0.25 m at Invergarry and 0.3 m at Glasgow) until 22 August when both groups were transferred to larger tanks (1 m x 1 m, water depth 0.3 m). The fish remained in these stock tanks until the end of the experiment except when they were being used in the tanks used to monitor behaviour (see below). The fish were fed to excess throughout the experiment on a pelleted diet of an appropriate size according to manufacturer's tables. Food was dispensed from clockwork belt feeders at a trickle rate 24 h per day.

Random samples of 150 fish (of the 1,150) from each group were measured on 29-30 May and 19-20 June, and all of the fish were measured on 7 August, 26 August, 24 September, 6 November and 23 December. The measured fish on 19-20 June included all the fish that were retained for use in the experiments. On each occasion, the fish were anaesthetised in an aerated water bath using Benzocaine in 95% alcohol, and then measurements were made of fork length (to 1 mm) and weight (to 0.01 g, after blotting with damp tissue paper to remove excess water). On 26 August, all the fish were measured and tagged with Passive Integrated Transponder (PIT) tags. Tagging was delayed until this date so that fish were large enough

(smallest fish on 26 August = 2.5 g). The PIT tags were inserted into the body cavity through an incision made in the body wall. The entry wound was dusted with a 50:50 mix of Cicatrin™ antibiotic powder (Wellcome Foundation Ltd, London, U.K.) and Orahesive™ Protective Powder (ER Squibb & Sons, Hounslow, U.K.).

After tagging, individual Specific Growth Rates in fork length (SGR) between measurement periods were calculated as:

$$SGR = 100 \times [\ln (FL_{t2}) - \ln (FL_{t1})] / (t_2 - t_1),$$

where t_1 = first sampling day, t_2 = second sampling day, and FL = fork length.

An indirect measure of aggression in salmonids is the prevalence of dorsal fin damage (as the fin is damaged as a consequence of bites made during aggressive attacks) (Turnbull et al., 1996, 1998). Therefore, on each of the measurement days, splitting of the dorsal fin was assessed as either absent or present.

Four sets of three replicate behavioural trials were conducted between 15 September and 23 December 1997. Table 3.1 shows the timing of the trials. Each trial took three weeks and there were six trials using control fish and five using LT fish (as the PIT tag monitoring equipment failed during the sixth LT trial). A plan view of the tank design for the behavioural experiments is shown in Figure 3.1. The tanks were 1 m x 1 m with a water depth of 0.3 m. Each was divided into three sections: a feeding area, a sheltering area and a corridor between the two. An antenna at either end of the corridor detected the identity of each PIT tag as the fish passed, and the time of day, date and individual ID number of the fish were automatically recorded on a computer file. A mesh screen prevented entry to the feeding area from the other direction, so all visits to the feeding area were logged. A pump at one end of the feeding area provided water circulation. Food pellets dropped into the tank 24 hours per day from a belt feeder situated at the upstream end of the feeding area, and the water flow was adjusted so that all uneaten pellets would drop to the tank floor and be collected by a low baffle rather than be carried in the current into the corridor. Therefore fish had to enter the feeding area to obtain food. The sheltering area was kept dark (1 lux during the day and < 0.01 lux at night) using a black plastic cover,

while the feeding area and the corridor were brightly lit during the day (180 and 130 lux respectively) and dimly lit at night (1.3 and 0.5 lux respectively).

On the first day of each trial, eight fish were selected from the stock tank, measured, assessed for dorsal fin damage, and given a unique combination of alcian blue dye fin marks. The fish used were within the size range 75-140 mm fork length, but within a trial they never varied by more than 31 mm in length. The fish were first confined by a mesh screen to the feeding area of the behavioural tank, and allowed to settle for 2-3 days. Dominance ranks were assigned on days 3 to 10, using a serial removal technique modified from Metcalfe et al. (1989). The procedure adopted each day was as follows. A single food pellet was dropped into the tank at the upstream end of the feeding area. The first fish to ingest the pellet (even if it was subsequently rejected) was given three points. Any fish that performed an aggressive act (nipping or chasing another fish) at the time of this feeding event was given two points, while the fish that was holding station nearest to the feeder at this moment was given one point. Each dominance ranking session involved ten repetitions of this procedure (i.e. 10 food pellets, separated by at least five minutes). The fish that scored the most points during a session was judged to be the dominant fish, and was removed from the tank. The procedure was then repeated on the remaining fish, with intervals of at least eight hours between sessions, until five fish of the eight in each tank had been assigned a rank. The remaining fish were given a joint rank of 6. If there was no clear dominant or if the fish were disturbed during the ranking procedure, no dominance rank was assigned and the session was repeated. At the end of the dominance ranking period, the mesh screen confining the remaining fish to the feeding area was removed, giving all eight fish access to all areas of the tank.

The eight fish were then allowed to feed in the tanks undisturbed until the end of the trial, and their activity patterns in the third week (as logged by the PIT tag antennae) were analysed. Since there were two antennae, the direction of travel of the fish could be computed and from this the total length of time each fish spent in the feeding area could be calculated. The nature of the data means that "time spent in the feeding area" is actually time definitely not spent in the sheltering area, and thus

includes some time spent in the corridor.

Seven fish died during the course of the trials. These fish were excluded from analysis and the dominance ranks of the remaining fish were adjusted accordingly. The PIT tags of a further two fish did not register reliably during the trials, and so these fish were excluded from analysis but, since they were present throughout the trials, the ranks of the other fish were not adjusted. Final group size was never less than six fish with functional tags per trial.

On the last day of each trial, the food supplied to the fish was swapped for the same food labelled with X-ray-dense ballotini beads (size 9, Jencons Ltd., Leighton Buzzard, UK) for the four hours between 0900 and 1300. The fish were then anaesthetised and X-rayed to determine the amount of labelled food ingested (Talbot & Higgins, 1983; Jobling et al., 1989; McCarthy et al., 1992). This allowed us to relate actual food intake to time spent in the feeding area while the labelled food was available. The fish were then measured and returned to the stock tanks; each fish was only used in one behavioural trial.

3.3 Results

Growth rates in stock tanks

Individual growth rates were not available prior to tagging so group growth rates were calculated using the following method. For each group, I calculated the natural logarithm of individual fork lengths at the start and end of each growth interval. I then calculated the regression equations of Ln (fork length) against the number of days since the start of the experiment. The slope (b) of the regression line is equivalent to SGR/100. The slopes of the LT and control regression lines could then be compared for each growth period (Table 3.2a). While the LT group was in cold water, its growth rate was (as expected) significantly lower than that of the control, but no further differences in growth rates were apparent prior to tagging.

After tagging, individual growth rates could be calculated and compared using analysis of covariance (ANCOVA) of SGR with fork length at the start of each growth period ("initial fork length") as a covariate (Table 3.2b). Only fish that survived to the end of the experiment and were immature smolts in December (minimum fork length of 105 mm) have been included, while fish that were sexually maturing, delayed smolting or died before the end of the experiment have been excluded. Fish that were used in behavioural experiments during a growth period have also been excluded from the analysis for that growth period. There was no difference between the growth rates of LT and control fish between 26 August and 24 September (Table 3.2b, Figure 3.2a). However, during the next growth period (24 September to 6 November) the LT group exhibited a clear compensatory growth spurt: fish in this group grew significantly faster on average than fish of the same size in the control group (Table 3.2b, Figure 3.2b). In the following period (6 November to 23 December) the LT group was still growing faster than the control (Table 3.2b), but the difference between the two groups was so small (Figure 3.2c) that it was likely to be of little biological significance.

At the end of the temperature-manipulation period, the LT group was smaller than the control group (LT mean fork length 50.4 mm $\pm$ 0.3; Control mean 55.1 mm $\pm$ 0.4; one-way analysis of variance (ANOVA): $F_{1, 299} = 71.3$, $p < 0.001$) and remained so up to and including 24 September (LT mean 91.0 mm $\pm$ 2.3; Control mean 103.5 mm $\pm$ 2.4; $F_{1, 63} = 12.0$, $p < 0.002$). However, the strong compensatory growth spurt between 24 September and 6 November resulted in the LT fish catching up with the controls, so that there was no difference in the mean lengths of the two groups from 6 November onwards (LT mean 115.8 mm $\pm$ 2.3; Control mean 117.7 mm $\pm$ 2.8; $F_{1, 57} = 0.3$, n.s.). Since there was a clear difference in the strength of the compensatory response in the growth periods before and after 6 November, the behavioural data have been split between these two periods, termed periods A and B respectively.

Use of Feeding Area

Although there was a positive correlation between time spent in the feeding area and food intake (Spearman rank correlation, $r_s = 0.61$, $n = 36$, $p < 0.01$), there was considerable spread in the data: while high food intake was only associated with longer periods in the feeding area, long periods did not always result in high food intake (Figure 3.3a). It should also be noted that some fish were able to obtain small amounts of food without entering the feeding area. There was a similar relationship between growth rates and the total amount of time spent in the feeding area during the final week of the trial (Figure 3.3b). Since SGR for a constant relative food intake is inversely related to body size (Jobling, 1985), the SGRs have been adjusted accordingly: SGR is expressed as the residual from the regression line of SGR on initial fork length for the control group in the stock tank between 24 September and 23 December. Residual SGR is therefore the deviation from the mean growth rate expected for a fish of that size. There was a significant relationship between residual SGR and time spent in the feeding area (square-root transformed to produce a linear regression) (r^2 (adjusted) = 34.4%, $p < 0.001$, d.f. = 77). However, the relationship was curvilinear, approaching a plateau in growth rate at longer durations of time in the feeding area.

After dominance ranking, the fish were classed as either dominant (dominance rank 1 or 2) or subordinate (all other ranks). Data from periods A and B were analysed separately. I compared the use of the feeding area by the different classes of fish using repeated measures ANOVA with group (LT or control) and rank (dominant or subordinate) as between-subjects factors and time of day (day or night) as the within-subjects factor (Table 3.3). The proportion of the available time that the fish spent in the feeding area over the course of the experiment (Figure 3.4 a-b) differed between the day and night, with fish spending larger proportions of the night in the feeding area. However, the effect of time of day on time spent in the feeding area did not differ between the four classes of fish. During period A, the LT and control groups did not differ in the proportion of time they spent in the feeding area,

but social rank had a strong effect: dominant fish spent a larger proportion of the available time there than did subordinates. During period B, the classes of fish did not differ significantly in the proportion of time they spent in the feeding area, although the effect of time of day was still in evidence.

In order to compare the relative use of the feeding area by dominant and subordinate fish in each class, and to standardise for differences between trials in the length of time spent in the feeding area, I converted the absolute length of time spent in the feeding area into an index of dominance of the feeding area (Figure 3.4 c-d). For each fish, the total length of time spent in the feeding area (by day and night separately) was divided by the sum of the time spent in the feeding area (by day or night) by all the fish in the same tank and then multiplied by 100. When there were fewer than eight fish per tank, the result was adjusted proportionately to account for the number of fish in the tank. A dominance index of 12.5 for all fish in a tank would indicate that time spent in the feeding area was equally shared between the fish, irrespective of the absolute length of time involved. Once this adjustment had been made, time of day had no effect on dominance index, but there were significant differences between classes of fish during period A (and not during period B) (Table 3.3). While there was no overall effect of group (LT or control) on dominance index, rank had a strong effect and there was a significant interaction between rank and group. Thus, the dominant fish in the LT treatment dominated the feeding area to a far greater extent than did their equivalents in the control groups, and the effect was equally strong during both the day and the night. This had the effect that the inequality between dominant and subordinate fish in time spent in the feeding area was greater amongst LT fish than amongst control fish.

During period A, there was a tendency for subordinate LT fish to grow more slowly than the other groups, while the LT dominants grew marginally faster than the controls (Figure 3.5). However, two-way analyses of variance (Table 3.4) revealed no significant effect of group or rank, although the effect of rank was nearly significant. At this time, in all classes except for the LT subordinate class, growth rates were elevated above those found in the control stock tank. During period B,

there were no significant differences between the growth rates of the four classes of fish, and growth rates were closer to those found in the control stock tank.

Residual SGR was positively correlated with social dominance rank (1 to 6) in the LT behavioural tanks during period A but not during period B or in the controls during either period (Table 3.5). Dominance of the feeding area was similarly correlated with social dominance rank amongst LT fish during period A, but not otherwise (Table 3.5).

Changing rates of aggression were indicated by variation in the incidence of fin damage. At the end of period A, there was a higher incidence of split fins in the LT stock tank than the control stock tank (50% of 36 LT fish had split fins, compared to 27% of 41 control fish; $\chi^2 = 4.4$, 1 d.f., $p < 0.04$). There were no such differences between stock tanks at the end of period B (9% of 22 LT fish; 9% of 35 control fish ; $\chi^2 = 0.9$, 1 d.f., n.s.). The incidence of split fins was considerably higher in the stock tanks than in the behavioural tanks. In the latter, although there was no difference between LT and control groups at the start of the trials (35% of 20 LT fish; 36% of 22 Control fish; $\chi^2 = 0.01$, 1 d.f., n.s.), a higher percentage of LT fish had split fins than controls at the end of trials during period A (30% of 20 LT fish; 5% of 22 Control fish; $\chi^2 = 4.9$, 1 d.f., $p < 0.03$), although most (four out of six), were in a single LT tank. The incidence of fin splitting did not differ between LT and control fish at the end of the trials in period B (21% of 14 LT fish; 27% of 22 Control fish; $\chi^2 = 0.2$, 1 d.f., n.s.).

The patterns of fin damage broadly corresponded to levels of observed aggression during the dominance ranking sessions. In period A, a mean of 1.1 aggressive acts were recorded per observation session in the LT tanks but only 0.02 in the controls. (It should be noted that most of the aggression, 16 out of a total of 20 aggressive acts, occurred in a single tank. This was also the tank that had the highest incidence in fin damage at the end of the trial). During period B, the equivalent numbers were 0.1 for LT and 0.4 for control fish. Thus, the evidence from fin damage and direct observation indicates that there was more aggression during

periods of compensatory growth than during periods of normal growth.

3.4 Discussion

As expected, the period in cooled water in the spring resulted in a significant setback in growth for the LT group. This setback was compensated for by a growth spurt later in the year, with the result that there was no difference between the LT and control groups in the mean lengths of immature smolts by the start of November. The growth spurt shown by the LT fish is a genuine example of compensatory growth, and is not simply an effect of the negative relationship between length and SGR that is common amongst fish (Jobling, 1985), since the LT fish grew faster than control fish *of the same size.*

Studies of compensatory growth after a period of starvation or food restriction do not show a prolonged delay between the end of the growth setback and the onset of compensatory growth (Bilton & Robbins, 1973; Dobson & Holmes, 1984; Jobling et al., 1993; Kindschi, 1988; Quinton & Blake, 1990; Nicieza & Metcalfe, 1997), and it has been suggested that the compensatory growth spurt occurs in response to an assessment of the ratio of storage tissues (e.g. fat deposits) to structural tissues (e.g. bone) (Jobling & Miglavs, 1993; Broekhuizen et al., 1994). However this does not explain why compensatory growth occurs after periods of low temperature or other types of growth setback, and it appears that compensatory growth is instead a response to an assessment of absolute body size in comparison with a target size for the time of year (Metcalfe et al., submitted). In previous studies of compensatory growth after temperature manipulation in salmonid fish, conducted under natural photoperiod, compensation was evident within a month of the end of temperature manipulation (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997; Chapter 2). However, in the present study, there was a delay of ca. 13 weeks before the onset of compensatory growth. It may be that the absence of seasonal cues meant that the fish had no means of comparing their body size to an expected size for the time of year. Thus, compensatory growth was only initiated when the fall in daylength at the start of the photoperiod winter gave them a cue as to the time of year.

The compensatory growth spurt was accompanied by significant behavioural differences between LT and control fish. These differences were evident during period A, when the fish in the stock tank exhibited strong compensatory growth, but not during period B (when compensation in the stock tank was statistically detectable but weak and probably of little biological significance). In the stock tanks during the compensating period (but not at other times), fin damage was significantly more common amongst LT fish than controls. Since dorsal fin damage in salmonids is largely inflicted during aggressive interactions (Abbott & Dill, 1985; Turnbull et al., 1998), its prevalence can be used as an index of the level of aggression within groups of fish (Christiansen & Jobling, 1990; Moutou et al., 1998). We can therefore conclude that the overall level of aggression increased during the period of compensatory growth. In contrast, Nicieza & Metcalfe (1997) found that fish that were growth-compensating after a period of low temperature were not more aggressive than controls, unlike fish that were growth-compensating after food restriction. Differences in experimental design may explain the discrepancy between the two studies. In Nicieza & Metcalfe's (1997) study, the LT fish shared a tank with the control population and the food-restricted fish, but due to their smaller size they were probably socially subordinate to the larger controls and food-restricted fish. In such a situation, it may have been unprofitable for the LT fish to engage in aggressive interactions with a population of dominant fish, while in the present experiment, there were no such restrictions on aggressive behaviour.

Nicieza & Metcalfe (1997) also found that fish that were growth-compensating after a period of low temperature fed for longer each day than controls, thereby (presumably) increasing their overall food intake. In contrast, I found that although dominant fish spent more time in the feeding area than subordinates, neither dominant nor subordinate LT fish spent longer in the feeding area than their equivalents in the control group. Again, the differences in experimental design may explain this disparity, as Nicieza & Metcalfe's (1997) fish may have altered their feeding strategy due to their lower social status. Such adjustments in the time of feeding have been demonstrated in Atlantic salmon post-smolts, where smaller fish

feed at different times of day from larger (dominant) fish (Kadri et al., 1997a). In my study, however, the total amount of time spent in the feeding area may not be a very good indicator of the time actually spent feeding. While food intake and residual growth rates were positively related to time spent in the feeding area, the relationship was far from perfect. This is to be expected as individual variations in metabolic rate, stress, activity, feeding efficiency and social status would all increase the variance in this relationship. However, it appears that some food was available outwith the feeding area, as some fish that never entered the feeding area did have low levels of food intake: water currents created by moving fish may have swept some food out of the feeding area into the corridor. Moreover, reasonable growth rates were attained even by fish that spent very little time in the feeding area, probably because when food is supplied in abundance, even short feeding excursions can result in a high rate of food intake and rapid growth (Metcalfe et al., 1999). However, I found that the highest levels of food intake and growth rates were only found amongst fish that spent long periods in the feeding area. Thus, although the feeding area certainly provided the most immediate and reliable access to food, time spent there is perhaps best understood not as time spent feeding *per se*, but as time in possession of the best feeding territory. Hence fish that spent longer periods in the feeding area were more successful competitors than fish that spent little time there. If we adopt this view, the partitioning of time spent in the feeding area between fish in the same tank may be a more useful indicator of social interactions than the absolute length of time spent there, and is similar in meaning to the share of group meal used by McCarthy et al. (1992).

Time in the feeding area was less equally apportioned between dominant and subordinate individuals in the LT groups than amongst controls. This indicates that the socially dominant fish in these groups monopolised the feeding area to a greater extent. In addition, there was more evidence of aggression amongst LT fish in the behavioural tanks, indicated by direct observation and the incidence of fin damage at the end of the trials. Thus, the increase in aggression appears to have led to a reinforcement of the social hierarchy amongst LT fish. This conclusion is reinforced by the existence of a positive correlation between social rank and both growth rate

and dominance of the feeding area only in groups of LT fish during period A, and not otherwise. Our results therefore suggest that during periods of compensation the dominant fish were more aggressive and less tolerant of the presence of subordinate fish in the feeding area, and monopolised the feeding area to a greater extent than dominant controls. This reinforcement of the social hierarchy may explain the trends in the growth rates of dominant and subordinate LT fish. While the dominant fish tended to approach the growth rates of compensating fish in the stock tanks, the subordinates clearly failed to compensate, presumably because they were unable to achieve the required food intake. The matter is somewhat complicated, however, by the lack of statistically significant differences between the growth rates of the different categories of fish, probably caused by the large variation in growth rates and the small sample sizes involved.

But why was there such a difference between the stock tanks and the behavioural tanks in the appearance of the compensatory response? Why did the vast majority of fish in the stock tank manage to compensate effectively, while only a few fish in the behavioural tanks approached comparable growth rates? The answer may lie in the design of the tanks themselves. The feeding area in the behavioural tanks was an easily defendable resource. In such conditions, one or two socially dominant individuals in a small group can easily monopolise the available food. For instance, decreasing the distance between food sources led to an increase in aggression and monopolisation of the food source by dominant convict cichlids *Cichlosoma nigrofasciatum* (Grant & Guha, 1993). In small groups of rainbow trout *Oncorhynchus mykiss* or Arctic charr *Salvelinus alpinus*, two or three fish may account for the majority of all feeding activity (Alanärä & Brännäs, 1993; Brännäs & Alanärä, 1993). Similarly, in groups of eight to ten rainbow trout, subordinate individuals may stop feeding altogether and lose weight (Li & Brocksen, 1977). Both greater access to the food supply and larger group size reduce the extent to which one or two fish can monopolise the food supply (Li & Brocksen, 1977; Jobling & Baardvik, 1994; Alanärä & Brännäs, 1996). Thus, in the LT stock tank, where larger numbers of fish competed for an easily accessible food supply, the majority of fish were able to put on a compensatory growth spurt.

In the face of increased aggression, subordinate fish in the LT tanks may have made an active "choice" not to compensate. Subordinate Atlantic salmon parr are known to adopt alternative feeding strategies that allow them to minimise their energy expenditure rather than maximising energy intake in the face of competition from dominant fish (Metcalfe, 1986). Instead of wasting time and energy competing (unsuccessfully) with the dominant fish, the subordinates in the current study may have opted to wait for small amounts of food to drift into the corridor or sheltering area (since some food was in fact available outside the feeding area). Such "sit-and-wait" strategies have been demonstrated in one-sea-winter Atlantic salmon (Kadri et al., 1996a). While the growth rates of the subordinate LT fish were not close to those of compensating fish, on the whole they sustained growth rates within the range of the controls in the stock tank throughout the behavioural trials. It may be that the extent of the compensatory response is dependent on trade-offs between the risk encountered or the energy used in obtaining extra food and the benefit of the extra size gain. The benefits of compensating may not always outweigh the extra effort or greater risk required to increase food intake. While there may also be physiological limits on the extent to which compensation can occur, the existence of such trade-offs may partly explain the failure of fish in some other studies to compensate completely (e.g. Nicieza & Metcalfe, 1997). Coho salmon (*Oncorhynchus kisutch*) that were growth-compensating after a period of food restriction were more likely to feed in a food patch that they perceived as risky (close to a predator) than controls (Damsgård & Dill, 1998). Such an increase in risk-taking is a common response to hunger, and is easily understandable, given the importance of replenishing lost energy reserves. I would predict that in a similar situation temperature-manipulated fish would also increase risk-taking behaviour, but possibly not to the same extent, as starvation is more immediately life-threatening than failure to attain a target size.

My results show that while juvenile Atlantic salmon have the capacity to compensate for a set-back in growth caused by a period of cool temperature, social factors may prevent them from achieving the growth rates necessary for full compensation. In the simple environment of fish-farms, where food is in plentiful supply and access is relatively unrestricted, most fish may manage to compensate

effectively for such a setback in growth. In the wild, however, compensation may not be assured. Even in the behavioural tanks, where food was supplied to excess, the subordinate fish were prevented from putting on a compensatory growth spurt when they were expected to. The behavioural tanks, although still far from natural, shared with the natural environment a degree of structural complexity that did not exist in the stock tanks. In the field, then, a similar situation may pertain: dominant fish presiding over good feeding territories may show growth compensation while subordinates in less profitable territories may not obtain the rates of food intake needed to compensate. Thus, the interaction of social and environmental factors clearly has a profound effect on the extent of compensatory growth exhibited by fish of differing social status. This in turn will reinforce the effects of social status on growth rates, with consequences for population structure and life-history decisions.

Table 3.1: Timing of behavioural trials. Fish in the LT group had been held at lower water temperatures than the control group from 30 May to 19 June 1997. The behavioural trials took place in four sets, using three replicate behavioural tanks at a time. * = no data obtained due to equipment failure.

Set of trials	Number of Behavioural Tanks		Start Date	End Date
	LT	Control		
1	2	1	15 Sep	6 Oct
2	1	2	8 Oct	28 Oct
3	2	1	11 Nov	1 Dec
4	1*	2	1 Dec	23 Dec

Table 3.2: Comparison of growth rates of two groups of juvenile Atlantic salmon **(a)** Growth during three time periods prior to individual tagging. Fish in the LT group were held in lower water temperatures than the control group (C) from 30 May to 19 June 1997. The slope of the regression line is equivalent to SGR/100. Statistics were calculated as in Fowler et al. (1998). **(b)** Growth during three time periods after tagging. Analysis of covariance of SGR by group with length as a covariate. The ANCOVA was performed with an interaction term (to test for differences in regression slopes) and then without the interaction term (to test for differences in elevations) when the slopes proved to be not significantly different.

(a)

Growth Period	Group	Slope (b) of regression line (SGR/100)	S.E. of regression line	Difference between slopes (b_1-b_2)	Standard error of the difference	T	p
30 May - 19/20 Jun	LT	0.0032	0.0004	0.0051	0.0007	7.8	<0.01
	C	0.0083	0.0005				
19/20 Jun - 7 Aug	LT	0.0055	0.0002	0.0005	0.0004	1.4	n.s.
	C	0.0060	0.0003				
7 Aug - 26 Aug	LT	0.0051	0.0009	0.0011	0.0014	0.8	n.s.
	C	0.0062	0.0012				

(b)

Growth Period	Effect	ANCOVA with interaction			ANCOVA without interaction		
		d.f.	F	p	d.f.	F	p
26 Aug - 24 Sep	Group	1	0.18	n.s.	1	2.93	n.s.
	Length	1	0.00	n.s.	1	0.03	n.s.
	Interaction	1	0.04	n.s.			
	Error	60			61		
24 Sep - 6 Nov	Group	1	4.48	<0.05	1	57.00	<0.001
	Length	1	20.60	<0.001	1	20.21	<0.001
	Interaction	1	1.16	n.s.			
	Error	54			55		
6 Nov - 23 Dec	Group	1	0.00	n.s.	1	8.92	<0.01
	Length	1	25.15	<0.001	1	53.22	<0.001
	Interaction	1	0.10	n.s.			
	Error	35			36		

Table 3.3: Repeated measures analyses of variance of use of the feeding area by juvenile Atlantic salmon. See text for definition of Dominance Index. All analyses were performed using the square root of the data (which gave a better fit to the normal distribution than the arcsine transformation).

Dependent Factor	Effects	Period A			Period B		
		d.f.	F	p	d.f.	F	p
Proportion of time spent in feeding area	***Within-subjects*:**						
	Time of day	1	8.4	0.006	1	5.0	0.032
	***Between subjects*:**						
	Group	1	3.1	0.085	1	0.7	0.408
	Rank	1	8.1	0.007	1	0.1	0.775
	Group x Rank Interaction	1	1.9	0.176	1	0.0	0.869
	Error	38			32		
Dominance Index	***Within-subjects*:**						
	Time of day	1	0.2	0.691	1	0.0	0.971
	***Between subjects*:**						
	Group	1	0.1	0.762	1	0.2	0.697
	Rank	1	12.6	0.001	1	0.0	0.979
	Group x Rank Interaction	1	4.3	0.046	1	0.1	0.714
	Error	38			32		

Table 3.4: Two-way analyses of variance of the effect of treatment group (LT or control) and social rank (dominant or subordinate) on the growth rates of juvenile Atlantic salmon in behavioural tanks during two growth periods.

Growth period	Effect	df	F	p
A	Group	1	0.41	0.525
	Rank	1	3.51	0.069
	Group x Rank Interaction	1	1.05	0.312
	Error	38		
B	Group	1	1.43	0.241
	Rank	1	0.01	0.913
	Group x Rank Interaction	1	0.53	0.471
	Error	32		

Table 3.5: Spearman Rank Correlations between dominance rank (on the original 1-6 scale) and either growth rate (residual SGR) or dominance of feeding area, in LT and control tanks during two growth periods.

Period and group	n	Residual SGR		Dominance of feeding area	
		r_s	p	r_s	p
A: LT	20	0.524	<0.05	0.529	<0.02
A: Control	22	0.091	n.s.	0.234	n.s.
B: LT	14	0.194	n.s.	0.128	n.s.
B: Control	22	0.055	n.s.	-0.110	n.s.

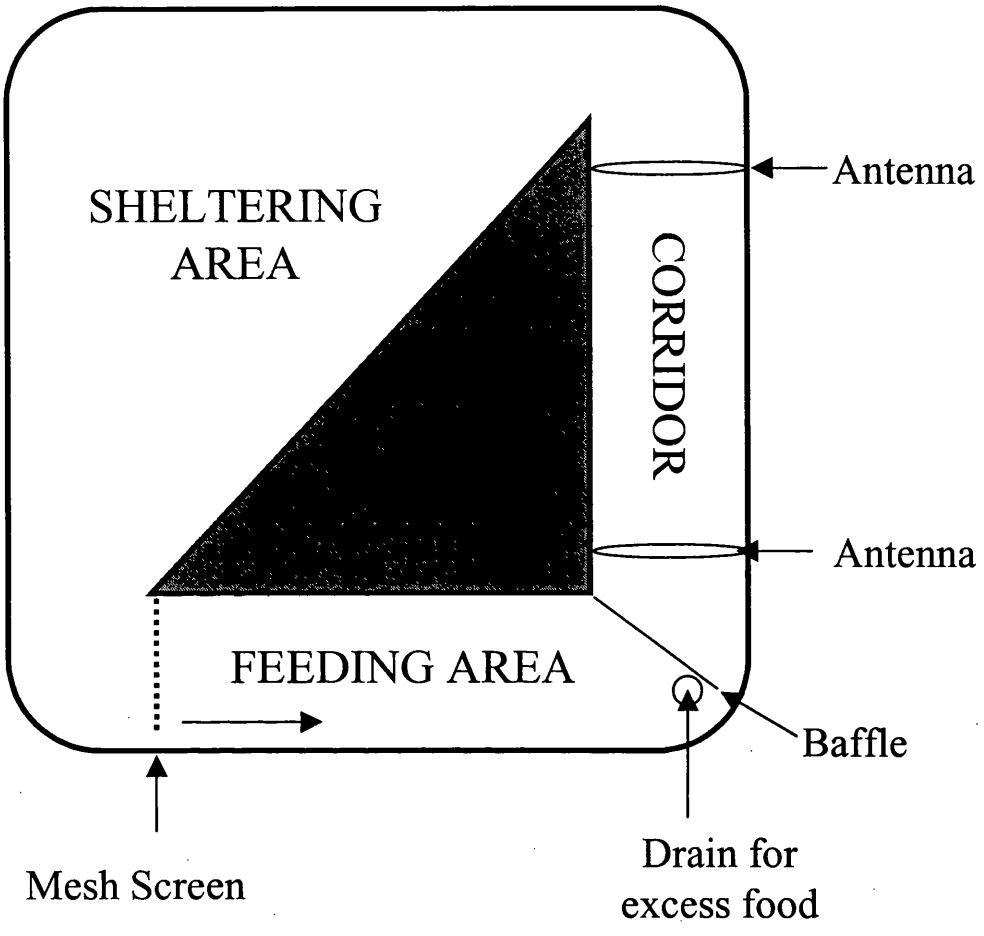

Figure 3.1
Plan view of tanks used in behavioural experiments. The shaded area in the centre of the tank was not accessible to fish. The arrow in the feeding area indicates the direction of water flow. See text for further details.

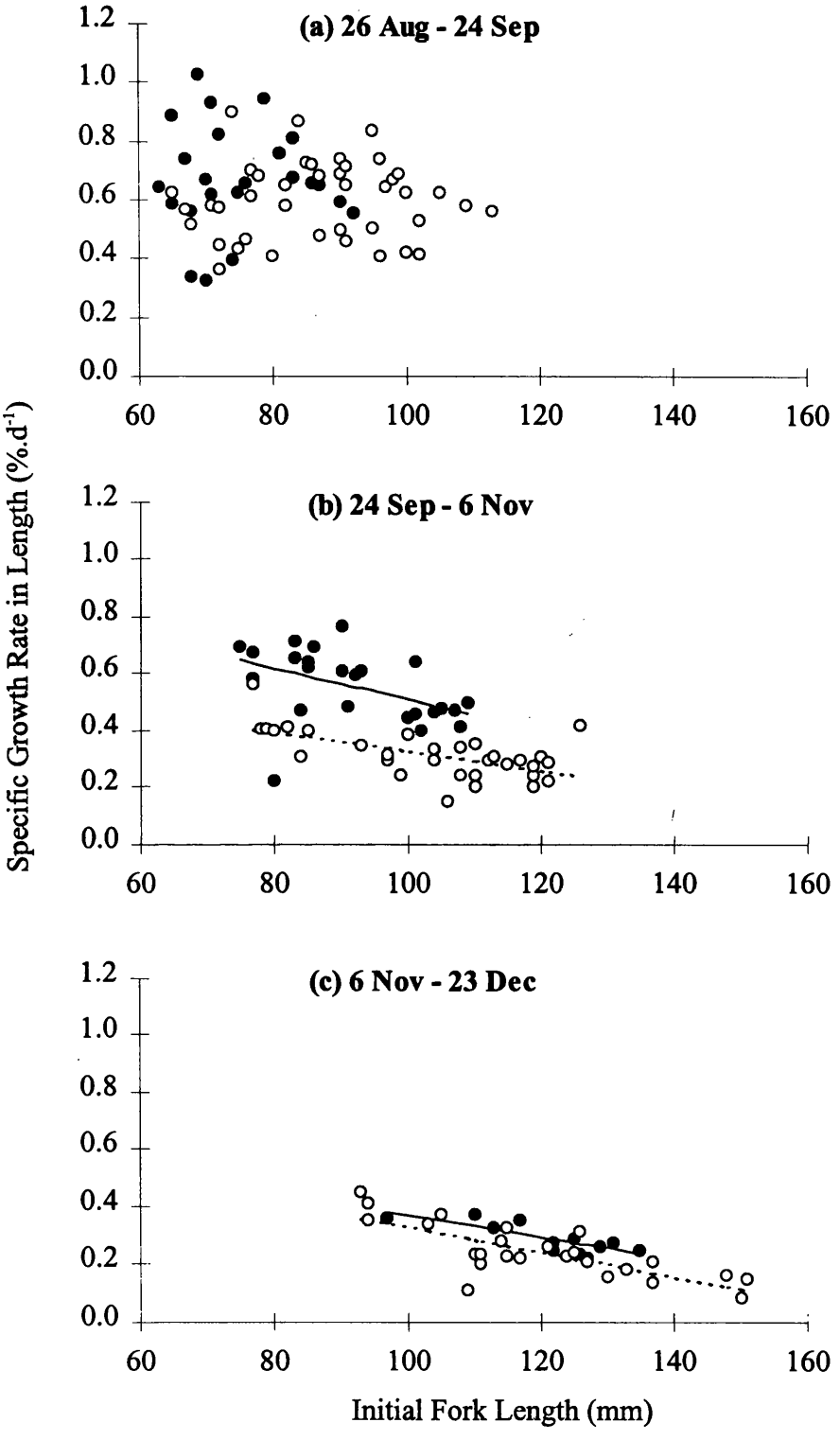

Figure 3.2
Specific Growth Rates (SGR) of juvenile salmon in LT (closed symbols and solid lines) and control (open symbols and dashed lines) groups plotted against initial fork length at the start of three growth periods: (a) 26 August to 24 September (b) 24 September to 6 November (c) 6 November to 23 December. Regression lines are shown where there was a significant effect of length on SGR.

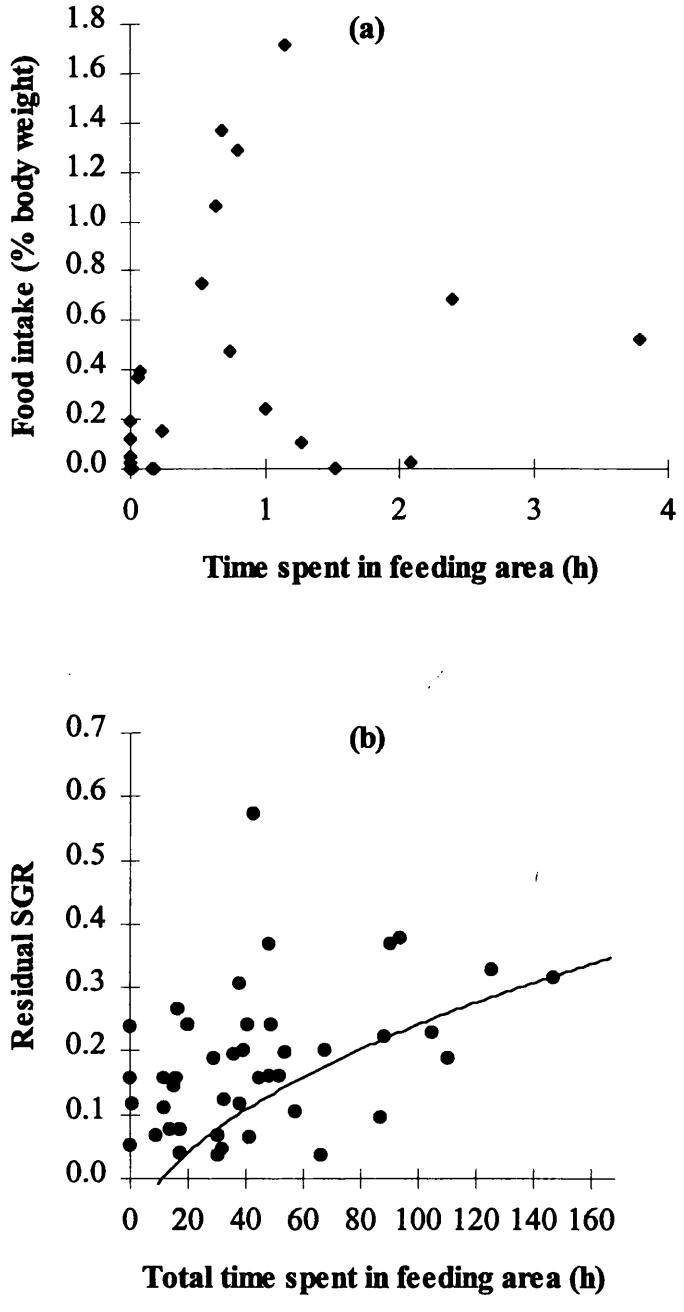

Figure 3.3
Relationship of food intake and growth to time spent by juvenile Atlantic salmon in the feeding area of behavioural tanks **(a)** Food intake as a percentage of body weight plotted against length of time spent in the feeding area over a four-hour period when labelled food was supplied. Only data for period B are presented due to technical problems in recording food intake during period A. **(b)** Growth rates (adjusted for initial size - see text for details) plotted against the total length of time spent in the feeding area of behavioural tanks during three week trials. The regression line is curved as it has been back-transformed from the linear regression of growth rate on the square root of total time spent in the feeding area.

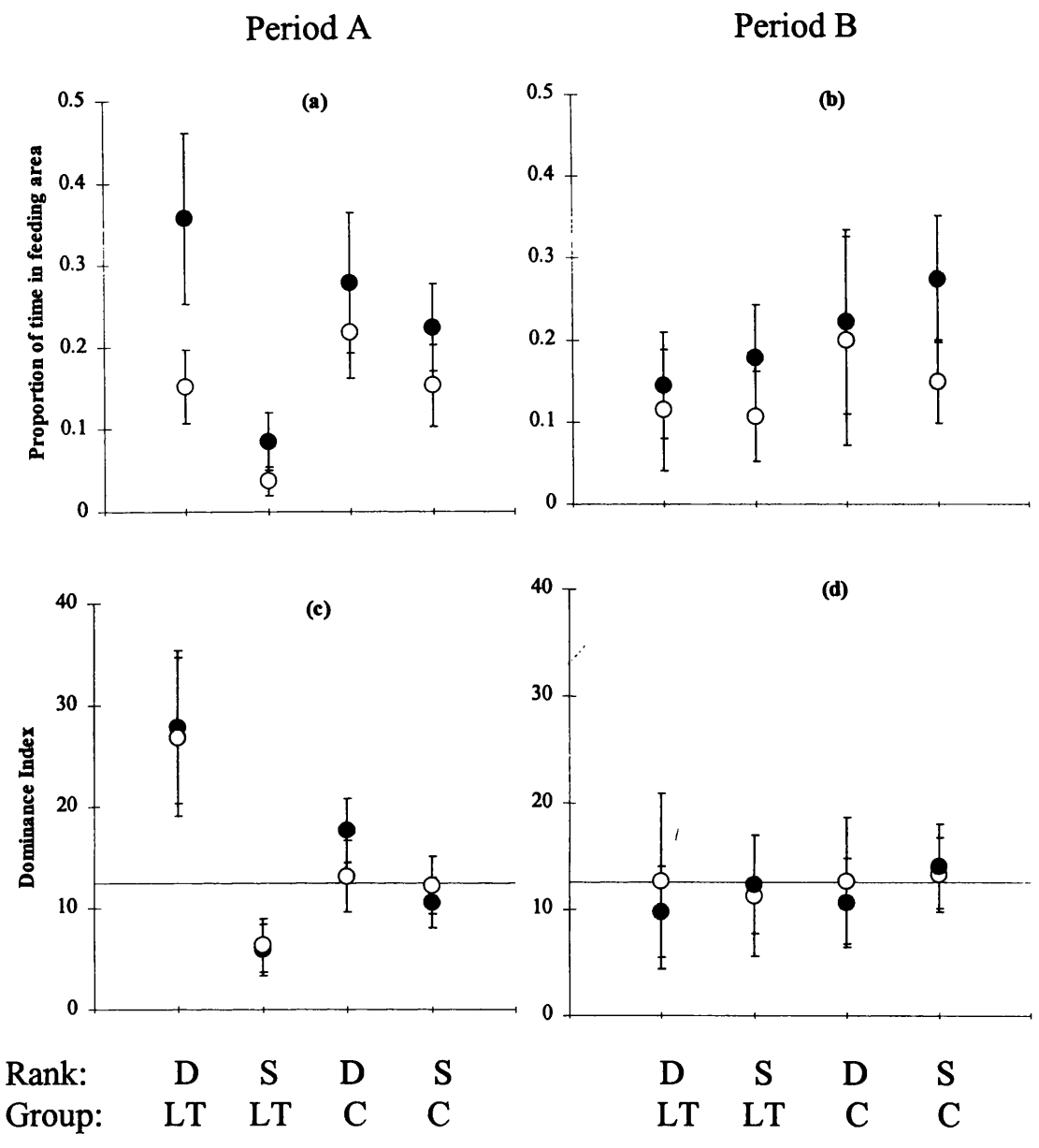

Figure 3.4
Use of the feeding area by dominant (D) and subordinate (S) juvenile Atlantic salmon belonging to LT and control (C) groups. Means ± SE are shown for day (open symbols) and night (closed symbols). Top graphs show proportion of available time spent in the feeding area during **(a)** period A and **(b)** period B. Bottom graphs show dominance of the feeding area during **(c)** period A and **(d)** period B. If all fish in a tank shared equal access to the feeding area, they would each have a dominance index of 12.5 (shown by horizontal lines in **(c)** and **(d)**).

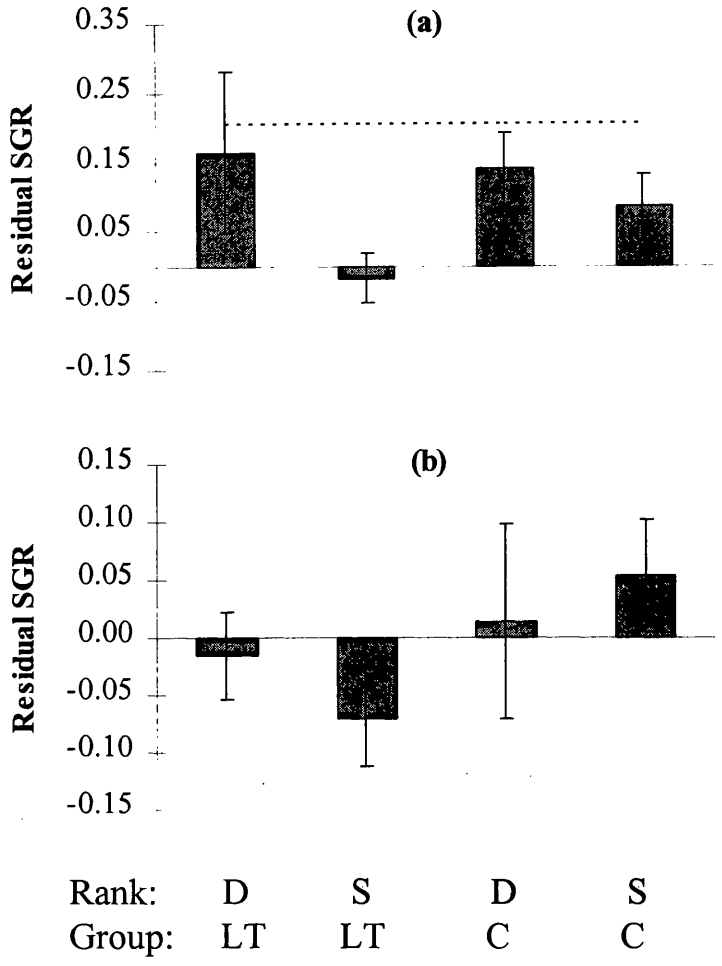

Figure 3.5
Comparison of mean specific growth rates (adjusted for initial size) of dominant (D) and subordinate (S) juvenile Atlantic salmon belonging to LT and control (C) groups during **(a)** growth period A and **(b)** growth period B. A residual growth rate of 0 is equivalent to the mean growth rate of the fish in the control stock tank. The mean residual growth rates of the fish in the LT stock tank during period A is indicated by the dashed horizontal line in (a).

Chapter 4: Sexual maturation in Atlantic salmon parr is not suppressed by low growth rates in spring

4.1 Introduction

When should an animal mature? The answer to this question is not straightforward. Life history theory predicts that an animal's reproductive strategy should maximise its lifetime reproductive success. Age and size at first maturity are believed to make important contributions to lifetime reproductive success, and can be reliably predicted for many fish species, on the basis of mortality and fecundity schedules (Roff, 1984). In species that have more than one reproductive strategy, such as the Atlantic salmon *Salmo salar*, the issue becomes more complex. Male Atlantic salmon exhibit two reproductive strategies: maturity at a small size in fresh water, or at a much larger size after a year or more in the sea. In Atlantic salmon, the strategy adopted by the individual male is not genetically fixed, and while genetic factors do come into play (Thorpe & Morgan, 1980; Thorpe et al., 1983; Wild et al., 1994), environmental variables can have as much influence as genetic factors on the strategy adopted (Rowe & Thorpe, 1990b). How, then, does the individual male choose which strategy to adopt? The answer to this question is not only of theoretical interest, but could also be of great value to the commercial sector. Increasing use of accelerated growth regimes (using elevated temperatures and long day lengths) in salmon culture has led to a higher than normal incidence of 0+ maturation. Maturation affects the quality of smolts, as it can reduce sea water adaptability (Lundqvist & Fridberg, 1982; Berglund et al., 1992). Thus, if parr maturation could be avoided, a major cause of inefficiency would be eradicated.

Maturation in salmonid fish (including Atlantic salmon) can be suppressed but not eradicated by periods of food restriction several months prior to maturation. The effect is often most pronounced during what appears to be a spring-time decision window: thus, in Atlantic salmon in sea-water, food restriction in February and

March (but not other months) significantly reduced the percentage of fish that became sexually mature later in the year (Thorpe et al., 1990). Similarly, a restriction of food intake in May reduced the percentage of female amago salmon *Oncorhynchus masou ishikawae* that matured (Silverstein & Shimma, 1994). The incidence of parr maturity in Chinook salmon *Oncorhynchus tshawytscha* can also be reduced by food restriction (Clarke & Blackburn, 1994; Hopkins & Unwin, 1997). In Atlantic salmon parr, food restriction during the month of June led to a reduction in the incidence of parr maturation the following autumn (Berglund, 1992). Similar results were obtained by Rowe & Thorpe (1990b), who found that the proportion of males that matured in groups of parr that grew rapidly during April/May and June/July was higher than for groups that grew rapidly in other months (February/March and August/September), while restricted feeding during the same months resulted in small, but statistically significant, decreases in the proportion of mature males. Studies such as these have led to the development of theoretical models in which sexual maturation is triggered only if some measure of the "state" of the fish (such as body size or lipid levels) exceeds a genetically determined threshold value during a crucial decision window (Metcalfe, 1998; Thorpe et al., 1998).

Certainly, spring growth does influence the decision to mature. However, other factors and other times of year must also be taken into consideration. Simpson (1992) found that the Gonado-Somatic Index (GSI, gonad weight as a percentage of body weight, and therefore a measure of investment in reproductive organs) in 0+ males was already bimodal by their first November, almost a year before they reached sexual maturity. This has important implications for the interpretation of other studies, as most have investigated maturation approximately 18 months after hatching, perhaps after a crucial decision point had already been passed. Indeed, differences between males that mature and fish that remained immature have been apparent from the start of many studies. Rowe & Thorpe (1990a) and Berglund (1992) found that males that became mature tended to be larger in the winter prior to maturation than fish that remained immature. In parr of Atlantic salmon (Simpson, 1992) and chinook salmon (Silverstein et al., 1998), differences in size and fatness could be discerned up to a year before maturation, while in brown trout *Salmo trutta*

condition factor in winter was a reliable predictor of maturity status the following year (Bohlin et al., 1994). Thus, it can be difficult to separate the effects of the maturation process from the factors that initiate it. By using 0+ fish instead of older fish, such prior effects can be eliminated.

In this chapter I investigate the effect on the incidence of maturation of periods of lowered temperature, using 0+ Atlantic salmon parr on an accelerated growth regime. Fish cannot feed rapidly at low temperatures even under conditions of high food availability, and so periods of lower temperature might prevent the high growth rates and rapid accumulation of body reserves that have been thought to trigger sexual maturation (Rowe & Thorpe, 1990b; Rowe et al., 1991). I expected that periods of lowered temperature would affect all fish in a group more or less equally. The limited effects of restricted feeding on maturation may be in part due to their unequal effects on fish of different size classes and social rank (Berglund, 1995). However, I hoped that the phenomenon of catch-up growth (whereby animals can compensate for periods of sub-normal growth by growing at a faster than normal rate when conditions for growth improve (Wilson & Osbourn, 1960; Lawrence & Fowler, 1997) would reduce or remove the effects of the periods of cold temperature on body size, so producing populations that would ultimately be of similar body size to the control but containing fewer mature male parr.

4.2 Materials and Methods

The experiments involved a population of farmed Atlantic salmon parr from pooled hatchery stock. The experiment started approximately two weeks after first-feeding, on 17 April 1997, when approximately 6,200 fish were transported from Marine Harvest McConnell's (MHM) hatchery at Inchmore to the MHM freshwater site at Invergarry. Here, the population was divided among four tanks labelled A-D with 1,550 ($\pm$ 7%) fish per tank. In order to manipulate growth rates, group D, the control, remained at Invergarry throughout the experiment, while groups A, B and C successively spent three weeks in colder water (mean of 8.3 $\pm$ 0.02°C, Figure 4.1a) in Glasgow University's aquaria (A from 17 April-8 May, B from 9-29 May and C from

30 May-19 June). The water at Invergarry was heated to ca. 12°C until mid-May when ambient temperatures reached that level. The fish were then kept at the ambient water temperature until the third week of October, when the water was heated to keep temperatures at ca. 8°C (Figure 4.1a).

From 17th April to 20th June, the fish at Invergarry were kept in small, circular tanks (diameter 0.6m, water depth 0.25m), and were maintained in similar size tanks (diameter 0.6m, water depth 0.3m) during the manipulation periods in Glasgow. On 20th June all four groups, now permanently at Invergarry, were transferred to larger, 2m square tanks (water depth 0.5m), where they remained until the end of the experiment.

Throughout the experiment the tanks were lit by overhead fluorescent strip-lights; the photoperiod regime was that used commercially to produce accelerated "S½" smolts, with long days separated by a photoperiod "winter" in the (real) early autumn (Figure 4.1b). The fish were fed to excess throughout the experiment on a pelleted diet of an appropriate size according to manufacturer's tables. Food was dispensed from clockwork belt feeders in the small, circular tanks and from hoppers in the large square tanks.

A random sample of 150 fish was measured on 18 April, the first day of the experiment. The fish were anaesthetised in an aerated water bath using Benzocaine in 95% alcohol, and then measurements were made of fork length (to 1 mm) and weight (to 0.01 g, after blotting with damp tissue paper to remove excess water). Random samples of 150 fish per group were then measured on 9 May, 29-30 May and 19-20 June. On 22-25 July, random samples of 100 fish per tank were measured and tagged with Passive Integrated Transponder (PIT) tags. The PIT tags were inserted into the body cavity through an incision made in the body wall. The entry wound was dusted with a 50:50 mix of Cicatrin™ antibiotic powder (Wellcome Foundation Ltd, London, U.K.) and Orahesive™ Protective Powder (ER Squibb & Sons, Hounslow, U.K.). The tagged fish were re-measured on 1-3 September, 3-9 November and 9 December.

Specific Growth Rate in length between measurement periods was calculated as:

$$SGR = 100 \times [\ln (FL_{t2}) - \ln (FL_{t1})] / (t_2 - t_1)$$

where t_1 = first sampling day, t_2 = second sampling day and FL = fork length.

Populations were thinned to 1,150 fish per tank on 29 May. Further thinning took place on 19 June when 135 $\pm$ 5 fish were removed from tanks C and D for use in other experiments (Chapter 3). On 2 September samples of 100 (untagged) fish per tank were culled and frozen for measurements of GSI (see below). On 3-9 November, all the remaining fish (both tagged and untagged) were measured and assessed for maturity by squeezing the body of the fish gently between thumb and forefinger and looking for the expression of milt. Mature fish were marked with an alcian blue dye fin mark before being returned to the tank. On 9 November, further samples of 100 unmarked (i.e. immature) fish per tank were culled and frozen. At a later date, the frozen fish were thawed, their length and wet weight were measured (to 1 mm and 0.01 g respectively) and the gonads were dissected and weighed (to 0.001g). Investment in reproductive tissue was expressed as Gonado-Somatic Index (GSI) = 100 x Gonad Weight (g)/Fish Weight (g). Male fish were judged to be maturing if their GSI was greater than 0.15. This value of GSI was chosen as Simpson (1992) found that in September in a population of 1+ parr, all mature males (those with running milt or white, thickened testes), and no immature males, had a GSI > 0.15.

On 29 August, while moving the fish between tanks, approximately 215 fish from Group D were accidentally mixed in with Group A. While the tagged fish from group D could be retrieved, the untagged fish could not, and from this time onwards Group A had more fish, and group D had fewer fish, than the other groups.

4.3 Results

Prior to tagging, individual growth rates were not available, so I used the following method to compare group growth rates. For each group, I calculated the natural logarithm of individual fork lengths at the start and end of each growth interval. The growth intervals are defined in Table 4.1. For each group and growth interval, I then calculated regression equations of Ln (fork length) against the number of days since the start of the experiment. The slope (b) of the regression line is equivalent to SGR/100. The slope of the control regression line could then be compared to the slopes of the other groups, for each growth period (Table 4.1). When groups A-C were in cold water, their growth rates were (as expected) significantly lower than those of the control. After their return to warmer water, compensatory growth was evident in groups A and B during the third growth period, when they grew respectively 25% and 29% faster than the control despite being kept in the same conditions (Figure 4.2). Group B grew more slowly than the control during the first growth period, despite being held in identical conditions.

By the end of the experiment in December, there was a clear bimodal split in all tanks between a Lower Modal Group (LMG) with maximum length 100 mm and an Upper Modal Group (UMG) with minimum length 110 mm. These two modal groups of the bimodal size distribution correspond to different smolt age groups, the UMG being composed of smolting fish and the LMG of fish delaying smolting until a later date (Thorpe, 1977).

After tagging, individual growth rates could be calculated and compared using analysis of covariance (ANCOVA) of SGR with fork length at the start of each growth period ("initial fork length") as a covariate (Tables 4.2 & 4.3). There were clear differences between the growth patterns of UMG, LMG and mature fish (Figure 4.3). Maturing male fish grew at slower rates than the immature fish in the UMG, especially during the photoperiod winter (Table 4.2). Thereafter, there was an interaction between maturity status and initial length.

For comparison of the growth rates of the four experimental groups (Table 4.3), only fish that survived to the end of the experiment and were immature smolts in December have been included, while fish that were sexually mature, delayed smolting (i.e. LMG fish) or died before the end of the experiment have been excluded. Amongst fish that ended in the UMG, growth rates were negatively related to initial size. However, during growth period 5, eleven of the smallest UMG fish had growth rates that were more characteristic of LMG fish (i.e. they grew more slowly than other UMG fish of similar size, Figure 4.3a), and have been excluded from analysis for that growth period.

Upper Modal Group fish in Group C exhibited a clear compensatory growth spurt during the growth period immediately following tagging (Figure 4.4a), growing at rates well above those of the control fish. Thus, all three experimental groups showed pronounced periods of compensatory growth. In the cases of groups A and C, the period of compensatory growth was delayed for three weeks and five weeks, respectively, after the return to warm water, while in the case of group B compensatory growth occurred during the growth period immediately after return to warm water. Although other differences between groups in growth rates were found during growth periods 5, 6 and 7 (Table 4.3), the difference from the control was small and likely to be of little biological significance.

The effects of the cold water treatment were enough to have a lasting effect on size. Compensatory growth did not result in a full recovery from the growth setback, as the mean fork lengths of the UMG fish in all three experimental groups at the end of the experiment in December were still significantly smaller than those of fish in group D (the control). Fish in group B, that had slower growth rates than the control during several growth periods, were significantly smaller at the end of the experiment than those in all other groups ($F_{3, 290} = 37.7$, $p < 0.001$; Tukey's pair-wise comparisons with family error rate of 0.05).

Overall, across all four groups, 10% of male fish were running milt in November (assuming a 1:1 sex ratio). Cold water treatment had no effect on the

incidence of maturation, as the percentage of fish that were running milt did not differ between groups (χ^2 = 1.18, 3 d.f., n.s.; Table 4.4 column d). In the sample of apparently immature fish killed at the same time, several fish showed significant development of the testes when they were dissected (i.e. they had a GSI of > 0.15; Figure 4.5). Although such fish had not yet reached full maturity, they had nevertheless taken the physiological decision to mature. Taking these fish into account and adjusting for sample size, we can estimate the numbers of fish that were maturing in each group (Table 4.4 column g). Again, the (estimated) proportions of maturing fish did not differ between the four groups (χ^2 = 6.96, 3 d.f., n.s.). Therefore the low temperature treatment was unsuccessful in reducing the percentage of fish that took the decision to mature.

However, there is some evidence that the low temperature treatment produced a delay in the maturation process. Of the fish sampled in September, 7% of 45 males in group A, 2% of 57 in group B, 22% of 50 in group C and 16% of 50 in group D were maturing (i.e. they had GSI's greater than 0.15) (Figure 4.5). Thus, the proportion of maturing males was significantly lower in group B than in control group D, but the other groups did not differ from the control (comparison of all four groups: χ^2 = 12.87, 3 d.f., n.s.; paired comparisons using sequential Bonferroni criteria (Rice, 1989)). However, as 17% of the fish in group A had originally belonged to group D, the percentage of maturing fish in group A may have been overestimated at this time. Thus reproductive investment in males started later in group B than in the other groups.

The GSI of immature fish (those with GSI < 0.15) in the UMG and LMG combined did not differ between groups in September (Kruskal-Wallis non-parametric analysis of variance, H = 1.83, 3 d.f., n.s.). By November, immature fish in group B had a lower GSI than the other groups (H = 31.28, 3 d.f., $p < 0.001$; paired comparisons to control group D), again suggesting that their greater growth setback influenced reproductive investment. However, the differences in GSI were small (Figure 4.5).

4.4 Discussion

While the fish that experienced the slowest growth rates overall (Group B) showed evidence of a reduced and delayed reproductive investment, I found no effect of periods of low temperature on the overall incidence of sexual maturation in male parr. Where negative results are concerned, it is not always possible to surmise whether they are the result of flaws in experimental design, low statistical power, or whether they demonstrate a genuine lack of effect of the treatment. In the present case, it is possible that the negative results arose because the growth rates experienced by the fish during the cold water treatment were not low enough to "switch off" maturation. Although temperatures during the cold water treatment were low in comparison to those experienced by the control, they were not exceptionally low. It is possible, therefore, that while the temperature reduction was sufficient to reduce growth rates to 34-38% of the control, it was not severe enough to have a negative effect on the maturation decision.

The reduction in growth rates was, however, effective enough to trigger compensatory growth in all three groups that experienced cold water treatment. Although compensatory growth can occur after a setback in growth caused by a period of unseasonably low temperature (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997; Chapter 2), it appears to be initiated only when the setback in growth causes body size to fall sufficiently below an expected target range for the size of year (Chapter 2). The fact that all groups showed compensatory growth in the present experiment is therefore indicative that their body size as a result of the cold water period was below the target range for the time of year. Since this was the case, we would also expect that maturation should have been switched off in at least some of the fish that experienced periods in cold water. Our results are somewhat unusual in that there was a delay in the compensatory response in two out of three groups tested. Usually, compensatory growth occurs during the three or four weeks immediately after return to warm water (Mortensen & Damsgård, 1993, Nicieza & Metcalfe,

1997; Chapter 2). The reasons for this delay may be due to the effects of the constant photoperiod, which prevented the fish from using photoperiod as a cue to indicate the time of year. Although the photoperiod winter acts as a strong seasonal cue that results in the completion of the maturation process (Eriksson & Lundqvist, 1980), there was nevertheless considerable asynchrony in male gonadal development in this experiment, with a large range in the values of GSI amongst fish that had decided to mature but were not yet running milt by early November. This was as expected, as maturation is less synchronised between individuals reared under constant conditions than under a natural photoperiod (Duston & Bromage, 1987).

The absence of photoperiodic cues prior to September could also explain why the period in the cold did not affect the proportion of male fish that matured. The timing of maturation in salmonids is subject to a circannual endogenous rhythm, entrained by photoperiod (Eriksson & Lundqvist, 1980; Duston & Bromage, 1987, 1988, 1991). If the absence of seasonal cues resulted in a less strictly defined maturation decision window, the three week period in the cold may not have had as much effect as it might have otherwise under natural conditions. Thus the rapid growth rates before and after may have negated the effects of the cold water treatment. Indeed, compensatory growth could have contributed to this effect, depending on the breadth of the decision window.

Alternatively, it could be that the low temperature treatment did not influence the maturation decision because it did not adversely affect the processes that lead to maturation being triggered. Most other studies of the control of maturation have involved the manipulation of growth rates through starvation or food restriction. While both starvation and reduced temperature cause a suppression of overall growth rates, they differ in their effects on body composition, particularly the levels of lipid deposits. In contrast with starved fish, our fish were well-nourished as they were fed to satiation throughout the experiment. Thus, their lipid stores should not have been depleted during the period of temperature manipulation. Rowe et al. (1991) found a

correlation between levels of mesenteric fat in the spring and the incidence of maturation, and suggested there was a causal relationship. While this has been called into question by Berglund (1995), if the decision to mature is indeed influenced in some way by the level of lipid deposits rather than growth rates, the triggering of maturation would have been unaffected by the low temperature treatment.

It has been suggested that maturation is in progress from the time of hatching onwards, but is suppressed by a failure to meet certain developmental criteria at critical times (Thorpe, 1994a; Thorpe et al., 1998). This view has been further strengthened by work on amago salmon, where differences in fat storage between early and late maturing strains have been detected as soon as one week after first feeding (Silverstein et al., 1997). In the present experiment, as the temperature manipulation had no effect on maturation and the conditions for growth were otherwise excellent, we would expect maturation to have remained switched on. However, maturation was switched off in all but 10% of the male fish. Bohlin et al. (1990) recognised that rapid growth rates in parr lead to a reduction in the age at maturity and the age at smolting, but need not necessarily increase the probability of maturing. Duston & Saunders (1995) have concluded that smolting, and not maturation, is the preferred developmental route for larger 0+ fish, and my results are in agreement with this finding (as the vast majority of fish in all groups would have smolted (see Chapter 5) and did not mature). This may be even more true in the case of farmed fish that have been artificially selected for several generations to smolt at an early stage, under conditions of enforced migration (i.e. smolt transport to seawater farms). Alternatively, the threshold growth rate or level of energy reserves that are required to trigger maturation at 0+ may be set so high that very few fish attained the threshold even under the very favourable growth conditions present in this experiment. It is noteworthy that in September the maturing parr were similar in size to typical immature UMG fish at the time of the first measurements of individual fish, despite having lower growth rates (Figure 4.3a), suggesting that their growth rate may previously have been higher than the mean for UMG fish.

In conclusion, the low temperature treatment had no effect on the percentage of male parr that matured, and I was unsuccessful in identifying a maturation decision window in 0+ male parr in this experiment. However, although compensatory growth occurred in the temperature-manipulated groups, UMG fish were still an average of 90% of the length and only 72% of the weight of the control fish at the end of the experiment. Thus, even if manipulation of growth rates using temperature had proved successful in reducing the prevalence of male parr maturation, it is doubtful whether this would be an acceptable technique in aquaculture.

Table 4.1: Comparison of growth rates of four groups of Atlantic salmon parr during four growth periods prior to individual tagging. Fish in groups A-C were held in lower water temperatures than the control group (D) for three week periods. The slope of the regression line is equivalent to SGR/100. P values for comparisons of groups A-C with D were judged to be significant using sequential Bonferroni criteria (Rice, 1989). Statistics were calculated as in Fowler et al. (1998).

Growth Period	Group	Slope (b) of regression line (SGR/100) x 10^{-3}	S.E. of regression line x 10^{-3}	Difference from slope of control (b_1-b_2) x 10^{-3}	Standard error of the difference x 10^{-3}	T	p
1	A	2.11	0.38	-4.06	0.52	7.77	<0.01
(17 April –	B	4.62	0.34	-1.55	0.51	3.04	<0.01
8 May)	C	6.66	0.36	0.50	0.52	0.94	n.s.
	D	6.17	0.38				
2	A	9.31	0.42	-0.14	0.59	0.23	n.s.
(9 May –	B	3.49	0.37	-5.96	0.56	10.65	<0.01
29 May)	C	10.09	0.39	0.64	0.57	1.17	n.s.
	D	9.45	0.42				
3	A	10.44	0.47	2.13	0.68	3.14	<0.01
(30 May -	B	10.74	0.41	2.43	0.64	3.78	<0.01
19 June)	C	3.18	0.43	-0.51	0.66	7.81	<0.01
	D	8.31	0.49				
4	A	11.70	0.36	-0.40	0.70	0.58	n.s.
(20 June -	B	12.05	0.40	-0.05	0.72	0.07	n.s.
22 July)	C	11.12	0.33	-0.98	0.68	1.43	n.s.
	D	12.10	0.60				

Table 4.2: Comparison of growth rates of maturing and immature Upper Modal Group Atlantic salmon parr during three growth periods after tagging, using analysis of covariance of SGR by maturity status with length as a covariate. The ANCOVA was initially performed with an interaction term to test for differences in regression slopes and then without the interaction term if it proved to be non-significant, to test for differences in elevations.

Growth Period	Effect	ANCOVA with interaction term			ANCOVA without interaction term		
		d.f.	F	p	d.f.	F	p
5	Maturity Status	1	0.1	0.757	1	13.3	<0.001
(22 July –	Initial Length	1	10.0	0.002	1	171.7	<0.001
1 Sept)	Maturity x Length Interaction	1	0.0	0.990			
	Error	292			293		
6	Maturity Status	1	5.5	0.020	1	184.2	<0.001
(1 Sept –	Initial Length	1	7.4	0.007	1	248.6	<0.001
3 Nov)	Maturity x Length Interaction	1	1.6	0.214			
	Error	303			304		
7	Maturity Status	1	11.9	0.001			
(3 Nov –	Initial Length	1	1.0	0.320			
9 Dec)	Maturity x Length Interaction	1	7.8	0.005			
	Error	303					

Table 4.3: Comparison of growth rates of four groups of immature Upper Modal Group Atlantic salmon parr during three growth periods after tagging. Analysis of covariance of SGR by experimental group with length as a covariate. The ANCOVA was initially performed with an interaction term to test for differences in regression slopes and then without the interaction term when it proved to be not significant, to test for differences in elevations.

Growth Period	Effect	ANCOVA with interaction term			ANCOVA without interaction term		
		d.f.	F	p	d.f.	F	p
5	Group	3	0.3	0.809	3	56.3	<0.001
(22 July -	Initial Length	1	77.2	<0.001	1	97.5	<0.001
1 Sept)	Group x Length Interaction	3	1.3	0.268			
	Error	275			278		
6	Group	3	0.2	0.902	3	3.0	0.030
(1 Sept –	Initial Length	1	196.6	<0.001	1	199.0	<0.001
3 Nov)	Group x Length Interaction	3	0.3	0.829			
	Error	286			289		
7	Group	3	1.0	0.390	3	14.0	<0.001
(3 Nov –	Initial Length	1	27.2	<0.001	1	27.3	<0.001
9 Dec)	Group x Length Interaction	3	0.7	0.552			
	Error	286			289		

Table 4.4: Male sexual maturity, assuming a 1:1 sex ratio, in four groups of Atlantic salmon parr. The percentage of male fish with running milt in November is given in column c. To calculate column e (estimated number of males without running milt but with GSI > 0.15 in November), I took the proportion of male fish with GSI > 0.15 in the November GSI sample (see Figure 4.5), and applied this to the estimated number of male fish minus the number that were running milt. In all cases, estimated numbers were rounded to the nearest integer.

Group	a Total number of fish checked for milt	b Estimated number of males (assuming 1:1 sex ratio)	c Number of fish running milt	d % of males running milt = c / b	e Estimated number of males without running milt but with GSI > 0.15	f Estimated number of males maturing (c + e)	g Estimated % of males maturing = 100 x f / b
A	1133	567	60	10.6	36	96	16.9
B	742	371	35	9.4	13	48	12.9
C	747	374	40	10.7	13	53	14.2
D	646	323	28	8.7	7	35	10.8

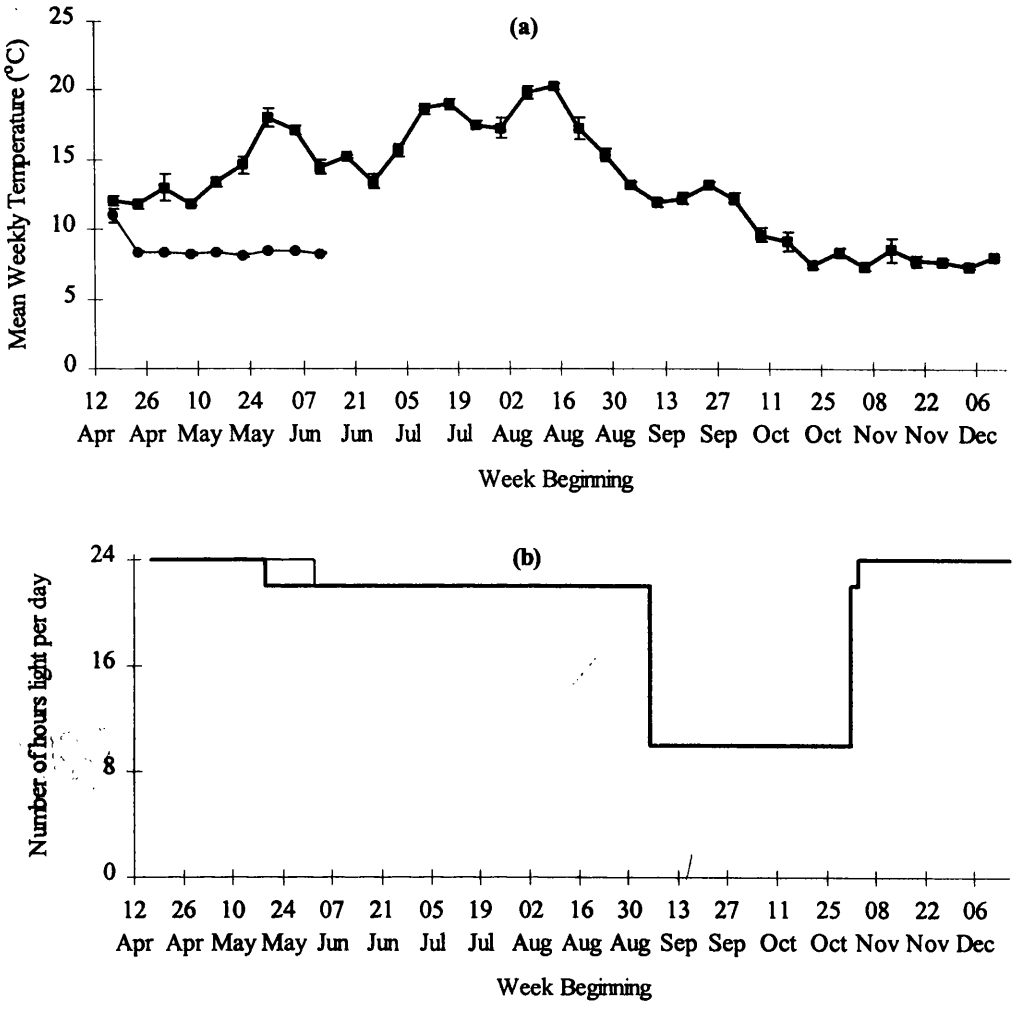

Figure 4.1

(a) Mean (± SE) weekly daytime temperatures and **(b)** photoperiod during the course of the experiment. Squares and bold lines indicate conditions experienced by group D (controls) throughout and by groups A-C except when subjected to the three-week cold water manipulation (circles and fine lines).

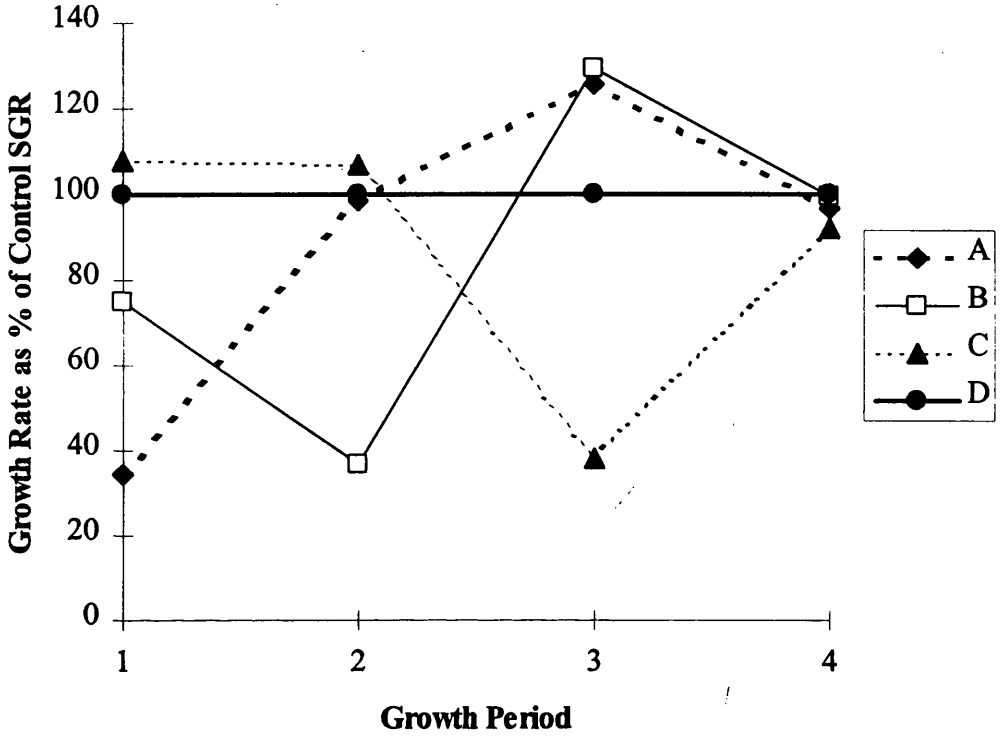

Figure 4.2
Mean growth rates of three groups of Atlantic salmon parr expressed as a percentage of that of control fish during four growth periods prior to tagging. See Table 4.1 for definition of growth periods and statistical analyses.

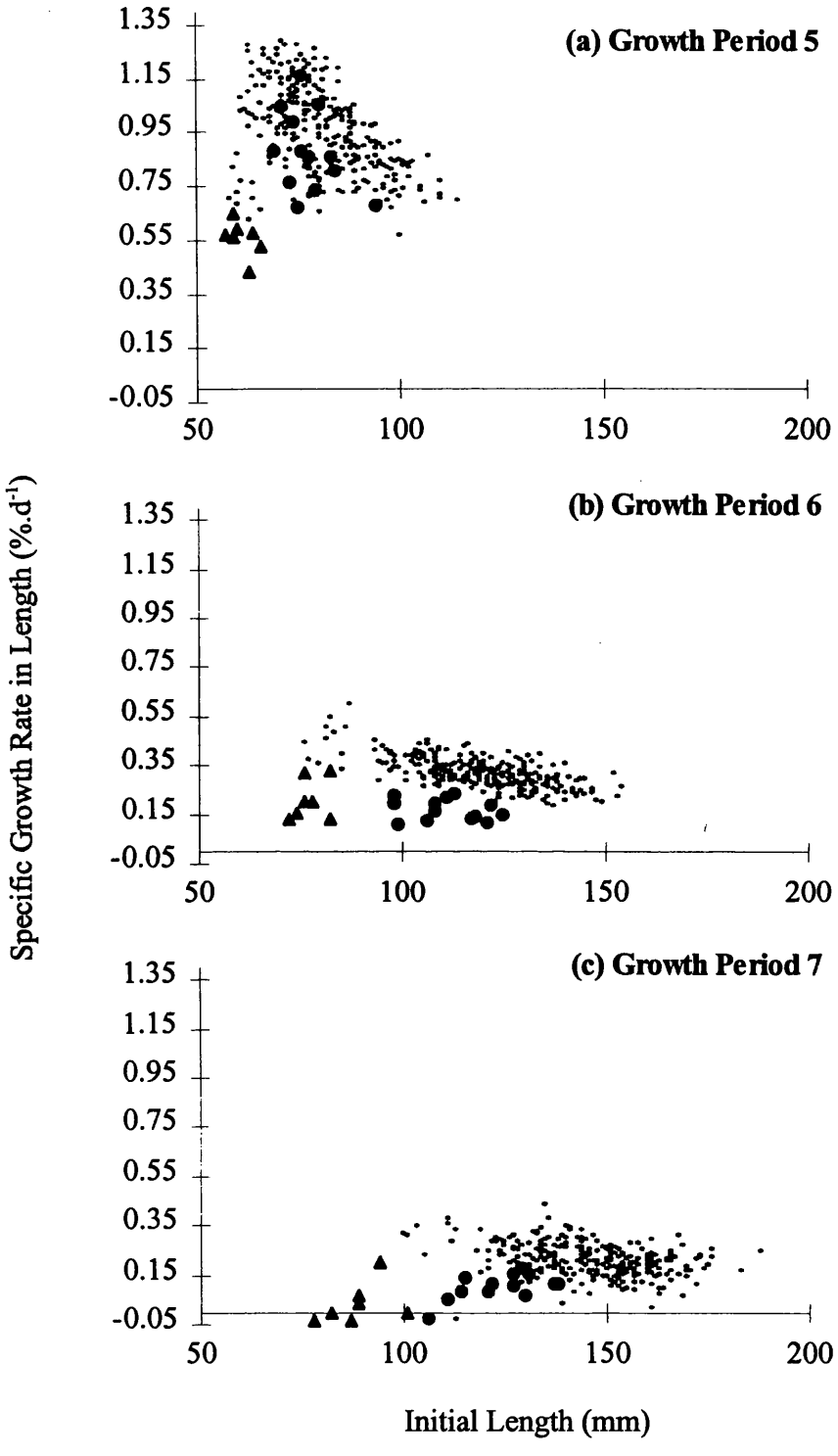

Figure 4.3
Growth rates of individually marked Atlantic salmon parr belonging to Upper Modal Group (points), Lower Modal Group (triangles) and Maturing (closed circles) categories during three growth periods (defined in Table 4.2).

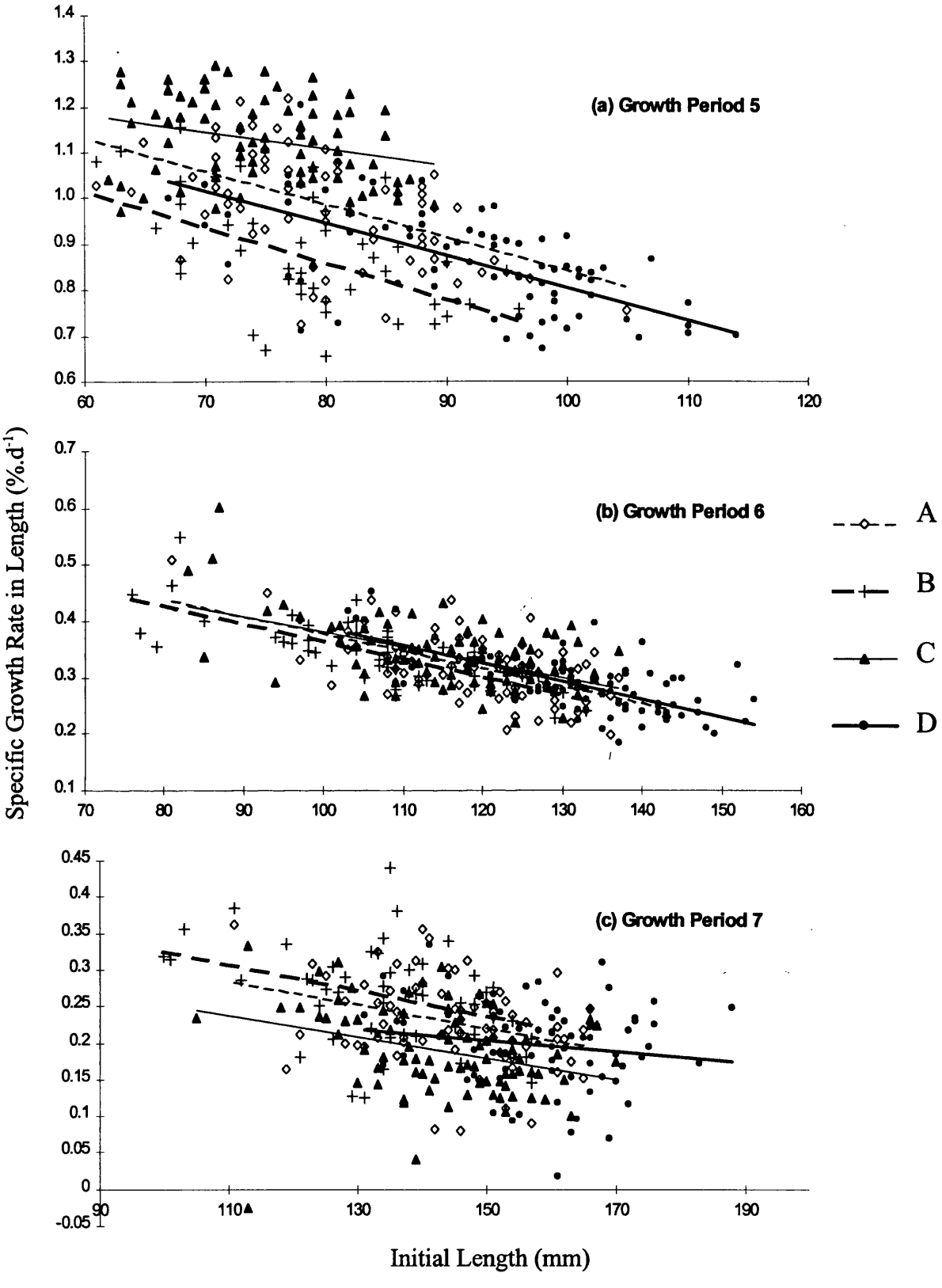

Figure 4.4
Growth rates of individually marked immature juvenile UMG Atlantic salmon in four experimental groups during three growth periods after tagging. The growth periods are defined in Table 4.3.

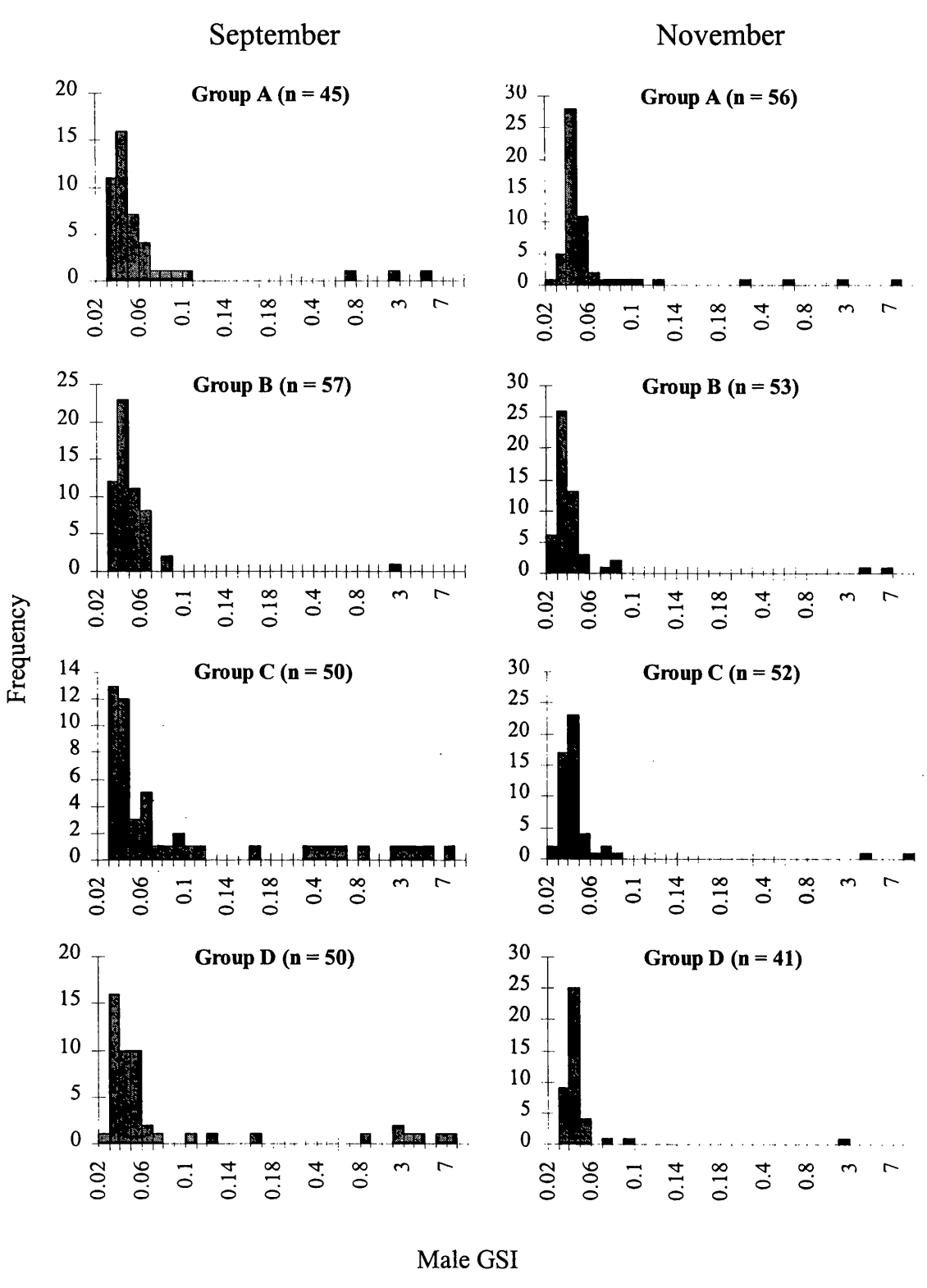

Figure 4.5
Frequency histograms of Gonado-Somatic index (GSI) of male Atlantic salmon parr from four groups sampled in September and November. Note that fish with high GSI values in September would almost certainly produce milt by November, and such fish were not included in the November samples, since only fish not producing milt were measured for GSI at that time.

Chapter 5: Sexual maturation inhibits smolting but does not prevent the physiological decision to smolt in Atlantic salmon parr

5.1 Introduction

The life-histories of all species have evolved under pressures of natural selection. While some species have rigid life history strategies that show little variation between individuals (e.g. annual plants, univoltine insects), others show a remarkable degree of phenotypic plasticity with regard to the life history strategies they adopt (Roff 1992; Stearns, 1992). This plasticity itself can be viewed as an adaptation to a variable or unpredictable environment. The Atlantic salmon *Salmo salar* is an example of a species that shows great variability in life-history strategies both within and between populations. In the wild, Atlantic salmon spawn in the autumn, and the eggs hatch in the late winter or early spring (Jones, 1950). The young fish, known as parr, remain in fresh water for a variable period of time before undertaking the seaward migration. In preparation for this they undergo the suite of physiological, morphological and behavioural changes known as smolting, that equip the fish for migration and life in sea water (Hoar, 1976).

During the period in fresh water, the parr may become sexually mature and spawn with anadromous salmon (Hutchings & Myers, 1988). While mortality rates among mature parr are high (Myers, 1984), surviving parr may eventually migrate to sea and become anadromous, returning to spawn again as full size adults. Maturation as parr is usually limited to males, as the fecundity of females is severely limited by size and the energetic requirements for maturation in females are too great to be supported by the freshwater environment, other than in exceptional circumstances. Over the geographical range of the species as a whole, the period in fresh water varies between one and eight years, while within a single population there are usually three or more year classes of smolts (as the seaward migrants are known) (Metcalfe & Thorpe, 1990). The period spent in the sea before returning to rivers to spawn is equally variable (Thorpe, 1994a).

Although genetic factors do come into play, to a large extent the life-history path followed by individual fish is determined by social and environmental factors that influence food acquisition, energy storage and growth rates (reviewed by Metcalfe, 1998). These factors have a profound influence on the age at which smolting and maturation occur, and on the phase of the life-cycle (freshwater or marine) at which they become mature. The decisions to smolt and to mature appear to depend on an assessment of whether or not the fish will exceed a genetically determined threshold by the time that smolting or maturation must be completed (Thorpe et al., 1998). The decisions are based on an assessment of past and current performance (such as state or rate of change of state, measured by body size, energy reserves or body condition) at critical times of year. For maturation, the critical times of year are thought to be November and April-June (one year and 5-7 months prior to spawning, respectively), while the decision to smolt is taken during the summer prior to emigration. Since both overwinter survival of mature fish and seawater survival of smolts are enhanced by large body size and energy reserves (Bilton et al., 1982; Holtby et al., 1990; Lundqvist et al., 1994; Smith & Griffith, 1994; Hutchings, 1996; Meyer & Griffith, 1997), and since both processes are in themselves energetically demanding, the assessment mechanisms prevent smaller fish or fish in poor condition from making life-history decisions that could seriously compromise their chances of survival and future reproduction.

The factors affecting the decision to smolt after one year in fresh water have been very well documented. During the first summer in fresh water, Atlantic salmon populations typically develop a bimodal size distribution. Fish that belong to the Upper Modal Group (UMG) will continue growth over the autumn and winter and migrate to sea the following spring at 1+, while those belonging to the Lower Modal Group (LMG) will arrest growth over the autumn and winter and remain in fresh water for at least a further year (Thorpe, 1977; reviewed by Saunders et al., 1994a). Maturation in the first autumn at 0+ is rare in the wild due to relatively poor growth conditions, but is becoming increasingly common in hatcheries where improved

growth conditions appear to allow more fish to fulfill the requirements for maturation in the first year (Adams & Thorpe, 1989).

Thorpe (1986, 1987, 1994b) has argued that, since sexual maturation is an absolute requirement for completion of the life-cycle, it should take precedence over smolting, which is not a necessary phase, and that smolting is a response to a failure to meet the conditions for maturation in fresh water, chiefly as a result of the low productivity of river environments. However, fish that smolt at the earliest opportunity (i.e. at 1+ under natural conditions) are usually amongst the larger fish in a population, and while the rearing environment in hatcheries, where food is available in abundance, frequently leads to an increase in the incidence of early maturation, the production of early smolts is likewise enhanced. Certainly, there is considerable evidence that the hormones associated with maturation have an inhibitory effect on smolting in salmonid fish. Castrated masu salmon *Oncorhynchus masou* developed smolt characteristics while sham-operated mature controls did not (Aida et al., 1984). Masu salmon did not develop smolt characteristics when fed the androgen methyltestosterone from February to April, although smolting was evident in controls fed a normal diet (Ikuta et al., 1985). Similarly, when groups of Atlantic salmon were given methyltestosterone they produced no smolts, while nearly 60% of fish in control groups smolted (Thorpe, 1987). Further inhibitory effects of androgens on the development of smolt characteristics in Atlantic salmon and Baltic salmon *Salmo salar* have been demonstrated by Lundqvist et al. (1989). Mature Atlantic salmon are less likely than immature fish to migrate to sea in the spring following maturation, although significant numbers of mature fish do migrate (Berglund et al., 1991; Hansen et al., 1989; Whalen & Parrish, 1999). Smolting is likewise thought to inhibit maturation the following autumn (Thorpe, 1986, 1987). Evidence of this sort has reinforced the view that the two processes are mutually incompatible, to the extent that in a recent model of Atlantic salmon life-history decisions, the decision to become sexually mature was judged to preclude the decision to smolt in the same calendar year (Thorpe et al., 1998).

However, while androgens clearly do inhibit the smolting process, mature parr can still smolt successfully in the spring following maturation (Järvi et al., 1991; Saunders et al., 1994b; Duston & Saunders, 1997). Therefore the view that maturation and smolting are mutually exclusive processes requires re-examination. In this chapter, I present evidence that, despite some inhibition of the smolting process in sexually mature parr, such fish nevertheless did make the decision to smolt as well as mature in the same year. I looked at the development of smolt characteristics (smolt coloration) and compared seawater adaptability in mature and immature Atlantic salmon parr, raised on an accelerated (S1/2) growth and smolting programme. I also manipulated the early growth rates of the fish (by altering water temperatures) in order to test whether this influenced the likelihood of successful smolting in either mature or immature fish.

5.2 Materials and Methods

The experiments involved a population of farmed Atlantic salmon parr from pooled hatchery stock. The experiment started approximately two weeks after first-feeding, on 17 April 1997, when approximately 6,200 fish were transported from Marine Harvest McConnell's (MHM) hatchery at Inchmore to the MHM freshwater site at Invergarry. Here, the population was split between four tanks labelled A-D with 1,550 ($\pm$ 7%) fish per tank. In order to manipulate growth rates, group D, the control, remained at Invergarry throughout the experiment, while groups A, B and C successively spent three weeks in colder water (mean of 8.3 °C $\pm$ 0.02) in Glasgow University's aquaria (A from 17 April-8 May, B from 9-29 May and C from 30 May-19 June), before being returned to Invergarry. The water at Invergarry was heated to 12.4°C ($\pm$ 0.2) until mid-May when ambient temperatures reached that level. The fish were then kept at the ambient water temperature (mean of 15.5 °C $\pm$ 0.3, minimum of 8.0°C, maximum of 21.6°C) until the third week of October, when the water was heated to keep temperatures at 7.9°C ($\pm$ 0.2).

From 17th April to 20th June, the fish at Invergarry were kept in small, circular tanks (diameter 0.6m, water depth 0.25m), and were maintained in similar

size tanks (diameter 0.6m, water depth 0.3m) during the manipulation periods in Glasgow. On 20th June all four groups, now permanently at Invergarry, were transferred to larger, 2m square tanks (water depth 0.5m), where they remained until the end of the experiment.

Throughout the experiment the tanks were lit by overhead fluorescent strip-lights. The photoperiod regime was that used commercially to produce accelerated "S½" smolts, with long days (24L:0D until the end of May, and thereafter 22L:2D) separated by a photoperiod "winter" (10L:14D) in the (real) early autumn (6 September - 1 November). This manipulation has the effect of producing fish that have undergone smolting by early winter, 6 months ahead of the earliest smolts produced under a natural photoperiod. For the purposes of this study, the protocol also allows exploration of whether fish can be smolting and maturing at the same time. The fish were fed to excess throughout the experiment on a pelleted diet of an appropriate size according to manufacturer's tables. Food was dispensed from clockwork belt feeders in the small, circular tanks and from hoppers in the large square tanks.

Populations were thinned to 1,150 fish per tank on 29 May. Further thinning took place on 19 June (135 $\pm$ 5 fish being removed from each of tanks C and D for use in the experiments reported in Chapter 3), 2 September (100 to 220 fish from each tank) and 9 November (100 fish from each tank) when fish were sampled for other purposes (reported in Chapter 4). On 29 August, while moving the fish between tanks, approximately 215 fish from Group D were accidentally mixed in with Group A. While 22 tagged fish from group D could be retrieved, the untagged fish could not, and from this time onwards Group A had more fish, and group D had fewer fish, than the other groups.

On 3-9 November, the fish were anaesthetised in an aerated water bath using Benzocaine in 95% alcohol, and then measurements were made of fork length (to 1 mm) and weight (to 0.01 g, after blotting with damp tissue paper to remove excess water). Maturity was assessed by squeezing the body of the fish gently between

thumb and forefinger and looking for the expression of milt. In mature fish this process was continued until milt was no longer expressed. The fish were then rinsed to remove excess milt, blotted and re-weighed. Milt production was measured in grams by subtracting the weight of the stripped fish from its unstripped weight. Mature fish were marked with an alcian blue dye fin mark before being returned to the tank. Silvering (as an indicator of smolting) was assessed using a visual scoring system developed by Graham et al. (1996), where:

1 = distinct parr marks (dark oval "thumb-prints" along the flanks), no silvering

2 = slight loss of parr marks, slight silvering

3 = distinct overall silvering, parr marks faint or nearly absent but with some pigmented areas

4 = complete overall silvering.

On this scale parr would typically score 1 and full smolts 4. On 8-9 December, 10 mature fish (that had been dye-marked in November) and 23 immature fish (spread as evenly as possible across the length distribution of each group) were selected from each treatment group. These fish were given a further alcian blue dye fin mark as a group identifier, and were kept together in a 2 m tank until 16 December, when they were transported to MHM's seawater challenge facility at Lochailort. Here, the fish were distributed evenly between six 1 m x 1 m tanks (water depth 30 cm), each of which contained fish from all treatment groups. The water in the tanks was ambient sea-water (31.5‰) made up to 35‰ with Sea-Mix artificial seawater. The water (temperature ca. 12°C) was aerated constantly. At six hourly intervals, dead and moribund fish were removed and measured (the moribund fish being killed prior to measurement). On 17 December, 24 hours after introduction to sea water, the remaining fish were killed and blood samples were taken and stored in heparin-coated eppendorf tubes. The tubes were centrifuged for 5 minutes at 2,500 rpm and plasma chloride levels were measured using a Jenway (Model PCLM3) chloride meter. Fish with a plasma chloride content of $\leq$ 160 mmol.l^{-1} were classified as successful smolts while those with a higher plasma chloride content were classified

as failures (according to MHM practice based on S. Stefansson & T. Hansen pers. comm.s; see also Sigholt & Finstad (1990), Staurnes et al. (1993)).

5.3 Results

No female parr were found to be mature in November. Assuming a 1:1 sex ratio, 10% of males in total (across all four treatment groups) were sexually mature and running milt in November. There were no differences between treatment groups in the percentage of males running milt (Chapter 4). A regression of Ln (milt weight) on Ln (fork length) showed a positive but not statistically significant relationship between the amount of milt produced by mature males and fork length (adjusted r^2 = 0.017, 162 d.f., $p < 0.10$, Figure 5.1a). As the regression coefficient of 0.82 was less than 1, the weight of milt produced did not increase in proportion to body size. Therefore when milt production was expressed as an (arcsine-transformed) percentage of body weight, it was negatively related to body size (fork length), and body size explained more of the variation in milt production (adjusted $r^2 = 0.163$, 162 d.f., $p < 0.001$, Figure 5.1b). Thus, there was overall a decreasing investment in milt with increasing body size. However, there were no differences between treatment groups in the effect of body size on milt production either in terms of amount produced or relative investment in milt production (Table 5.1).

In November, when all fish were measured, there was a clear bimodal split with an antimode at a fork length of 100 mm (Figure 5.2). Fish with a fork length below this threshold were classified as belonging to the LMG while larger fish were classified as belonging to the UMG. Most of the mature fish clearly fell into the size range of the UMG, with only 3.1% of 163 mature fish belonging to the LMG (Figure 5.2). Within the UMG, mature fish were on average smaller than immature fish, and fell into the smaller half of the UMG length distribution (Two-way ANOVA on body size in UMG fish, effects of maturity status: $F_{1, 3150} = 261.0$, $p < 0.001$; experimental group: $F_{3, 3150} = 20.0$, $p < 0.001$; interaction between maturity status and group: $F_{3, 3150} = 2.1$, n.s.; Figure 5.2).

Logistic regression analysis showed that the probability of surviving in sea water was strongly influenced by body size (Figure 5.3; fork length, $\chi^2 = 89.2$, $p < 0.001$) and maturity status (improvement in $\chi^2 = 28.4$, $p < 0.001$), but not by experimental group (improvement in $\chi^2 = 0.0$, n.s.). The lines in Figure 5.3 are logistic regression lines showing the probability of failing to survive transfer to sea water. There was a sharp distinction between UMG and LMG fish: smolting was 0% successful for fish with a fork length below 100 mm but 100% successful for those of 125 mm or longer. A similar relationship between body size and seawater performance was evident amongst mature fish, but the logistic regression line was shifted to the right. Thus mature fish of a given size had lower chances of smolting successfully than immature fish of the same size, although larger mature fish had a high probability of success.

Smolt coloration had begun to develop in both immature and mature fish by the end of the photoperiod winter in November (Table 5.2), when the majority of fish in the immature UMG and mature categories had a score of 3 or 4, while few LMG fish had a score above 2 ($\chi^2 = 66.6$; d.f. = 2; $p < 0.001$; categories 1 and 2 combined for analysis). This was still the case in December ($\chi^2 = 40.9$; d.f. = 2; $p < 0.001$; categories 1 and 2 combined for analysis). Smolt characteristics became more enhanced in all groups between November and December. Thus, the percentage of immature UMG fish with full smolt coloration (score of 4) increased from 45% in November to 100% in December. Likewise, the percentage of fish with a score of 4 increased from 22% to 50% amongst mature fish. Logistic regressions showed a strong influence of fork length (November $\chi^2 = 41.2$, 1 d.f., $p < 0.001$; December $\chi^2 = 52.0$, 1 d.f., $p < 0.001$) and maturity status (November improvement in $\chi^2 = 9.4$, 1 d.f., $p < 0.01$; December improvement in $\chi^2 = 24.4$, 1 d.f., $p < 0.001$) on the probability of having a score of 4 in both months (Figure 5.4). As with seawater survival, the proportion of fish with full smolt coloration increased with body size, and mature fish were less likely to show full smolt coloration than immature fish of the same size. Although, unusually, some larger LMG fish showed full smolt coloration in December, the colour scores of mature fish (of all sizes) were generally higher than those of immature LMG fish ($\chi^2 = 13.8$; d.f. = 2; $p < 0.001$), and the

seawater challenge results (Figure 5.3) showed that the larger LMG fish were not true smolts despite signs of being silvery.

5.4 Discussion

As expected, I found that smolt coloration and seawater adaptability were impaired in mature fish in comparison with immature fish of the same size. However, mature fish did show signs of smolting: the percentage of mature fish with full smolt coloration increased between November and December, while the larger mature fish showed good adaptation to sea water. Moreover, the majority of mature fish would be classified as UMG rather than LMG fish on the basis of their size. Therefore, although smolting was less complete in mature fish, the majority of fish that were maturing were also smolting at the same time.

While our results show, as expected, that smolting is *impaired* in fish that have reached sexual maturity, they indicate that the physiological decision to mature does not *preclude* the decision to smolt. If the timing of the two events was similar we would expect that androgen production in mature fish would nearly always prevent smolting. However, as there is usually a gap of several months between spawning and the smolt migration, there is ample opportunity for the testes to be resorbed and for the effects of androgens on the smolting process to be ameliorated. This is particularly the case when the fish experience warmer temperatures over the winter (Berglund et al., 1991). Indeed, a number of studies have found no difference between mature and immature fish in their smolt characteristics or seawater adaptability in the spring. For instance, Saunders et al. (1982) reported no adverse effect of prior maturation on seawater survival in previously mature males. Similarly, Järvi et al. (1991) found full smolt coloration in previously mature males by the time of transfer to sea water. Saunders et al. (1994b) found that mature and immature fish did not differ in gill Na^+-K^+-ATPase activity at the time of smolting or in growth rates during the first three months in sea water. However, this effect was restricted to fish that belonged to the UMG, while both mature and immature fish that belonged to the LMG had low levels of Na^+-K^+-ATPase activity and poor survival in sea water. In

June, at the time of optimal seawater adaptability, Berglund et al. (1992) found that males that had matured the previous autumn fell into two groups: one group consisted of fish with small, regressing testes, and hypoosmoregulatory ability equal to that of immature smolts, while the other group consisted of fish with larger, re-maturing testes and poor osmoregulatory ability. This bimodal smolting pattern in previously mature males demonstrates that the inhibition of smolting by maturity can arise not because of the previous reproductive attempt but because the fish is in the process of maturing for a second time and so will remain resident in fresh water for at least another year. Thus, rising androgen levels in the spring may act to suppress smolting in fish that are re-maturing but not in those that will migrate (Mayer et al., 1990). Conversely, smolting may act to suppress maturation in fish that undergo the smolt transformation. For instance, Berglund (1995) found that at age 1+, although increasing body size had a positive effect on maturation, the largest males smolted but did not mature.

Even within the UMG, osmoregulatory ability and seawater survival increase with body size. The poorer osmoregulatory abilities of mature fish may be associated partly with their smaller body size (see Thorpe & Metcalfe, 1998), but this effect is often more pronounced in mature males than immature fish (Berglund, 1992), as indeed it was in the present study. One possible reason for this is that smaller fish invest relatively more in the maturation process, exemplified by their greater investment in milt production in relation to body size. This may result in less energy being available for the smolting process, or the effect may be caused by higher androgen levels in smaller fish, although the latter hypothesis has yet to be tested.

Since the processes of smolting and maturation are both energetically demanding, fish that cannot meet the energetic requirements for both processes may be forced to adopt only one. Although parr that mature are often amongst the largest and fastest growing fish in a population (Saunders et al., 1982; Berglund, 1992; Herbinger & Friars, 1992), their somatic growth slows during the summer and autumn prior to maturation as energy is diverted to gonadal growth, so that they are amongst the smallest of their cohort by the late autumn (Berglund, 1992; Herbinger

& Friars, 1992; Rowe & Thorpe, 1990a; Saunders et al., 1982). It may be that in many populations, maturing fish do not smolt because their small size and slow somatic growth rates during the relevant decision period cause smolting to be "switched off". This appears to be the case in many natural situations where food supplies are limiting, with the result that mature fish are often in the LMG by the winter. This view is supported by the work of Saunders et al. (1994b), who found that male parr that become mature are capable of also completing smolting as long as they reach certain size thresholds near the time of final maturity. Normally, however, rates of food acquisition and growth rates are limiting to the extent that mature fish do not reach the minimum size of fish in the UMG (Saunders et al., 1982). I suggest that in nature, since resources are often limited, fish that have made the decision to mature will not usually be large enough or have enough surplus energy to meet the requirements for smolting. They will then defer smolting for another year, during which time they may re-mature (Myers, 1984; Berglund, 1995) In the present study, where food was abundant and there was good opportunity for growth, nearly all the fish joined the UMG and even fish that were maturing were large enough and had enough surplus energy for smolting.

Evidence is emerging that the life-history decisions of Atlantic salmon may be more flexible than is currently acknowledged. Lundqvist & Fridberg (1982) demonstrated that in previously mature males that smolted in the spring following first maturation, 100% re-matured when retained in fresh water, whereas only 7% re-matured after transfer to brackish water (despite experiencing more rapid growth thereafter than those retained in fresh water). In this case, the inhibition of re-maturation by smolting was to a large extent dependent on the environment (fresh water or marine). A further example of flexibility in life-history strategies has been reported recently by Utrilla & Lobón-Cerviá (1999). They reported that, in a wild Spanish population, some LMG fish (that have previously been thought of as exclusively non-migrant) developed smolt coloration and migrated downstream approximately 1.5 months after UMG smolts, after a period of rapid spring growth. It is likely that the excellent conditions for growth in this population of Atlantic salmon, located at the southern end of the species' geographic distribution, were

responsible for this unusual observation, since Duston & Saunders (1997) have shown that warm winter conditions can induce apparently LMG fish to become smolts. A similar result was obtained in the present experiment, where atypically warm conditions may have been responsible for full smolt coloration developing in 22% of the LMG fish by December. This silvering was restricted to the larger LMG fish (see Figure 5.4), and although they failed the seawater challenge test in December, they may have been on course to complete smolting shortly afterwards.

This flexibility in the life-history decisions of Atlantic salmon highlights the difficulties involved in attributing cause and effect to observed life-history patterns. The inhibition of smolting by maturation may occur for a number of different reasons. Smolting may not be adopted as the life history path of many fish that are maturing, either because their size or energetic status does not allow them to pursue both processes at once, or because they then re-mature the following year and remain resident in fresh water, rather than migrating to sea. This effect of the maturation decision on later life-history decisions should be distinguished from the impairment of smolting by androgens in fish that have made the decision to smolt (as in the present experiment).

In summary, I have demonstrated that the decision to mature does not preclude the decision to smolt in 0+ juvenile salmon, although maturation does limit or delay the smolting process in those fish that have taken both physiological decisions.

Table 5.1: Analysis of covariance of production of milt by sexually mature male Atlantic salmon parr from different treatment groups, with **(a)** Ln (fork length) or **(b)** fork length as a covariate. Three treatment groups had experienced periods of colder temperatures compared to the control (see text for details). Reproductive investment was expressed as **(a)** Ln of absolute weight of milt produced, and **(b)** milt produced as a percentage of body weight (arcsine-transformed). The ANCOVA was performed first using an interaction term between group and the covariate and, if the interaction term was not significant, was repeated without it.

	Effects	ANCOVA with interaction term			ANCOVA without interaction term		
		d.f	F	p	d.f.	F	p
(a)	Group	3	0.7	0.569	3	1.3	0.479
	Ln (Length)	1	4.0	0.047	1	3.8	0.053
	Interaction between group and Ln (length)	3	0.7	0.571			
	Error	155			158		
(b)	Group	3	0.6	0.632	3	1.7	0.160
	Length	1	28.9	<0.001	1	31.3	<0.001
	Interaction between group and length	3	0.5	0.700			
	Error	155			158		

Table 5.2: Percentage of immature LMG, immature UMG and Mature fish with each parr/smolt score in November and December. UMG and LMG fish were classified on the basis of their body size (see text).

			Parr/smolt status:			
Month	Category of fish	n	1	2	3	4
November	Immature LMG	17	47%	35%	18%	0%
	Immature UMG	584	0%	2%	53%	45%
	Mature	162	1%	14%	63%	22%
December	Immature LMG	18	17%	44%	17%	22%
	Immature UMG	70	0%	0%	0%	100%
	Mature	38	0%	13%	37%	50%

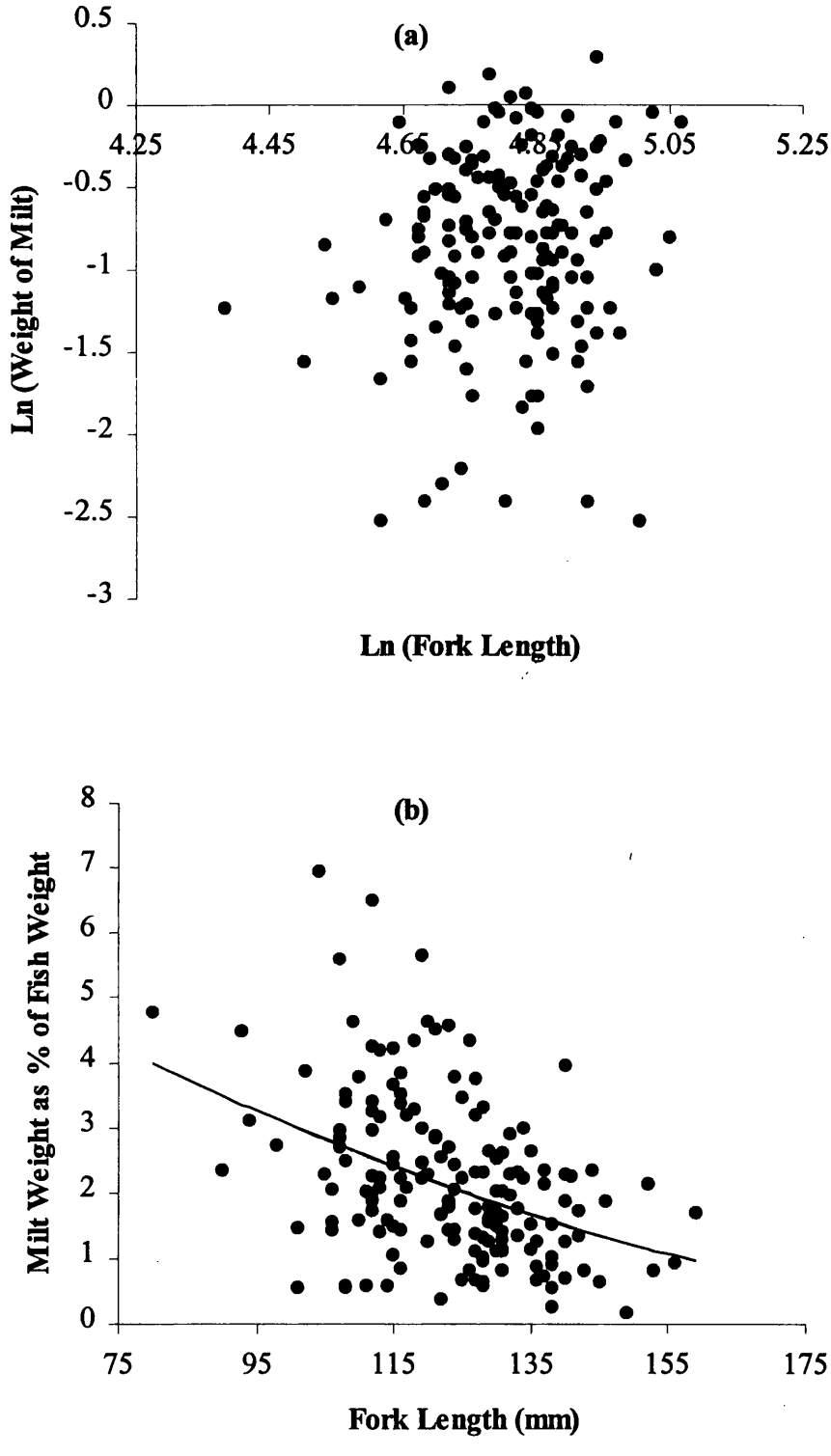

Figure 5.1
Production of milt (sperm) by Atlantic salmon parr **(a)** Weight of milt produced in relation to fork length; **(b)** weight of milt produced as a percentage of body weight, in relation to fork length. The line is the back-transformed regression line of arcsine (milt weight/fish weight) on fork length. See text for statistical analysis.

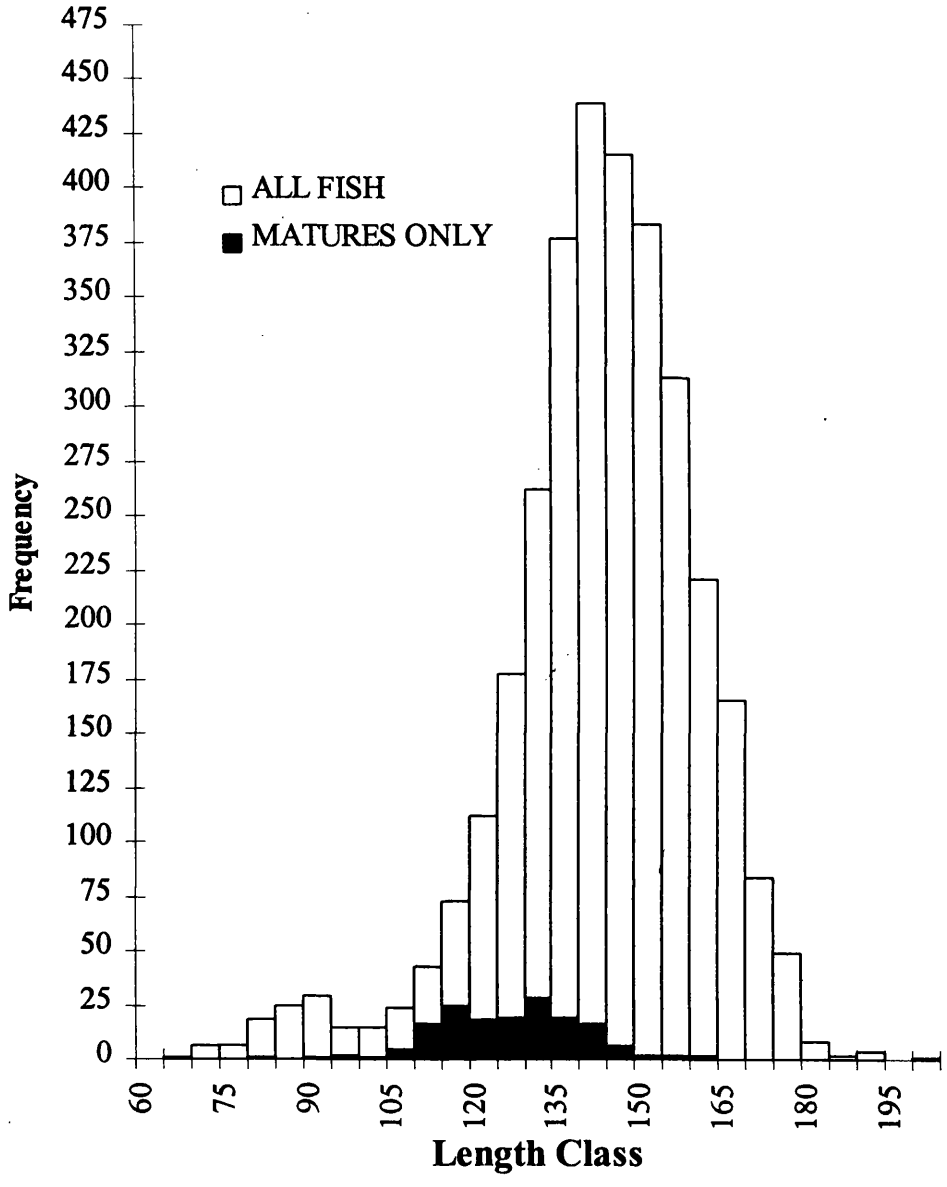

Figure 5.2
Length-frequency distribution of fork length (in 5 mm length classes) of mature and immature Atlantic salmon parr in November (all treatment groups combined, n = 3268 fish)

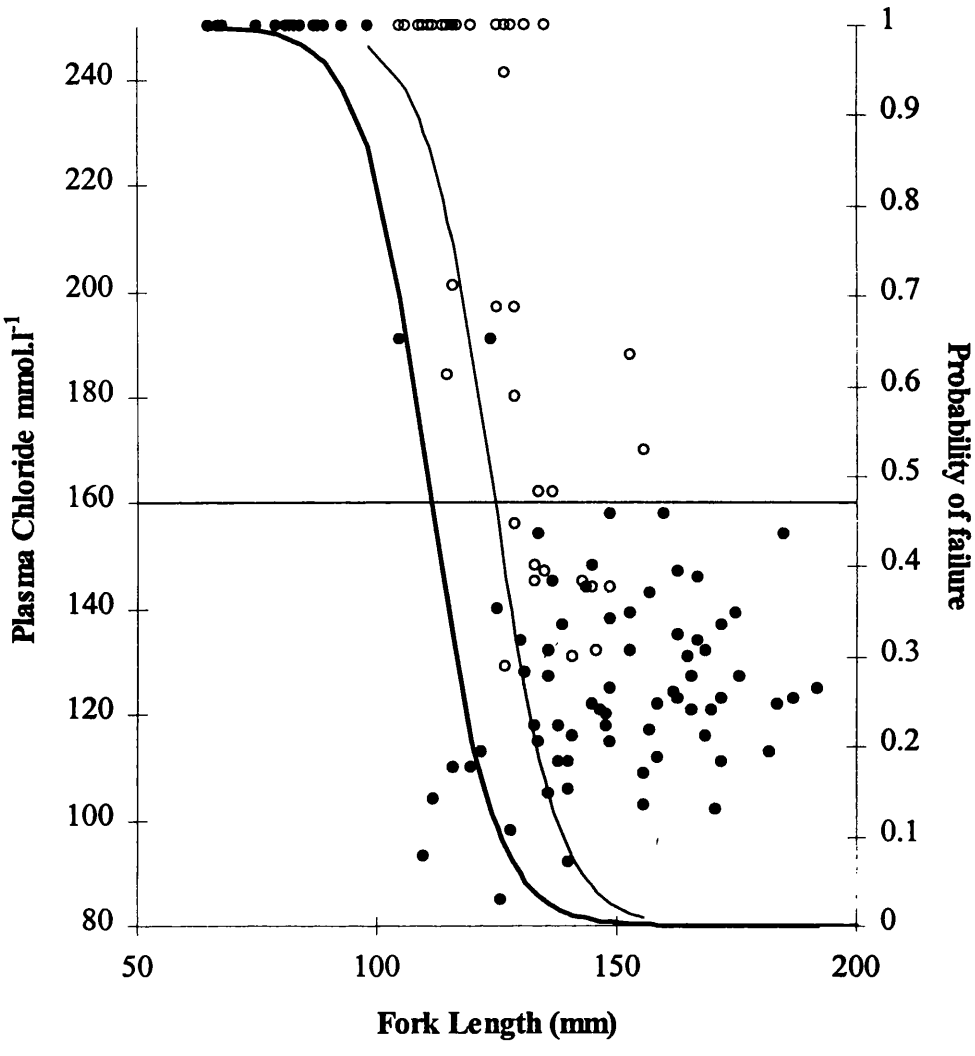

Figure 5.3
Osmoregulatory ability in sea water in relation to fork length in Atlantic salmon parr in December. Symbols represent the plasma chloride concentrations of individual fish after 24 h in 35‰ sea water. Fish with a plasma chloride level of over 160 mmol.l^{-1} (indicated by the horizontal line) were deemed to have failed the seawater challenge. For illustrative purposes, fish that died during the experiment are given a plasma chloride level of 250 mmol.l^{-1} (higher than that of any fish that was alive at the end of the experiment). The curved lines are those derived from the logistic regression analysis (see text), and have the equation: $Y = a/(1+a)$, where $a = e^{b+cX+d}$, Y = probability of failing to survive, X = fork length, b = 18.0366, c = -0.1457, and d = -1.9041 for mature fish and 0 for immature fish. The bold line and closed circles represent immature fish; the fine line and open circles represent mature fish.

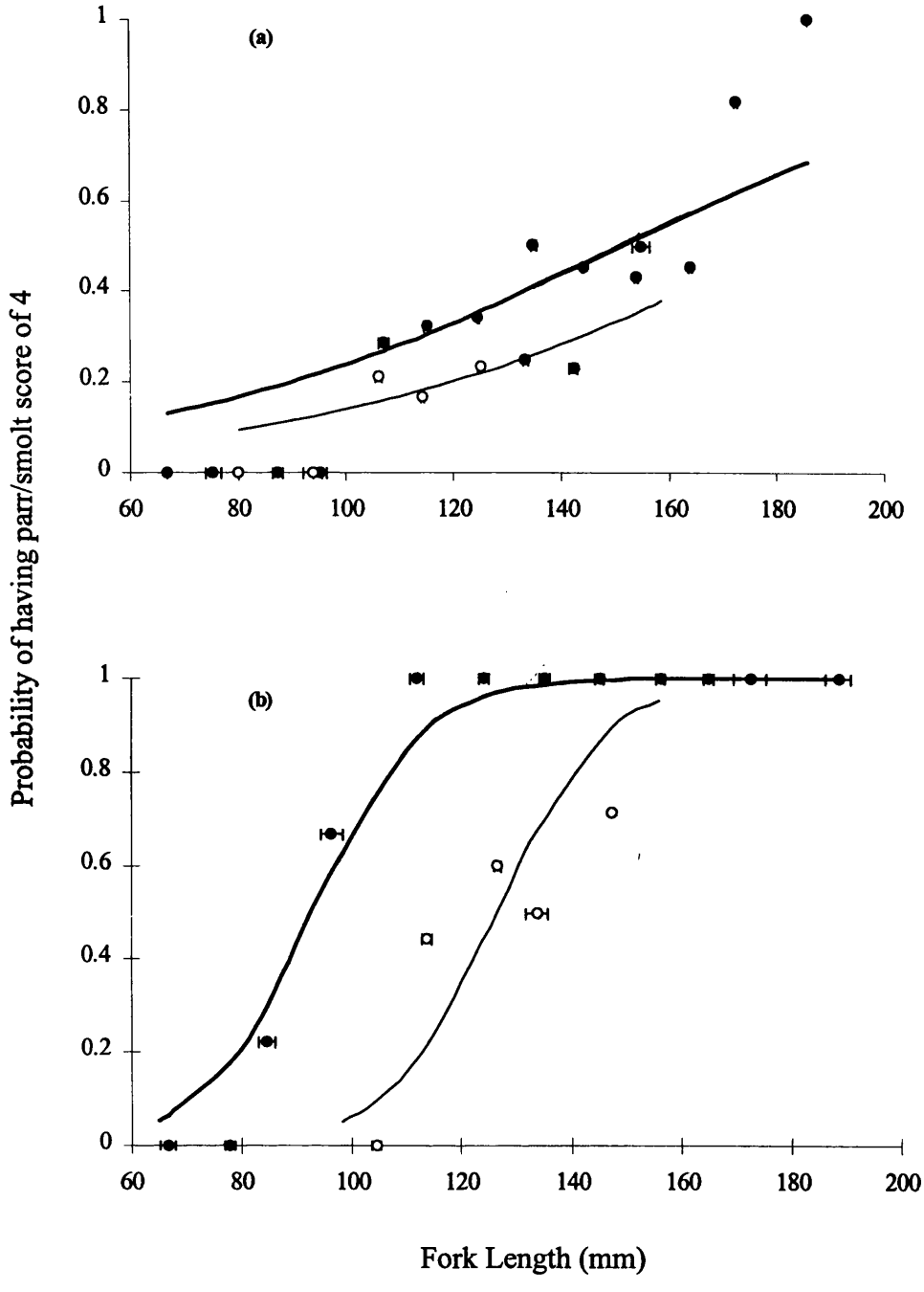

Figure 5.4
Logistic regressions of the probability of an Atlantic salmon parr having full smolt coloration (parr/smolt score of 4) in relation to its fork length in **(a)** November and **(b)** December. Symbols are the actual proportion of fish that had a colour score of 4, within each 10 mm size range. The analyses are based on 763 fish in November and 126 in December. Lines are the logistic regression line given by the equation: $Y = a/(1+a)$, where $a = e^{b+cX+d}$, Y = probability of having full smolt coloration and X = fork length. In November, b = -3.408, c = 0.0225, and d = -0.6693 for mature fish and 0 for immature fish. In December, b = -6.0396, c = 0.1017, and d = -6.8328 for mature fish and -3.4164 for immature fish. The bold lines and closed circles represent immature fish; the fine lines and open circles represent mature fish.

Chapter 6: Alternative competitive strategies in juvenile Atlantic salmon: evidence from fin damage

6.1 Introduction

In the wild, Atlantic salmon (*Salmo salar*) spend at least a year and usually more in fresh water. During this time they are territorial and engage in frequent bouts of agonistic behaviour (Kalleberg, 1958; Keenleyside & Yamamoto, 1962). Densities are much higher in aquaculture than in the wild and thus the physical damage caused by aggression is more frequent and severe in culture than in the wild: so much so, in fact, that fin condition can be used to distinguish between farmed fish and wild stocks (e.g. Bosakowski & Wagner, 1994). Aggression has a negative impact on growth and welfare in farmed fish. Growth depensation - the phenomenon whereby initial small size differences within a group become more pronounced as time goes on - can arise from inequalities in food intake that result from social hierarchies maintained by aggression (Jobling, 1985; Jobling & Wandsvik, 1983). Aggression can result in physical damage, which may lead to secondary infections with pathogens such as *Aeromonas salmonicida*, the causative agent in furunculosis (Schneider & Nicholson, 1980; Turnbull et al., 1996). Furthermore, social subordination is associated with chronic stress, which can have detrimental effects on health and growth (Schreck *et al.*, 1997; Wedemeyer, 1997).

Most studies of aggressive interactions in fish have been carried out using pairs, triads, or small groups of fish (fewer than twenty). In such groups, there are usually pronounced social hierarchies dominated by one or two aggressive individuals that monopolise the food supply and reduce the feeding activity and thereby the growth of their social subordinates (Jobling & Wandsvik, 1983; Koebele, 1985; Huntingford et al., 1993; Adams et al., 1998). Dominant fish perform more aggressive acts than subordinates and the subordinates usually receive more aggressive nips and exhibit more

fin damage than dominants (Abbott & Dill, 1989; Fenderson & Carpenter, 1971; Gregory & Griffith, 1996; Moutou et al., 1998). However, the conclusions reached from such studies may not necessarily hold true for larger groups of fish. For instance, in paired encounters between juvenile Arctic charr (*Salvelinus alpinus*), the more aggressive fish of the two usually acquired more food, but in culture conditions the same individuals were no more likely to grow well than their subordinate partners (Adams & Huntingford, 1996). This may be because the social hierarchy is less stable in larger groups (Fenderson & Carpenter, 1971). A difficulty in studying aggressive interactions in culture conditions is the large number of fish involved, which make it practically impossible to observe the behaviour of known individuals. It is here that the damage caused by aggressive behaviour can be used as an indicator to shed light on the subject.

The best-known physical damage caused by aggression is inflicted on the fins and is termed fin damage, fin erosion or fin rot. These terms cover a range of symptoms including splitting of the fin rays, tissue loss and pale nodular thickening of the distal portion of the fin (Turnbull et al., 1996). Fin erosion has been attributed to a plethora of factors, such as abrasion, trauma, malnutrition, under-feeding, sunburn, poor water quality, rough handling, high pH, infections, dissolved toxins and even abrasion through contact with other fish (listed by Winfree et al., 1998). While these factors can be involved, there is considerable evidence that the principle cause of fin erosion in farmed salmonids - especially when it occurs on the dorsal fin - is aggressive behaviour. While other fins may be damaged, the dorsal fin is the most commonly and most severely damaged fin. In paired encounters between rainbow trout, the dorsal fin was frequently attacked and incurred more damage than other parts of the body (Abbott & Dill, 1985). Similarly, in small groups of Atlantic salmon, the dorsal fin was attacked more frequently, was contacted more often, and incurred more damage than other parts of the body (Turnbull et al., 1998). Further evidence comes from the work of Turnbull (1992): fish that had damaged dorsal fins showed immediate improvement in fin condition when placed in isolation, while injuries similar to fin-rot could be produced by simulating bites

with the head of a dead salmon parr, but not by other means. Most importantly, scanning electron micrographs of damaged fins from fish-farms showed clear tooth marks and an absence of bacterial infection (Turnbull et al., 1996).

Given that fin damage is caused by aggression, a study of its prevalence should give valuable insights into the nature and extent of aggressive interactions in farmed salmonids. Fin damage has been used as an indicator of the strength of the social hierarchy by Christiansen & Jobling (1990) and Moutou et al. (1998), providing useful insights into the dynamics of aggression within larger groups of fish than can easily be studied otherwise. Fin splitting is the primary symptom of fin damage, and repeated splitting eventually leads to tissue loss. Splitting heals rapidly, whereas re-growth and reduction in thickening take longer to occur (Turnbull, 1992). Therefore, splitting is likely to be the best indicator of current levels of aggression. Tissue loss may be used as an indicator of the overall severity of fin damage, but not of current rates of aggression. Thickening is associated with the healing process but may also be more severe when damage is inflicted before previous wounds have healed (Turnbull, 1992).

The aim of this chapter is to examine the effect of body size on the incidence of fin damage in large groups of fish kept under culture conditions. By using data from individually-marked fish previously subjected to different manipulations of growth rates, I was able to compare the effects of both relative (to other group members) and absolute body size on the timing and duration of fin damage. I demonstrate a strong and consistent effect of relative body size, which indicates the existence of alternative strategies of aggression and feeding within groups of fish.

6.2 Materials and methods

The experiments involved a population of farmed Atlantic salmon parr from pooled hatchery stock. The experiment started approximately two weeks after first-

feeding, on 17 April 1997, when approximately 6,200 fish were transported from Marine Harvest McConnell's (MHM) hatchery at Inchmore to the MHM freshwater site at Invergarry. Here, the population was split between four tanks labelled A-D with 1,550 (± 7%) fish per tank. In order to manipulate growth rates, group D, the control, remained at Invergarry throughout the experiment, while groups A, B and C successively spent three weeks in colder water (mean of 8.3 ± 0.02 °C , Figure 6.1) in Glasgow University's aquaria (A from 17 April-8 May, B from 9-29 May and C from 30 May-19 June) before being returned to Invergarry. The water at Invergarry was heated to ca. 12°C until mid-May when ambient temperatures reached that level. The fish were then kept at the ambient water temperature until the third week of October, when the water was heated to keep temperatures at ca. 8°C (Figure 6.1).

From 17th April to 20th June, the fish at Invergarry were kept in small, circular tanks (diameter 0.6m, water depth 0.25m). During the manipulation periods in Glasgow, they were kept in similar-sized tanks (diameter 0.6m, water depth 0.3m. On 20th June all four groups, now permanently at Invergarry, were transferred to larger, 2m square tanks (water depth 0.5m), where they remained until the end of the experiment.

Throughout the experiment the tanks were lit by overhead fluorescent strip-lights; the photoperiod regime was that used commercially to produce accelerated "S½" smolts, with long days separated by a photoperiod "winter" in the (real) early autumn (Figure 6.1). The fish were fed to excess throughout the experiment on a pelleted diet of an appropriate size according to manufacturer's tables. Food was dispensed from clockwork belt feeders in the small, circular tanks and from hoppers in the large square tanks.

Populations were thinned to 1,150 fish per tank on 29 May. Further thinning took place on 19 June (135 ± 5 fish being removed from tanks C and D), 2 September (100 to 220 fish from each tank) and 9 November (100 fish from each tank) when fish were

sampled for use in experiments reported in Chapters 3 & 4. On 29 August, while moving the fish between tanks, approximately 215 fish from Group D were accidentally mixed in with Group A. While the tagged fish from group D could be retrieved, the untagged fish could not, and from this time onwards Group A had more fish, and group D had fewer fish, than the other groups.

A random sample of 150 fish was measured on 18 April, the first day of the experiment. The fish were anaesthetised in an aerated water bath using Benzocaine in 95% alcohol, and then measurements were made of fork length (to 1 mm) and weight (to 0.01 g, after blotting with damp tissue paper to remove excess water). Random samples of 150 fish per group were then measured on 9 May, 29-30 May and 19-20 June. On 22-25 July, random samples of 100 fish per tank was measured and tagged with Passive Integrated Transponder (PIT) tags. The PIT tags were inserted into the body cavity through an incision made in the body wall. The entry wound was dusted with a 50:50 mix of Cicatrin™ antibiotic powder (Wellcome Foundation Ltd, London, U.K.) and Orahesive™ Protective Powder (ER Squibb & Sons, Hounslow, U.K.) to help prevent infection and close the wound. The tagged fish were re-measured on 1-3 September, 3-9 November and 9 December. Since from July onwards only the tagged fish were assessed for fin damage, and data are presented only for the tagged fish that survived to the end of the experiment and were assessed on all sampling dates (n = 314), the changes in frequency should represent actual healing or incurring of damage rather than sampling error.

On each of the measurement days, damage to the dorsal fin was assessed by comparison with Figure 6.2. Three separate measures were assessed: tissue loss (judged by fin size), splitting and thickening. Fin size was scored on a five-point scale (Figure 6.2). There was no 100% class as it was difficult to distinguish whether a fin was intact or slightly reduced in size; thus the 90%+ category was taken to be undamaged and the lower categories were classed as damaged. Splitting and thickening were both classed as

either absent (0), mild (1) or severe (2). Fin splitting categories 1 and 2 were combined for analysis as the distinction between the two was judged in retrospect to be unsatisfactory. Thickening category 2 was never seen.

Specific Growth Rate in length between measurement periods was calculated as:

$$SGR = 100 \times [\ln (FL_{t2}) - \ln (FL_{t1})] / (t_2 - t_1)$$

where t_1 = first sampling day; t_2 = second sampling day and FL = fork length (mm).

6.3 Results

Severity and frequency of fin damage

Fin splitting was seen in more fish than the other types of damage: 91% of the fish had split fins at some time between July and December, while only 25% had fin thickening and 37% had fin tissue loss. The fin condition of individual fish changed over time, so that not all fish affected on one sampling date were affected on the next, and *vice versa*. Fin thickening and tissue loss were closely associated with splitting: in 87% of the instances when thickening was recorded (n = 107), the fish had split fins at the same time, and in 9% the fish had had split fins on a previous sampling date. Similarly, in the majority of instances when tissue loss was recorded (83% of 228), the fish either currently or previously had split fins. Figure 6.3 shows the percentage of fish in each group exhibiting each symptom of fin damage, while comparisons between groups of the frequencies of fin damage at each sampling date are given in Table 6.1.

No fin damage of any kind was visible until the end of May, when it was evident in small numbers of fish in groups A, B and C. There was a rapid rise in the prevalence of damage between June and July. From July onwards, splitting was the most prevalent type of fin damage. In July and September, it affected between 50% and 75% of fish (depending on the treatment group). It then declined during the photoperiod winter so

that by November 6% to 38% of fish were affected. It increased to previous levels by December, affecting between 47% and 76% of fish.

Thickening was less prevalent than splitting. It was never seen in more than 33% of the fish in any group at one time. In July, it affected significantly more fish in the control group (D) than in the other groups. In November, group B was more affected than the other groups. On both of these occasions, the most affected group also had the highest incidence of splitting at the time.

Tissue loss was also less prevalent than splitting. With the exception of Group B, it never affected more than 28% of the fish in a group at any one time. Group B showed a gradual increase in the numbers of fish affected from July onwards, and by the end of the experiment 50% of the fish in this group were affected. Tissue loss was not particularly severe. Only two fish ever had less than 30% of the dorsal fin remaining, and most of those affected (130 out of 139) had 60% to 90% remaining.

Since fin splitting was the most common category of damage and it is the best indicator of current levels of aggression, all subsequent analyses are based only on this measurement.

Relationship between Fin Splitting, Body Size and Growth Rate

By November, 2.2% (7 fish) were very small parr that had failed to smolt, while 4.1% (13 fish) were sexually mature male parr. As the growth patterns of both of these categories of fish differed from the majority of the fish (which were immature smolts) they have been excluded from the analysis. As cold water treatment had a significant effect on growth rates and hence body size, the four groups of fish are treated separately.

Table 6.2a gives the mean fork length (± SE) of fish with and without split fins in July, September and December when fin splitting was evident in large numbers of fish in all groups. There was a significant effect of experimental group at all times due to the effect of the experimental manipulation (Table 6.2b). There were also significant differences in length between fish with and without split fins in July and September, when fish that had split fins were on average larger than fish without split fins. In December, there was a significant interaction between group and fin condition, as the fish in group C that had split fins were still larger than those without split fins, but there was no longer any such relationship within the other groups. Fin condition of a given fish in September was not associated with its fin condition in December (all groups combined, n = 296, χ^2 = 1.872, 1 d.f., n.s.).

The relationship between fin damage and length within each group of fish in July was very strong. Figure 6.4 shows the logistic regression lines relating fin splitting to fish length for each group in July; the corresponding statistical analyses and the values of the coefficients of the logistic regression equation are given in Table 6.3. The data are presented separately for each group as the size range of fish varied as a result of the cold temperature treatment. In all groups, there was a strong and significant positive relationship between the probability of having split fins and the size of the fish, with the probability rising from less than 0.15 for the smallest fish in each tank, to over 0.9 for the largest fish. The effect was related to the size range within the tank, not the absolute size of the fish: for instance, an 85 mm fish would be one of the largest fish in the tank in Group B or C, and would have a 0.86 or a 0.88 probability of having split fins, respectively. A fish of the same size but in Group A or D would be in the middle of the size range and would be considerably less likely to have split fins (probabilities of 0.62 and 0.61 respectively).

Figure 6.5 shows the same data for all four tanks combined. Statistical analyses and the coefficients of the logistic regression equation are given in Table 6.3. To adjust

for the differences in size between the four groups, fish size is expressed as the deviation from the mean length of the group as a proportion of the mean. Data points representing the parr that would not smolt and the mature male fish have been added to Figure 6.5 for comparison but were not included in the logistic regression analysis. The non-smolting parr were as likely as other fish of their size to have fin damage. The mature male parr were somewhat more likely to have fin damage than immature fish in the same relative size range ($\chi^2 = 4.42$, 1 d.f., n = 122, $p<0.05$).

Table 6.4a shows for each group the mean SGR (±SE) for each growth period after tagging (July to September, September to November and November to December), according to fin condition at the end of the growth period (split or not split). Since SGR in fish is inversely related to body size (Jobling, 1985) the SGRs have been adjusted by expressing them as the residual from the regression line for control fish of SGR on initial fork length at the start of each measurement period. Table 6.4b gives the results of two-way analyses of variance of growth rates by group and fin condition. The only growth period in which there was a difference in the growth rates of damaged and undamaged fish was July to September. Fish that had split fins in September had been growing more rapidly, on average, than fish that did not have split fins. This was not the case for the following growth periods.

6.4 Discussion

When it first appeared, fin damage was strongly associated with size. The largest fish in a tank were up to six times more likely to have damaged fins than the smallest fish. This is the first time that such a relationship has been demonstrated, and indeed it appears to contradict some previous findings. Turnbull (1992) found that larger fish had less fin damage than smaller fish, but the fish were sampled from fish farms over a period of several months and so do not accurately reflect the relationship of fin damage

to relative size within a group. Turnbull et al. (1998) found no connection between size and fin damage in groups of eight Atlantic salmon parr.

Most previous studies have demonstrated that social subordinates bear the brunt of the aggressive attacks of dominant fish (Abbott & Dill, 1989; Gregory & Griffith, 1996; Moutou et al., 1998). If this was the case, it would imply that the smaller fish in this study were actually dominant. Was this likely? Huntingford et al. (1990) found that social rank in Atlantic salmon was not always correlated with size, although when there was a large size differential the larger fish was usually dominant, and concluded that size is a consequence of dominance rather than a cause of it. At first feeding, social dominance in salmonids is not related to body size, but to aggressiveness, which is itself related to metabolic rate (Titus & Mosegaard, 1991; Metcalfe et al., 1992, 1995). Since social hierarchies in salmonids appear to be quite stable over time (Abbott et al., 1985), the advantage that dominance confers in terms of increased feeding opportunity soon leads to increased growth rates and hence greater body size (Metcalfe et al., 1992). Thus the larger fish in the current experiment were almost certainly aggressive fish that could compete effectively for food and therefore achieve rapid growth. Indeed, fish that had fin damage did have higher growth rates than undamaged fish between July and September. Further confirmation of this view comes from the fact that the fish that had delayed smolting, that are usually socially subordinate (Metcalfe et al., 1989), had the same low levels of fin damage that were observed in pre-smolts of the same size.

Why, then, was fin damage more prevalent in the larger fish? As the size of a group increases, it becomes increasingly difficult for a single fish to monopolise the food supply and a group of dominant fish emerges (Alanärä & Brännäs, 1996). Furthermore, in small groups of Atlantic salmon held at production densities, the fish with the highest food intake also received the largest number of aggressive attacks (Adams et al., 1998). Therefore in large groups of hundreds of fish, it is likely that there are many large, aggressive, dominant fish that fight amongst themselves for food. Abbott & Dill (1985)

found that in the case of steelhead trout, the dorsal fin was more frequently nipped in reciprocal bouts of fighting than in bouts where the attacked fish escaped. If the same is true in farmed Atlantic salmon, this would add weight to the conclusion that the larger fish were fighting amongst themselves.

What about the less aggressive fish? They may have adopted alternative feeding strategies that reduced the risk of injury. One possible alternative strategy could be sneaking in to feed while others fight (Pettersson et al., 1996). Adams et al. (1998) found that some fish managed to obtain food without fighting at all, by darting in and out of the feeding area as soon as food became available. Another possibility is a sit-and-wait strategy. Kadri et al. (1996a) found that in a sea-cage of one sea-winter Atlantic salmon, the most successful fish - those that achieved the greatest food intake - fed at the water's surface and contested many pellets (although there was little overt aggression). The less successful fish avoided contests by staying well below the surface and feeding on pellets that dropped down through the water column. Subordinate fish might also avoid competition by feeding at different times of day, thus avoiding interaction with dominants, as suggested by studies of post-smolts (Kadri et al, 1997a). Since parr are more aggressive than salmon in sea-water, the risks of direct competition for food should be greater and so, while for aggressive fish the greater growth rates attained may outweigh the costs of injury, alternative strategies may well be more profitable for less aggressive fish.

When food is available in excess these less aggressive strategies should still result in viable, if lower, rates of food acquisition and growth while reducing the risk of injury. However, when food is scarce, such strategies might not pay off and fin damage should be more evenly distributed between size classes, or even concentrated amongst the smaller fish if they are forced to compete with dominant fish. Subordinate fish may have begun to compete with dominants, but for other reasons, later in the year. By December, fin damage was no longer related to body size in three out of the four groups,

although the group with the smallest mean fork length had the highest prevalence of fin damage. This may indicate that the end of the photoperiod winter induced the smaller fish to compete more actively for food in order to increase their growth rates and smolt at a larger size. Indeed, a field study of Atlantic salmon has shown that relatively small pre-smolts grow more during the spring than larger pre-smolts from the same population (Nicieza & Braña, 1993). However, in the present study, the increase in aggression in the "spring" suggested by the increased incidence of fin damage was not accompanied by any detectable increase in growth rates and fin condition was not related to growth rate. The increase in fin damage after the end of the photoperiod winter is nevertheless typical of fish in such culture conditions (C. Cox, MHM, pers. comm.).

The reduction in incidence of fin damage during the photoperiod winter suggests that there was a decrease in aggression, which also coincided with the autumnal decline in water temperature. It is possible that the decreased demand for food at lowered temperatures coupled with the photoperiodic cue for winter resulted in a decline in appetite and therefore rates of aggression. However, temperatures were still low between November and December, when fin damage (and therefore aggression) increased once again. For any given temperature, gut evacuation rate (and therefore the maximum potential food intake) of juvenile Atlantic salmon is lower in the autumn than in the spring (Higgins & Talbot, 1985). Thus, the increase in day length after the photoperiod winter (analogous to spring) may have been a cue for a resurgence in appetite.

Overall, the fin damage observed in this experiment was less severe than is often encountered on fish farms, where tissue loss can be so severe that the dorsal fin is almost entirely eroded (Turnbull et al., 1996). Fin splitting was common in this experiment, but tissue loss was considerably less so. This may have been because stocking densities were generally lower than is usual on commercial farms, resulting in lower encounter rates between fish. The gross thickening of the fin tissue that is the classic symptom of "fin rot" was not observed, although mild thickening was evident in some cases. Thickening

is part of the healing process and involves the migration of epithelial cells to the damaged area (Turnbull, 1992; Turnbull et al., 1998). Thickening is more severe when damage occurs regularly, without time to heal, as it results in an accumulation of pathological changes. This is most noticeable in the cold, as the healing process is slowed (Turnbull, 1992). Thus the combination of warm water and the relatively low severity of damage may have prevented severe cases of "fin rot" from developing.

The absence of fin damage at the start of the experiment and the low levels of damage prior to July could be because the fish were not yet exhibiting aggressive behaviour. However, wild salmonids become aggressive within days of emergence from the redd (Kalleberg, 1958; Dill, 1977; Gustafson-Greenwood & Moring, 1990; Titus & Mosegaard, 1991). The appearance of fin damage in large numbers of fish occurred after a marked decrease in stocking density. It is possible that this change in stocking density coincided with an ontogenetic shift in aggressive behaviour. Kalleberg (1958) noticed that agonistic behaviour in Atlantic salmon parr went through a qualitative change when the fish were 60-70 mm in length. At smaller sizes, agonistic encounters mainly involved frontal attacks, with the dorsal fin lowered close to the back, while larger fish tended to use lateral displays with the dorsal fin erect. Clearly the latter posture would expose the dorsal fin to a far greater chance of damage. In the present study, the rapid appearance of fin damage coincided with the majority of the fish passing the 60mm threshold. Possibly, then, the increased tendency to exhibit lateral display behaviour exposed the dorsal fin to damage for the first time.

In conclusion, I have used fin damage as an indicator of aggressive interactions in large groups of juvenile Atlantic salmon in culture conditions. Fin damage was strongly related to relative body size, indicating the existence of alternative feeding strategies within groups of fish. The present study serves as a warning against uncritically extrapolating the findings of small-scale studies to culture conditions: social interactions may differ markedly according to group size. Fin damage can give valuable

insights into the nature of aggressive interactions in large groups of fish, and could be useful in comparing the success of feeding regimes in reducing the level of aggression in cultured populations.

Table 6.1 Comparisons by χ^2 test of frequencies of three categories of fin damage between groups of Atlantic salmon of different mean length on five sampling dates. χ^2 values were regarded as invalid and are omitted if cross-tabulation yielded expected frequencies of less than five in one or more cells. D.f. = 3 in all cases. Levels of statistical significance: * = $p<0.05$, ** = $p<0.01$.

Category	Sampling Date	n	χ^2
Splitting	June	600	0.69
	July	314	7.70
	September	314	10.73 *
	November	314	25.84 **
	December	314	15.54 **
Thickening	July	314	18.19 **
	November	314	31.74 **
	December	314	2.41
Tissue Loss	June	600	7.99 *
	July	314	15.25 *
	September	314	2.58
	November	314	37.96 **
	December	314	28.38 **

Table 6.2: (a) Mean fork length (± SE) of fish with and without split fins in groups A to D in July, September and December. **(b)** Two-way analyses of variance of fork length by fin condition (split or intact) and treatment group (A-D). Statistical significance: * = $p < 0.05$ ** = $p < 0.01$

(a)

Month	Group	n	Mean Fork Length (mm) ± SE of fish with fins: intact	split
July	A	76	77 ± 1.3	83 ± 1.2
	B	53	70 ± 1.8	80 ± 1.5
	C	82	70 ± 1.1	78 ± 0.9
	D	83	82 ± 2.0	95 ± 1.2
Sept.	A	76	116 ± 2.2	122 ± 1.5
	B	53	98 ±3.4	111 ± 2.0
	C	82	113 ± 2.1	118 ± 1.7
	D	83	126 ± 3.1	133 ± 1.4
Dec.	A	76	159 ± 2.9	156 ± 1.5
	B	53	146 ± 5.8	146 ± 2.0
	C	52	147 ± 2.4	157 ± 1.7
	D	83	168 ± 2.1	169 ± 1.8

(b)

Month	Factor	F	d.f.	p
July	Splitting	81.8	1	**
	Group	46.4	3	**
	Interaction	2.5	3	n.s.
	Error		286	
Sept.	Splitting	24.9	1	**
	Group	37.5	3	**
	Interaction	1.1	3	n.s.
	Error		286	
Dec.	Splitting	1.6	1	n.s.
	Group	34.8	3	**
	Interaction	3.1	3	*
	Error		286	

Table 6.3 Values of χ^2 and coefficients defining the logistic regression line of the probability of having split fins in relation to fork length in July in groups A to D, and of the probability of having split fins in relation to deviation from the group mean in all groups combined (see also Figures 6.4 and 6.5). The logistic regression equations are given by the formula Y = a/(1+a) where Y = probability of having split fins, X = fish length and $a = e^{b+cX}$.

Group	b	c	χ^2	p
A	-7.9442	0.0994	10.56	0.0012
B	-9.4217	0.1322	14.04	0.0002
C	-12.5787	0.1720	22.39	<0.0001
D	-11.0913	0.1357	25.59	<0.0001
Combined	0.4002	10.4572	71.44	<0.0001

Table 6.4 (a) Mean adjusted SGR (± SE) of fish with and without split fins in groups A to D during three growth periods. **(b)** Two-way analyses of variance of adjusted SGR by fin condition (split or intact) and treatment group (A-D). Statistical significance: * = $p < 0.05$ ** = $p < 0.01$

(a)

Growth period	Group	n	Mean SGR (adjusted) ± SE of fish with fins: intact	split
July-Sep	A	76	0.018 ± 0.02	0.059 ± 0.02
	B	53	-0.169 ± 0.04	-0.098 ± 0.02
	C	82	0.081 ± 0.02	0.159 ± 0.02
	D	83	-0.023 ± 0.02	0.012 ± 0.01
Sep-Nov	A	76	-0.006 ± 0.01	-0.017 ± 0.01
	B	53	-0.025 ± 0.01	-0.024 ± 0.01
	C	82	-0.003 ± 0.01	0.016 ± 0.01
	D	83	0.001 ± 0.01	-0.01 ± 0.02
Nov-Dec	A	76	0.031 ± 0.01	0.019 ± 0.01
	B	53	0.042 ± 0.02	0.049 ± 0.01
	C	82	-0.006 ± 0.01	-0.027 ± 0.01
	D	83	0.007 ± 0.01	-0.007 ± 0.01

(b)

Growth Period	Factor	F	d.f.	p
Jul-Sep	Splitting	13.4	1	**
	Group	42.6	3	**
	Interaction	0.5	3	n.s.
	Error		286	
Sep-Nov	Splitting	0.0	1	n.s.
	Group	3.9	3	**
	Interaction	1.0	3	n.s.
	Error		286	
Nov-Dec	Splitting	1.7	1	n.s.
	Group	11.9	3	**
	Interaction	0.5	3	n.s.
	Error		286	

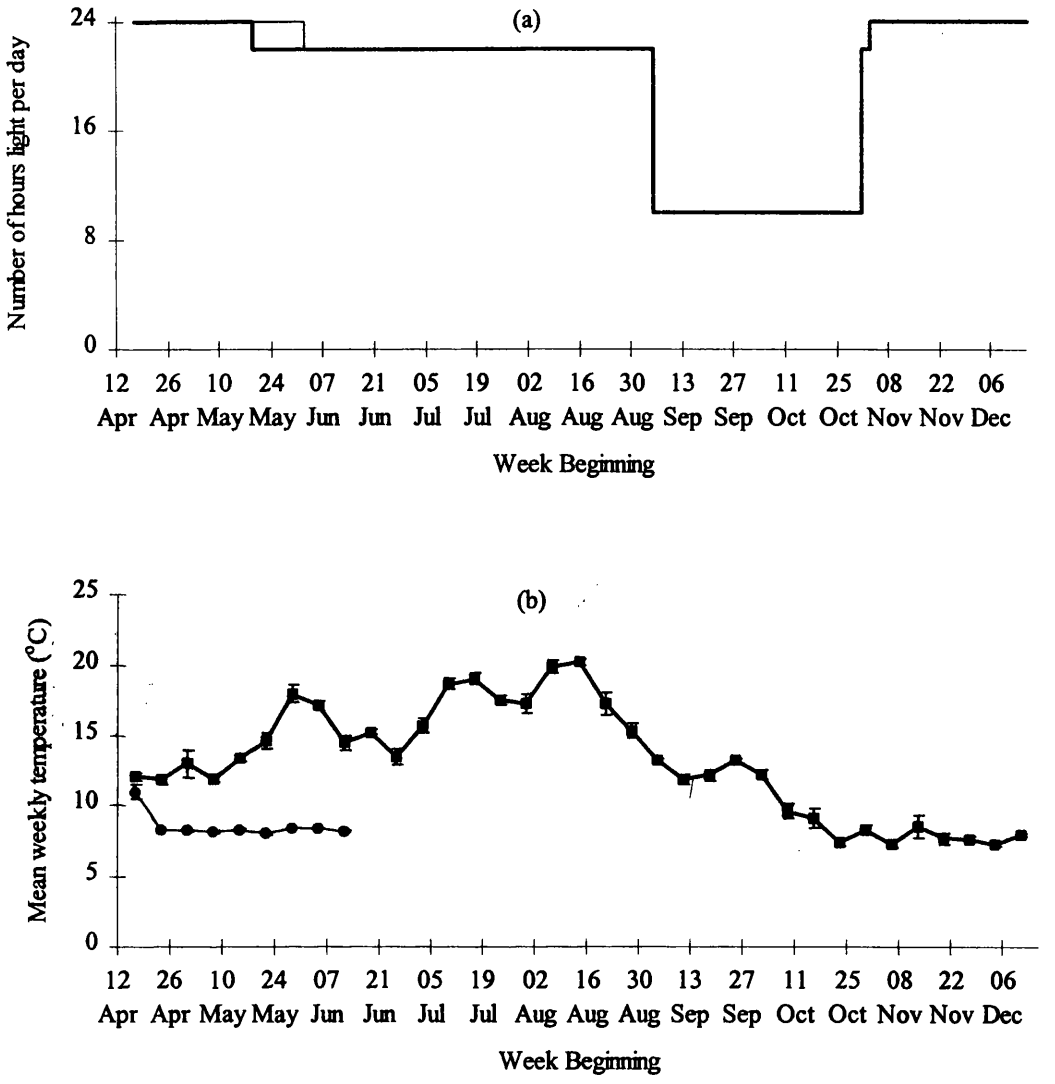

Figure 6.1
(a) Photoperiod and (b) mean (± SE) weekly daytime temperatures during the course of the experiment. Squares and bold lines indicate conditions experienced by group D (controls) throughout and by groups A-C except when subjected to the three-week cold water manipulation (circles and fine lines).

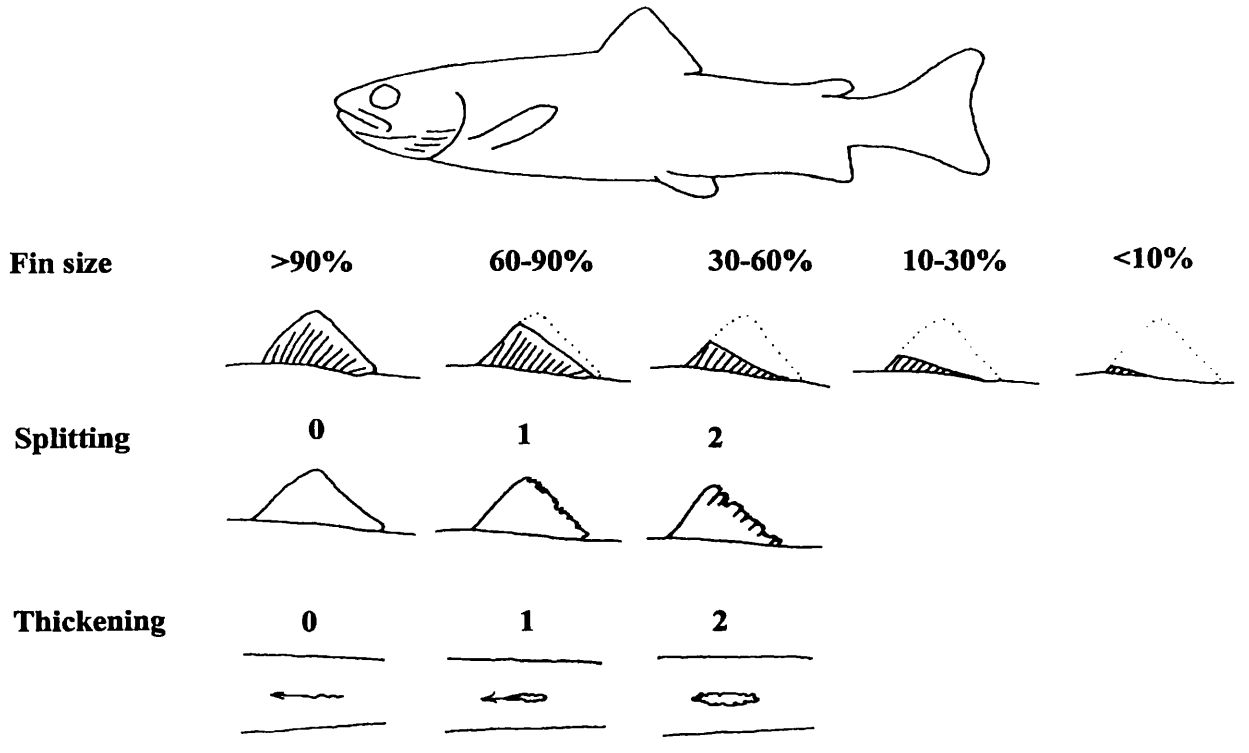

Figure 6.2
System used to score three categories of dorsal fin damage in juvenile Atlantic salmon. The size of the drawing was reduced or enlarged to approximate to the mean fork length of the fish being assessed. Fin damage was assessed in three categories: tissue loss, splitting and thickening. Tissue loss was scored on a five-point scale depending on the amount of the fin left. Splitting and thickening were both scored as either absent (0), mild (1) or severe, independently of the size of the fin. The fish was viewed laterally to score tissue loss and splitting, and dorsally to score thickening.

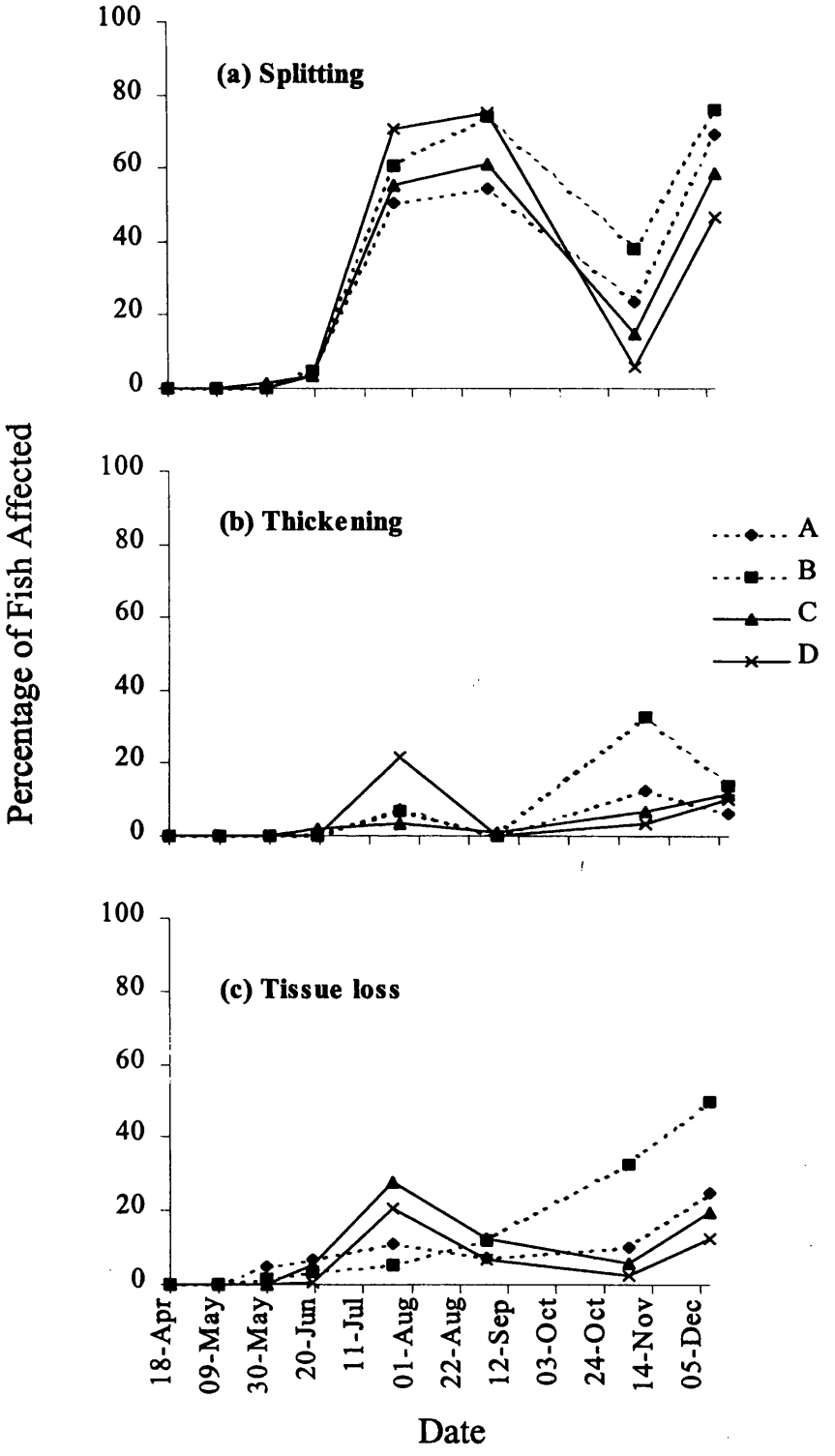

Figure 6.3
Changes over time in the percentage of fish affected by three measures of dorsal fin damage in four groups of juvenile Atlantic salmon: (a) splitting, (b) thickening and (c) tissue loss.

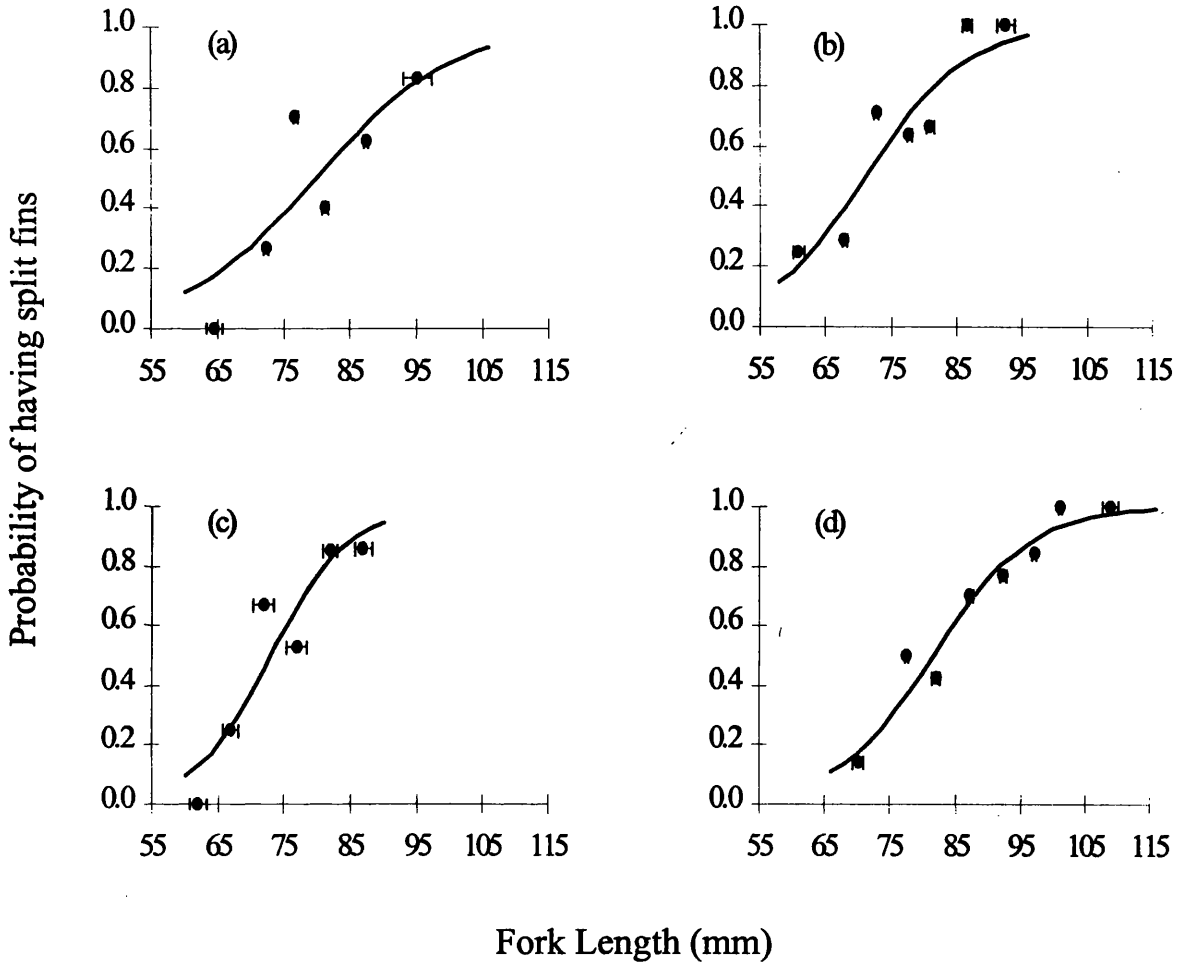

Figure 6.4
The effect of fork length on the probability of having split fins in four groups of juvenile Atlantic salmon in July (a) Group A (b) Group B (c) Group C (d) Group D. Symbols are the actual proportion of fish that had split fins, within each 5 mm size range. Where a 5 mm size range included fewer than 5 fish, it was combined with a neighbouring size range. Lines are the logistic regression line given by the equation: $Y = a/(1+a)$ where $a = e^{b+cX}$. The values of b and c are given in Table 6.3.

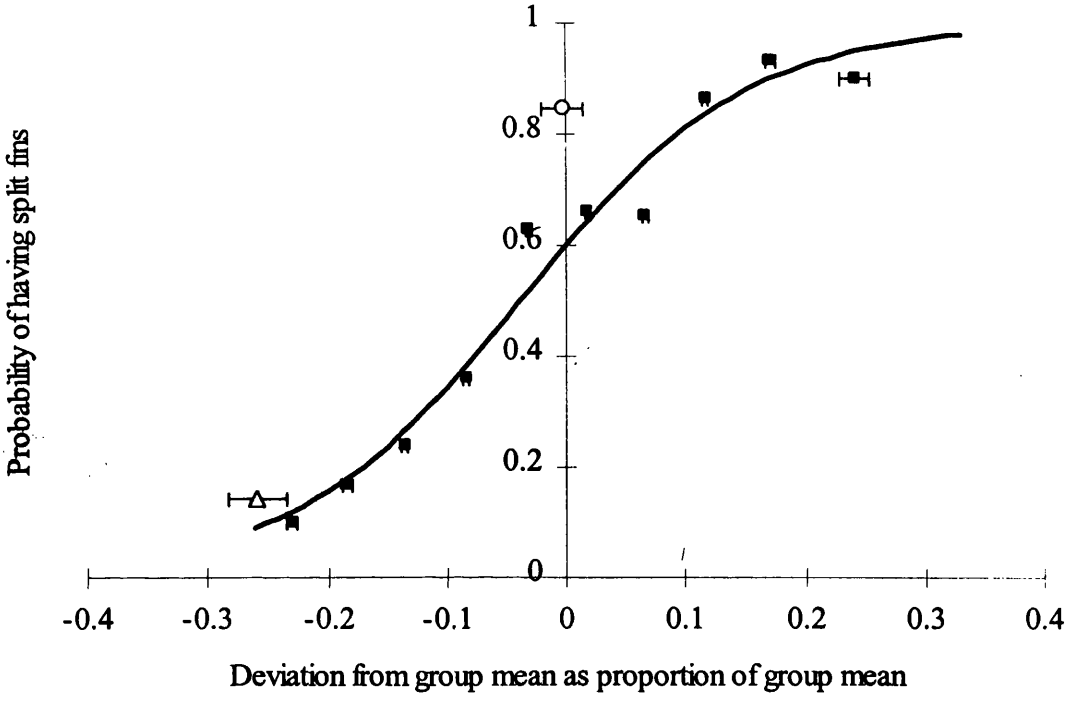

Figure 6.5
The effect of deviation from mean fork length for fish in a group (see text for explanation) on the probability of having split fins in Atlantic salmon in July. The logistic regression line is given by the equation: $Y = a/(1+a)$ where $a = e^{b+cX}$. The values of b and c are given in Table 6.3. Symbols (squares) are the actual proportion of fish that had split fins, within each 0.05 range. The logistic regression line has been calculated using only immature smolts (n = 294), but symbols representing non-smolting parr (n = 7; open triangle) and mature male parr (n = 13; open circle) have been added for comparison.

Chapter 7: Is aggression the cause of opercular erosion in cultured Atlantic salmon parr?

7.1 Introduction

The operculum plays a major part in the ventilation of the gills of fish. Flaring of the operculae produces negative pressure in the opercular cavity, drawing water from the buccal cavity over the gills (Marshall, 1965). A reduction in the size of the operculum can therefore result in poor gill ventilation, which can cause respiratory difficulties when fish are under oxygen stress. Opercular erosion – a condition in which the operculae become shortened, exposing the gills – is often observed in cultured fish, and can lead to respiratory stress (J.F. Turnbull, pers. comm.), but has been largely ignored in the published literature. Although the size of the operculae is generally recognised as an indicator of fish health (Roberts & Shepherd, 1997), and eroded operculae have been reported in several species, little is known about its causes.

Vitamin deficiency is most often cited as a cause of opercular deformity or foreshortening. Vitamin A deficiency is associated with warped operculae in carp *Cyprinus carpio* (Aoe et al., 1967), while Korean rockfish (*Sebastes schlegeli*) fed a diet deficient in vitamin E, develop shortened operculae (Bai & Lee, 1998). In both these cases, the problems were associated with other, severe, symptoms of vitamin deficiency, such as exophthalmia, poor growth, fin haemorrhages and foreshortening of the gill arch in carp (Aoe et al., 1967) and muscular dystrophy, exophthalmia, reduced appetite and slow growth in the Korean rockfish (Bai & Lee, 1998). Opercular deformation has also been reported as a result of vitamin C deficiency, but again this was in conjunction with other severe symptoms (Roberts, 1989a). Ostrowski et al. (1996) reported shortened or flared operculae in hatchery-reared Pacific threadfin (*Polydactylus sexfilis*), and suggested that it was related to nutrient deficiency. However, other factors may also be involved. For instance, exposure to pulp mill effluent leads to an increase in the frequency of shortened or cratered

operculae in perch *Perca fluviatilis* (Lindesjoo et al. 1994). Congenital abnormalities cannot be ruled out in cases where the operculum is permanently reduced in size (Roberts, 1989b) or severely deformed (Tchernavin, 1937). Bacterial infection could also be a causative agent, but in histological studies of the operculae of Atlantic salmon, J.F. Turnbull (pers. comm.) could find no bacteria or other pathological symptoms other than a thickening of the epithelium at the distal margin of the operculum. When healthy, well-fed, fast-growing fish in unpolluted water still develop eroded operculae, other explanations must be sought.

In this chapter, I describe the development of opercular erosion during the freshwater cycle in cultured Atlantic salmon parr, and suggest that it may have been caused by agonistic interactions resulting in physical injury.

7.2 Materials and methods

The experiments involved a population of farmed Atlantic salmon parr from pooled hatchery stock. The experiment started approximately two weeks after first-feeding, on 17 April 1997, when approximately 6,200 fish were transported from Marine Harvest McConnell's (MHM) hatchery at Inchmore to the MHM freshwater site at Invergarry. Here, the population was split between four tanks labelled A-D with 1,550 ($\pm$ 7%) fish per tank. In order to manipulate growth rates, group D, the control, remained at Invergarry throughout the experiment, while groups A, B and C successively spent three weeks in colder water (mean of 8.3 °C $\pm$ 0.02) in Glasgow University's aquaria (A from 17 April-8 May, B from 9-29 May and C from 30 May-19 June) before being returned to Invergarry. The water at Invergarry was heated to ca. 12.4°C ($\pm$ 0.2) until mid-May when ambient temperatures reached that level. The fish were then kept at the ambient water temperature (mean of 15.5 °C $\pm$ 0.3, minimum of 8.0°C , maximum of 21.6°C) until the third week of October, when the water was heated to keep temperatures at ca. 7.9°C ($\pm$ 0.2).

From 17th April to 20th June, the fish at Invergarry were kept in small, circular tanks (diameter 0.6m, water depth 0.25m). During the manipulation periods in Glasgow, they were kept in similar-sized tanks (diameter 0.6m, water depth 0.3m).

On 20th June all four groups, now permanently at Invergarry, were transferred to larger, 2m square tanks (water depth 0.5m), where they remained until the end of the experiment.

Throughout the experiment the tanks were lit by overhead fluorescent strip-lights. The photoperiod regime was that used commercially to produce accelerated "S½" smolts, with long days (24L:0D until the end of May, and thereafter 22L:2D) separated by a photoperiod "winter" (10L:14D) in the (real) early autumn. The fish were fed to excess throughout the experiment on a pelleted diet of an appropriate size according to manufacturer's tables. Food was dispensed from clockwork belt feeders in the small, circular tanks and from hoppers in the large square tanks.

A random sample of 150 fish was measured on 18 April, the first day of the experiment. The fish were anaesthetised in an aerated water bath using Benzocaine in 95% alcohol, and then measurements were made of fork length (to 1 mm) and weight (to 0.01 g, after blotting with damp tissue paper to remove excess water). Random samples of 150 fish per group were then measured on 9 May, 29-30 May and 19-20 June. On 22-25 July, random samples of 100 fish per tank was measured and tagged with Passive Integrated Transponder (PIT) tags. The PIT tags were inserted into the body cavity through an incision made in the body wall. The entry wound was dusted with a 50:50 mix of Cicatrin™ antibiotic powder (Wellcome Foundation Ltd, London, U.K.) and Orahesive™ Protective Powder (ER Squibb & Sons, Hounslow, U.K.). The tagged fish were re-measured on 1-3 September, 3-9 November and 9 December.

On each of the measurement days, each fish was scored for opercular erosion using the following scale:

0 - operculum complete (illustrated in Figure 7.1a)

1 - reduction in size of operculum but gills filaments not visible

2 - two-thirds or more of operculum remaining but gill filaments visible

3 - one-third to two-thirds of operculum remaining (illustrated in Figure 7.1b)

4 - one-third or less of operculum remaining

5 - none of operculum remaining (illustrated in Figure 7.1c)

The left and the right operculum were given independent scores. The scoring was always done by the same author.

Populations were thinned to 1,150 fish per tank on 29 May. Further thinning took place on 19 June (135 ± 5 fish being removed from tanks C and D), 2 September (100 to 220 fish removed from each tank) and 9 November (100 fish removed from each tank), the removed fish being used in the experiments reported in Chpaters 3 & 4. On 29 August, while moving the fish between tanks, approximately 215 fish from Group D were accidentally mixed in with Group A. While the tagged fish from group D could be retrieved, the untagged fish could not, and from this time onwards Group A had more fish, and group D had fewer fish, than the other groups.

7.3 Results

There was no opercular erosion on the first two measurement dates, but it then increased dramatically in frequency from 0% on the 9th of May to 88% of all fish by the middle of June (Figure 7.2). Thereafter, the frequency of erosion began to decline, and by the end of the experiment in December only a very small percentage (0.6% of all fish) were affected by it. There were some differences between groups in the frequency of erosion (Table 7.1), but all four groups exhibited the same pattern of rapid increase in the spring followed by a decline in the autumn months.

When opercular erosion first appeared at the end of May, it was fairly mild, with scores of 1 in all but one of the affected fish (Figure 7.3). By June, when erosion was most frequent, it was also most severe: more than half (53%) of the affected fish scored 2 or 3. No score higher than 3 was recorded at any time. By July, although the overall incidence of opercular erosion was nearly as high as before, fewer (43%) of the affected fish scored 2 or 3, while from September onwards the decline in frequency of opercular erosion was matched by a decline in severity, with very few fish (4% or less of those affected) scoring 2 or 3 and the vast majority scoring 1. Since from July onwards, the data presented include only the tagged fish that survived to the end of the experiment and were assessed on all sampling dates, the subsequent decreases in the frequency and severity of opercular erosion are

indicative not of mortality but of a process of healing, which was complete by early December. While healing was in progress, a transparent layer of epithelial tissue was visible on the posterior edge of the operculum in many fish. Nevertheless, a small number of fish did develop opercular erosion after July. Table 7.2 shows the percentage of fish whose right operculum scores improved, worsened or were unchanged between measurement dates. Scores worsened in a small number of fish between July and September (in 14 without, and one with, opercular erosion in July) and between September and November (in 4 fish without opercular erosion in September). All other fish showed either an improvement or no change over the same period. The right operculum tended to be more severely affected than the left operculum. Of those fish affected by opercular erosion in June (n = 529), the left operculum was the more severely affected in only 14% of cases (n = 75), while the right operculum was more severely affected in 44% of cases (n = 233) (Goodness of fit test, assuming no lateral bias in damage, $\chi^2 = 81.05$, 1 d.f., $p < 0.01$).

There was no overall significant correlation between stocking density and the percentage of fish with opercular erosion (Figure 7.4, r = 0.13, 31 d.f., n.s.), but the correlation was positive (r = 0.84, d.f. = 15, $p < 0.01$) during the period when most of the opercular erosion developed (18 April to 20 June, represented by closed symbols in Figure 7.4). Importantly, however, there was no such relationship between the two variables on any one measurement date, indicating that the correlation between them was non-causal.

I examined the effect of body size on opercular erosion on the two sampling dates (30 May and 19-20 June) during the period when most damage was developing. Not surprisingly, there was a significant effect of treatment group on body size on both dates due to the effect of the experimental manipulation (Table 7.3). However, there was also a relationship between opercular erosion and body size: fish with opercular erosion were on average larger than unaffected fish (Table 7.3). On both occasions, the probability of a fish having eroded operculae was related to its relative size within each group: larger fish within each tank were more likely to have opercular erosion (Table 7.4, Figure 7.5). However, the increase over time in the probability of having opercular erosion was not related to absolute size. This is

best exemplified by group C, where the mean fork length changed little between the two dates (due to the cold water treatment, see Table 7.3), but the incidence of opercular erosion nevertheless increased as dramatically as it did in the other, faster-growing groups (Figure 7.2). Thus, although larger fish were more likely to be affected, the increase in frequency of opercular erosion between May and June was not simply an effect of the fish growing in size.

7.4 Discussion

The causes that are often implicated in the development of opercular erosion did not appear to be involved in the present study. During the summer months, when opercular erosion was developing, the fish were generally healthy with low levels of mortality and good growth rates. Opercular erosion was no more common in the three groups that spent time in Glasgow than it was in the controls, despite *Saprolegnia* infection and poorer water quality in Glasgow leading to a slightly greater mortality (4.9 - 8.4% over a three week period, compared to < 1.7% in controls). No other symptoms of vitamin deficiency were in evidence, and water quality was good throughout most of the experiment. There is therefore no reason to suppose that disease, vitamin deficiency or poor water quality were responsible for the opercular erosion in this study. What, then, caused it?

I suggest that the opercular erosion seen in this study could have arisen as a result of agonistic interactions between fish. While the results are by no means conclusive, they do justify further investigation in this direction. Several strands of evidence lead me to this tentative conclusion. Turnbull et al. (1998) have demonstrated that Atlantic salmon parr may attack the head region during agonistic interactions. Others have observed contact with the operculum during aggressive encounters between Atlantic salmon parr both in small groups and in culture (A. MacLean, pers. obs.; D. Cahill, K. Greaves, K. O'Connor, pers. comm.). In addition, observations of steelhead trout (*Oncorhynchus mykiss*) show that attacks are often concentrated on the anterior end of the body during reciprocal bouts of aggression (Abbott & Dill, 1985). Although none of these workers examined the operculae for damage as a result of these encounters, there does seem to be considerable

opportunity for Atlantic salmon parr to inflict damage on this part of the body.

In the present study, fish that had opercular erosion tended to be larger than fish that did not. Later in the year, the same groups of fish showed a similar (but stronger) relationship between fork length and the probability of having dorsal fin damage (Chapter 6), which is now known to be caused primarily by aggression (Abbott & Dill, 1985; Turnbull et al., 1996, 1998). The formation of a class of large, dominant fish that competed aggressively for food, while smaller fish avoided competition, appears to have led to the development of the positive relationship between size and fin damage (Chapter 6). Although caution should be applied in attributing similar results to a common cause, it is not unreasonable to suggest that the greater tendency for larger fish to have opercular erosion was caused by more intense aggression between them. The relationship between size and opercular erosion was not particularly strong, however. At the time of first-feeding, social dominance in salmonids is determined not by size but by aggressiveness, which is associated with a high metabolic rate (Titus & Mosegaard, 1991; Metcalfe et al., 1992, 1995). The increased food intake associated with social dominance leads to faster growth rates and eventually to larger size some time after first-feeding (Metcalfe et al., 1992). The weakness of the relationship between size and opercular erosion may therefore have been because the size advantage of socially dominant fish was only just beginning to develop.

If opercular erosion was indeed caused by aggression, why was so little inflicted after July, allowing it to heal completely by the end of the experiment? The fish were certainly aggressive after July, as evidenced by a sharp increase in the frequency of dorsal fin damage (Chapter 6). Kalleberg (1958) reported that agonistic behaviour in Atlantic salmon parr went through a qualitative change when the fish were 60-70 mm in length. At smaller sizes, agonistic encounters mainly involved frontal attacks with the dorsal fin lowered close to the back. Above this size threshold, frontal attacks were less common and the fish tended to use lateral displays with the dorsal fin held erect. In the present study, the fish were all below the 60-70 mm size threshold prior to July. If frontal attacks cause damage to the operculum, a size-dependent cessation of this behaviour would explain why

opercular erosion began to heal after July (when the majority of fish had passed the size threshold).

Since salmon parr become territorial and exhibit aggressive behaviour within a few days of first feeding (Kalleberg, 1958; Dill, 1977; Gustafson-Greenwood & Moring, 1990; Titus & Mosegaard, 1991), why was opercular erosion absent during the first six weeks of the experiment? It may have taken some time for enough damage to accumulate to produce noticeable results. In addition, the small initial size of the fish may have meant that they were physically unable to cause damage to the operculae. As time passed, the effect of repeated attacks by increasingly stronger fish could have led to the increasing frequency and severity of erosion that I observed.

A justified criticism of this interpretation is that direct attacks on the operculum could be expected to cause damage to the entire operculum, not just the trailing edge. An alternative explanation could be that the fish collide with each other during feeding (rather than direct aggression), resulting in damage to the trailing edge when the operculum flares during food-handling. If this is true, opercular erosion should be more prevalent at higher stocking densities. However, stocking density did not have a direct effect on opercular erosion, and healing occurred at similar stocking densities to those that saw the development of opercular erosion earlier in the year. There was a significant correlation between stocking density and the frequency of opercular erosion while it was developing, but this appears to be a non-causal correlation: no such relationship was evident on individual dates and, moreover, there was a large increase in the frequency of opercular erosion in group C at the same time as in the other groups, despite the fact that stocking density remained virtually unchanged due to slow growth in cooled water at the time.

Another suggestion might be that the damage was caused by abrasion on the sides of the tanks. This might explain why opercular erosion was more severe on one side than on the other, since salmon parr hold station facing the current and might rub one side against the tank wall more often than the other. However, it seems unlikely since the fish were held in smooth fibre-glass tanks throughout the experiment. Alternatively, aggression could be directed more often to one side of a

fish than the other in a circular tank. Unfortunately, I kept no record of the direction of flow and so could not relate it to the frequency of damage on either side of the fish.

I must stress that I did not look directly at aggression during this study, and therefore the conclusions I have reached are tentative and based on circumstantial evidence only. Clearly, further research is required to confirm or refute the hypothesis that opercular erosion in farmed salmonids is caused by aggressive interactions between fish. However, I am not aware of any evidence that contradicts the hypothesis. Turnbull et al. (1998) found that when making aggressive attacks, Atlantic salmon parr preferentially attack the dorsal and caudal fins. The head region was attacked less frequently, but the study involved only fish that were well above the 60-70 mm size threshold. Steelhead trout under 54 days old (and presumably under Kalleberg's size threshold) frequently aim attacks at the head region of an opponent, occasionally inflicting damage (although the part of the head that was injured was not identified) (Abbott & Dill, 1985). This may also be the case in salmon, but further work on this aspect is required, since trout and salmon differ in their behavioural patterns (Kalleberg, 1958).

Table 7.1: Comparisons by χ^2 test of frequency of opercular erosion between four groups of Atlantic salmon of different mean length on five sampling dates (see also Fig. 2). The sample size is that of the total number of examined fish; χ^2 values were regarded as invalid and are omitted if cross-tabulation yielded expected frequencies of less than five in one or more cells. d.f. = 3 in all cases. Levels of statistical significance: * = $p < 0.05$, ** = $p < 0.01$.

Sampling Date	n	χ^2
30 May	600	12.7 **
June	314	2.7
July	314	10.4 *
September	314	2.8
November	314	13.1 **

Table 7.2: Changes in opercular erosion scores in individual juvenile Atlantic salmon between three measurement dates (n = 314 for each period). Unaffected fish were defined as those with a score of 0.

Period	% of unaffected fish at start of period that ended with:		% of affected fish at start of period that ended with:		
	Same score	Worse score	Same score	Improved score	Worse score
Jul-Sep	76%	24%	50%	49%	1%
Sep-Nov	96%	4%	35%	65%	0%
Nov-Dec	100%	0%	1%	99%	0%

Table 7.3: (a) Mean fork length (± SE) of fish with and without opercular erosion (OE) in groups A to D on 30 May and 19-20 June. **(b)** Two-way analyses of variance of fork length by opercular condition (damaged or intact) and treatment group (A-D). Statistical significance: * = $p < 0.05$ ** = $p < 0.01$

(a)

Month	Group	Fish without OE		Fish with OE	
		Mean Fork Length (mm) ± SE	n	Mean Fork Length (mm) ± SE	n
May	A	41.8 ± 0.3	91	43.1 ± 0.5	59
	B	39.1 ± 0.3	94	40.0 ±0.4	56
	C	47.2 ± 0.4	90	47.4 ± 0.4	60
	D	45.6 ± 0.5	67	46.7 ± 0.4	83
June	A	49.6 ± 0.8	21	52.6 ± 0.4	129
	B	47.6 ± 0.9	14	49.6 ± 0.3	136
	C	48.5 ± 1.0	15	50.6 ± 0.4	135
	D	53.8 ± 1.0	21	55.3 ± 0.5	129

(b)

Month	Factor	F	d.f.	p
May	Opercular condition	10.1	1	**
	Group	172.4	3	**
	Interaction	0.7	3	n.s.
	Error		592	
June	Opercular condition	14.4	1	**
	Group	22.1	3	**
	Interaction	0.3	3	n.s.
	Error		592	

Table 7.4: Results of logistic regression analyses of the probability of having opercular erosion in relation to body size on 30 May and 19-20 June (see also Figure 7.5). To adjust for differences in size between the four treatment groups, relative body size is defined as the deviation from the mean fork length (mm) of each group, expressed as a proportion of the mean fork length for that group. The logistic regression equations are given by the formula $Y = a/(1+a)$ where Y = probability of having opercular erosion, X = relative body size and $a = e^{b+cX}$. Treatment group (A-D) had an additional significant effect on the logistic regression in May ($p = 0.006$) but not in June ($p = 0.49$).

Date	b	c	χ^2	p <
30 May	-0.29	3.75	23.21	0.001
19-20 June	2.12	5.98	17.96	0.002

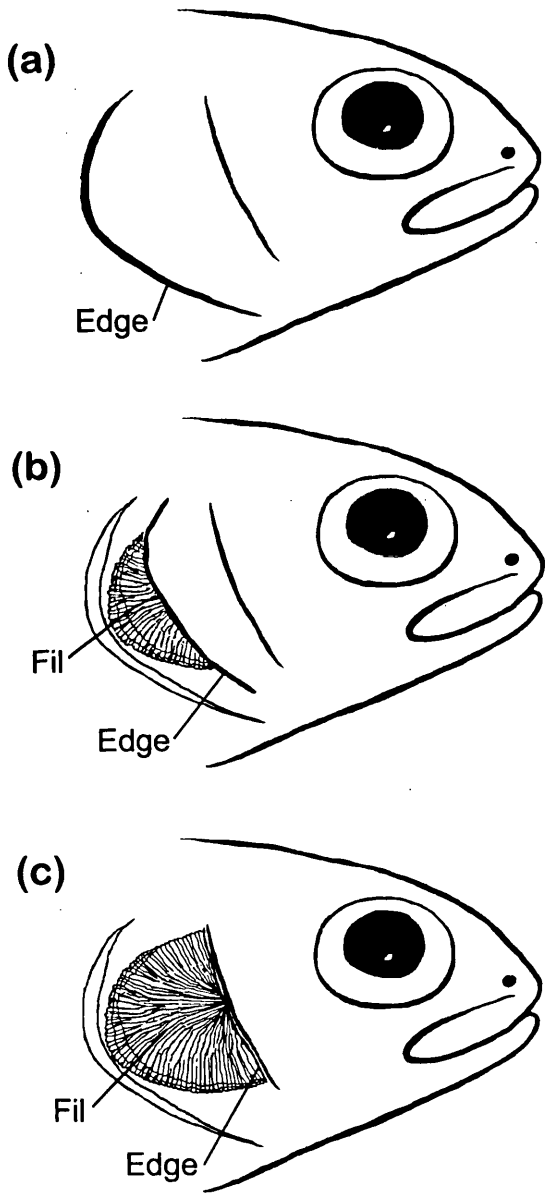

Figure 7.1
Illustrations of opercular erosion in Atlantic salmon parr **(a)** complete operculum (score 0) **(b)** half of operculum remaining, gills partly exposed (score 3) **(c)** none of operculum remaining, gills fully exposed (score 5). Edge = edge of operculum; Fil = gill filaments.

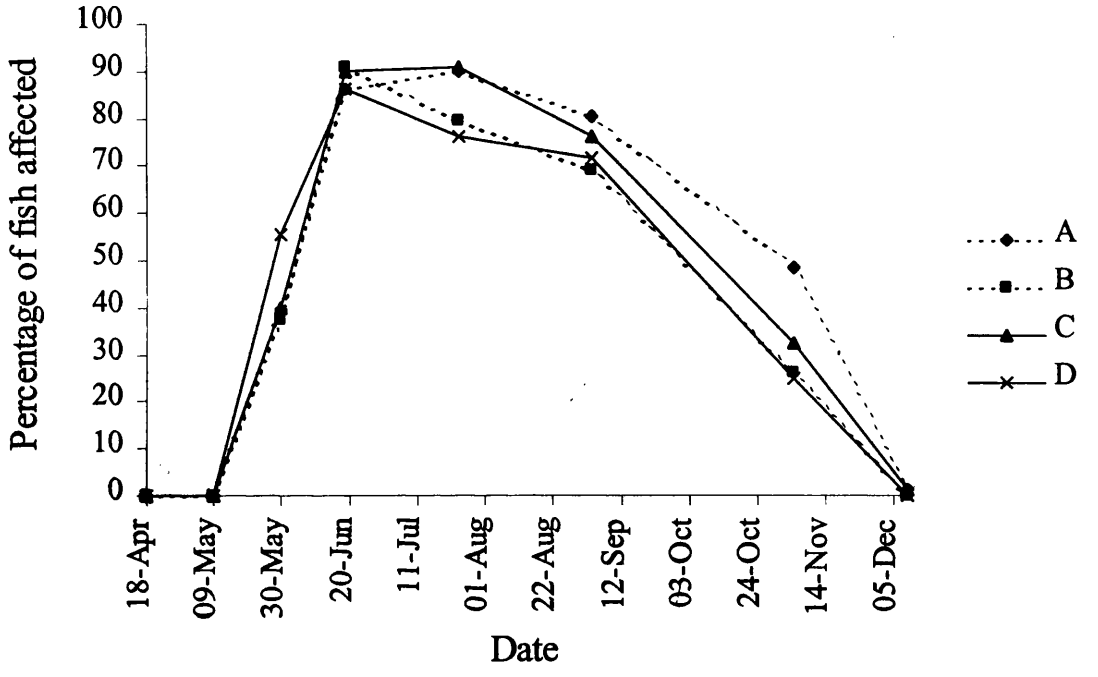

Figure 7.2
Changes over time in the percentage of fish affected by opercular erosion in four groups of juvenile Atlantic salmon. A total of 600 fish were examined on each sampling date during April-June. From July onwards, data are presented only for the tagged fish that survived to the end of the experiment and were assessed on all sampling dates (n = 314).

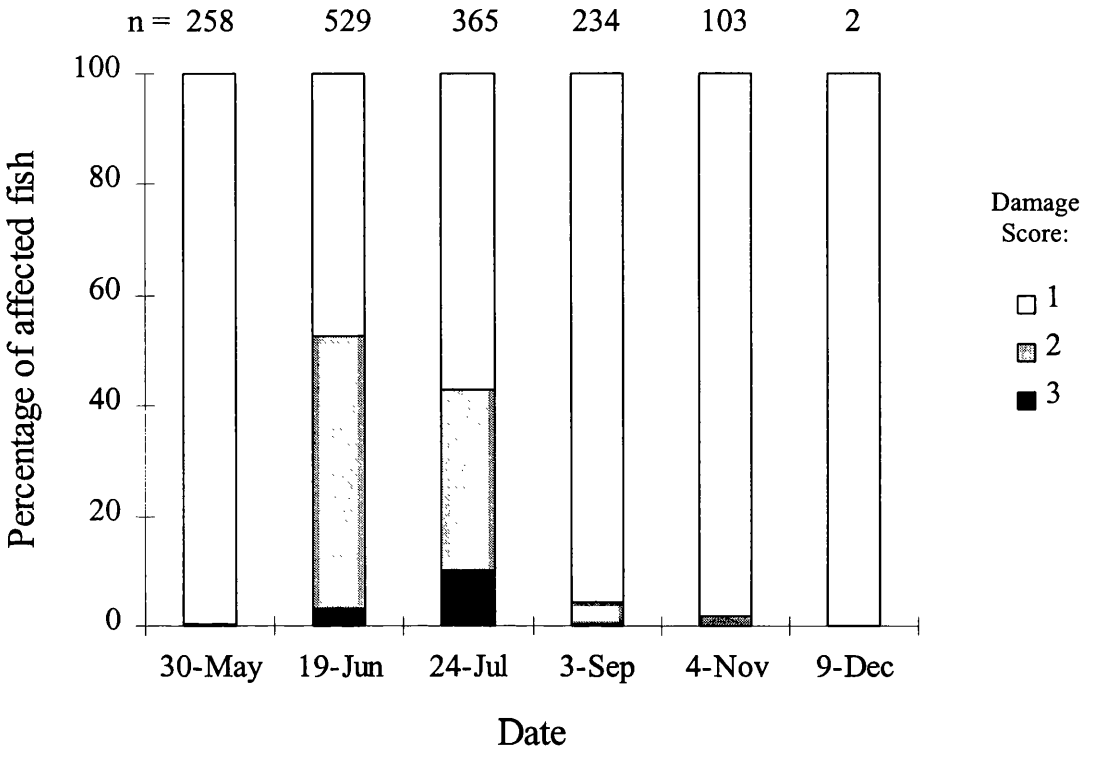

Figure 7.3
Changes over time in the severity of opercular erosion in juvenile Atlantic salmon. The higher of the two scores (left and right operculum) is used for each fish. Data from groups A-D have been combined. The number of fish in each category is expressed as a percentage of the number of fish with some opercular erosion on each date (given above each column); extent of damage increases from category 1 to 3 (see text for details).

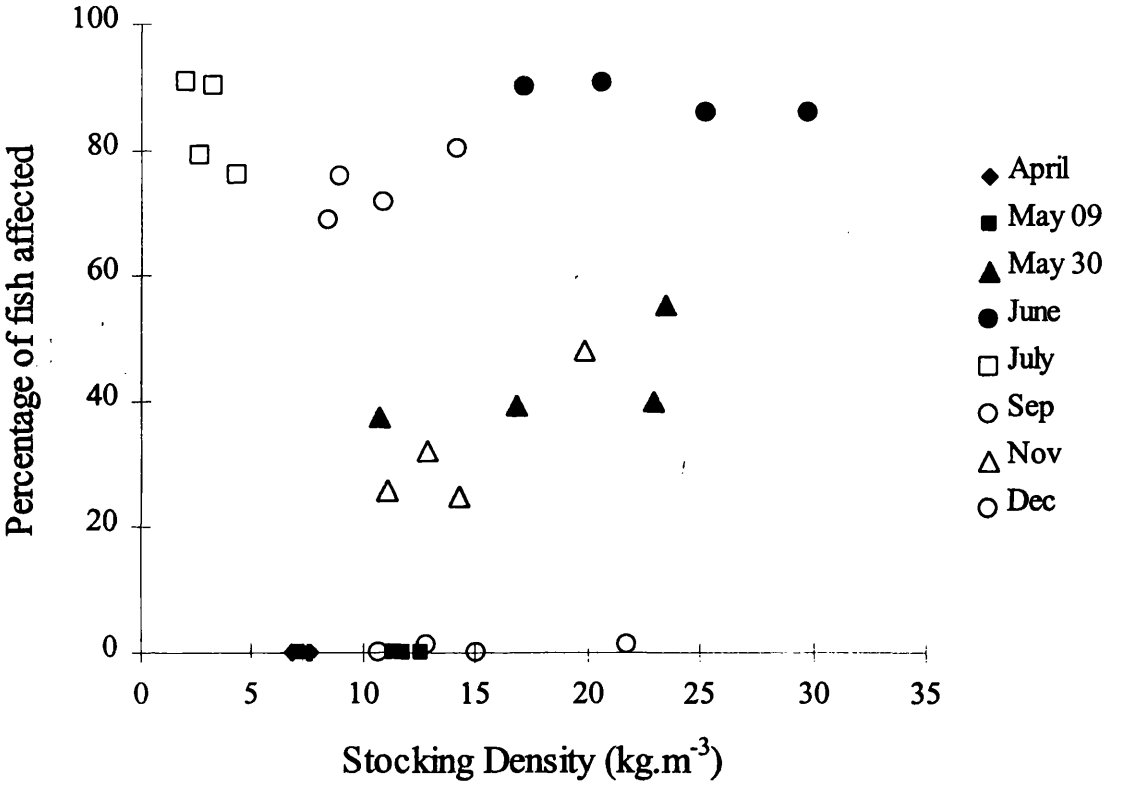

Figure 7.4
Relationship between stocking density and the percentage of fish with opercular erosion in four groups of juvenile Atlantic salmon measured on 8 occasions. Closed symbols represent samples taken during the period when most opercular erosion was inflicted; open symbols represent samples taken thereafter.

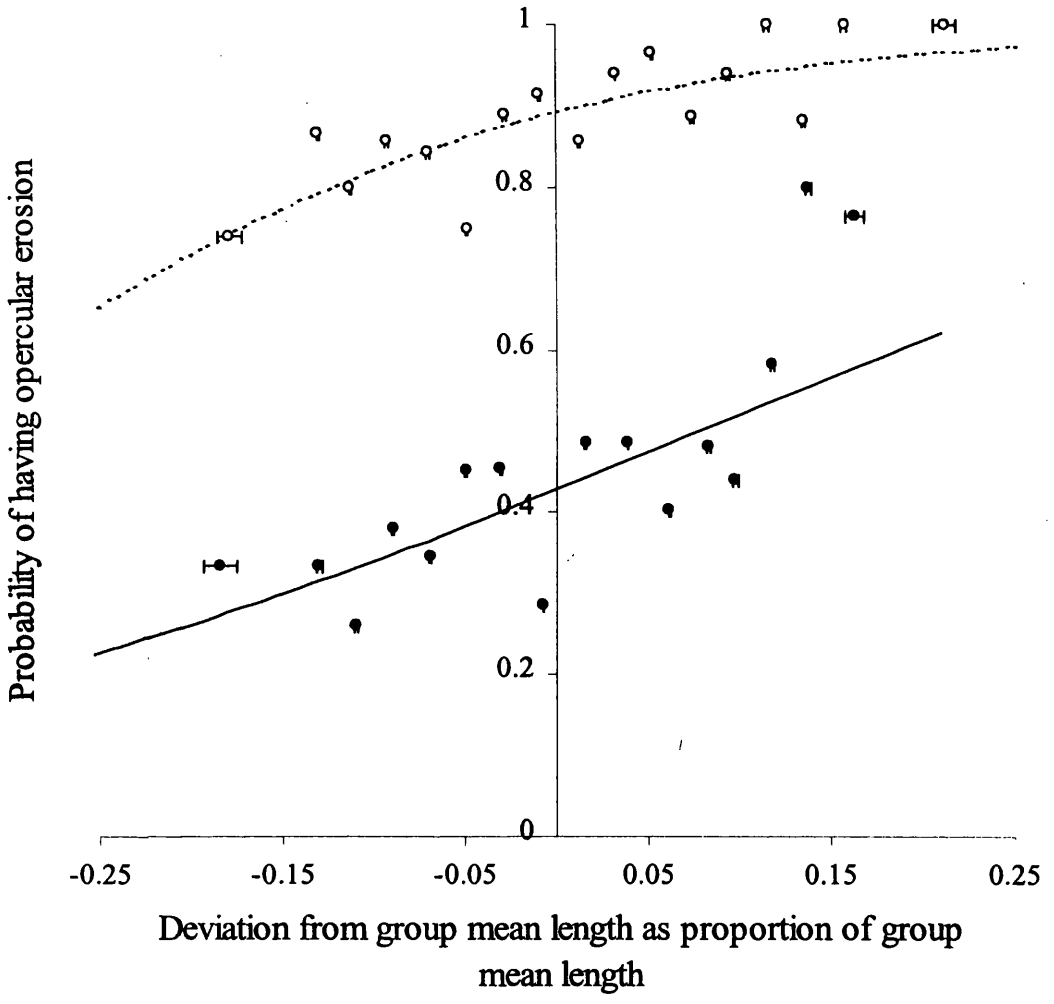

Figure 7.5
The effect of relative body size on the probability of having opercular erosion in juvenile Atlantic salmon in May (closed symbols and solid lines) and June (open symbols and dashed lines). Relative body size is defined as the deviation from the mean fork length within each treatment group, expressed as a proportion of that mean fork length. Symbols are the actual proportion of fish that had opercular erosion. Lines are logistic regression lines given by the equation: $Y = a/(1+a)$ where $a = e^{b+cX}$. The values of b and c and statistical analyses are given in Table 7.4.

Chapter 8: General Discussion

8.1 Summary of main findings

By slowing the growth rates of 0+ Atlantic salmon during spring and early summer, I was able to demonstrate that they show a compensatory growth response after being returned to warmer water (Chapters 2-4). Within a group, fish of all sizes showed this response to a similar extent. However, the appearance of compensatory growth within a group appeared to be dependent on the extent to which they had fallen behind their growth schedule, as groups of fish that emerged from cold water at a larger mean size (and had therefore experienced a less severe setback in growth) than other groups did not show compensatory growth (Chapter 2). Fish that were undergoing a period of compensatory growth were more aggressive than controls (as indicated by levels of fin damage), and dominant individuals within the compensating group were able to gain more exclusive access to a feeding area (Chapter 3). The ability of fish to exhibit compensatory growth was therefore dependent on their social status and on the ability of the dominant fish to monopolise the food patch.

Contrary to expectation, the temperature manipulations had no effect on the proportion of fish that became sexually mature, although there was some evidence that it caused a delay in reproductive investment in terms of testis growth (Chapter 4). As expected, sexually mature fish were less well adapted to sea water than immature fish. However, they did show signs of smolting (smolt coloration and seawater adaptability), although at the time of assessment the process of smolting was less complete than in immature fish of the same size (Chapter 5). Therefore the majority of the fish in these experiments made the life-history decision to smolt at the first opportunity, irrespective of their maturity status.

By using fin damage as an indicator of aggressive interactions between fish, I demonstrated that in large groups of fish in culture conditions, there was a strong and consistent positive relationship between the relative size of a fish within a group and its likelihood of having fin damage (Chapter 6). This suggests that, in large groups, a class of larger, dominant fish compete aggressively amongst themselves for food while less aggressive, subordinate fish adopt alternative feeding strategies, thus avoiding overt competition and reducing the risk of injury. There was a similar relationship between fish size and the incidence of opercular erosion (Chapter 7). Opercular erosion may have also been caused by aggressive interactions, as there was no evidence that other possible causes (e.g. vitamin deficiency) were involved.

8.2 Compensatory growth and behavioural adaptations

For fish that had been subjected to unseasonably cold temperatures, compensatory growth occurred immediately on return to warm water on a natural photoperiod cycle (Chapter 2), but was usually delayed when daylength was held constant (Chapters 3 & 4). Furthermore, compensatory growth did not occur in groups of fish that had experienced less severe growth setbacks (Chapter 2). Clearly, compensatory growth is not simply an automatic response that is exhibited when conditions for growth improve, but is initiated in response to an assessment of body size in relation to a target size for the time of year. If compensatory growth were simply a consequence of the retention of physiological adaptations to the cold for some time after return to warm water, we would expect that all groups in Chapter 2 would have shown compensatory growth irrespective of their size, and that there would be no delay in the compensatory response in Chapters 3 & 4. If physiological adaptations to colder temperatures (or to other factors such as food deprivation) are retained, they may be involved in compensatory growth when it occurs without a prolonged delay, but they are clearly mediated by the organism's size with regard to its expected growth trajectory, perhaps through endocrine control.

A distinction should be drawn between these adaptations and other, more generalised responses that allow increased growth rate at any time. One could

speculate as to whether the alterations in behaviour that we observed during a compensatory growth spurt after a considerable delay (Chapter 3) would have been found in fish that compensated immediately upon return to warm water. If physiological adaptations to colder water are retained for some time after return to warmer water, increased aggression might not be necessary to increase growth rate. Indeed, Nicieza & Metcalfe (1997) did not find an increase in aggression in fish that showed no delay in compensatory growth after periods in cold water. Although social factors and experimental design are most likely to account for the differences between studies (as discussed in Chapter 3), there remains the possibility that the discrepancies resulted from a physiological difference between fish.

The physiological mechanisms underlying compensatory growth could of course be studied directly using techniques such as the injection of tritiated amino acids or use of stable isotopes in food to measure the rates of protein synthesis and degradation (reviewed by Houlihan et al., 1995). In minnows *Phoxinus phoxinus* (Russell & Wootton, 1992) and Arctic charr *Salvelinus alpinus* (Miglavs & Jobling, 1989), hyperphagia and improved growth efficiency are both known to contribute to compensatory growth after periods of food restriction. However, little is known of the relative contributions of physiological and behavioural adaptations to compensatory growth. In all the studies of which I am aware, food has been provided to excess during periods of compensatory growth. The contribution of behaviour and increased food intake to compensatory growth could be evaluated by comparing compensatory growth in animals fed to excess with animals fed the same ration as the control. Any compensatory growth in the latter regime should be due to physiological factors and not to increased food intake (although differences in activity levels might also have an impact).

Paradoxically, if compensatory growth is primarily a response to deviations from a target size range for the time of year, it is doubtful that it would have been observed if the fish had been growing at slower rates. Most studies of compensatory growth in fish and other taxa have involved very good conditions for growth except during the period when growth was being restricted. The organisms used in such

studies were probably within the target size range for the time of year prior to the growth setback, but were put out of the target size range by the growth rate manipulation. However, we might see a lessened compensatory growth response, or even none at all, if the control growth conditions were already poor: if the fish were already significantly below the target size for the time of year, they should already be making as much effort as possible to achieve the target size.

In cases where growth is time-limited, we would expect organisms to meet any deviation from their expected growth rates by a greater compensatory growth response than when growth is less limited by time factors. For instance, populations of a species at high latitude must make the most of a shorter summer growing season and prepare for a harsher winter with stronger size-selective mortality than at lower latitudes. Nicieza et al. (1994) demonstrated that northern and southern populations of Atlantic salmon have markedly different innate patterns of growth when raised under identical conditions. Fish from a northern (Scottish) population grew more rapidly over the summer, began to develop a bimodal length distribution at an earlier stage and virtually ceased growth over winter, while fish from a southern (Spanish) population continued growth over the winter and smolted at a larger size, despite having been smaller than the northern population in the autumn. These growth patterns matched the seasonal changes in growth opportunity in the two locations, and demonstrate that they had become adapted, through natural selection, to their respective expected conditions. Since growth opportunity in Spain is less restricted to the summer months than it is in Scotland, compensatory growth in fish from the southern population should be less intense, or of shorter duration, than compensatory growth in the northern population. In the latter case a growth setback would have a more marked effect on the size of fish at the end of the growing season and as smolts the following spring. This should also apply to any species with a distribution that covers a broad range of latitudes with large variations in seasonality (Metcalfe et al., submitted).

Similar effects should be found when comparing populations that live at high and low altitude. For instance, the wood frog *Rana sylvestris* must reach a minimum

size before it undergoes metamorphosis into the adult form (Berven & Gill, 1983). We would expect stronger bouts of compensatory growth in high altitude populations of this species than in lowland populations, as they experience cooler daytime temperatures (even though they have faster growth rates anyway, a feature that helps to offset the cooler temperatures they regularly experience (Berven & Gill, 1983)). The same should be true of species or populations where migration is time-limited and size-dependent (Arendt, 1997). A comparison of the intensity of compensatory growth in populations of anadromous and non-anadromous salmon (such as sockeye versus kokanee salmon *Oncorhynchus nerka* (Wood & Foote, 1990)) should reveal stronger compensatory growth in the former. Similarly, migratory forms of three-spined stickleback *Gasterosteus aculeatus* (Snyder, 1991) should show more intense compensatory growth than non-migratory forms.

Wieser et al. (1992) found that the duration and intensity of compensatory growth in starved cyprinids was positively related to the duration of the starvation period, while Bull & Metcalfe (1997) showed that compensating salmon adjusted the duration but not the intensity of their response in relation to the period of deprivation. Although I was able to identify the existence of periods of compensatory growth, I was unfortunately unable to compare the exact duration and intensity of compensatory growth between groups of fish, as logistics dictated that the intervals between measurements varied somewhat. Other factors, such as the time of year, may also affect the duration and intensity of compensatory growth. Metcalfe et al. (submitted) found that in the summer, Atlantic salmon parr subjected to starvation exhibited compensatory growth in structural tissues (measured as fish length) and storage tissues (lipid reserves), whereas after a similar deprivation in the winter they restored lost lipid reserves but did not compensate in terms of length. This was attributed to the fact that skeletal growth rates are normally low in winter, and thus little opportunity for skeletal growth had been lost. Furthermore, food is more scarce in winter and foraging may be more risky, so the increase in length may not have been worth the extra risk that would have been involved in obtaining the necessary food. This study highlights the importance of season for compensatory growth. However, that study involved only LMG fish that would not have smolted the

following spring, and thus whose survival over the winter was paramount, while growth in length was not a requirement during the winter.

We would therefore expect that manipulations of growth rate at different times of year might result in different degrees or intensities of compensatory growth. It could be argued that fish that are close to the end of the growing season and have already put on a large proportion of their necessary (and expected) growth may not need to compensate to the same extent as fish near the start of the growing season that have still to put on most of the year's growth and, in an unpredictable environment, cannot rely on good growth conditions in the future. Indeed, in such circumstances compensatory growth may act as an "insurance policy" against future poor growth conditions, by allowing faster growth than normal in order to offset the possibility of future growth setbacks, as well as making up for poor growth in the past. However, a model of compensatory resource allocation predicts exactly the opposite scenario: manipulations closer to the end of the growing season should result in stronger compensatory growth due to the reduced time available for growth (Metcalfe et al., submitted). Likewise, a reduction in growth rates in the spring, close to the time of the smolt migration, should result in greater compensatory growth than a reduction in growth rates earlier in the year.

8.3 Sexual maturation and smolting

Although most fish studied in this thesis joined the UMG, fish that were subjected to long periods in colder water were less likely to do so than fish from other groups (Chapter 2), presumably because they had not achieved a target size by the time of the decision to smolt. However, most fish did not make the physiological decision to mature (Chapter 4). While this may have been because the developmental target for maturation was set too high even for most of the fish in these experiments to achieve it, it may indicate instead that smolting, rather than maturation, is the preferred developmental route for 0+ Atlantic salmon as suggested by Bohlin et al. (1990). This conclusion is supported by the fact that the majority of fish that did become sexually mature nevertheless showed signs of smolting (Chapter 5).

It is possible that maturation as parr has different consequences for the individual fitness of 0+ parr as opposed to 1+ parr. Amongst 0+ parr, it is usually the largest, socially dominant fish that become smolts and migrate to sea a year after hatching, while smaller, socially subordinate fish remain resident in freshwater for at least a further year (Metcalfe et al., 1989). Of these remaining 1+ fish, it is usually the largest males, or those in best condition, that mature the following autumn (Myers et al., 1986; Berglund, 1992, 1995). While most of those that do not mature may smolt the following spring at age 2+, the mature males may not smolt for at least a further year (at 3+ or older). Social factors are known to have a strong influence on the decision to migrate to sea in Atlantic salmon (Metcalfe et al., 1995), and I now suggest that this concept be extended to the understanding of the maturation decision. In the wild, anadromous males compete aggressively with each other for matings with females, while the mature male parr take advantage of their small size to sneak in to secure matings (Hutchings & Myers, 1988). Since fish that migrate to sea at 1+ are usually socially dominant, they may be more likely to succeed in aggressive competition with other males on the spawning grounds. Thus for these fish, the optimal life-history strategy may be to migrate to sea as soon as possible (whether or not they mature at age 0+), and return as large, anadromous males. For subordinate fish, that may be unable to compete as successfully as anadromous males, the optimal strategy may be instead to remain in fresh water and mature as parr, which they are able to do as long as they meet the necessary energetic requirements for maturation.

However, the nature of the maturation decision in 0+ fish was not clarified by the experiments reported here. While in 1+ and older fish the decision appears to be based at least in part on condition and growth during decision periods one year and 6-7 months prior to spawning (reviewed in Thorpe et al., 1998), 0+ fish have no such prior growth history to influence the decision. Silverstein et al. (1997) found differences in lipid deposition rates between early and late-maturing strains of amago salmon as early as one week after first-feeding. If such differences were also present in Atlantic salmon that later matured or remained immature, this would strongly

suggest that the decision is taken very early in the life-cycle. Although growth rates during the first months of life have a crucial role in the smolting decision, differences can be detected between LMG and UMG fish very soon after first-feeding. Fish that later join the UMG tend to hatch earlier, have larger otoliths relative to their body size, have a higher standard metabolic rate and are more aggressive than fish that later join the LMG (Metcalfe et al., 1989, 1990, 1992, 1995). The decision to mature in 0+ fish may have a similarly strong basis in such innate characteristics, while environmental effects on growth rates and body condition are likely to have more influence on decisions to mature that are taken at age 1+ or older.

8.4 Social interactions

There was evidence that the growth rates of the smallest fish were strongly socially suppressed, as their growth rates were well below those of other fish of their size (Chapters 2 & 4). However, the negative relationship between growth rate and body size during most growth periods indicated that the growth rates of most fish were not suppressed to the same extent (Chapters 2, 3 & 4). More socially subordinate fish appear to have avoided direct competition with the larger, aggressive, dominant fish, by adopting alternative feeding strategies, as evidenced by the relationship between fin damage and body size (Chapter 6). This would allow them to avoid injury, although at the cost of reduced energy intake. Alternative, sit-and-wait feeding strategies may have allowed them to minimise energy expenditure (Metcalfe, 1986), enabling them to make more efficient use of the food they did obtain.

Fin damage was not the only indicator of aggressive interactions between fish. It seems likely that the presence of opercular erosion was also due to aggressive competition between fish (Chapter 7), although this has yet to be proven conclusively. The possibility of a size-dependent, ontogenetic shift in aggressive behaviour, from frontal attacks that principally injure the operculum to lateral displays where the dorsal fin is more vulnerable, should be investigated further. Why should this change in behaviour occur? One possibility is that the lateral display is

only an adequate signal of strength or intent once a fish has reached a minimum size. On the other hand, in nature, the more aggressive frontal attacks may occur during the period when feeding territories are still under dispute. Once territories have become firmly established, lateral displays may serve to indicate possession, without the need for physical contact. In the more crowded environment of fish-farms, however, the adoption of lateral displays with the dorsal fin held erect is more likely to result in injury.

Although dominant individuals reap benefits in terms of better territories and increased access to food and mates, there is mounting evidence that dominance can have costs as well as benefits. Although most of the work in this field to date has focused on the costs of dominance in birds and mammals, it seems likely that the same principles will apply in other groups, including fish. In willow tits *Parus montanus*, social dominance entails additional energetic costs (Hogstad, 1987), while in starlings *Sturnus vulgaris* dominant individuals are more prone to exhibit fluctuating asymmetries (Witter & Swaddle, 1994). There is also mounting evidence that dominant individuals suffer from hormonal stress. For example, although high social status has some reproductive benefits for females in troops of baboons *Papio cynocephalus anubis*, high-ranking females also incur reproductive costs in terms of an increased likelihood of miscarriage (Packer et al., 1995). Dominant individuals (from species as disparate as the black-capped chickadee *Parus atricapillus* (Ficken et al., 1990), the African wild dog *Lycaon pictus* and the dwarf mongoose *Helogale parvula* (Creel et al., 1996)) may be involved in more frequent agonistic encounters than subordinates, which may increase levels of stress hormones and can involve injury, irrespective of the outcome of encounters. The finding that larger, dominant salmon parr are more prone to injury in culture (Chapters 6 & 7) is a further example of a cost of dominance.

8.5 Implications for aquaculture

It has still to be proven conclusively that aggression is the primary cause of opercular erosion in healthy parr (Chapter 7). Future work should include a comparative study of the frequency of frontal attacks, lateral displays, and the location of nips in fish above and below Kalleberg's (1958) size threshold. Future work must also demonstrate a direct causal relationship between attacks on the operculum and opercular erosion, if this hypothesis is to be proved correct. Of course, other factors may still be involved: weaknesses in the opercular tissue caused by malnutrition could make it more prone to injury, and damage could be exacerbated by bacterial infection.

The incidence of fin damage (and possibly also opercular erosion) could be used as an indicator of levels of aggression within tanks. An increase in either might indicate that the rate of food input should be increased or food deliveries should be dispersed in space and time. Such alterations to farming practice could reduce the degree to which fish suffer from injury and from the chronic stress associated with agonistic interactions (Schreck et al., 1997; Wedemeyer, 1997).

Since periods in colder water in the spring failed to reduce the incidence of sexual maturation in male parr (Chapter 4), it may be difficult to eradicate the problem, as food restriction often has only a small, though significant, effect on maturation rates (summarised by Berglund, 1995). Further disadvantages associated with this approach are that periods of reduced growth in the spring increased the proportion of fish that joined the LMG (Chapter 2), and fish that had experienced periods of colder temperatures did not catch up with the controls in size, despite periods of compensatory growth. Other approaches, perhaps involving the manipulation of photoperiod, may be more successful in reducing the incidence of maturation in 0+ parr. However, if mature fish that are unlikely to survive transfer to sea water (i.e. the smaller mature fish in the UMG) could be graded out at the time of smolt transfer and retained in fresh water for some weeks or months, they might complete smolting successfully. Traditional methods of grading out mature fish by

hand might be too labour-intensive to justify this approach, but methods of passive grading, based on differences in behaviour between maturing and immature fish, might yet be developed. Maturing one-sea-winter Atlantic salmon show a surge in appetite in the spring, followed by a loss of appetite during the summer (Kadri et al., 1996b, 1997b). Since seasonal differences in the appetite of maturing and immature parr may also exist (Simpson et al., 1996), it may be possible to use behavioural differences to separate maturing and immature fish, although reliable methods have yet to be developed (F.A. Huntingford, pers. comm.).

The existence of compensatory growth could be exploited to enhance growth rates in culture. Although most of my results show incomplete compensation after periods in cold water, one group of fish did compensate completely and caught up in size with the control group (Chapter 3). Hayward et al. (1997) used cycles of feeding and starvation to double the growth rates of hybrid sunfish, by starving the fish as soon as the hyperphagia during compensatory growth periods returned to normal. Whether such a technique - using either starvation or periods of lowered temperature - could be adapted for salmonids would be worth investigating. However, care should be taken due to the possibility of increased levels of aggression associated with compensatory growth (Chapter 3).

8.6 Closing remarks

Compensatory growth is exhibited by many organisms when conditions improve after periods of poor growth. It is an important adaptation because, even though patterns of appetite and growth can evolve to match prevailing seasonal conditions, the environmental conditions encountered by individual animals can vary from the expected pattern. Growth rates are dependent on temperature, the availability of food and social rank, and compensatory growth has evolved to allow organisms to attain developmental targets despite such environmental unpredictability.

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