THE ROLE OF GROWTH AND SEASONAL FAT DYNAMICS IN THE MATURATION OF ATLANTIC SALMON (*SALMO SALAR*) PARR

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by

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ABSTRACT

Growth studies of individually tagged 1+ Atlantic salmon parr revealed no difference in specific growth rates between maturing and non-maturing male parr. However, maturing parr had lower mean condition factors than non-maturing males during March, and were characterized by greater increases in condition factor during April. Changes in condition factor during April were related to feeding opportunity during that month, and a relationship between April increases in condition factor and maturation rates of males was confirmed in 8 sibling populations of salmon parr. A relationship between condition factor increases during April and maturation in autumn was also confirmed for grilse and reconditioned kelts.

Maturing male parr replenished non-mesenteric fat stores during April, and the mesenteric store in May. In comparison lipid replenishment and deposition in non-maturing fish was delayed until May and June respectively. The April increases in condition factor of maturing males are therefore symptomatic of the earlier replenishment of lipids depleted during winter.

The mesenteric fat store is an important reserve utilized during maturation in male parr and contains up to 40% of the total lipid content of salmon parr. Its size decreases significantly during the later stages of gonadal

development, while the relative size of females and non-maturing males's increases. Mesenteric fat levels are highest in maturing males in July, just before gonadal growth accelerates, and there is a strong correlation between GSI and mesenteric fat levels at this time. Because feeding in maturing male parr is depressed between August and October, the size of the mesenteric store is likely to be important in sustaining gonad differentiation, as well as in the elaboration of secondary sexual characters.

Seasonal manipulations of growth rate resulted in variations in the maturation rate of male parr. Increased feeding and growth during April and May increased maturation rates whereas decreased feeding resulted in delayed replenishment of fat reserves and lower maturation rates compared with controls. Changes in growth during other months had little effect on maturation rates.

The results indicate that maturation is initiated in a proportion of male parr as early as in winter, but is suppressed if fat deposition into the mesenteric store is below a genetically determined level by the end of May. However, the timing of fat deposition into the mesenteric store is dependendant on the prior replenishment of other body stores, and so is particularly sensitive to fat dynamics in April.

The manipulation of maturation rates by altering growth opportunity in April and May occurs despite the fact that physiological changes leading to maturation are already in train. Thus maturation is switched off in many male parr by reduced feeding and growth during spring months. This maturation suppression switch, related to growth in fat reserves during spring months, provides the means by which growth exerts some control over maturation, and is likely to be responsible for much of the correlation between fast growth and early age of maturation in salmonids. The switch is time specific and is believed to be adaptive. It is likely to prevent maturation in the autumn if the winter is long and spring is late. A late spring shortens the growing season, and the probability of acquiring sufficient fat reserves for successful spawning and overwintering would be low in such summers.

The physiological mechanisms by which growth in fat reserves during spring could affect maturation are discussed, and a hypothetical model for the role of fat stores in the hormonal control of maturation, is presented.

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The role of growth and
seasonal fat dynamics in the
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(*Salmo salar* L)

CHAPTER 3

EFFECTS OF SEASONAL CHANGES IN FEEDING ON MATURATION RATES OF MALE SALMON PARR

CHAPTER 4

THE ROLE OF FAT STORES IN MATURATION

CHAPTER 5

SYNTHESIS AND GENERAL DISCUSSION

CHAPTER 1

General Introduction

Life history variation in salmonids

The Salmonidae are characterized by species of fish which have highly variable life-history patterns, and which are generally opportunistic and phenotypically plastic (Thorpe 1986). However, this versatility is countered by the effects of reproductive isolation. Many salmonid species develop discrete spawning stocks, with life history patterns narrowly adapted to specific environmental conditions.

Giesel (1976) indicated that, for many vertebrates, choice of reproductive strategy is governed by the nature of their environment, and that phenotypic plasticity is likely to be of great importance for species living in rapidly and unpredictably changing environments. Thorpe (1986) pointed out that most salmonids introduced throughout the world readily acclimatise to their new habitats, despite the specialisation of populations to native habitats. These species therefore retain sufficient genetic heterozygosity to colonize new environments. It is apparent that, while reproduction and early rearing in freshwater encourages specialist adaptations and genetic divergence, the tendency to specialise is tempered by the maintenance of phenotypic plasticity in life history pattern, and the ability to

respond to changing or different environmental conditions.

Balon (1983) categorized species of fish as altricial (generalists), or precocial (specialists), and it is clear that the Salmonidae adopt both tactics. Their principal adaptations, for reproduction and early life in protected freshwater environments, have enabled populations of these primitive species to thrive (Thorpe 1986), but the ability to change life history pattern by varying developmental rates is likely to have been equally important in ensuring the spread and survival of the species. Of particular significance to biologists today is the ability of the various salmonid species to vary growth rates and age of first maturation. An understanding of when and how such developmental decisions are made is of fundamental importance to the management of fisheries.

Problems with early age of maturation

Salmonids form the basis for many important commercial and recreational fisheries, and maturation in these fish is accompanied by reduced growth or, in semelparous species, by death. A reduction in the average age of first maturation can therefore have far-reaching adverse effects on salmonid fisheries (Porter *et al.* 1986).

A number of authors have indicated that increases in the proportion of Atlantic salmon males maturing in freshwater

as parr decrease the subsequent production of salmon returning from the sea (Schiefer 1971; Myers 1983; Thorpe 1986). This is believed to occur because of the high mortality rates of mature parr (Osterdahl 1969; Leyzerovich 1973; Mitans 1973; Dalley et al. 1983), and the reduced probability of emigration of mature parr from the river (Hansen et al. 1989). Myers (1984) modelled the demographic consequences of high mortality rates for mature male parr in the Little Codroy River and concluded that parr maturation could account for the loss of up to 60% of male production.

Early maturation clearly has the potential to reduce returns in wild fisheries, but is also a problem in commercial fisheries. Thorpe (1986) summarized evidence for the developmental conflict that exists between parr maturation and smolting, and indicated that while maturation does not prevent smolting it is likely to impede it, and to reduce survival following smolting. Hatchery rearing tends to increase proportions of mature parr (Murphy 1980), and these are likely to reduce the return on hatchery fish for sea-ranching, cage-rearing, or enhancement and rehabilitation purposes.

Nevertheless, the economic cost of early maturation in cage-rearing of salmon is the main problem at present and has provided the main impetus to control age of maturation. Grilse are smaller and less valuable than salmon which

mature after 2 or more years in sea-cages. Thus high rates of grilse maturation limit profitability by reducing numbers of later maturing salmon. Furthermore, the secondary sexual characteristics accompanying maturation decrease the value of fish, and so grilse must be culled, before these develop, and marketed within a restricted period of time.

Current approaches to controlling maturation

The high rates of grilse maturation in cage-reared salmon have led to a variety of artificial methods to prevent maturation in Atlantic salmon. These include auto-immune castration (Ellis 1981), pharmacological blocking of sex hormone production (Murphy 1980), production of monosex, all-female fish, or sterile triploids by hormone treatment (Johnstone et al. 1978), and sterilization by irradiation of eggs (Thorpe et al. 1986).

Artificial selection programs to produce late maturing stocks have also been developed. Genetic differences play a major role in determining age of maturation in salmonids (Ricker 1972; Gjedrem 1985; Thorpe et al. 1983; Glebe & Saunders 1986), and Gjedrem (1985) pointed out the scope for selection of late maturing strains of Atlantic salmon. However, he noted that this might not be compatible with selection for increased growth rate, and Thorpe et al. (1983) indicated that selection for rapid growth and late

maturity are likely to prove incompatible objectives, as fast growth and early maturation are genetically coupled.

Environmental control of maturation: a role for growth?

Whereas the role of fast growth in maturation has long been suspected, the segregation of genetic from environmental effects in the control of maturation has taken many years. However, despite clear proof that early maturation is a heritable trait, a number of studies have found evidence for a genetic-environmental interaction in the control of maturation in Atlantic salmon (Glebe et al. 1978; Saunders et al. 1983). These studies imply that growth does affect age of maturation, and proffer some hope that a simple, effective method for controlling maturation will be found.

Alm (1959) was the first to show that there was a positive correlation between growth rate and maturation in salmonids. Since then this general relationship has been reported for a wide range of species in the genus Salmo, Oncorhynchus, and Salvelinus (Thorpe 1986), and has provided the basis for much speculation about the role of environmental factors, particularly those influencing growth rate, in the control of maturation (Gardner 1976; Scott 1979; Wootton 1982; Naevdal 1984; Billard 1985; Power 1986). Nevertheless, the precise nature of the link between growth and control of age of maturation has remained enigmatic.

Recently some light was thrown on this problem by a series of reviews of studies on the maturation of Atlantic salmon (Meerburg 1986). Maturing male parr are generally the largest parr and a number of authors have suggested that fast growth leads to the earlier attainment of a critical size needed for maturation (Elson 1957; Refstie et al. 1977; Bailey et al. 1980; Myers et al. 1986). However, Saunders et al. (1982) found that accelerated incubation and rearing in warmer waters resulted in maturation of parr at age 0+, instead of 1+ which occurs under natural conditions. He suggested that a developmental threshold could be involved, rather than a size threshold. Thorpe (1986) also discounted a critical size. He argued that this begs the question of a reference point: how does the organism know how large it is?

Thorpe (1986) proposed that current growth performance was a better basis for developmental decisions. It had been noted that the developmental decision "not to smolt" was effectively taken, in mid-summer, 10 months prior to the smolting season. Fish which subsequently failed to smolt reduced appetite and growth from mid-July onwards, despite favourable conditions for growth. He suggested that this developmental decision was made on the basis of energetic considerations related to growth performance around mid-summer and, by analogy, developed an hypothesis for the initiation of maturation. This incorporates the known

effects of genetic factors on maturation, with both growth and photoperiod, the latter having been shown to affect the seasonal timing, if not the onset, of the maturation process (Lundqvist 1983; Scott & Sumpter 1983).
This hypothesis states that:

"salmon are physiologically aware of their growth-rate through their rate of acquisition of surplus energy (sensu Ware 1980), and the hormone kinetics associated with its storage. Provided this rate is above a genetically determined level in early spring, when the fish are sensitive to photoperiodic stimulation of their gonadotrophic hormone systems, gonadal maturation will be triggered and reallocation of energy resources to include maturation will be set in train." He cited evidence from field studies of salmonids to indicate that improved conditions for individual growth (eg. reduced density or improved productivity) would increase the rate of energy acquisition, so initiating maturation.

Aims of this study

Thorpe's (1986) proposition raises a number of testable hypotheses for the role of growth rate in maturation, specifically;

1) that within a sibling stock of male parr, only fish whose growth performance is above a certain threshold in spring will mature

2) that the period, during which the assessment of growth performance is made, is limited to a particular time of year (spring)

3) that the size, or rate of increase in fat stores during this time of year is the basis for the assessment.

4) the implication of 3) above, is that maturation is an energy demanding process, and that fat stores built up prior to maturation are depleted during the spawning season.

These working hypotheses form the basis of studies outlined in Chapters 2, 3 and 4 respectively. Chapter 2 examines seasonal differences in growth performance between individually marked maturing and non-maturing male parr. (NB. The term non-maturing is adopted in preference to immature to avoid confusion between fish in transition between the two states. For example fish can be maturing but still show no sign of increased gonad growth. Maturation is thus defined as a process, rather than as an all or nothing state indicated by the relative size or development of the gonad).

Correlations between fast growth performance of individual fish at a particular time of year, and their maturation in autumn, may show when fast growth performance is likely to

be important to the maturation process. However, such correlations do not establish cause and effect, and could be coincidental, or symptomatic of maturation. Proof of the importance of growth, and its seasonal timing, in the control of maturation can only be obtained experimentally. Chapter 3 therefore investigates the effect of manipulations in seasonal growth rates on maturation rates of sibling populations of parr.

In Chapter 4 the effects of maturation in male parr on feeding rates and fat stores are examined to establish the importance of lipid reserves in the maturation process. The seasonal pattern of lipid depletion and deposition in the major fat stores of maturing and non-maturing parr is then established, and the effects, on this pattern, of growth restriction during different months determined.

Chapter 5 provides a synthesis of the results, and a discussion of their implications, for both advancing our understanding of the way in which growth affects maturation, and further studies.

CHAPTER 2

Growth differences between mature and immature parr

2.1 INTRODUCTION

A positive correlation between growth rate and age of first maturation has been established for many salmonids (Thorpe 1986), and is generally based on reports of differences in age-specific size between maturing and non-maturing fish. Maturing male parr of Atlantic salmon are no exception. They are usually the largest parr (Leyzerovich 1973; Simpson & Thorpe 1976; Naevdal *et al.* 1978b; Bailey *et al.* 1980; Murphy 1980; Dalley *et al.* 1983; Thorpe *et al.* 1983; Bagliniere & Masse 1985; Pepper *et al.* 1985), and this association between maturation and size has led to a succession of hypotheses to explain the role of growth in maturation.

Elson (1957), Refstie *et al.* (1977), Bailey *et al.* (1980) and Myers *et al.* (1986) have all proposed that a minimum threshold size was needed for maturation of male *Salmo salar* parr. Furthermore, Thorpe *et al.* (1980) and Saunders *et al.* (1982) both argued that the size threshold for maturation was larger than the threshold for smolting. However Myers *et al.* (1986) indicated that a size threshold was too simplistic and Saunders *et al.* (1982) proposed a developmental threshold to explain the high

incidence of maturation at age 0+, when incubation and early rearing of Atlantic salmon eggs and fry were accelerated by increasing water temperatures.

A genetic basis for the early maturation trait has now been established (Gjedrem 1985), and (Thorpe et al. 1983) indicated that fast growth and early maturation were genetically linked. The correlation between fast growth and early maturation may therefore represent genetic differences between families, with fast growth being symptomatic of developmental rates which result in early maturation. However, Saunders et al. (1982) cited evidence for phenotypic variation in maturation rates linked to developmental rates. Later, Saunders (1986) proposed a mechanism for the genetic control of maturation which incorporates developmental rates. He suggested that the genetic determination of maturation may specify the physiological and biochemical conditions to be met before maturation is initiated, and not the season or year for the event.

Thorpe (1986) approached this problem from a different perspective and proposed that a genetically determined rate of development must be exceeded during a specifc time of year. Thorpe (1977) described the bimodal distribution of sizes that develops in hatchery populations of 0+ Atlantic salmon parr after summer. This

bimodality arises because certain fish reduce their food intake after summer and cease growing during winter, while others do not, and continue to grow (Higgins 1985; Higgins & Talbot 1985). The fish which continue feeding and growth during winter become the upper modal group and smolt at age 1+ (S1 fish). Those that cease growing do not smolt until age 2+ or older (PS2 fish). Thorpe et al. (1980) and Villarreal (1983) demonstrated that the decision to reduce food intake in the prospective lower modal group (PS2) fish occurs in early July, and Thorpe (1987) indicated that this decision can occur in fish of all sizes. (ie. S1 and PS2 parr occur throughout the unimodal size distribution that exits in July). He proposed that the decision to curtail or continue feeding, and hence to smolt or not, is not dependent on a size threshold in July, but on growth performance at this time.

The decision to mature or not is believed to be made on the same basis as the decision to smolt (ie. growth performance) (Thorpe 1986). However, maturation in Atlantic salmon is photoperiod regulated (Lundqvist 1980, 1983) and is believed to be initiated under increasing photoperiods (Scott & Sumpter 1983). The initiation of maturation is therefore likely to be based on growth performance in winter or spring, rather than July, which is the time for the developmental decision for smolting.

Thorpe (1986) argued that size is a measure of past growth performance, whereas instantaneous or specific growth rates measure current performance, and provide a better basis for making developmental decisions. A high specific growth rate during spring may therefore be associated with maturation, whereas specific growth rates below a stock specific threshold would inhibit it. This study was designed to test the null hypothesis, that the specific growth rates, of maturing and non-maturing male Atlantic salmon parr, do not differ during spring months.

Most studies of the relationship between growth and maturation have been compromised because of failure to restrict comparisons to maturing and non-maturing fish of the same age, sex and family. Others have not encompassed growth differences during the spring and summer months before maturation, or have compared growth over the whole growing season prior to maturation. A test of the specific growth rate hypothesis must be carried out at the level of individual sibling fish grown under the same conditions, and monitored at an appropriate time interval. It therefore requires the measurement of growth, from individually tagged fish in the controlled conditions of a hatchery, at appropriate time intervals throughout the growing season.

Although spring is believed to be the most likely period during which growth performance is assessed by fish, the

duration of the assessment period is not known, and spring is too vague a concept as a time base. A month was therefore chosen as a suitable time period over which to measure growth performance, and the null hypothesis tested for each spring month, as well as for late-winter and early summer months.

2.2 MATERIALS AND METHODS

2.2.1 Fish stocks and culture conditions

There is a significant genetic component influencing variation in the maturation rates of Atlantic salmon (Salmo salar) parr (Thorpe 1975; Naevdal et al. 1978a, 1978b; Gjedrem 1985; Thorpe et al. 1983; Gjerde 1984). A sibling stock was therefore used for all experiments comparing effects of growth on maturation rates. Eggs were stripped from a 57cm FL, 3 year old (2.1 +) female salmon, from the River Almond (Perthshire), on 5th November 1985. These were fertilised with the milt of six, 1 year old (1 +) male parr, ranging in length from 12 to 19cm FL. Eggs were incubated at the Almondbank Hatchery and hatched on 17th March 1986. Yolksac fry were transferred to a 2m diameter radial flow tank (Thorpe 1981) on 2nd May, just before first feeding, and were reared under natural photoperiods and water temperatures on EWOS dry-pelleted salmon food.

Proportions of PS2 parr in sibling populations of salmon parr vary, as do the proportions of these which mature (Thorpe 1975). The stock of Atlantic salmon at Almondbank was therefore monitored in October and December 1986 to establish the cut-off size for selecting PS2 fish, and the proportion of PS2 parr from the length frequency distribution. There was no change in the proportion of PS2 fish, or in the cut-off point (80mm FL) between October

1986 and January 1987, when fish were selected for the growth experiments (Fig. 1).

Differences in the experimental fish associated with size were examined by taking a sample of salmon parr from the stock population in January 1987, before experiments began. These fish were sorted into three size groups viz. small (57-61mm FL), medium (62-66mm FL), and large (67-71mm FL). The sex, length, weight and gonad weight were measured for each fish, and % water calculated by drying to constant weight at 70 °C. Sex ratios, mean gonadosomatic indices, % water and condition factor were compared between the three size groups.

After tagging (see 2.2.2 below), experimental fish were transferred to 1m diameter radial flow tanks. These are designed so that water, travelling down a perpendicular pipe to the centre of the tank, flows radially along the bottom of the tank to its perimeter, where it is drained. Flow rates are adjusted to produce a water velocity of approximately 5 body lengths/second, the optimum for maximising proportions of S1 parr (Thorpe & Wankowski 1979). Individual fish orientate themselves facing the direction of flow, and sit or maintain station near the bottom of the tank, below a 15cm wide ring cover suspended below the water surface. The net effect is that fish become evenly distributed in a circle around the tank,

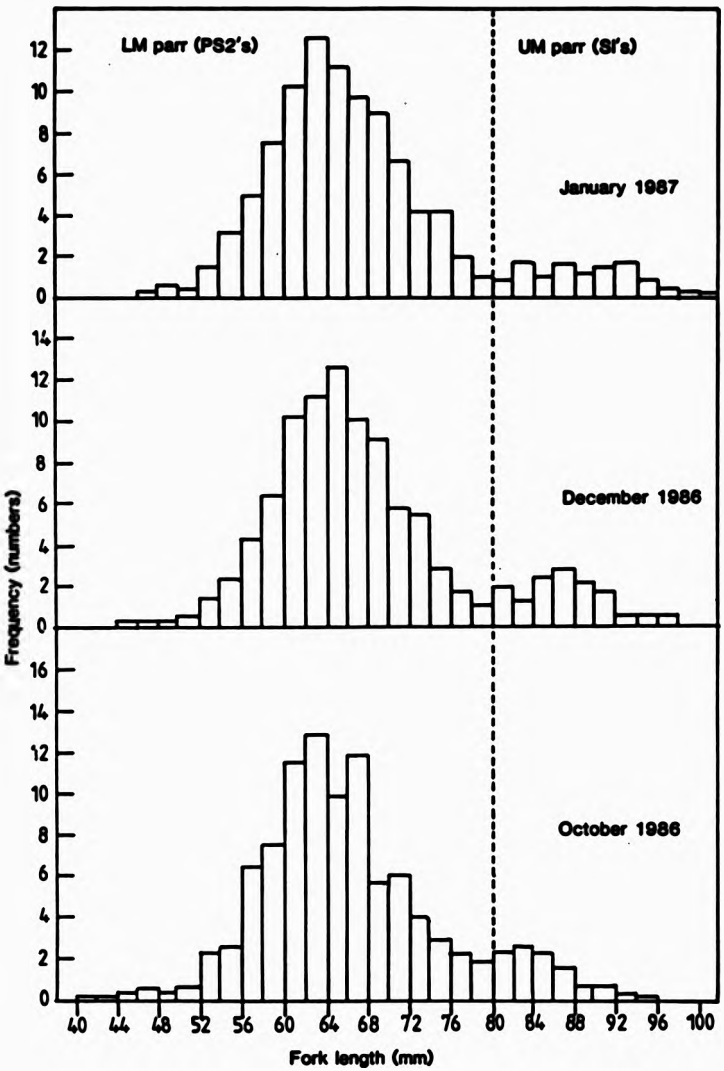


Fig. 1. Bimodal distribution of salmon parr and cutoff point for separation of lower modal (LM) group and upper modal (UM) group fish.

facing the centre. Food is dispensed from automatic feeders into the inlet water and is dispersed radially to the waiting fish. This design reduces feeding hierarchies and so decreases competition for food and space. Mean coefficients of variation in length are significantly reduced in radial flow tanks compared with tangential flow tanks (Thorpe & Wankowski 1979), indicating a reduction in the variation between individual growth rates. Experimental fish were reared under natural photoperiods and water temperatures (Fig. 2).

Salmon parr not used for growth experiments were returned to a 2m diameter radial flow tank. Here, the automatic feeder dispensed food at approximately 15min intervals, and daily ration was set to exceed the optimum, so that food supply did not limit growth. Maturation rates (proportion of age 1+ males maturing) for the PS2 male parr populations can be expected to be as high as 80-90% under this feeding regime. Lower rates were required to obtain data on non-maturing male parr as well as on maturing males. The automatic feeders supplying experimental populations were therefore adjusted to restrict daily ration to approximately 1% of fish biomass, which is less than the optimum ration recommended in feeding schedules for Atlantic salmon parr (Farmer et al. 1983). Feeding frequency was adjusted to provide some food every 15 minutes, except during hours of darkness. Experimental populations of individually tagged fish were

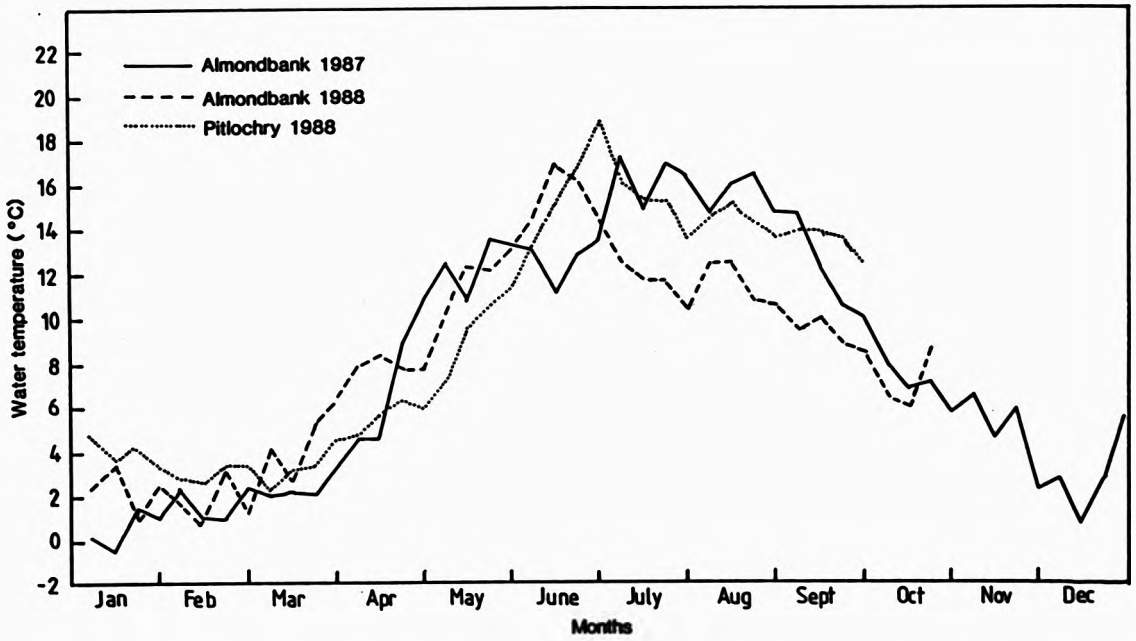


Fig. 2. Water temperatures at the Almondbank and Pitlochry hatcheries during the study period (points are 7 to 8 day means).

maintained on identical rations up until July.

2.2.2 Tagging Methods

Higgins (1985a) described a way of marking individual fish with X-ray microtags. This involved insertion of a 1.6mm long stainless steel tag into the dorsal musculature of fish, just anterior to the dorsal fin. One edge of each tag was notched, with the number and position of notches indicating a binary code. The face of the tag was aligned with the side of the fish and, when the anaesthetised fish was X-rayed on its side, the binary code could be read from the radiograph. Problems in reading codes arose because muscle growth in some fish twisted the tags out of alignment, and the notches could not be distinguished on radiographs. Higgins (1985b) tested this tagging method on Atlantic salmon parr growing from 1 to 8g, but maturing 1+ parr can be expected to grow from 2 to 20g. Because of uncertainty about the long term readability of dorsally inserted microtags in the larger maturing parr, a second method of tagging individual fish was developed. This involved attaching two coloured bands to fish, different colours coding for a different number.

In January 1987, 104 PS2 parr, ranging in size from 60-70mm FL, were taken from the stock population, and were microtagged following Higgins (1985a) method. Only 52

Plate 1. Bandtags attached to salmon parr by a surgical suture through the dorsal musculature, below the dorsal fin. A colour code identifies individual fish. (Scale bar is 3cm long).

different codes were available so pairs of fish were given tags with the same code, and one fish from each pair was distinguished by clipping its adipose fin. Microtagged fish were all anaesthetised in MS-222, and were weighed (to the nearest 0.1g), and measured (to the nearest 0.1cm) before tagging. All fish survived tagging and only 4 died over the next month.

Another group of 94 PS2 parr, ranging in size from 60-80mm FL, were bandtagged. After anaesthetising, weighing and measuring, each fish was placed in a holder made from a piece of split rubber tubing with an elongate hole in the top, exposing the fishes back. Coloured bands were made by cutting 1mm long lengths, from the hollow insulation cables (0.8mm external diameter) of very fine electric wires that are used in telecommunications. Two bands were fixed to each fish, one on each side of its back; the combination of two colours giving the fish an individual number in accordance with the international colour code for resistors. Bands were attached to the fish with very fine (0.1mm diameter) polypropylene sutures (Prolene 6/0). These are made for microsurgical applications and combine strength and durability with resistance to bacterial colonisation. A suture, with a colour-band threaded onto it, was passed through the fish's back, anterior to the dorsal fin, from one side of the fish to the other. A second colour-band was threaded onto the suture, which was then passed back, through the muscle blocks, to the other

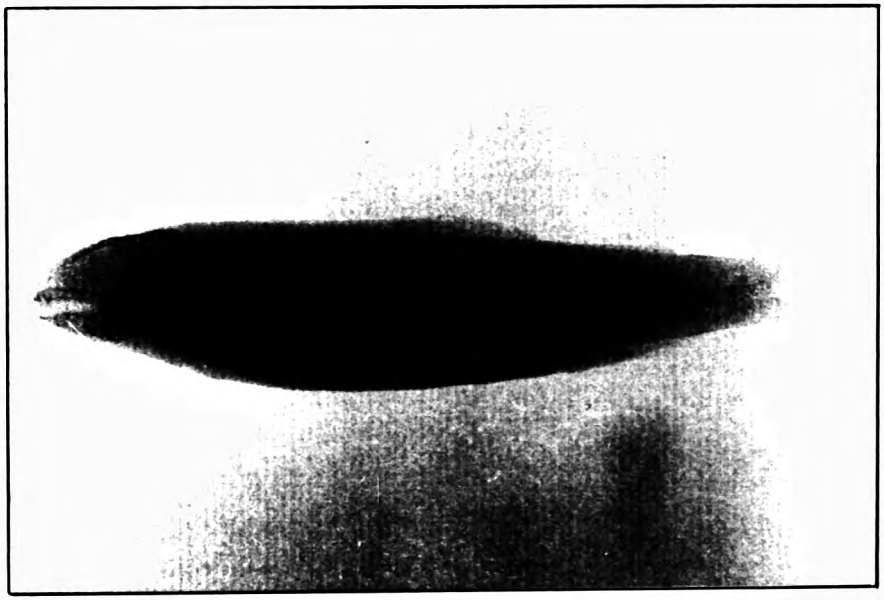


Plate 2. Radiograph of salmon parr showing stainless steel microtag in dorsal musculature and iron particles in anterior region of gut. White shadow is air bladder.

side. The ends of the suture were then tied using a flat and 2 square throws to increase knot security. The operation took less than 1 minute and there were 2 mortalities over the next month. This method of tagging also proved successful, with sites of exit and entry for the sutures providing no problems. When required fish were treated with methylene blue to check fungal infections. No tags were lost due to knot failure or breakage of the suture, and the main problem proved to be embedding and tissue rejection of tags because insufficient room was left for growth when tying the suture. This affected approximately 30% of the fish between July and September.

2.2.3 Data collection and analysis

Lengths and weights of individual fish, in both microtagged and bandtagged populations, were measured to the nearest 0.1cm and 0.1g respectively, once a month starting in January 1987.

The colours and position (left or right) of the two bands were recorded as each of the anaesthetised bandtagged fish were weighed and measured. Later, each fish was identified by the number assigned to its colour code. Identification of microtagged fish proved more problematical. Each fish was individually anaesthetised and X-rayed after it had been weighed and measured. Radiographs (Kodak Industrex MX

film) were exposed with a Todd Research Triton 300, set to provide 75-80 Kv (penetration power of X-rays) and 100mA (quantity of X-rays), for 0.6 sec, using a fine point focus and a source to object distance of 100cm. The radiograph was later developed and placed in a microfiche reader where microtags could be viewed at a magnification of x60. In practice notches forming the binary code were clearly visible in only 80% of tags. Remaining fish were identified by noting the shape of the tag, because even though codes could not be completely read, each tag had a unique shape.

Mature male parr can be identified from late September through to December by the expression of milt, when gentle pressure is applied to the abdomen. However, not all maturing parr are ripe in September, and non-maturing males cannot always be distinguished from females by external signs. Relative gonad size of maturing male parr, as measured by changes in GSI, peaks in August (Murphy 1980) and provides an earlier means of assessing maturational status of all male fish. Bandtagged fish were sacrificed in September and microtagged fish in November. After weighing and measuring, gonads were removed from the freshly killed fish and weighed.

The gonadosomatic index (GSI) was calculated for each fish as follows:

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

Length frequency distributions for maturing and non-maturing males, and females, were back-calculated to March, and compared before the growing season started (Fig. 3A,B). Most of the non-maturing male parr and females were in the size range 50-70mm FL, and parr longer than 70mm FL were predominantly males all of which matured. Comparisons of growth between maturing and non-maturing male parr were therefore restricted to fish of comparable initial size (ie. less than 71mm FL). Male parr larger than 70mm FL were all bandtagged fish, and were treated separately from the smaller maturing males.

Data were routinely tested for normality using a modified Shapiro-Wilk statistic (Lee et al. 1986), and when departures from normality occurred, outliers were deleted, or logarithmic transformations were used. Mean lengths and weights were calculated for each month between January and September for mature male parr, non-maturing males and females. Specific growth rates were also calculated as these provide an indication of size-related growth performance over a given time interval. Specific growth rates were calculated between months for both changes in

length (G_l) and weight (G_w) as follows:

$$G = \frac{(\log S_2 - \log S_1)}{T_2 - T_1} \times 100$$

where G is specific growth rate
S_n is length or weight at time n
T_2-T_1 is number of days between time 1 and 2

Condition factors were calculated using the formula:

$$K = \frac{W}{L^b} \times 100$$

where K is condition factor
W is weight (g)
L is length (cm)
b is the slope coefficient calculated from the least squares regression for the log transformed length weight data.

Differences in mean size, specific growth rate and condition factor between mature males and non-maturing males and females, were tested by analysis of variance for each month and between months.

2.3 RESULTS

Variation in maturation rates resulting from genetic differences was minimised by using a sibling stock of Atlantic salmon parr. In addition only the PS2 fraction of the sibling parr population was used. This restriction served to maximise maturation rates, and to minimise any variation that might have occurred as a consequence of the different developmental rates of upper (S1) and lower (PS2) modal groups of fish during the 0+ year.

The PS2 parr varied in size from 50-80mm FL, and these differences in size reflect differences in past growth performance. Larger fish may be more easily "triggered " to mature than smaller fish, particularly if size reflects an underlying difference in gonad size, chemical composition or physiology. Size-related differences in the stock of PS2 parr were examined in January before the experiment was started. There was no significant difference in sex ratio, condition factor, or in mean GSI for male and female fish between the small (57-61mm FL), medium (62-66mm FL) and large-sized (67-71mm FL) fish (Table 1). However, the smallest PS2 parr had a significantly higher % of water, indicative of a relatively lower nutritional status. This difference may have affected their chances of maturing compared with larger parr.

TABLE 1. Variation in sex ratio, gonad size, mean water content, and mean condition factor (K) with size of fish for PS2 parr in January 1987

Variable	Size class (fork lengths in mm)		
	57-61	62-66	67-71
Sex ratio (% males)	50.0	49.0	55.0
Mean GSI (males)	<0.05	<0.05	<0.05
Mean GSI (females)	0.30	0.31	0.30
Water content (%)	77.1*	76.3	76.1
Condition factor (K)	0.49	0.49	0.48

* significantly higher ($p<0.05$) than in larger size groups.

If nutritional status influences a fish's chances of maturing it is least likely to affect fish in the range 60-70mm FL during January. Most of the individually tagged fish fell within this range. Mortality rates and summary statistics for microtagged and bandtagged populations are given in Table 2.

2.3.1 Seasonal differences in size

The largest fish by March (ie. over 70mm FL) were mainly males, all of which matured (Fig. 3). However, mature males occured throughout the size range of PS2 parr in both March and June, with fish as small as 57mm FL in March, subsequently maturing.

There were no significant differences in mean size between non-maturing males and females for any month (Table 3), and maturing males were only significantly larger than other fish, in the microtagged population in May and June. However, the mean size of maturing male parr was consistently larger than that for non-maturing male parr up until August (Figs. 4-7). This size differential decreased from September onwards, in both populations, with growth rates declining faster in maturing males than in non-maturing parr.

TABLE 2. Summary statistics for populations of individually tagged Atlantic salmon parr.

	Microtagged	Band tagged
No. of fish tagged	104	94
Mortalities pre-July (%)	15.7	9.6
Total fish recovered	63	43
Males recovered	34	25
Mature males (% males)	73.5	76.0

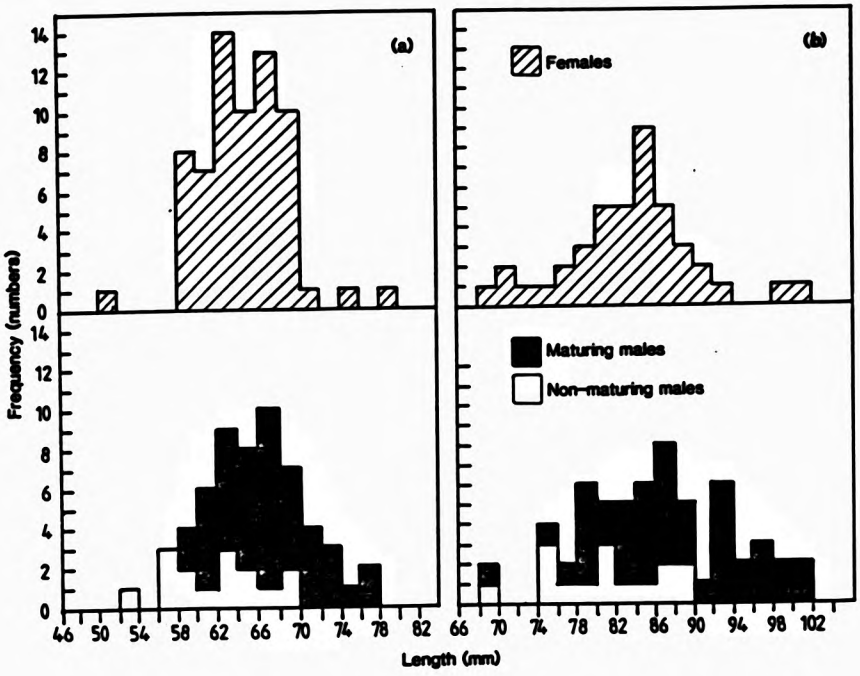


Fig. 3. Size distributions of individually tagged salmon parr in (a) March 1987 (b) June 1987.

TABLE 3. Significance of differences in mean size between maturing male parr and non-maturing parr of both sexes. (Results of ANOVA to determine F-ratios for 2,59 degrees of freedom (Jan-Jun), and 2,52 degrees of freedom (Jul-Nov)).

Month	Mean weights	Mean lengths
Jan	N.S.	N.S.
Feb	N.S.	N.S.
Mar	N.S.	N.S.
Apr	N.S.	N.S.
May	$p<0.01$	$p<0.01$
Jun	$p<0.01$	$p<0.01$
Jul	N.S.	N.S.
Aug	N.S.	N.S.
Sep	N.S.	N.S.
Oct	N.S.	N.S.
Nov	N.S.	N.S.

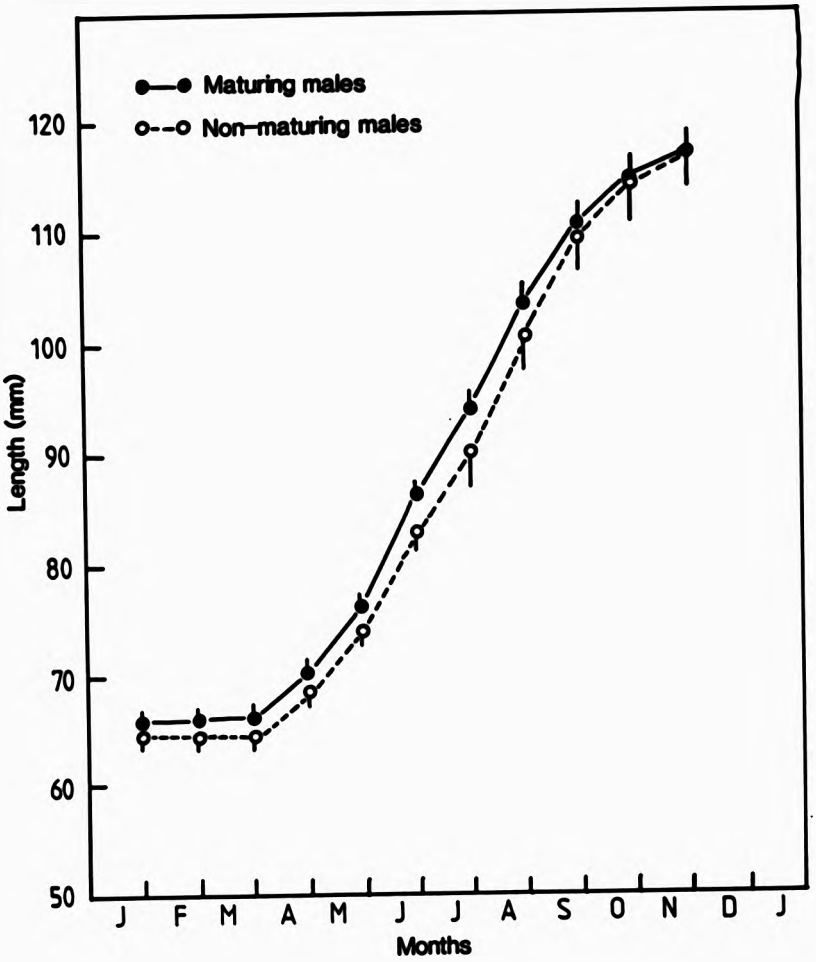


Fig. 4. Monthly changes in mean length of 1+ microtagged fish (vertical bars are ± S.E.).

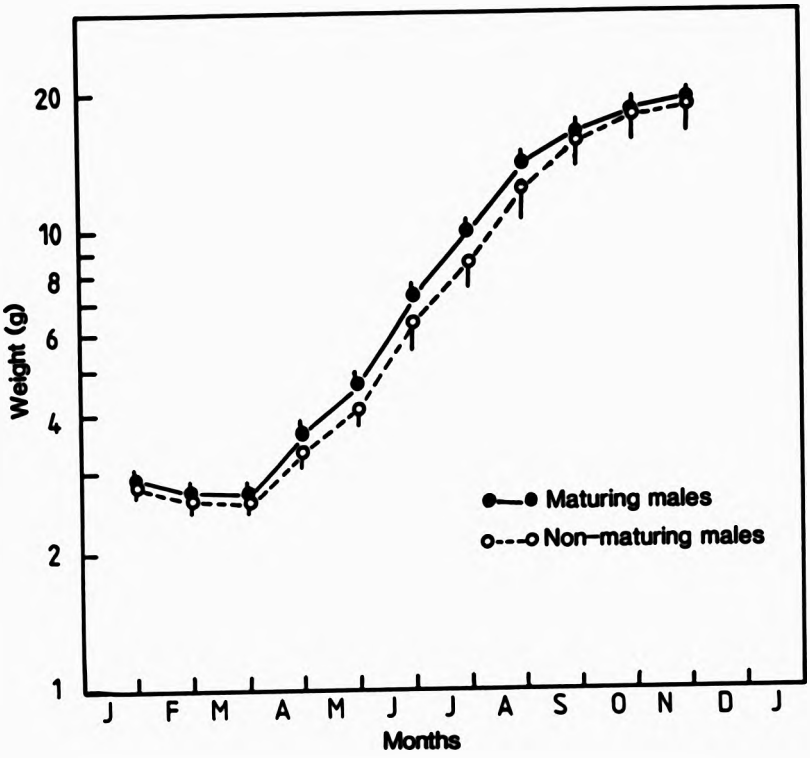


Fig. 5. Monthly changes in mean weight of 1+ microtagged fish (vertical bars are ± S.E.).

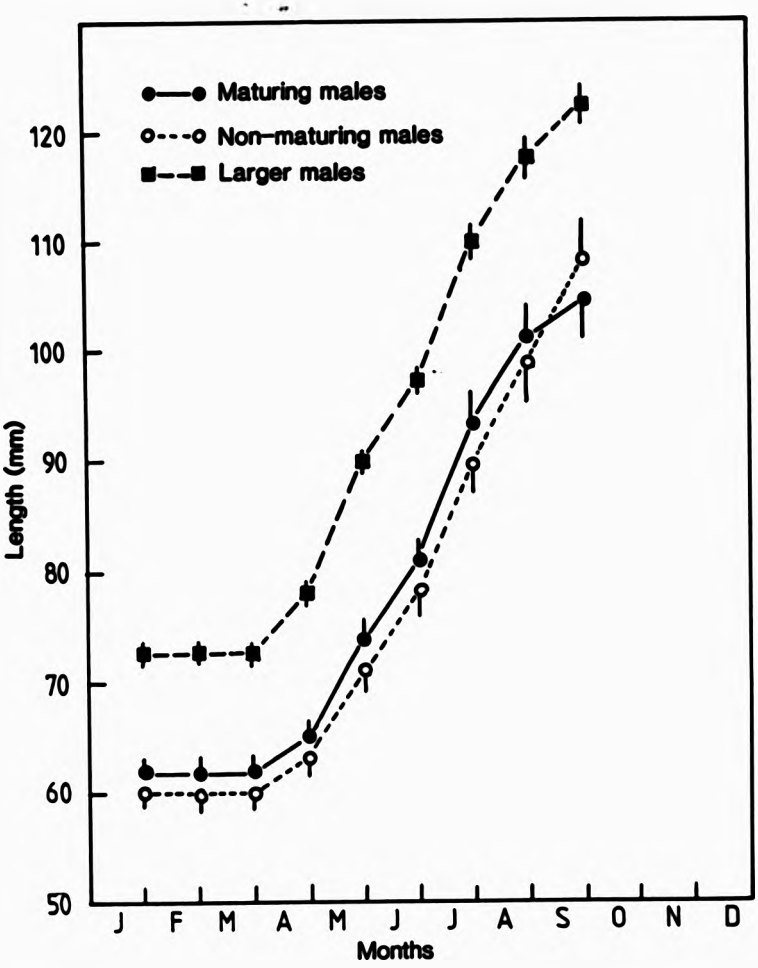


Fig. 6. Monthly changes in mean length of 1+ bandtagged fish (All larger males matured, vertical bars are ± S.E.).

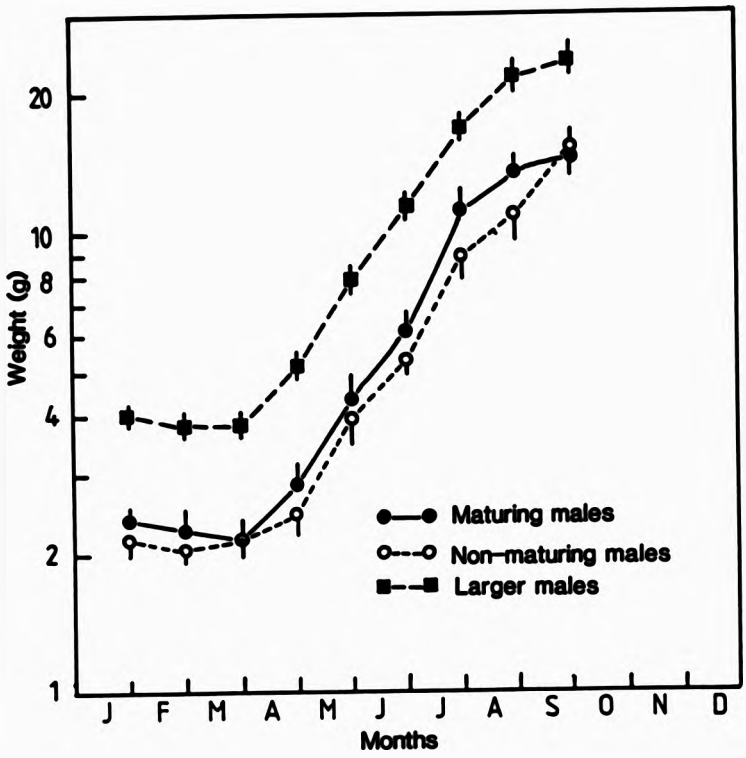


Fig. 7. Monthly changes in mean weight of 1+ bandtagged fish (All larger males matured, vertical bars are ± S.E.).

2.3.2 Seasonal differences in specific growth rate

Mean values for specific growth rate in length (G_l) followed a seasonal pattern. From values marginally above 0.0 between January and March, they started to increase in April, reaching a maximum value close to 5.0 in June for the microtagged fish, and May for the bandtagged fish (Figs. 8 & 9). Mean G_l remained relatively high between May and August, and declined during autumn to values of around 1.0 by December.

Mean specific growth rates for weight (G_w) also varied on a seasonal basis (Figs. 10 & 11). Negative values in January and February, as well as in March for the bandtagged population, indicate weight loss during winter months. G_w's increased significantly in April in both populations, reaching maximum values of 1.6-1.8 for the bandtagged population in May, and for the microtagged fish in June. In general G_w's fluctuated between May and August, but were declining in both populations by September.

There were no significant differences in mean specific growth rates between non-maturing males and females in any month, except May, when they were lower for females in the bandtagged population. The only statistically significant differences between maturing and non-maturing male parr that occurred in both populations, were the

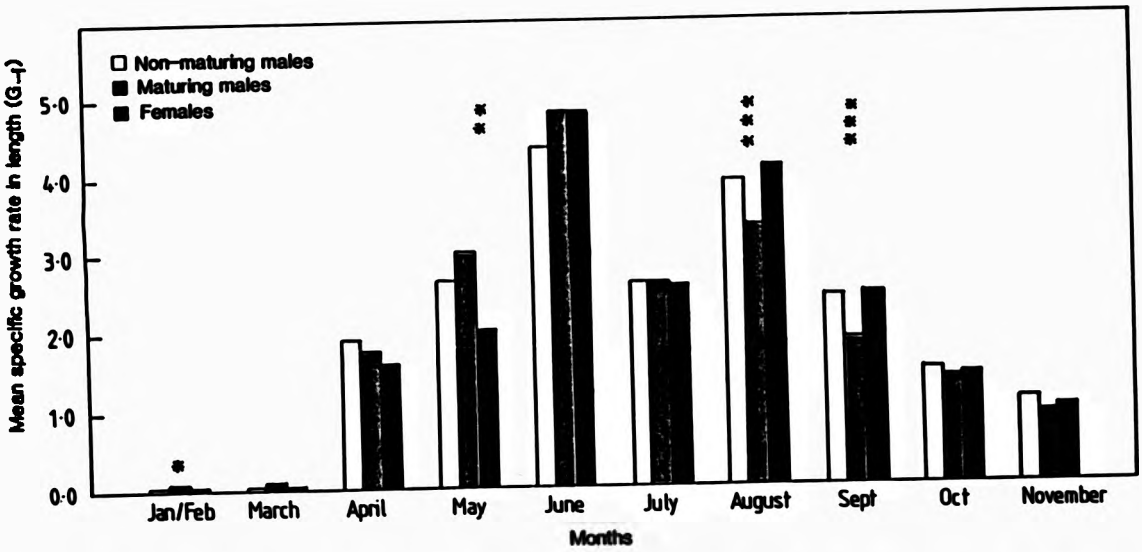


Fig. 8. Monthly differences in specific growth rates in length of microtagged fish (mean value below asterisks is significantly different to other two means; *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

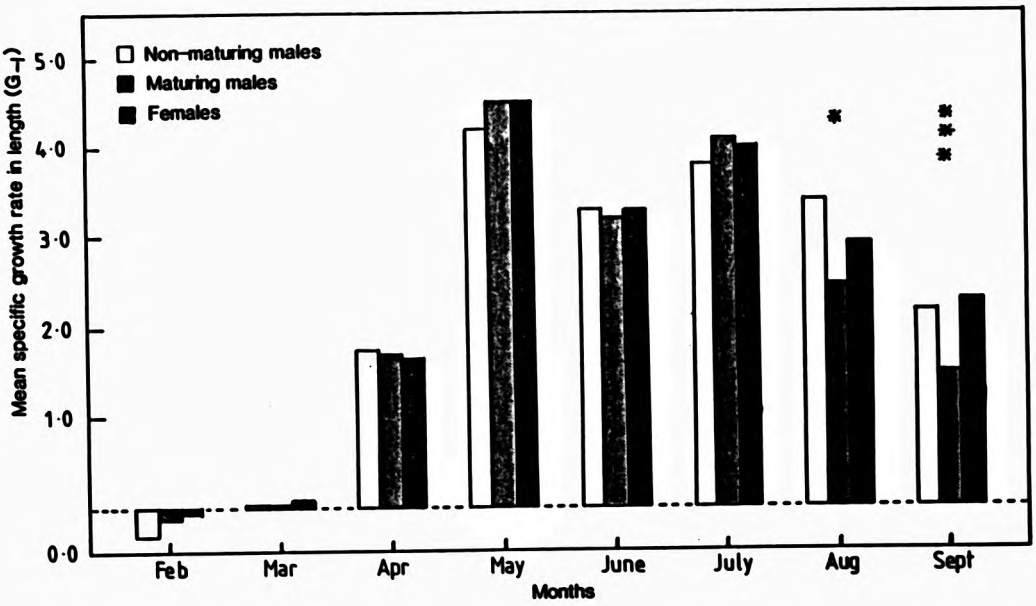


Fig. 9. Monthly differences in specific growth rates in length of bandtagged fish (mean value below asterisks is significantly different to other two means; *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

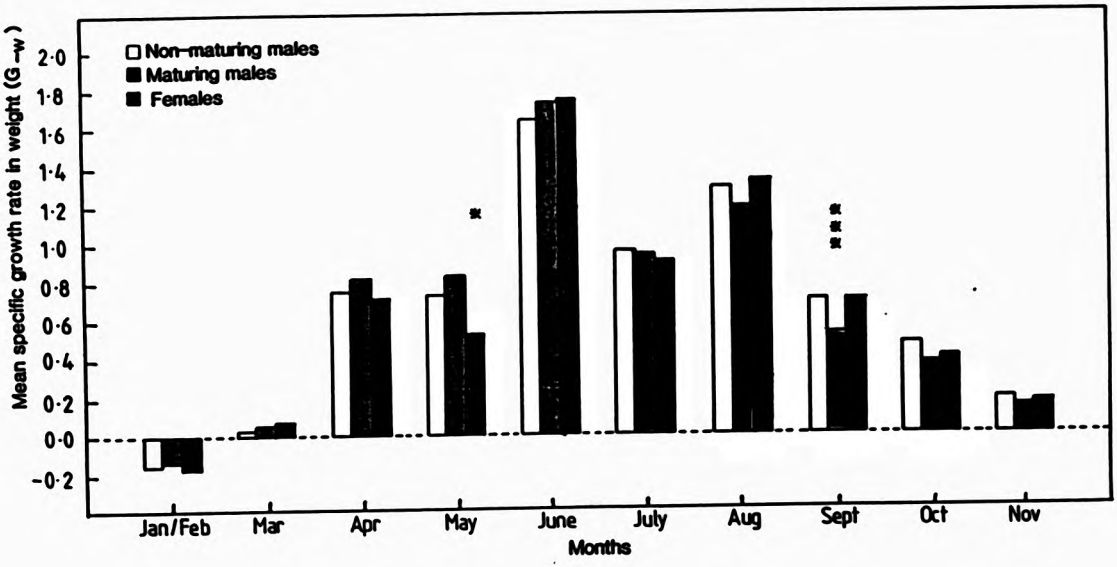


Fig. 10. Monthly differences in specific growth rates in weight of microtagged fish (mean value below asterisks is significanly different to other two means; *** $p < 0.001$, ** $p < 0.05$, * < 0.10).

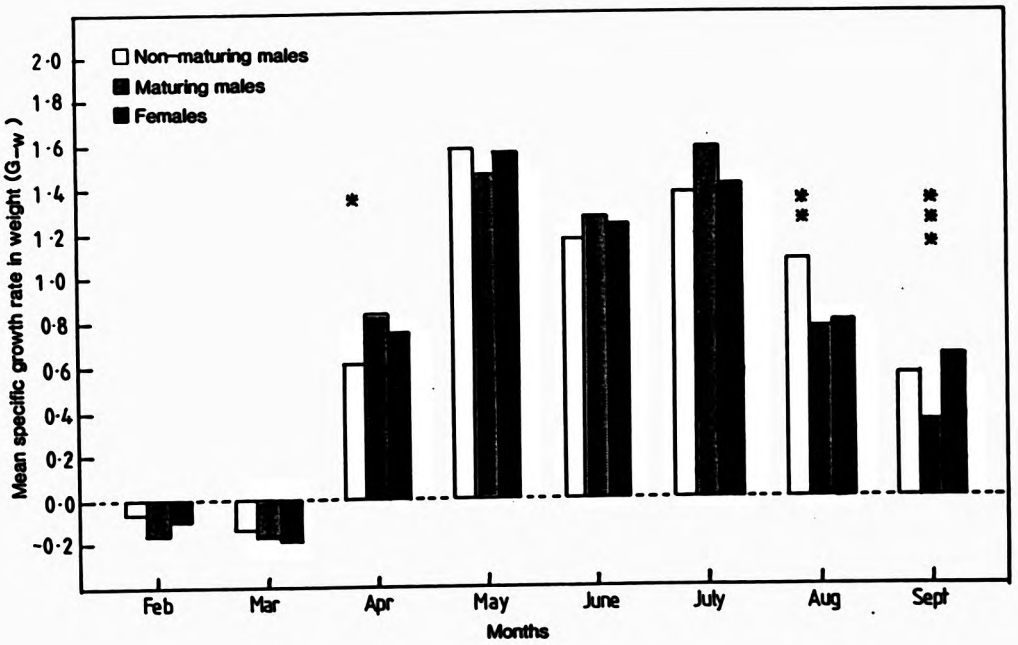


Fig. 11. Monthly differences in specific growth rates in weight of bandtagged fish (mean value below asterisks is significantly different to other two means; *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

relatively low values of G_1 for maturing parr in August and September, and for G_w in September.

2.3.3 Seasonal differences in condition factor

Kane (1988) examined the length weight relationship of hatchery reared Atlantic salmon parr and found that the slope coefficient was close to 3.0. He therefore calculated condition factors using Fulton's formula which is a special case of the empirical length-weight relationship, used when slope coefficients are close to 3.0.

Monthly logarithmic regressions of weight on length for microtagged and bandtagged fish revealed a marked seasonal trend in the slope coefficient, with values exceeding 3.0 in all months, and reaching peak values of 3.4-3.6 by the end of April (Fig. 12). When the data for all months between January and September were pooled for microtagged and bandtagged fish respectively, the slopes of the regressions were 3.42 and 3.45 (Table 4). There was no change in slope when data for the period July to September (ie. when condition factors of maturing males increase), were deleted from the analysis. A mean value of 3.43 was therefore chosen as the best basis for calculating condition factors.

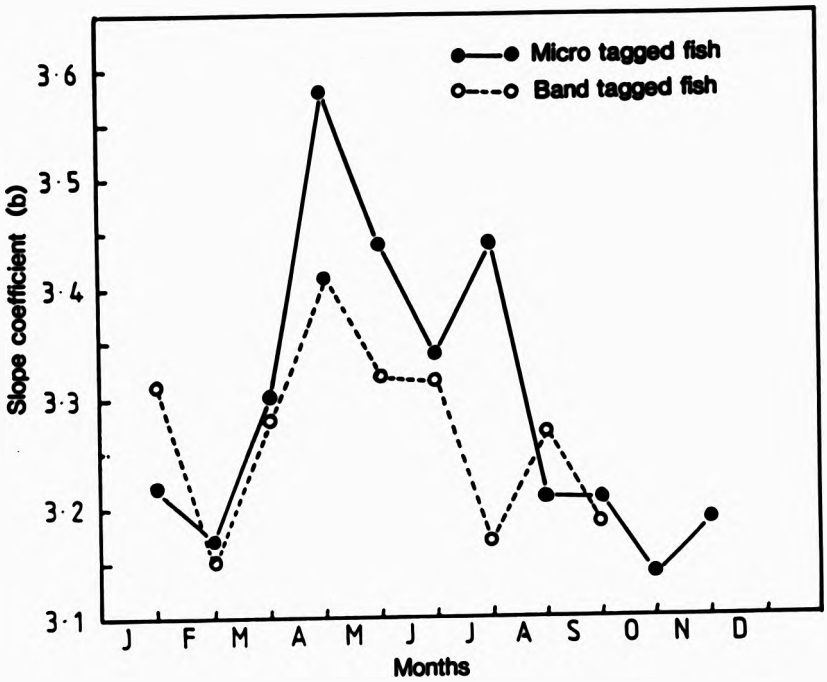


Fig. 12. Seasonal changes in the slope coefficient (b) for the logarithmic regressions of weight on length for the individually tagged populations of 1+ salmon parr in 1987.

TABLE 4. Results of regression analysis for pooled length - weight data from microtagged and bandtagged populations of Atlantic salmon parr.

Period for pooling data	Number of fish	Correlation Coefficient (r)	Regression parameters		ANOVA Results	
			intercept (a)	slope (b)	F statistic	P value
		Microtagged parr				
Jan to Jun	372	0.999	-13.31	3.42	17989.67	<0.001
Jan to Sept	395	0.997	-13.30	3.42	67674.02	<0.001
		Bandtagged parr				
Jan to Jun	234	0.994	-13.40	3.45	19655.21	<0.001
Jan to Sept	339	0.997	-13.40	3.45	53776.62	<0.001

In general, mean condition factor declined during winter months, increased in spring and summer, and declined again in autumn (Figs. 13 & 14). Kane (1988) also recorded increases in condition factor during summer and decreases in autumn. There were no statistically significant differences in mean condition factor between maturing and non-maturing males during any month for the microtagged fish, but maturing bandtagged parr had a significantly lower mean condition factor than non-maturing parr at the end of March, and a significantly higher one by August. This pattern of lower mean condition factor during winter months, increasing to a higher one in autumn, also occurred in the maturing microtagged fish. There was a significant difference in the mean increment in condition factor between maturing and non-maturing males during April (Table 5). It is apparent that in February and March, condition factors of maturing males decline, and then during April, they increase at a significantly faster rate than in non-maturing males. By mid to late August condition factors of maturing males are higher than for non-maturing males, even though condition factors are all declining by this time.

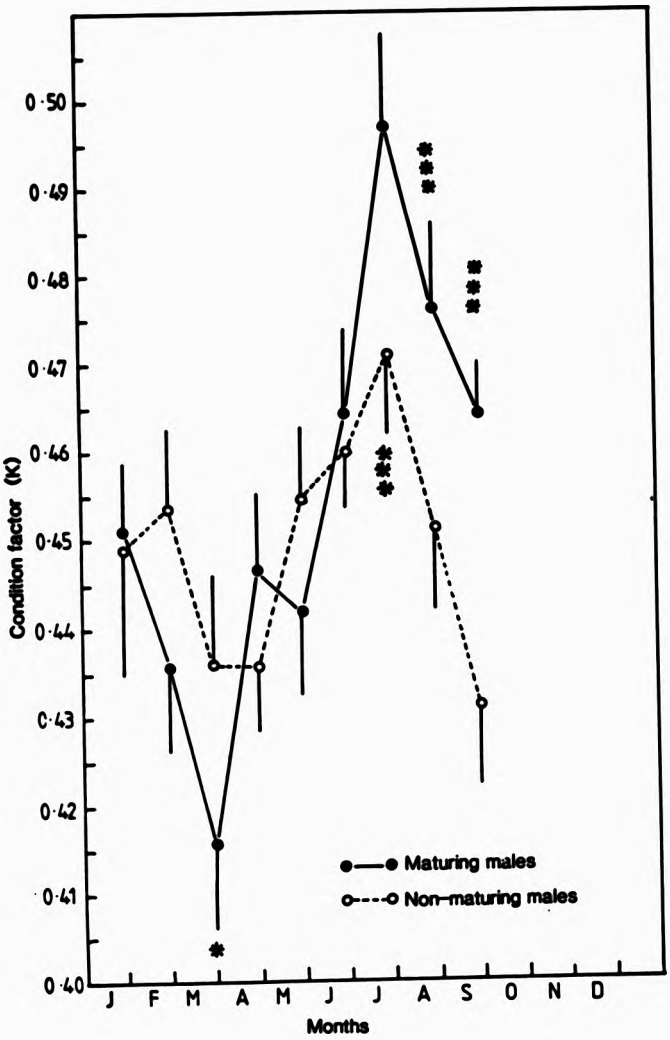


Fig. 13. Monthly changes in condition factor of male bandtagged fish (means ± S.E.).

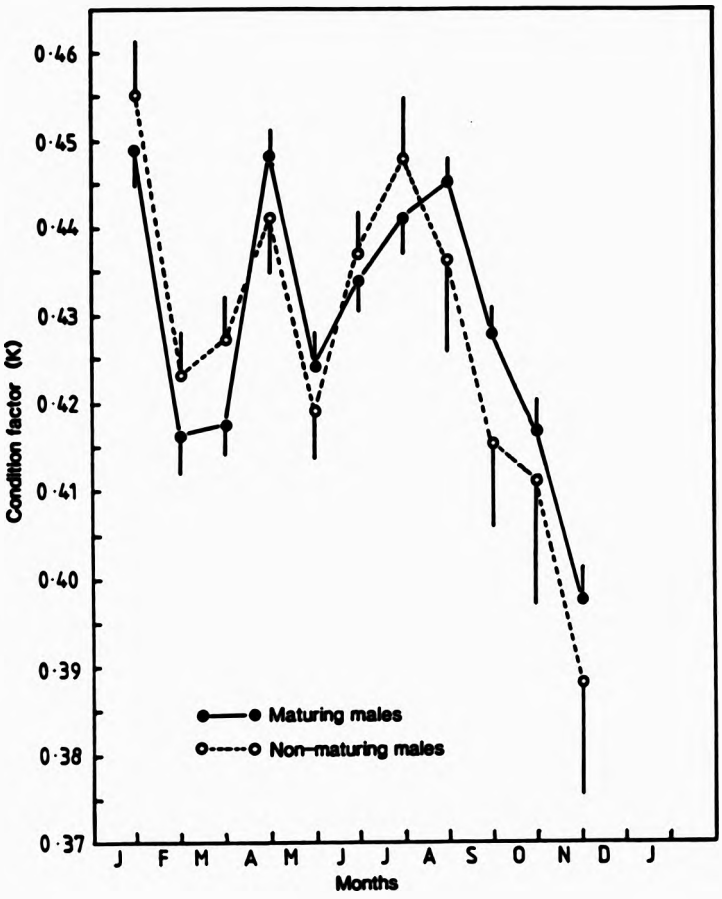


Fig. 14. Monthly changes in condition factor of male micro-tagged fish (means ± S.E.).

TABLE 5. Significance of differences in mean monthly increment or decrement of condition factor (Figs. 13 and 14) for maturing male, non-maturing male and female parr. (Results of ANOVA to determine F ratios for equality of means).

Month	Microtagged population	Bandtagged population
Feb	N.S.	N.S.
Mar	N.S.	N.S.
Apr	$p<0.05$	$p<0.001$
May	N.S.	$p<0.05$
Jun	N.S.	N.S.
Jul	N.S.	$p<0.05$
Aug	$p<0.001$	N.S.
Sep	N.S.	N.S.
Oct	N.S.	--
Nov	N.S.	--

2.4 CONCLUSIONS

Many studies of wild and hatchery-reared Atlantic salmon have found that maturing parr are generally the largest fish, and have concluded that they are therefore the fastest growing fish (Leyzerovich 1973; Simpson & Thorpe 1976; Naevdal *et al.* 1978b; Bailey *et al.* 1980; Murphy 1980; Dalley *et al.* 1983; Thorpe *et al.* 1983; Bagliniere & Masse 1985; Pepper *et al.* 1985). However, Naevdal *et al.* (1978b) indicated that the correlation between age of first maturation and growth rate was small for Atlantic salmon. Several other studies have found that maturing jacks are the smallest parr (Saunders & Sreedharan 1977; Gjerde 1984).

These apparently contradictory results would be reconciled if maturing males were larger to begin with, and then, during autumn, experienced slower growth than non-maturing parr. This has been suggested by Leyzerovich (1973), Dalley *et al.* (1983), Gjerde (1984), and more recently Herbinger (1987), but none of these studies ruled out the possibility of a size-selective mortality for mature males, depressing estimates of mean size. Mitans (1973) and Leyzerovich & Melnikova (1979) have documented the increased mortality of mature male parr of Atlantic salmon following spawning, and Gross (1985) indicated that the larger mature jacks of coho salmon (*Oncorhynchus kisutch*) fought more with adult male salmon, and are less

successful in spawning than smaller parr. Even in the more sheltered environment of hatcheries, mature males have comparatively higher mortality rates, and larger males may be more susceptible to this. Murphy (1980) found that maturing males had a higher mortality rate than females because of their greater susceptibility to fungal infections. He noted that, during autumn months, the linear growth rate of all parr declined, with the decline being greatest for maturing males. Unfortunately he had no immature male parr on which to base his comparison, only females, so the difference in growth rates could have been due to either sex or to a size-selective mortality of males, rather than to maturation. Saunders et al. (1982) tagged individual parr and recorded a decreased growth of maturing males relative to immature parr, but like Murphy (1980) did not eliminate sex differences.

Confirmation of an autumnal decrease in growth rates of maturing males requires a comparison between individually tagged, maturing and non-maturing, sibling males grown under the same conditions. Such a comparison is provided by this study. The results show that maturing males are generally the largest parr, and that this size difference which is established before January, continues up until August-September. After this, growth rates of maturing parr are lower than those of non-maturing males. As a consequence, the size difference is eliminated or reversed

by November. The autumnal decrease in growth rates of mature male parr, relative to non-maturing males, is therefore confirmed. Results reported later (Chapter 4) show that feeding rates of mature males are lower than non-maturing males and females in August. Although it is clear that feeding and growth of maturing males is reduced by maturation, this conclusion does not rule out the possibility that a size-selective mortality of mature male parr may occur, particularly in the wild, and could exaggerate estimated decreases in growth rate of maturing males.

Although the male parr, that eventually matured at 1+, were on average larger than non-maturing males during spring and summer months, there was no evidence for a size threshold for maturation. Length frequency distributions of maturing and non-maturing males showed considerable overlap, both in March, before summer growth starts, and in June, just before gonadosomatic indices increase. This overlap cannot be explained by stock differences and the size threshold hypothesis is therefore rejected.

The only difference between specific growth rates of maturing and non-maturing male parr during spring and summer months occurred in April, when the specific growth rate in weight of maturing males was significantly higher than that for non-maturing males. However, this difference did not occur in the microtagged population, and there was

no difference in the specific growth rates of maturing males and females during April for this population. There was thus little evidence of a difference in specific growth rates for weight or length, between maturing and non-maturing male parr, during spring or summer months. The null hypothesis that specific growth rates of maturing males are no different to those of non-maturing males, during late winter, spring, or early summer months is therefore accepted. However, the possibility that a seasonal difference in specific growth rates occurs during the first year of life, and results in the size difference noted between maturing and non-maturing males at the end of the first winter, still remains. Naevdal et al. (1979) found that the increased size of maturing fish could be traced back as far as the summer of the previous year.

Although there were no consistent seasonal differences in specific growth rates in length or weight between maturing and non-maturing males, there were significant differences in mean condition factor between the two groups of fish.

Leyzerovich (1973) found that Atlantic salmon parr populations in the Neva hatchery had lower growth rates than salmon in the Narva hatchery, but had higher condition factors and significantly more mature males. He excluded genetic effects as a cause for the differences and cited the opinion of Yevropeytseva (1957; 1960a;

1960b) that changes in condition factors, reflecting differences in weight gain relative to length, were of great importance to the development of mature male parr. More recently, Murphy (1980) found that maturing male parr had a higher mean condition factor than immature parr in April, but a lower one from August onwards. The lower condition factors in autumn contradict results obtained by Saunders et al. (1982) and Naevdal (1983) that mature male parr had a higher mean condition factor than immature males, but Murphy's immature parr were all females, so his result may be due to differences between the sexes. More recently, Herbinger (1987) followed the growth of individually tagged Atlantic salmon, and found that prospective maturing salmon had higher post winter condition factors than non-maturing salmon. He concluded that of all growth measurements condition factor was the best predictor of future maturation status.

Saunders et al. (1982) attributed the higher condition factors of mature male parr to the large mass of testes present, but Naevdal (1983) discounted higher gonad weights as a cause of increases in condition factor. The GSI's of mature male parr peak during September and October (Section 4.3, Murphy 1980), and the higher autumnal condition factor of maturing male parr in the microtagged and bandtagged populations is thus due in large measure to the weight of gonads present at this time. However, the increases in condition factor of male

microtagged and bandtagged fish during April are not due to gonadal growth as GSI's of maturing male parr at this time are less than 0.5% (Murphy 1980). The spring increase in condition factor therefore reflects a greater gain in somatic weight relative to length for all fish, with this fattening effect being significantly greater in maturing male parr during April.

The results show that there is a possible relationship between increases in condition factor during spring months, particularly April, and maturation during the following autumn. Examination of the published literature on maturation in Atlantic salmon has revealed three other independent studies in which growth of individually tagged maturing and non-maturing fish has been measured (Hunt et al. 1982; Johnston et al. 1987; Herbinger 1987). In the two former studies data on monthly condition factors were recorded. Monthly changes in mean condition factors of maturing and non-maturing fish were therefore compared, particularly during April (Figs. 15 and 16). In both cases maturing salmon had comparatively greater increases in condition factor during April than non-maturing salmon. Herbinger (1987) monitored fish on a seasonal basis and found that high post winter condition factors were the best predictors of maturation in autumn. These results corroborate the conclusion of this study that, during April, maturing males increase their condition factor at a

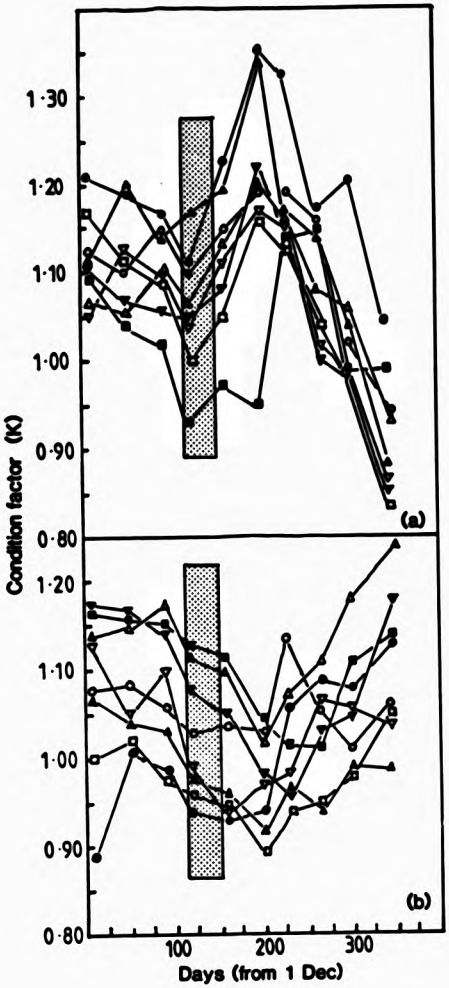


Fig. 15. Differences in condition factors (K) between (a) maturing and (b) non-maturing grilse of Atlantic salmon. (Shaded area denotes April). Data from Hunt et al. (1982).

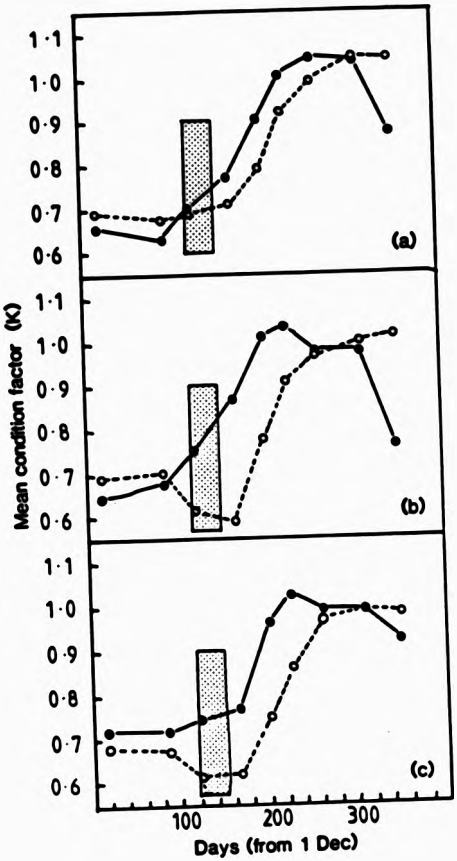


Fig. 16. Differences in mean condition factor (K) of maturing (●—●) and non-maturing (o--o) kelts grown under (A) simulated natural temperature and photoperiod (B) simulated natural photoperiod and 12°C, and (C) advanced photoperiod and 12°C. (Shaded area denotes April). Data from Johnston et al. (1987).

faster rate than non-maturing males. The question remains as to whether maturation is causally related to the comparatively greater increases in condition factor, or whether the increases are merely symptomatic, reflecting the fact that maturation has already been initiated in these fish.

The male parr which mature tend to be the larger fish as early as January, and have significantly lower condition factors than non-maturing males in March. These morphological differences distinguish maturing males during their first winter and may indicate that maturation has already been initiated. However, they may simply reflect differences in feeding opportunity during the first summer and winter, which increase the probability of maturation being initiated in these fish later. The larger size of maturing rainbow trout (Salmo gairdneri) males could be traced back to the previous year (Naevdal et al. 1979). Furthermore, Magri et al. (1985) found that only a proportion of rainbow trout parr matured at age 0+ after testosterone implants when 5 months old. This result indicates that at the time of treatment there were two populations of male fish with different susceptibilities to the steroid treatment. Some males were apparently already primed for maturation, whereas others were not.

It is apparent that the initiation of maturation, or an increased susceptibility to it, occurs in some male fish at

a very early stage in their development. In Atlantic salmon there is also evidence that maturation is initiated at least as early as the winter before. Abdullah (1981), Hunt et al. (1982) and Youngson & McLay (1985) all recorded small peaks in plasma testosterone levels in a proportion of Atlantic salmon during February to March. All these fish matured during the following autumn, whereas salmon not showing these seasonal elevations in testosterone did not mature. Testosterone promotes gonad growth, and the occurrence of higher levels of this hormone in prospective maturing males at this time, provides good evidence that the physiological changes leading to maturation have already been initiated in a proportion of male parr by the end of their first winter.

Purdom (1979) suggested that sex hormones could accelerate somatic growth during some parts of the sexual cycle, and the elevations in plasma testosterone levels in late winter may act anabolically. Pharmacological doses of 17a-methyltestosterone have a dose-dependent anabolic effect on growth of Atlantic salmon parr, with low doses increasing both growth rates and condition factor, and higher doses having little effect or depressing growth (Saunders et al. 1977). There was no difference between growth rates of maturing and non-maturing parr during May to July, when natural levels of testosterone in Atlantic salmon parr were low but increasing (Murphy 1980).

However, it is possible that the smaller spring elevations during February to March are responsible for the April increases in condition factor. The spring increase in condition factor cannot be accounted for by gonad growth as can the autumn increase (Naevdal *et al.* 1981), and represents an increase in somatic weight for length. Gjerde (1984) pointed out the need to determine whether the size differences between maturing and non-maturing male parr are caused by differences in growth ability *per se*, or whether the maturation process accelerates growth. It is apparent from these results that growth rate is not accelerated by the early or advanced stages of maturation, but the possibility that the small elevations in testosterone in February and March stimulate growth at this time, resulting in better weight gains for length in maturing males, needs to be considered.

During autumn months, when testosterone levels rise to peak values, growth rates of maturing males actually decreased. Saunders *et al.* (1977) and Simpson (1976) found that high doses of methyltestosterone administered in the food of Atlantic salmon depressed growth rates, and it is likely that the naturally high levels of testosterone in maturing male parr during autumn are responsible for the reduced feeding of parr at this time. A hormonal suppression of feeding and growth during maturation emphasizes the need for an energy store to support the costs of maturation.

Several authors have speculated that the initiation of maturation may be linked to the accumulation of fat reserves during spring (Thorpe 1986; Myers *et al.* 1986 Herbinger 1987). Hoar (1939) indicated that the coefficient of condition would be a valid index of fat content in Atlantic salmon, and it appears probable that the April increase in condition factor in mature males reflects an increased deposition of fat in these fish.

Evidence of a relationship between condition factor and fat content in Atlantic salmon parr is provided by Pinder & Eales (1969). They found that mean levels of ether-extractable fat were significantly correlated with mean condition factor, from July to April. Groves (1970) and Parker & Vanstone (1966) indicated that body fat is the primary variable determining condition factor in immature sockeye salmon (*Oncorhynchus nerka*), and the correlation between condition factor and fat content has also been confirmed for parr of Arctic charr (*Salvelinus alpinus*) (Dutil 1984). It is therefore probable that the spring increases in condition factor of immature Atlantic salmon parr are due in large measure to increases in lipid content. If so this fattening effect is greatest in maturing males and may be due to anabolic affects of testosterone on growth at this time.

CHAPTER 3

Effect of seasonal changes in feeding on maturation

3.1 INTRODUCTION

The relative importance of genetic versus environmental influences on the variation in maturation rates of Atlantic salmon parr has been a source of speculation for many years. A number of studies have established a genetic basis for much of the variation (Naevdal et al. 1978a; Naevdal 1983; Thorpe et al. 1983; Gjerde 1984) but the role of environmental influences is still enigmatic.

The correlation between growth rate and maturation noted for many salmonids, including the parr of Atlantic salmon (Thorpe 1986), suggests that environmental factors may have a role in the initiation of maturation through their effects on growth rate. However, Myers et al. (1986) found no evidence that increased growth rates during the first year of life increased the proportion of mature male parr in wild populations. The correlation between growth rate and maturation may simply reflect genetic differences in developmental tempo, with faster growing strains maturing earlier than slower growing ones (Thorpe et al. 1983).

Segregating genetic and environmental influences on maturation rates has proved difficult and the evidence for

phenotypic variation in response to different environmental conditions is sparse. A number of authors have reported variation in maturation rates within wild stocks of Atlantic salmon parr, and indicated that this variation cannot be due entirely to genetic factors (Saunders & Sreedharan 1977; Glebe et al. 1984; Myers et al. 1986). However, Naevdal (1983) reported significant variation between families, and Thorpe et al. (1983) established that parr maturation is an heritable trait. Gjedrem (1985) noted the high heritability rates for this trait from both sire and dam components, and it is conceivable that large variations in maturation rates between years for the same stock are due to changes in both mating and mortality patterns. Comparatively higher survival rates of faster growing parr, sired by early maturing males, could result in higher than expected maturation rates. Variation in maturation rates of wild stocks is therefore of limited value in establishing a role for environmental factors. Evidence for phenotypic variation must be sought from sibling populations.

Leyzerovich (1973) provided the first evidence that fast growth altered the phenotypic expression of maturation rates. He reported differences in the maturation rates of Atlantic salmon parr populations between hatcheries, and, as the populations came from a common stock, discounted genetic effects. The differences in maturation rate were

attributed solely to the different foods and rearing conditions between hatcheries. Saunders et al. (1982) and Sutterlin & MacLean (1984) also concluded that early rearing practices affected maturation rates of male parr. They accelerated incubation and early rearing of Atlantic salmon fry by increasing water temperatures, and obtained relatively high proportions (30-60%) of mature 0+ fish, whereas under normal hatchery practices male parr rarely mature before 1+. The high proportion of mature 0+ male parr in the accelerated stock was thought to be due to their faster developmental rate, but the differences may also have been due to a change in male parentage.

Glebe & Saunders (1986) pointed out that there is a high probability that grilse sires have already matured as parr. Such fish would be expected to produce more maturing parr than fish which matured first as grilse. As there is no way of discerning whether grilse have matured as parr or not, the high proportion of 0+ mature parr obtained after accelerated rearing could be due to hereditary differences between sires. This explanation could account for results obtained by Saunders et al. (1984), and Sutterlin & MacLean (1984), but is less likely in the latter case. Sutterlin & MacLean (1982) found that an F1 generation derived from a wild stock of landlocked Atlantic salmon, produced no 0+ mature males when reared under natural temperatures. However significant numbers of

0+ mature males were produced in an accelerated F2 generation derived from the F1 stock. The F1 generation was sired by a wild male likely to have matured first at 12-15cm FL (age unknown). The sire for the F2 generation was a 3+ fish of 25-30cm FL. As less than 5% of the F2 generation matured at the same size as the wild males, the sire for the F2 generation is unlikely to have matured first at a smaller size than that of the F1 sire. These results thus provide evidence, but not irrefutable proof, that exposure to increased temperatures at the egg and fry stage increases the maturation rate at age 0+.

Sower et al. (1984a) provided more convincing evidence for phenotypic differences in maturation rates. A population of Atlantic salmon parr, grown under natural temperatures but subject to a 12L:12D photoperiod from the time of first feeding, produced significant numbers (70%) of 0+ mature males. The stock population grown under natural temperatures and photoperiod produced none. Eggs and fry were not accelerated and the main difference in treatment was the rearing conditions, and in particular photoperiod. Increased daylengths have been reported to increase growth rates of Atlantic salmon parr (Saunders & Henderson 1970; Lundqvist 1980; Saunders et al. 1985), and Adams & Thorpe (1989) have demonstrated a close relationship between growth rates of Atlantic salmon parr and the product of mean daily temperature and daylight hours up to

July. Salmon parr do not feed extensively at night (Higgins & Talbot 1985) and so the 12L:12D may have increased the spring growth rates of the control fish relative to fish in the stock population. Alternatively, the stock population may have received more food than the 12L:12D population.

Other evidence for an effect of photoperiod on maturation rates is provided by Saunders & Henderson (1988). They found significant differences in maturation rates between sibling populations exposed to simulated natural light (LDN) and 16L:8D photoperiods. However, there was no difference in maturation rates between populations exposed to 12L:12D and 24L:0D photoperiods. Mean size at August, and hence growth rates of the respective populations varied, and the effects of photoperiod on maturation through growth rates cannot be distinguished from direct effects on maturation. Saunders & Henderson (1988) noted that some males matured under each photoperiod and concluded that as with other salmonids, photoperiod manipulations can extend or delay maturation, but do not initiate it. Their results thus confirm an environmental effect on maturation rates of Atlantic salmon parr, but the respective contributions of growth and photoperiod are still confounded.

These results indicate that there is a significant environmental component associated with maturation rates of Atlantic salmon parr. Longer daylengths and higher water temperatures both increase maturation rates, and it is believed that growth integrates the effects of these environmental factors at the level of individual fish. It follows that, whatever the cause, increases in growth rate above a stock specific level, should increase maturation rates and produce phenotypic variation in this trait.

Maturation in Atlantic salmon is photoperiod regulated (Lundqvist 1980; Lundqvist 1983), and in salmonids is believed to be initiated under increasing photoperiods (Scott & Sumpter 1983). The timing of increased growth rates is therefore likely to be just as important as the extent of the increase, and Saunders et al. (1982) have argued that in 0+ parr, fast growth at or near the time of first feeding is critical for maturation to be initiated. Thorpe (1986) proposed a more general model, indicating that maturation will occur if the rate of accumulation of surplus energy exceeds a genetically determined threshold in early spring.

It is apparent that experiments to determine the effects of increased photoperiod and water temperature on maturation will be limited because these variables also influence growth. The role of growth alone needs to be established. This study was therefore designed to test the

hypotheses that phenotypic variation in maturation rates can be caused by differences in growth rate alone, and that spring is the time when such increases affect the decision to mature. The converse of this, that reduced growth during spring would depress maturation rates, was also tested.

3.2 MATERIALS AND METHODS

Three experiments were carried out to determine whether changes in seasonal growth patterns would affect maturation rates of populations of sibling male parr. The first, the optimal feeding experiment, was designed to restrict growth for all but two months of the growing season, during which feeding opportunity was optimised. The timing of the two monthly period of optimal feeding, and therefore relatively faster growth, was varied for different populations of salmon parr (Fig. 17). The restricted feeding experiment, the converse of the first, was designed to allow optimal feeding, and therefore relatively fast growth, throughout the entire growing season, except for a 2 monthly period when food was restricted. The time for the 2 monthly period of food restriction was also varied between populations (Fig. 18).

The third experiment, the April starvation experiment, was designed to simulate the effect of a prolonged winter, or late spring on feeding and growth, and tested the effect of starvation during April on the condition factor and maturation rates of parr populations.

All experiments were carried out under natural photoperiod and water temperatures (Fig. 2).

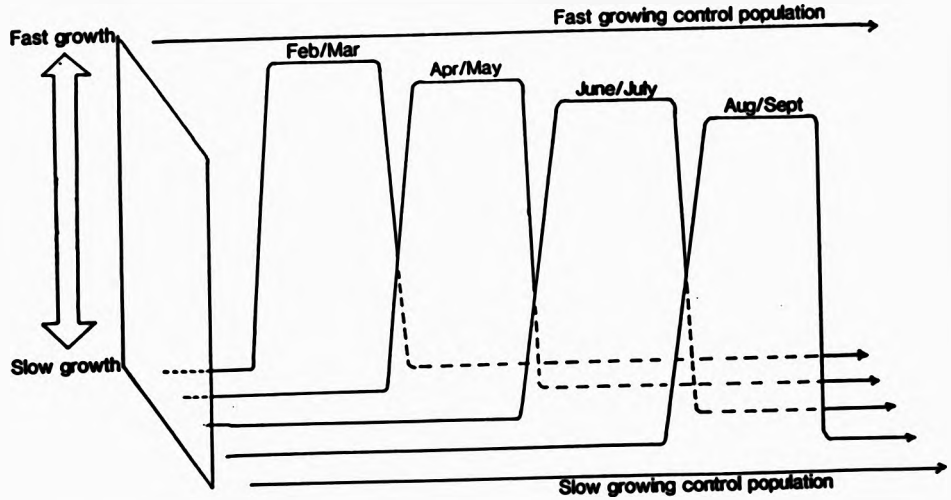


Fig. 17. Design of the optimal feeding experiment. Times of comparatively fast and slow growth for the four experimental and two control populations.

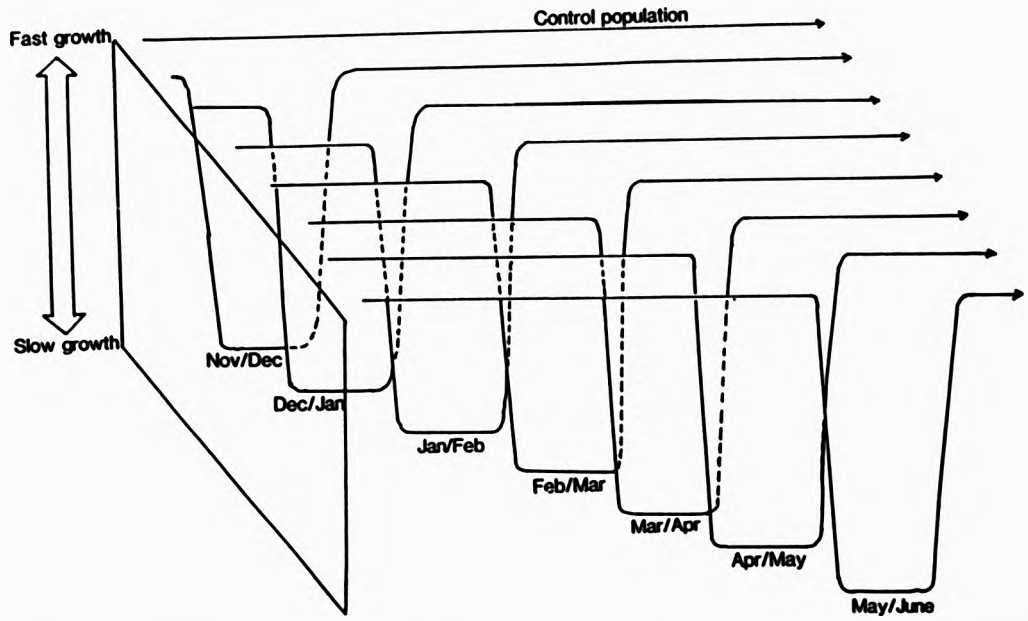


Fig. 18. Design of restricted feeding experiment. Times of comparatively fast and slow growth for the seven experimental, and one control population.

3.2.1 The optimal feeding experiment

Optimal growth opportunities were created during different 2 monthly periods by reducing densities of fish, while maintaining food supply at the level set for the higher density. Suppression of growth during the remainder of the experimental period was achieved by increasing fish densities to the point where the population was receiving slightly less than the recommended ration for optimal growth.

Control over growth of fish populations in hatcheries is usually achieved by altering ration size or feeding frequency. A major limitation with this method is that intraspecific aggression increases when food supply is discontinuous or limited (Symons 1971). Dominance hierarchies are based predominantly on size, and growth of dominant parr can be 30% greater than that of subordinates. This leads to variations in individual growth rates. Another approach is to modify fish densities, while maintaining constant rations (Refstie & Kittelson 1976), but this also leads to variation in individual growth rates. A way of reducing growth is needed which also reduces individual variation in growth rates caused by dominance hierarchies. Such a method is suggested by results of a growth study by Jobling (1983). He found that the feeding of most small fish was inhibited in the presence of larger ones, and that this effect was

not due entirely to competition, as fish were fed to satiation. This suggests that the addition of relatively large numbers of large (S1) Atlantic salmon parr to a population of smaller (PS2) ones would depress the growth rate of the PS2 parr and reduce competition among them. Accordingly this method was used to suppress growth rates and it was assumed that the larger parr would uniformly suppress food intake of the experimental PS2 parr, thus reducing variation in individual growth rates.

This assumption was tested in July and August when water temperatures were at their maximum (Fig. 2), and fish metabolism and activity were high. Mean feeding rates for the experimental PS2 parr populations, with and without the larger S1 parr, were tested to determine whether the addition of the larger parr depressed meal size of the smaller, experimental fish. In addition the frequency distributions of feeding rates for the experimental parr were tested for normality to check the assumption of uniform suppression in meal size, and hence in growth restriction.

Meal size of the experimental parr was measured using the iron-labelling method (Talbot & Higgins 1983; Talbot *et al.* 1984). During summer, the proportion of Atlantic salmon parr that are feeding is lowest during hours of darkness and greatest around dawn and dusk (Higgins &

Talbot 1985). Populations of the experimental PS2 parr, both with and without S1 parr, were therefore given iron-labelled food, from before dawn until 11am, when they were anaesthetised, weighed and X-rayed. The calibration curve for calculating meal size is given in Figure 19. Because meal size is proportional to fish size (Fig. 20), the estimates of meal size for each fish were transformed to feeding rates (ie. mg food consumed/g fish weight/hour).

The optimal feeding experiment was started in early January 1987 and terminated in late September, after maturing males could be readily distinguished from non-maturing ones on the basis of gonadosomatic indices (GSI). Approximately 300 PS2 parr, selected at random from the same sibling stock as used in the individual tagging experiments (See 2.2.1), were identified by clipping the adipose fin. Fifty of these were transferred to each of six 1m diameter radial flow tanks in the Almondbank hatchery. One hundred S1 parr were then added to 5 of the tanks as shown in Figure 21. The 100 S1 parr kept in one tank throughout the experiment created a comparatively slow-growing control population of PS2 parr. The tank with 50 PS2 parr, and no S1's, was kept as a relatively fast-growing control for the duration of the experiment. The 100 S1 parr were temporarily removed from the remaining 4 tanks at different times, to provide a period of reduced density and increased growth opportunity. These times were January-February, March-April, May-June and July-August

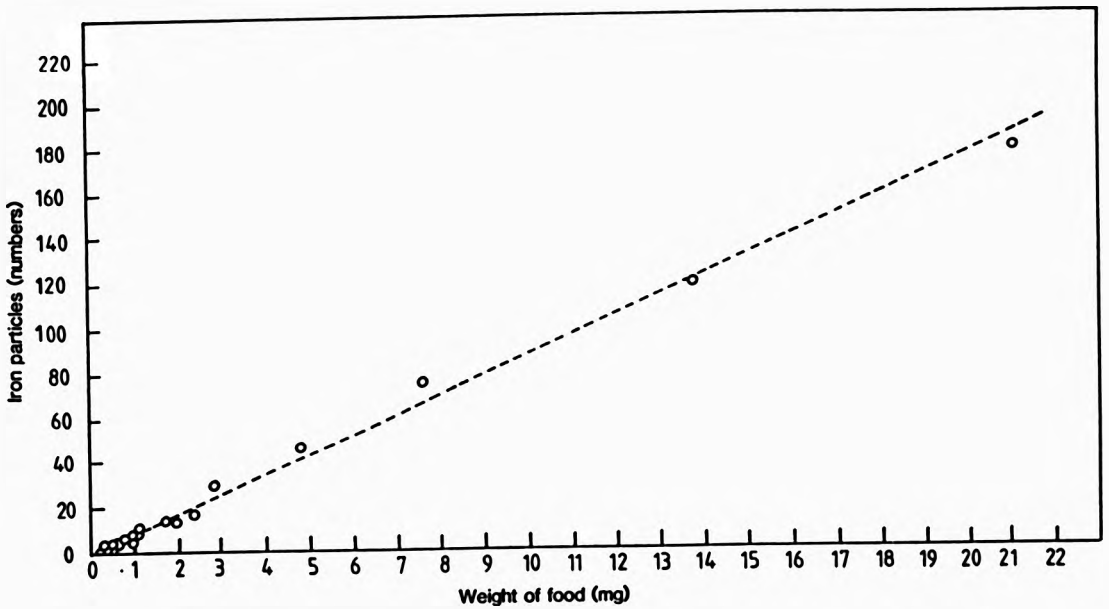


Fig. 19. Calibration curve for calculating meal size from counts of iron particles. Regression parameters (r = 0.996, b = 1.13, a = -0.56) were calculated using weight of food as independent variable.

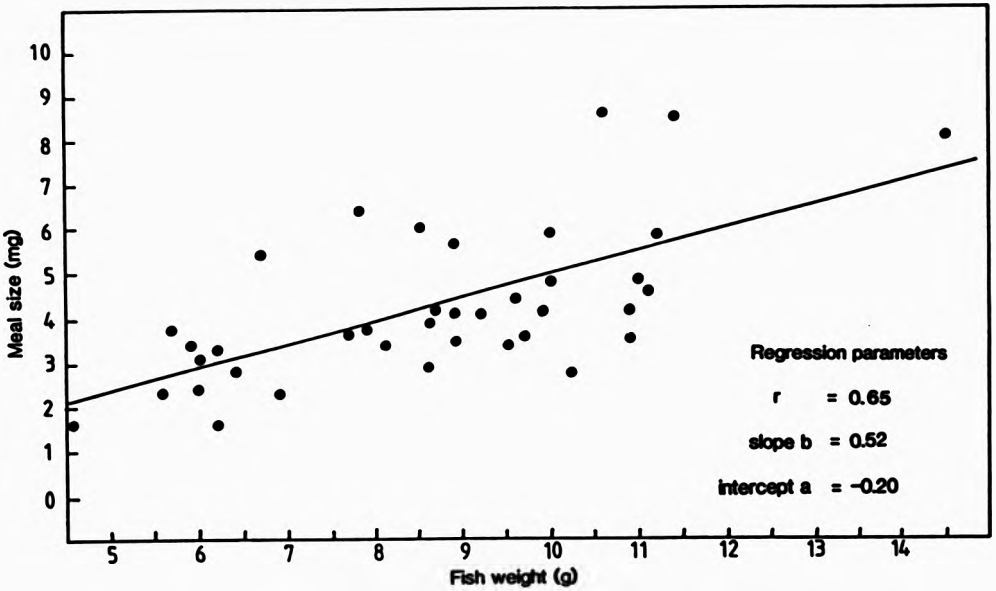
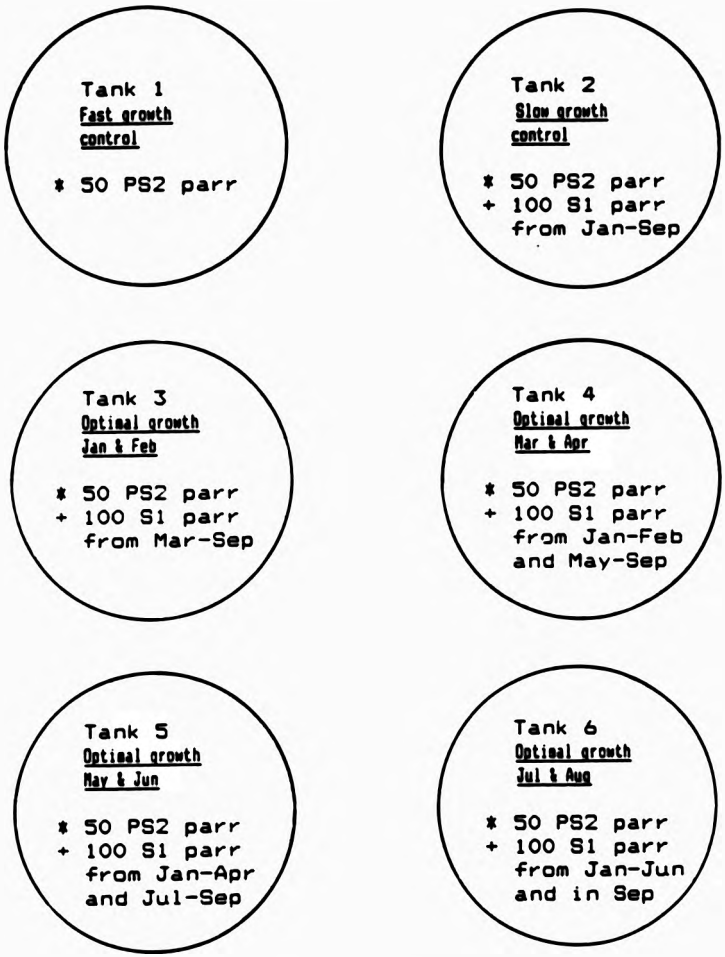


Fig. 20. Relationship between meal size and fish weight for 1+ PS2 parr.

Figure 21. Numbers of fish per tank and times of stocking for the PS2 and S1 parr in the optimal feeding experiment.



respectively. Daily ration size for all tanks was kept constant at approximately 1% of the total biomass (PS2 plus S1 parr) of the slow-growing control population. Mortalities of S1 parr were compensated for with replacement fish, and mortalities among the PS2 parr kept for sexing and determination of GSI's.

Experimental fish were removed each month, anaesthetised, weighed and measured. All experimental fish were sacrificed at the end of September, and their sex, weight and gonad weight recorded. Mean specific growth rates were calculated for each month to determine the effect of the manipulations in fish density on growth. Mature males were readily distinguished by visual inspection of gonads and by their higher gonadosomatic index. Differences in maturation rates both overall, and between control and experimental populations, were tested using the Chi-square statistic calculated as follows:

$$X^2 = \frac{1}{pq} \sum_{i=1}^{m} n_i (p_i - p)$$

where X^2 is Chi-squared

p is the overall proportion of mature males

q is 1-p

m is the number of samples (ie. populations)

n_i is the sample size (ie. males per population)

p_i is proportion of mature males per sample

3.2.2 The restricted feeding experiment

Approximately 180 PS2 parr from a sibling stock of fish, originating from the Kyles of Bute salmon farm, Argyll, were placed into eight 2m radial flow tanks at Pitlochry. These fish were fed excess rations except for a period of 2 months when they were subjected to alternating weeks of fasting and the standard excess ration (Table 6). No fish could feed during the week when no food was provided, and therefore feeding and growth of all fish were curtailed during this time, irrespective of differences in density. The two monthly periods of food restriction were varied for each tank, and overlapped by one month from November 1987 to June 1988. One tank was kept as a control, and received an excess food supply throughout the duration of the experiment.

Tanks were cleaned routinely and treatment of fish for fungal and bacterial infections was applied to all tanks as required. Mortalities were noted and those from 16th June onwards were kept as maturing males could be determined from GSI's at this stage (See 4.3.2). Subsamples of 20 fish were removed from tanks before and after the 2 monthly period of food restriction and analysed for lipid content (See 4.3.3). Changes in density would not affect fish growth rates as food was supplied to excess or not at all. All fish were sacrificed in late August and sex, weight and gonad weight recorded.

TABLE 6. Design of the restricted feeding experiment (F indicates week of fasting during which respective populations were denied food).

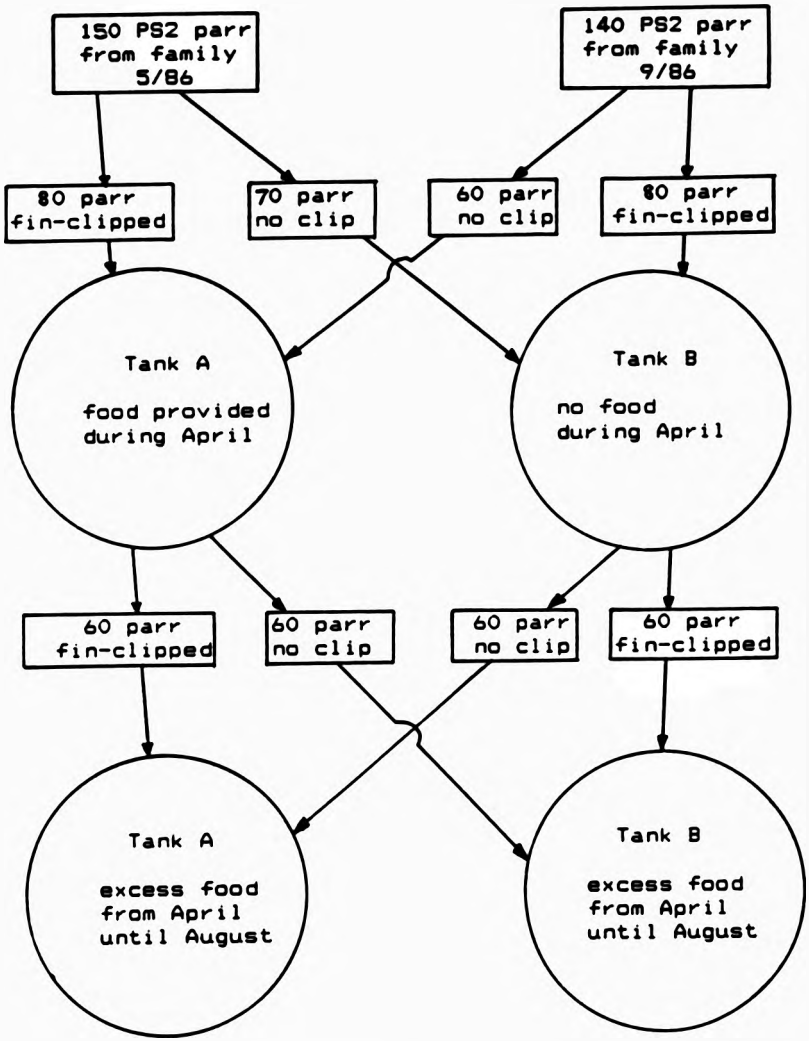
Month	Week No	Tank No							
		1	2	3	4	9	10	11	12
Nov	1								
	2		F						
	3								
Dec	4		F						
	5								
	6		F	F					
	7								
Jan	8		F	F					
	9								
	10			F	F				
	11								
Feb	12			F	F				
	13								
	14				F	F			
	15								
	16				F	F			
Mar	17								
	18					F	F		
	19								
	20					F	F		
Apr	21								
	22						F	F	
	23								
	24						F	F	
May	25								
	26							F	F
	27								
	28							F	F
	29								
Jun	30								F
	31								
	32								F
	33								

Maturation rates of experimental populations were compared with the control to determine the effect of different periods of growth restriction on the maturation process.

3.2.3 The April starvation experiment

In March 1988 approximately 150 PS2 parr, aged 0+ were removed at random from each of two different stock populations of Atlantic salmon parr (family 5/86 and 9/86). The 150 parr from each family were split into two groups, one of which was marked with an adipose fin clip (Fig. 22). The marked group from the 9/86 family were placed, together with the unmarked group from the 5/86 family, in a 2m diameter radial flow tank at the Almondbank hatchery. This tank received no food between 23 March and 5 May. The other two groups were placed in a separate tank and received food to excess. Lengths and weights of all fish were recorded on 23 March and 5 May, and condition factors and growth rates calculated for each group. After 5 May starved and fed fish were re-combined as families (Fig. 22) and placed in 2m radial flow tanks, where they were fed excess rations until the end of August. At this time all fish were killed and sorted into the groups that were starved and fed during April. Fish were weighed, measured, sexed and gonad weights recorded. Maturation rates of the male parr were calculated for each group and the significance of differences between starved and fed groups tested using Chi-square analysis.

Figure 22. Origin and treatment of fish in the April starvation experiment.



3.3 RESULTS

3.3.1 Effects of seasonal variations in optimal feeding

The increase in fish density, produced by the addition of 100 larger S1 parr to the experimental populations, reduced feeding rates of the PS2 parr (Fig. 23). Reductions were greater in July, than in August, but the difference in feeding rates between PS2 parr, at comparatively low and high densities, was significant ($p<0.05$) in both months.

The distributions of meal size for experimental PS2 parr were normal when these fish were at low densities, but were negatively skewed at high densities (Fig. 24). A few of the PS2 parr in the high density populations maintained feeding rates, at or above the mean value for parr at low densities. Consequently, suppression of growth was not achieved for all PS2 parr. Proportions of experimental fish, in the high density populations, with feeding rates above mean values for low density parr, were small, ranging from 10.5% in July to 15.4% in August. Suppression of feeding rates and hence growth was achieved in the majority of PS2 parr.

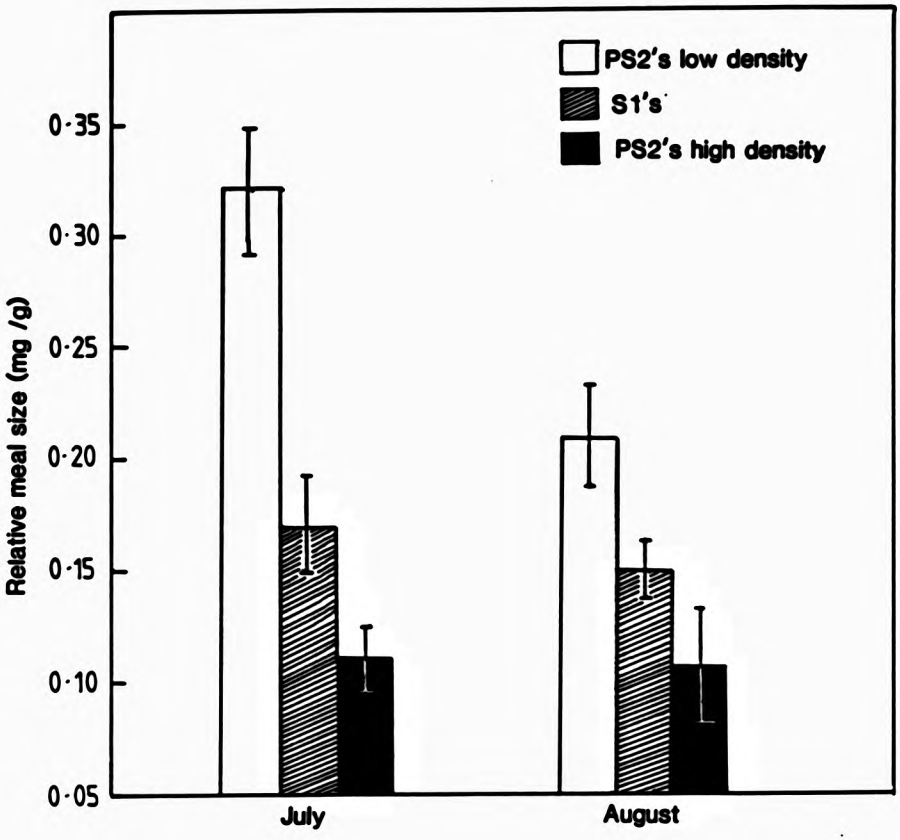


Fig. 23. Effect of fish density on mean meal size of PS2 parr. (Low density is 50 PS2 parr, high density is 50 PS2 parr plus 100 S1 parr. Differences between mean meal size of PS2 parr at high and low density were both significant at $p < 0.001$).

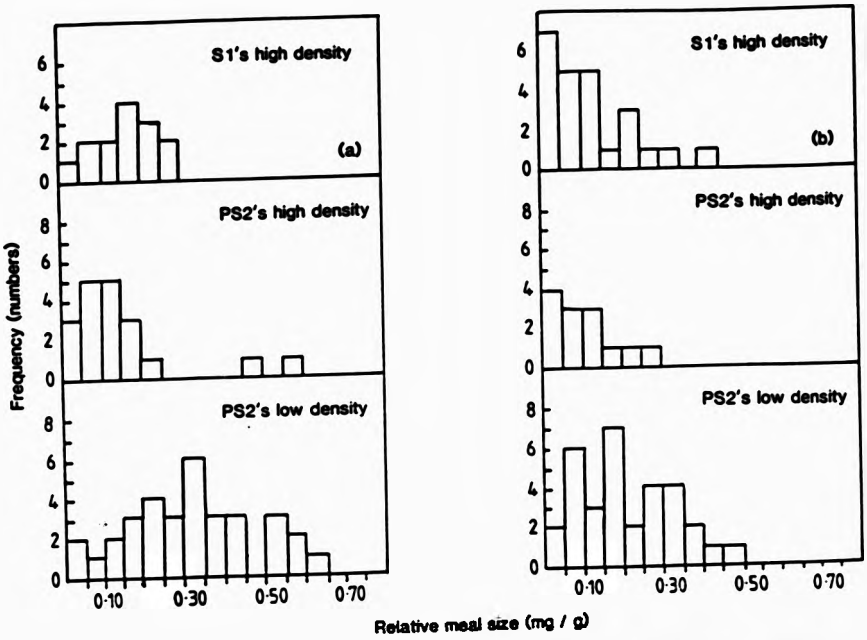


Fig. 24. Distribution of relative meal sizes for experimental (PS2) parr at high and low density stockings.(a) July, (b) August.

Effects of seasonal variations in feeding on growth rates

The seasonal differences in optimal feeding opportunity had no effect on mean size of fish by September (Fig. 25). There was a significant ($p<0.05$) difference in mean length between slow and fast-growing control populations (FL of 103mm versus 115mm), but mean lengths of fish in experimental populations differed from one another by less than 4mm.

In general, specific growth rates of PS2 parr increased as water temperatures increased, and were depressed by the addition of larger S1 parr (Figs. 26, 27). Specific growth rates of the fast-growing control population were generally higher than those of the slow-growing control population (Fig. 26A), the one exception occurring at the end of May, when bacterial infections affected some fish in several tanks, and are likely to have reduced their feeding and growth.

During the periods of reduced density, specific growth rates of the experimental fish populations were higher than those of both the slow and the fast-growing control populations (Figs. 26-29). In general these periods of relatively higher growth corresponded with the periods of reduced density (Table 7).

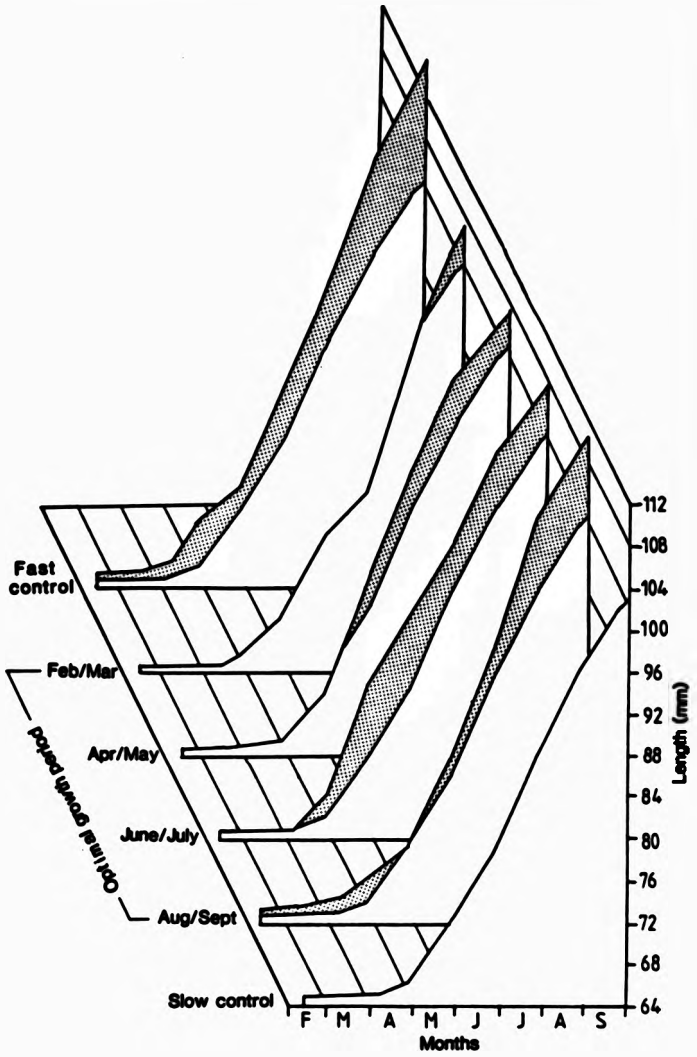


Fig. 25. Growth curves for experimental and control populations in the optimal feeding experiment. (Shaded areas indicate when mean lengths exceeded those of the slow-growing control population).

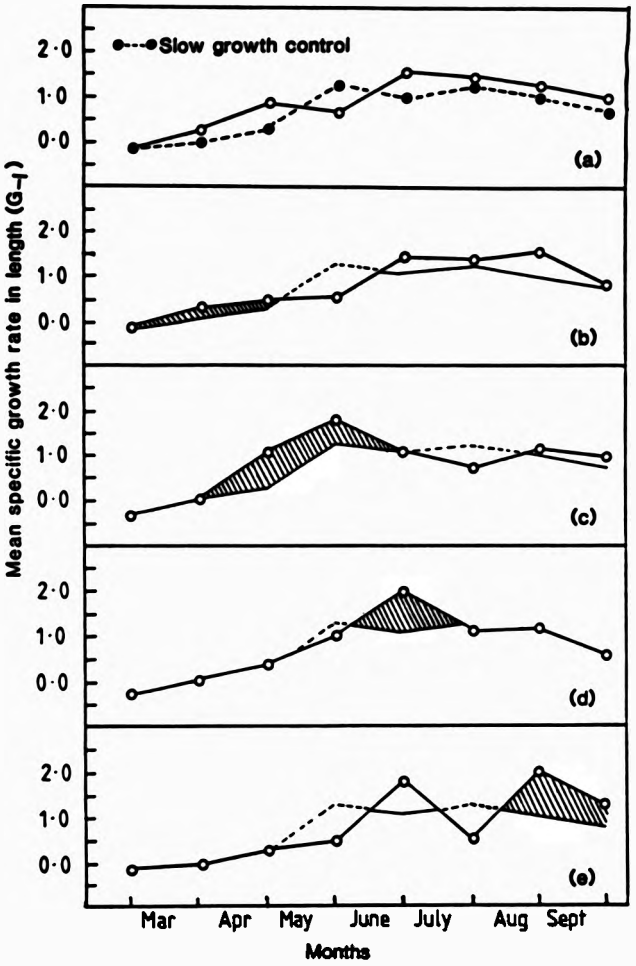


Fig. 26. Seasonal changes in mean specific growth rates in length for (a) fast-growing control population and experimental populations allowed optimal feeding during (b) Feb-Mar, (c) Apr-May, (d) Jun-Jul, (e) Aug-Sep 1987. (Shaded areas indicate when reduced density increased growth rates relative to slow-growing control population).

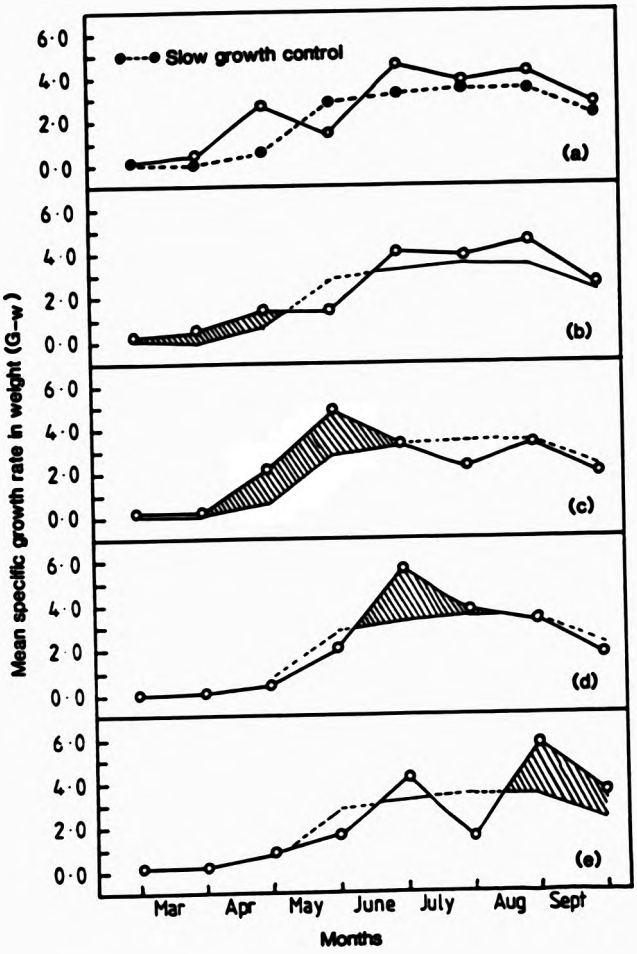


Fig. 27. Seasonal changes in mean specific growth rates in weight for (a) fast-growing control population and experimental populations allowed optimal feeding during (b) Feb-Mar, (c) Apr-May, (d) Jun-Jul, (e) Aug-Sep 1987. (Shaded areas indicate when reduced density increased growth rates relative to slow-growing control population).

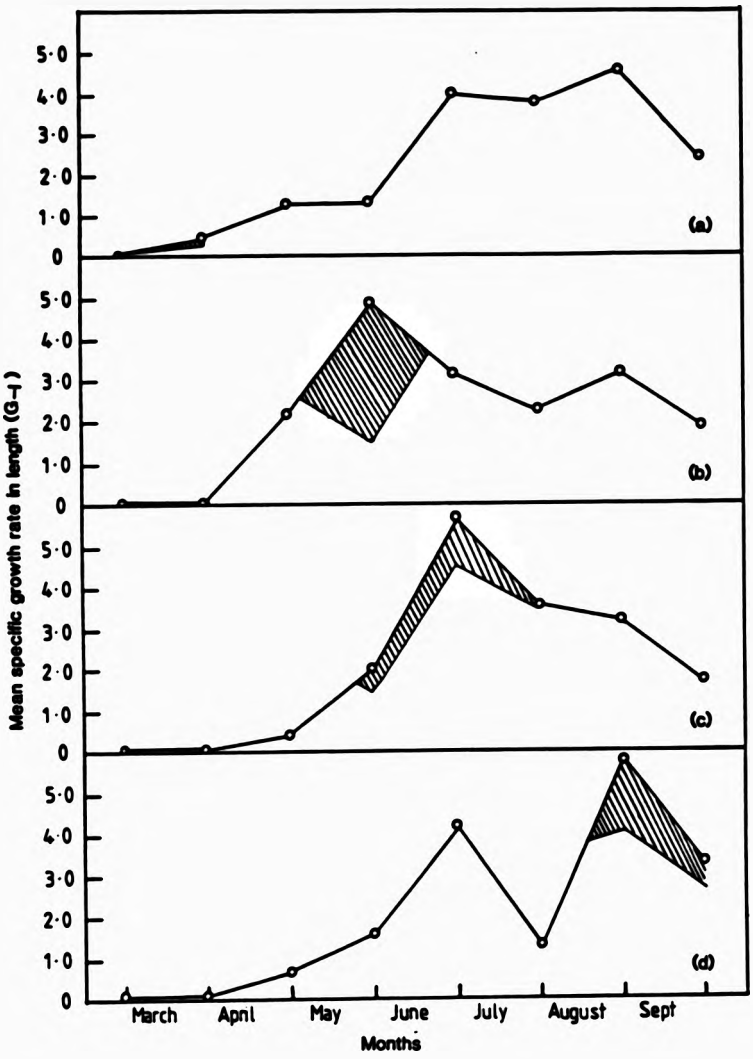


Fig. 28. Periods when specific growth rates in length of experimental populations exceeded values for fast-growing control population (shaded areas). Experimental populations allowed optimal feeding during (a) Feb-Mar, (b) Apr-May, (c) Jun-Jul, (d) Aug-Sep 1987.

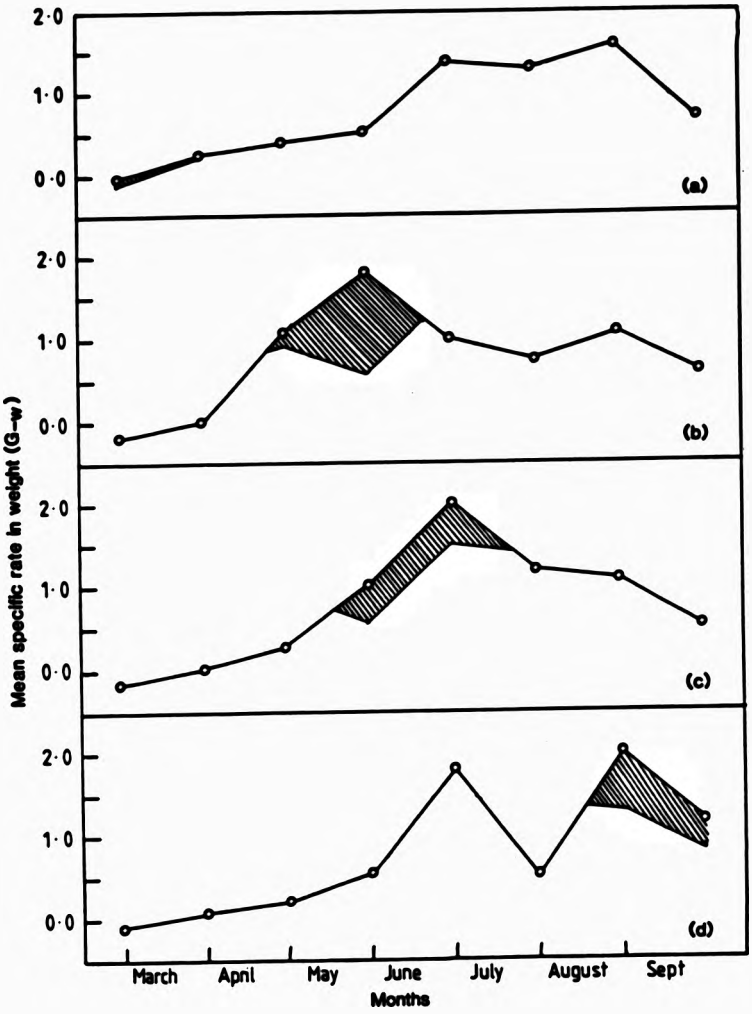


Fig. 29. Periods when specific growth rates in weight of experimental populations exceeded values for fast-growing control population (shaded areas). Experimental populations allowed optimal feeding in (a) Feb-Mar, (b) Apr-May, (c) Jun-Jul, (d) Aug-Sep 1987.

TABLE 7. Corresspondence between times of optimal feeding and months when mean specific growth rates were above those of the slow-growing control population.

Period of optimal feeding opportunity	Months when mean G_1 and G_w exceeded control values
February and March	March and April
April and May	April and May
June and July	June
August and September	August and September

The faster specific growth rates compared with the fast-growing control population are surprising as fish densities and food supply were the same. Faster than expected growth following periods of suppressed growth, could represent a compensation response to growth suppression. This phenomenon may also explain anomalously high specific growth rates that occurred on other occasions in the experimental populations. In June and August, mean specific growth rates for two of the populations whose growth was supposed to be suppressed at this time, exceeded values recorded for the slow-growing control (Fig 26B & E, Fig. 27B & E). This difference in specific growth rates occurred despite the fact that fish densities and food supply were the same. Such periods of anomalously faster growth all occurred the month after bacterial infections depressed feeding and resulted in mortalities in these tanks (Table 8). The time-lagged correlation between bacterial infections and faster specific growth rates indicates that compensation growth in affected fish raised the mean specific growth rates of experimental populations in June and August.

Differences in maturation rates between populations

Murphy (1980) found that mature male parr were more susceptible to fungal diseases than immature males or females. Selective mortality of maturing males would

TABLE 8. Mortalities per month among the populationsof salmon parr in the optimal feeding experiment.

Month	Sex	Period of optimal feeding for the respective populations					
		Nil slow control	Feb-Mar	Apr-May	Jun-Jul	Aug-Sep	Feb-Sep fast control
April	M	0	2	2	7	0	1
	F	2	3	3	3	3	1
May	M	1	2	0	3	6	4
	F	1	7	0	0	2	4
Jun	M	0	0	0	0	2	0
	F	0	0	0	0	0	0
Jul	M	1	5	1	1	4	5
	F	1	6	0	1	10	5
Aug	M	0	0	1	0	0	0
	F	0	0	0	0	1	0
Total	M	2	9	4	11	12	10
	F	4	16	3	4	16	7

depress maturation rates estimated from surviving male fish, therefore both sex and maturation status of all mortalities was recorded. Males with GSI's over 0.05% were deemed to be maturing, as this was the upper limit for the GSI of non-maturing males (see Section 4.2.2). There was no evidence that males suffered a greater mortality rate than females (Table 7). Total numbers of males recovered from each population ranged from 20-28 (Table 9), which is close to the expected number of 25, based on a 1:1 sex ratio established for PS2 parr in this stock (Table 1).

Maturation rates between the fast- and slow-growing control populations differed, and rates for the experimental populations varied (Fig. 30). The value of chi-squared, used to test differences between proportions of mature males in the six populations was 11.00 (5 degrees of freedom), which is close to the significant value of chi-square (11.07) at p= 0.05. Populations allowed optimal feeding during April/May, and June/July, had high maturation rates, comparable with that of the fast-growing control population. Populations allowed optimal growth during February/March, and August/ September had low maturation rates, comparable with that of the slow-growing control.

Differences in mean gonad size between populations

Mean GSIs (Fig. 31) of mature males in the experimental and control populations were generally lower (5-7%) than

TABLE 9. Summary statistics for male parr in the optimal feeding experiment.

Statistic	Optimal feeding period for each population					
	Nil slow control	Feb-Mar	Apr-May	Jun-Jul	Aug-Sep	fast control
No. males surviving	27	17	27	19	17	15
No. these mature	14	7	21	14	9	12
No. mortalities	1	6	0	1	11	5
No. these mature	0	3	0	0	4	2
Total males	28	23	27	20	28	20
Total mature	14	10	21	14	13	14
% males in population	44.0	67.0	57.7	45.0	66.0	63.0

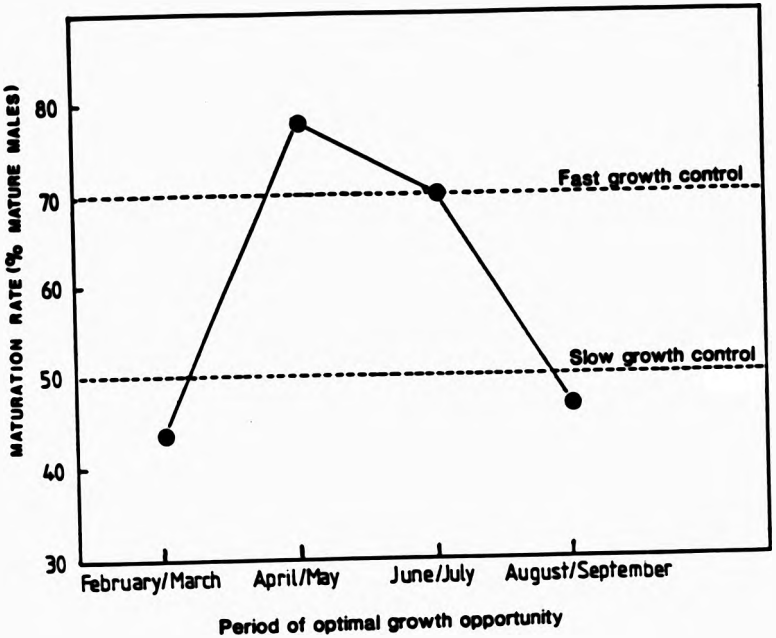


Fig. 30. Differences in maturation rate between control and experimental populations in the optimal feeding experiment.

expected from this stock (see section 4.3.2). This difference probably reflects their generally lower rations compared with the stock population. Even so, the mean GSI's of the experimental populations, subjected to optimal growth in February/March and August/September, were significantly lower ($p<0.01$) than the GSI's of the other populations, including fast and slow-growing controls.

There was no difference in mean gonad weight between populations, except for the fish subjected to optimal growth in August/September. The mean gonad weight for mature males in this population was significantly lower ($p< 0.05$), and thus the lower mean GSI for this population was due to their relatively smaller gonads. However, this was not the case for the population subject to optimal growth in February/March. The lower mean GSI of males in this population was due to their proportionately greater weight of somatic tissue.

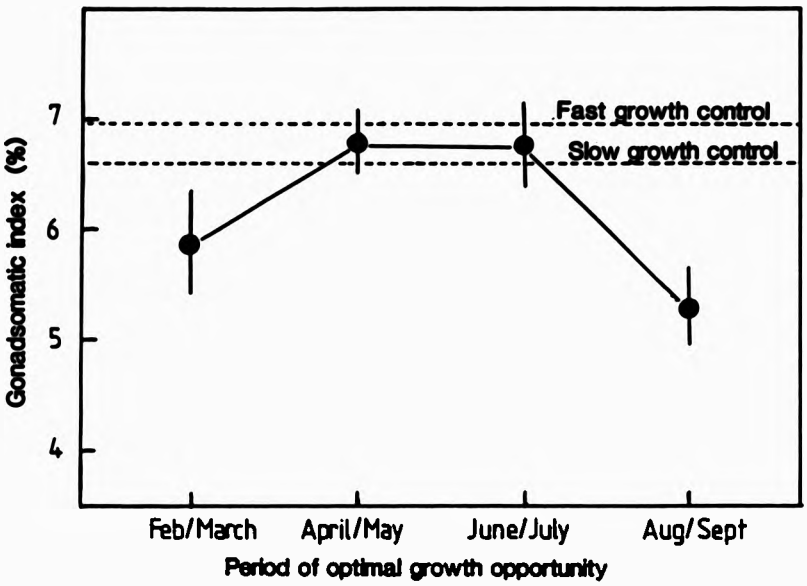


Fig. 31. Differences in mean (± S.E.) GSI of mature males in control and experimental populations of the optimal feeding experiment.

3.3.2 Effects of seasonal variations in food restriction

Gonad resorption and recrudescence in fish mature at 0+

Examination of sub-samples of fish taken for lipid analysis in November 1987 (See Chapter 4) revealed that some male parr were already mature. Gonads were relatively small (average GSI was 0.95%), but were nevertheless larger than those of immature males (0.07%). They were also ripe; a small amount of milt could be expressed by applying gentle pressure to the abdomen. These 0+ mature males were larger than immature ones, and constituted approximately 30% of the male parr population. Changes in mean size and GSI of the two groups were recorded from November until July to determine the pattern of gonadal resorption and recrudescence, and differences in gonadal growth between the two groups (Fig. 32).

Mean GSIs of parr maturing first at 0+ did not change between November and December, and resorption did not occur until after January. GSI's then decreased from just over 0.4%, to 0.25% by the end of April. In May GSI's began to increase again, and by July were 1.25%. In comparison, the mean GSI of the males which matured first at age 1+ did not change between November and May. During June, however, significant gonad growth occurred, with GSI's increasing to 1.5% by the beginning of July.

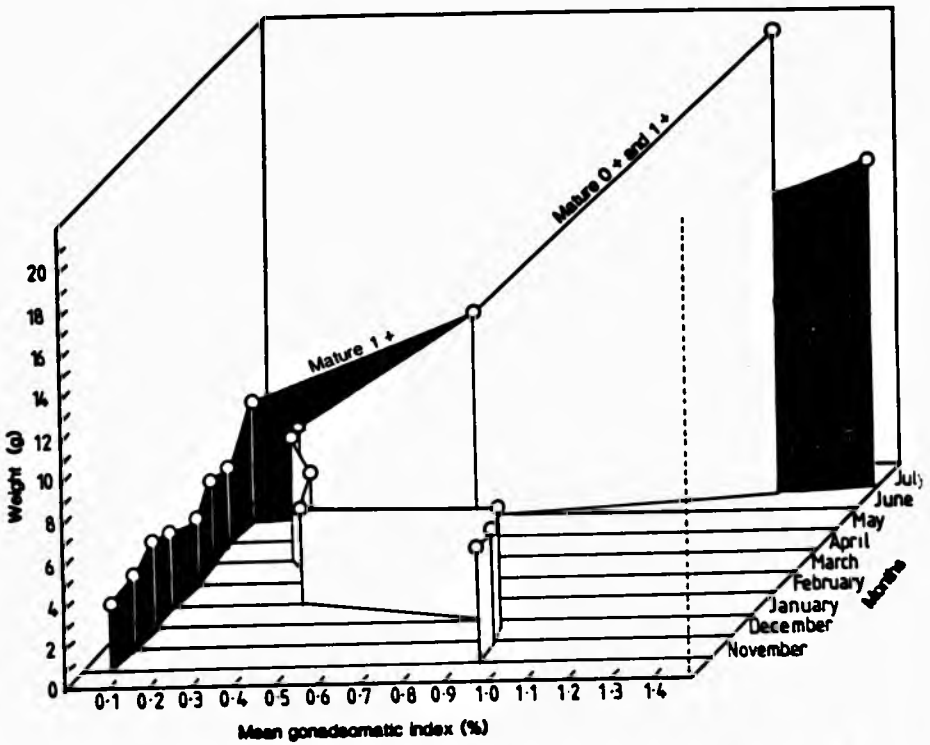


Fig. 32. Gonadal resorption and recrudescence of male parr maturing at age 0+, compared with gonadal growth of parr maturing first at age 1+, during 1988.

During June, growth in weight of the larger males, which had matured first at 0+, was faster than that of their smaller later maturing siblings. But their rate of increase in mean GSI was slower. The differences in growth rate, and the positive but curvilinear relationship between gonad weight and fish weight in July (Fig. 33), indicate that this discrepancy between increases in somatic growth and GSI, was due to faster gonad growth during June in the smaller, later maturing males, rather than to greater somatic growth of the larger, 0+ maturing males.

Effects of seasonal food restriction on growth rates

Not surprisingly, food restriction in winter months (December, January, February, March), when water temperatures are below 4°C and growth minimal, had little effect on size of female fish. However, food restriction during some winter months (January, February, March) did increase subsequent growth of male fish (Fig.34).

Water temperatures at Pitlochry were below 4°C during February and March, and weights of PS2 parr decrease under such conditions, despite an excess of food (See Figs. 10 and 11). Food restriction at this time can only have exacerbated weight loss and reduced condition. The larger

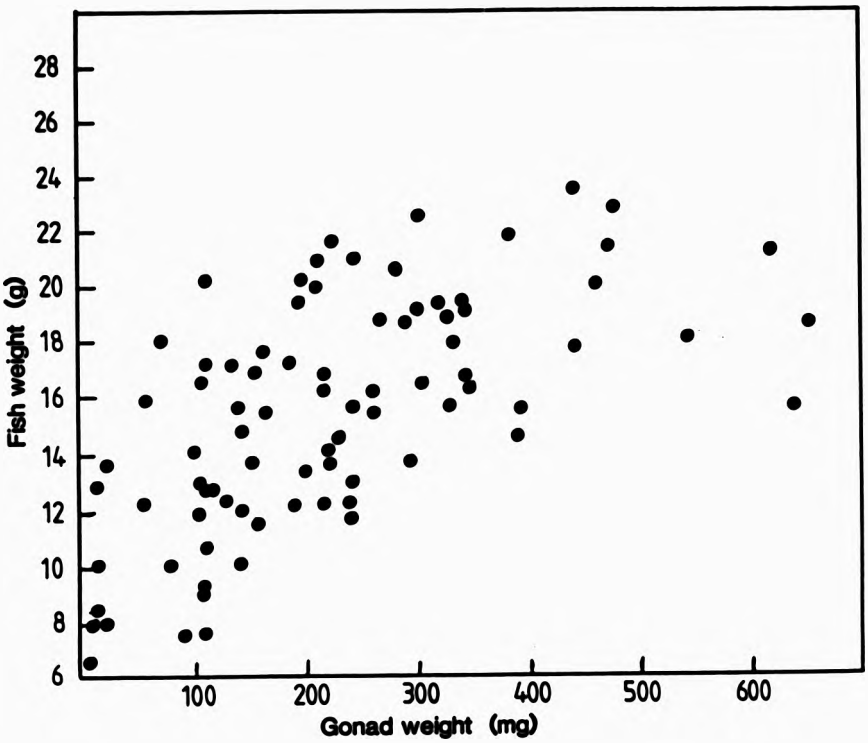


Fig. 33. relationship between gonad weight and fish weight for mature male parr.

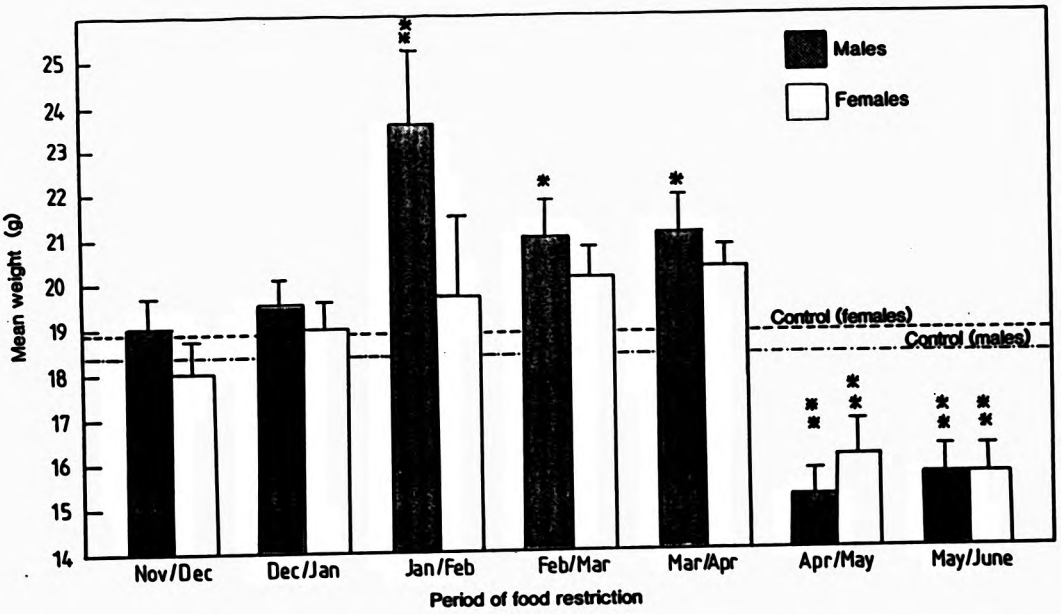


Fig. 34. Effects of differing periods of restricted feeding during 1988 on mean size of 1+ salmon parr by mid-August. (Asterisks indicate significant differences to control, *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

July size, of the males, whose food was restricted during late winter months, is therefore likely to be due to some stimulatory effect on subsequent growth rates, associated with weight loss in late winter.

Food restriction during spring and summer months (April, May, June), when water temperatures were increasing (Fig. 2) and faster growth is possible, reduced the mean size of both male and female parr by July (Fig. 34).

Differences in maturation rates between populations

There were twice as many mortalities in tanks 2 and 4 as in the other tanks (Table 10), and the numbers of maturing males may have been reduced, so biasing estimates of maturation rates. This possibility was checked by examining sex ratios of surviving fish. Selective mortality of maturing males would be expected to bias sex ratios towards females, but there was no significant difference between the sex ratios of surviving fish in the eight tank populations ($p > 0.10$). Sex ratios were all close to the expected population value of 1:1. Selective mortality of male fish can therefore be discounted.

The maturation rate of the control population was 92.5%, and there was no statistically significant difference between this and the maturation rates for populations

TABLE 10. Summary statistics for populations of parr in the restricted feeding experiment (♦ No. includes mortalities occurring after 16th June).

Tank No.	Restriction period	Mortality rate (%)	Sex ratio (% males)	Males (Nos.)
1	Nil (Control)	17.3	53.5	53
2	Nov-Dec	40.8	51.4	51♦
3	Dec-Jan	17.4	50.4	58
4	Jan-Feb	53.4	49.2	46♦
9	Feb-Mar	25.7	56.2	59
10	Mar-Apr	21.5	48.6	54
11	Apr-May	24.8	56.7	59
12	May-Jun	33.7	56.5	72♦

experiencing food restriction during the months November to March (Fig. 35). Because of the results of the optimal growth experiment, populations experiencing food restriction between April and June were expected, *a priori*, to have low maturation rates. The value of Chi-square, used to test the significance of the difference between maturation rates, was significant ($p < 0.005$). Maturation rates of populations experiencing food restriction during late winter months were generally higher than that for the control population, but the differences were not statistically significant.

Effects of seasonal food restriction on mean gonad size

Mean GSI's of male fish were positively correlated with fish size in all populations, significantly so for populations whose food was restricted during December/January, March/April, and April/May (Table 11). In contrast, GSI's of female fish were negatively correlated with fish size in all populations except the one subject to food restriction in February/March (Table 12).

Food restriction suppressed GSI's of males in a systematic fashion, with least effect occurring during winter months, when growth in body size is negligible, and greatest effect during late autumn and spring months, when water temperatures were somewhat higher (Fig. 36). GSI's of

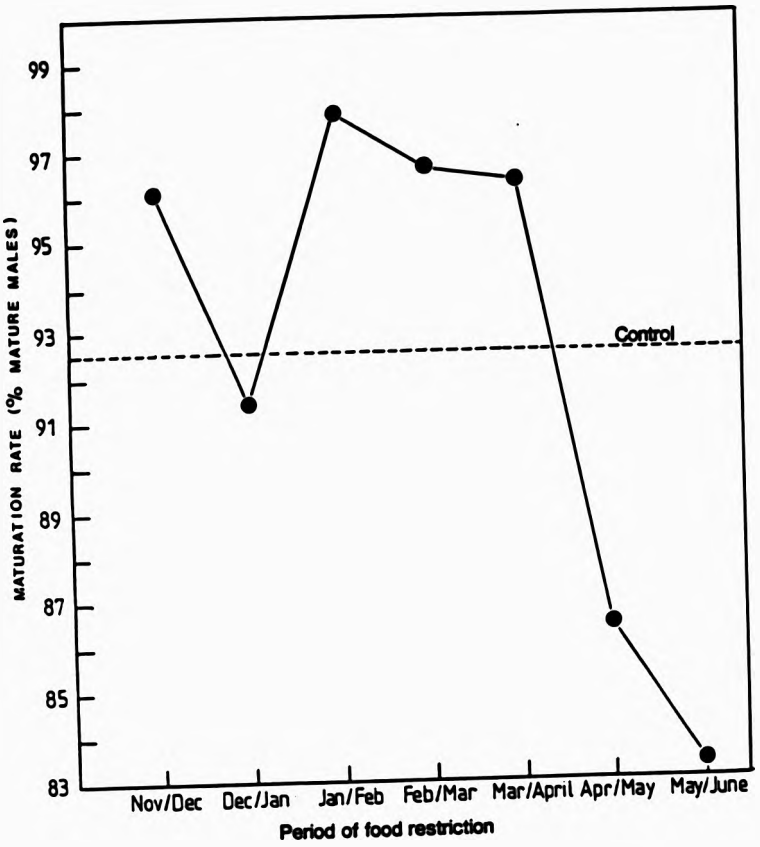


Fig. 35. Effect of differing periods of food restriction during 1988 on maturation rates of male parr.

TABLE 11. Correlations between GSI and fish weight for mature male parr in the restricted feeding experiment (Significance levels are *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

Tank No.	Restriction period	No. of fish	Departure from normality*	Correlation coefficient (r)
1	Nil (Control)	36	0.97	0.13
2	Nov-Dec	23	0.96	0.18
3	Dec-Jan	38	0.95*	0.51 ***
4	Jan-Feb	9	0.96	0.31
9	Feb-Mar	37	0.96	0.26
10	Mar-Apr	40	0.97	0.31 **
11	Apr-May	39	0.98	0.57 ***
12	May-Jun	39	0.97	0.01

* modified Shapiro-Wilk statistic (Lee et al. 1986).

TABLE 12. Regressions of GSI on fish weight for female parr in the restricted feeding experiment (Significance levels are: *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

Tank No.	Restriction period	No. of fish	Departure from normality♦	Correlation coefficient (s)	Regression parameters intercept (a)	slope (b)
1	Nil (control)	32	0.96	-0.55 ***	0.52	- 7.37
2	Nov-Dec	22	0.94	-0.72 ***	0.51	- 8.79
3	Dec-Jan	42	0.98	-0.60 ***	0.47	- 5.63
4	Jan-Feb	7	0.94	-0.75 **	0.44	- 5.58
9	Feb-Mar	35	0.97	-0.08	0.35	- 7.01
10	Mar-Apr	43	0.97	-0.43 ***	0.41	- 4.11
11	Apr-May	35	0.97	-0.74 ***	0.55	-10.25
12	May-Jun	40	0.97	-0.46 ***	0.50	- 6.56

♦ modified Shapiro-Wilk statistic (Lee et al 1986).

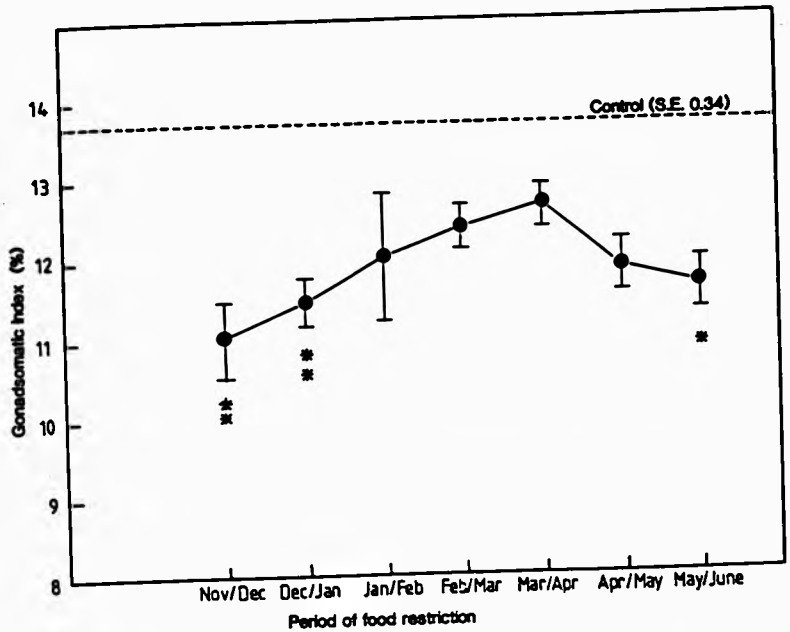


Fig. 36. Effects of differing periods of food restriction on mean (± S.E.) GSI of mature male parr by mid-August. Significance of differences between means determined by ANOVA.

female fish were only suppressed in populations whose food was restricted in mid winter (January to March) (Fig. 37). However, they were increased in the population whose food and growth was restricted in May and June.

3.3.3 Effects of starvation during April

Sex ratios for the two families differed, but were the same for fed and starved groups within each family (Table 13). Mortality rates in starved groups were higher than in fed groups, but males and females were equally affected.

Feeding during April increased condition factor and starvation decreased it (Fig. 38). Mean lengths of starved fish did not change during April (Fig. 39), and fish deprived of food lost weight. Conversely when food was available, size increased. The increases in condition factor indicate that feeding fish gained weight faster than length. The decline in condition factor in starved fish indicates weight loss. It is apparent that changes in condition factor during April are due primarily to increases or decreases in weight, and to the extent of feeding at this time.

Maturation rates of fed groups in both families were high and close to 90%. Starvation during April reduced these by 19% and 29% respectively (Fig. 40). Differences were tested using Chi-square analysis and were significant ($p < 0.025$) for the 5/86 stock.

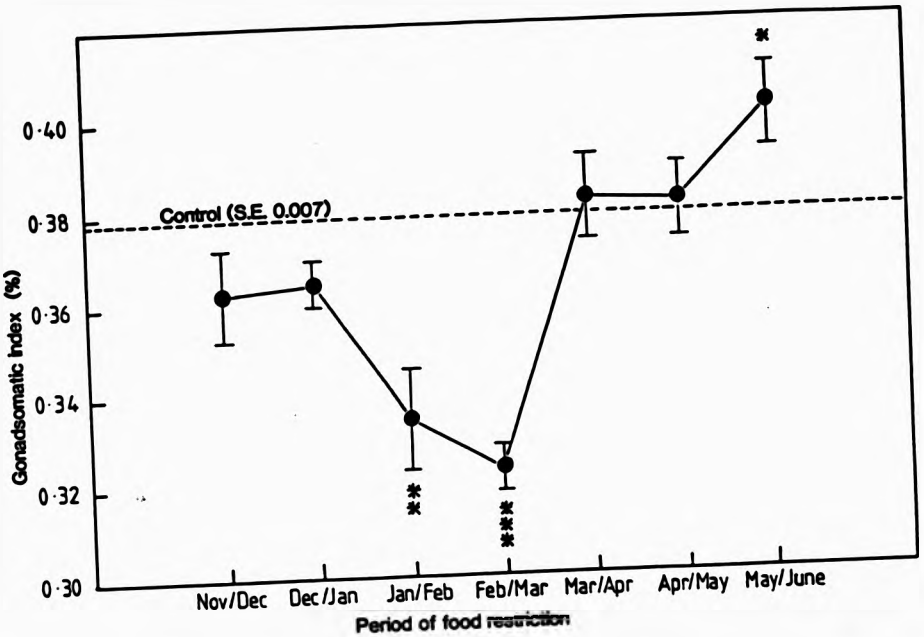


Fig. 37. Effects of differing periods of food restriction on mean (± S.E.) GSI of female salmon parr by mid-August. Significance of differences between means determined by ANOVA.

TABLE 13. Summary statistics for the populations in the April starvation experiment.

Family	Treatment	No. of fish	Sex ratio (% Males)	Mortality (%)
9/86	Fed	54	44.4	10.0
9/86	Starved	61	44.3	23.8
5/86	Fed	74	54.1	7.5
5/86	Starved	55	56.4	21.4

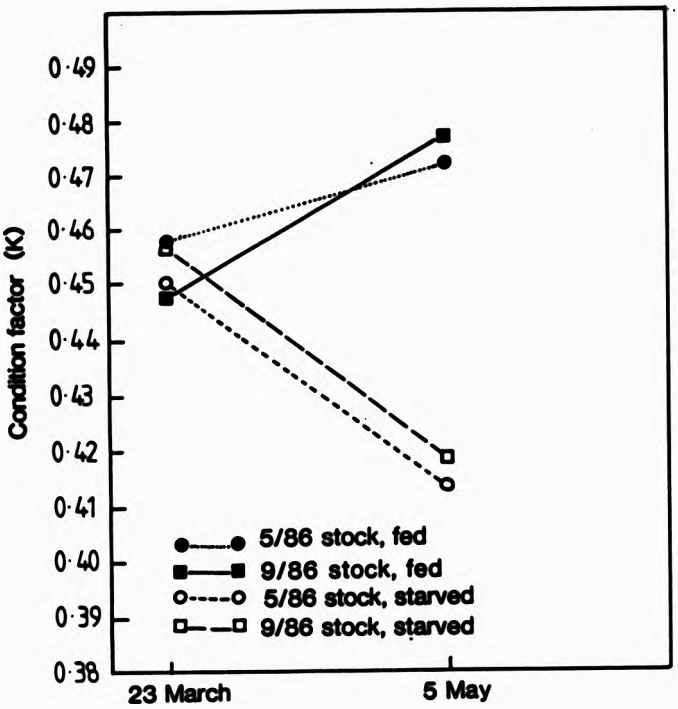


Fig. 38. Changes in condition factor (K) for starved and fed groups of two stocks of 1+ salmon parr during April 1988.

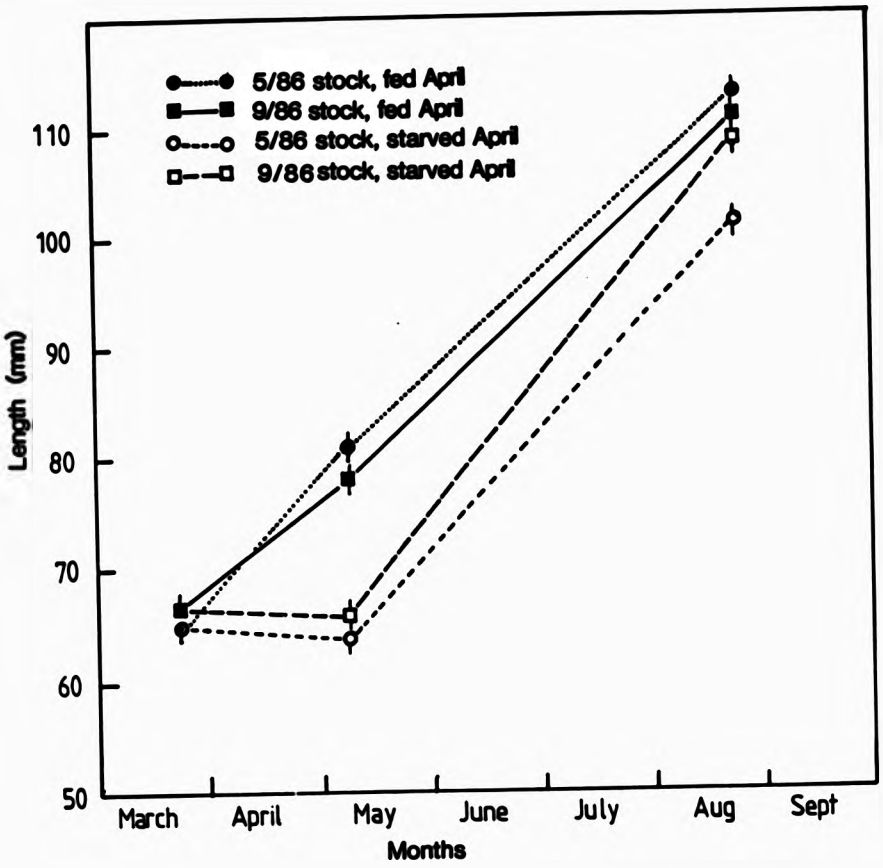


Fig. 39. Effects of starvation during April 1988 on growth of 1+ salmon parr and mean size by mid-August.

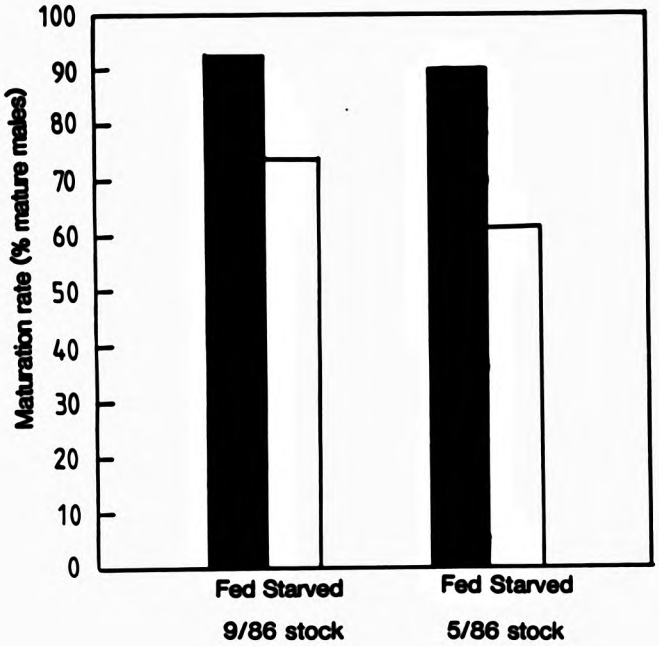


Fig. 40. Effect of starvation during April on maturation rates of 1+ male parr in 2 stocks of salmon parr.

3.4 CONCLUSIONS AND DISCUSSION

The maturation rate of the 1+ male parr in the optimal feeding experiment was significantly higher in the low density, fast-growing, control population, than in the high density, slow-growing, control population. Furthermore, maturation rates were increased relative to the slow-growing control population when growth during spring months was increased. They were decreased, relative to the control population, by restricting growth during these months. The results show that differences in feeding opportunity and growth during spring to early summer are responsible for the phenotypic changes in the expression of the early maturation trait in Atlantic salmon parr. It follows that increased daylengths, elevated water temperatures, decreases in density and relative increases in ration have all increased maturation rates of Atlantic salmon parr, through their positive effects on feeding and growth.

The maximum difference in maturation rates obtained within the sibling stock of Atlantic salmon (77.5% versus 43.0%) indicates that environmental factors affecting feeding and growth can have just as significant an effect on maturation rates as genetic differences between families. The fact that improved feeding and growth have also resulted in differences in maturation rates for brook trout (Salvelinus fontinalis), rainbow trout (Salmo

gairdneri), and sockeye salmon (Oncorhynchus nerka) (Kato 1978; McCormick & Naiman 1984; Skarphedinsson et al. 1985), indicates that this phenotypic plasticity is widespread in the Salmonidae.

For the four experimental populations in the optimal feeding experiment, the highest maturation rate occurred in the population allowed optimum feeding opportunity, and which had comparatively high specific growth rates, in April and May. However, comparably high maturation rates also occurred in the fast growing control population, and in the experimental population given optimum feeding opportunity from June to July. The two former populations were characterised by comparatively high specific growth rates during April, but the latter population had a low specific growth rate at this time. Its growth rates were high in June when those of the former populations were low. It is apparent that while some aspect of feeding and growth during April to May is correlated with maturation, specific growth rates are not. The hypothesis that increases in growth rate during spring months result in increased maturation rates must therefore be rejected.

This finding concurs with that obtained in the study on individual growth rates (Chapter 2). There, spring increases in condition factors, rather than specific growth rates, were correlated with maturation. When mean

monthly condition factors for the respective populations in the optimal feeding experiment were examined the populations with high maturation rates were all distinguished by greater increases in condition factor during April (Fig. 41). Fish put on more weight for length when provided with good growing conditions in April, and this fattening effect is greatest in male parr which mature. It is also apparent that the growth of many male parr in the experimental population subjected to optimal feeding conditions from May to June, was not completely suppressed during April.

The timing of the period of optimal feeding and growth is important, and failure to consider this has limited the interpretation of many studies of growth and maturation of male parr (eg. Naevdal et al. 1978b; Riley & Power 1987; Saunders & Henderson 1988).

In the optimal feeding experiment, the maturation rates of all populations, whose feeding and growth were restricted during the period April to July, were suppressed. Similar results occurred in the experiment which restricted food intake of fish on a seasonal basis, while maintaining fish densities constant. These results show that, while increases in feeding and growth during April increase maturation rates, suppression occurs when feeding opportunity is reduced between April and June. Starvation of fish during April significantly reduced maturation

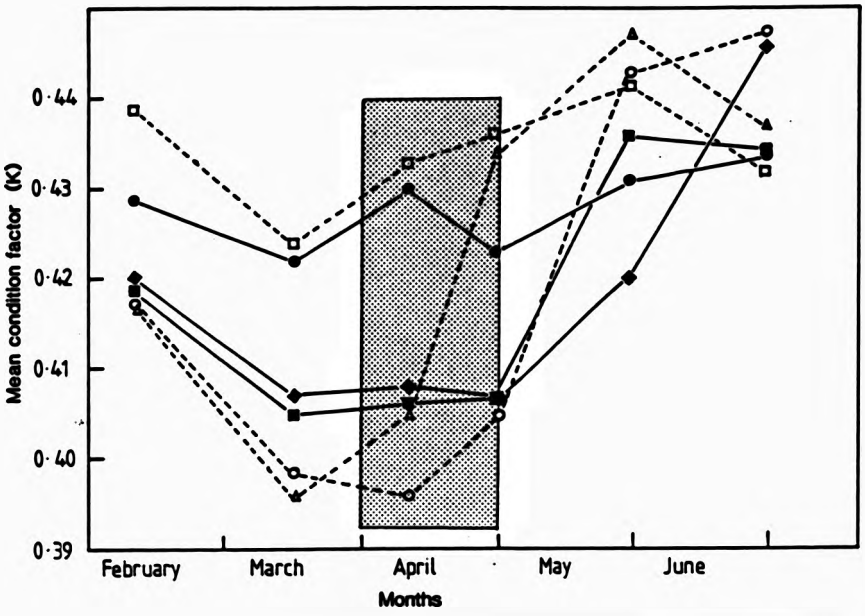


Fig. 41. Changes in mean condition factor during April 1987 for experimental and control populations of salmon parr in the optimal feeding experiment. Populations allowed optimal growth during Feb-Mar (●—●), Apr-May (Δ---Δ), Jun-Jul (o---o), Aug-Sep (◆—◆). Fast-growing control (□---□), slow-growing control (■—■).

rates of Atlantic salmon male parr, indicating that the absence of feeding opportunity during this month has a significant effect. However, the fact that maturation rates were reduced, rather than completely suppressed, indicates that feeding opportunity in other months is also likely to be important in the control of maturation.

The finding, that maturation rates can be increased by enhancing feeding during April, and decreased by restricting feeding between April and June, indicates the existence of a time-specific control mechanism for maturation, linked to feeding opportunity in spring months.

The existence of a specific season or "time window" during which feeding and growth may influence the control of maturation has also been suggested in rainbow trout. Burger (1985), cited by Herbinger (1987), began experiments with rainbow trout populations on different feeding regimes in July. These produced differences in mean size between populations by December, but there were no differences in maturation rates. When the experiments were repeated, but starting in April, differences in both size and maturation rate occurred, the faster growing populations having higher maturation rates. Rainbow trout generally spawn in late winter through spring, whereas Atlantic salmon spawn in late autumn through winter.

Despite the differences in time of spawning between the two species, the seasonal "window", during which feeding opportunity influences the control of maturation, is apparently the same.

Although it is apparent that feeding and growth in April and May are important for maturation, growth restriction during late winter months may also affect the control of maturation, by stimulating growth in spring. Alternate weeks of fasting during February to March resulted in a subsequent improvement in the growth of male fish, and higher, though not statistically significant maturation rates. This pattern of growth also occurred in the individually identified male fish. The maturing males all showed a greater decline in condition factor compared with non-maturing males until March, followed by better growth in weight for length during April. Abdullah (1981) concluded that stress and handling, and growth restriction of Atlantic salmon in winter months increased maturation rates. This may be due to a growth rebound effect in spring, as is likely to have occurred here.

While variations in seasonal growth patterns affected maturation rates, they also influenced the gonadosomatic indices of females and maturing males. In the optimal feeding experiment, mean GSI's were significantly depressed in the populations whose growth was restricted during April to July, but not in the slow-growing control

population whose growth was suppressed in all months. In the restricted feeding experiment GSI's were suppressed in all populations experiencing periods of fasting, with this effect being least in midwinter, and greater in autumn than in spring months. These results may reflect differences in the timing of gonad growth, but this is unlikely as this process has been shown to be under photoperiod control in Atlantic salmon parr (Lundqvist 1983), and all populations experienced the same natural photoperiod.

The comparatively high mean GSI of male fish in the slow growing control population for the optimal feeding experiment is difficult to explain if restrictions in ration reduce gonad size. Fish in this population were, as expected, smaller than in all other populations (Fig. 25). However, the high GSIs were not due to proportionately larger gonads. Gonad weights were significantly lower (0.987g, S.D.=0.05) than in the fast growing control population (1.125g, S.D.=0.09), but no different to the gonad weights of males in the experimental populations. Proportionately less somatic growth than gonad growth occurred in male fish in the slow-growing population, and was responsible for their comparatively high GSIs.

The mean GSI of the immature females was also influenced by seasonal variations in food restriction; fasting

between January to March resulting in lower mean GSIs, and between May and June in an increased mean GSI. Greatest suppression occurred in mid winter. Effects of seasonal variations in food restriction on female GSI tended to be the reverse of the changes in mature males. No histological data were collected and gonad size may not reflect developmental stage. However, Sutterlin & MacLean (1984) found no difference in GSI of immature female salmon aged 16.5 to 23.5 months, despite large variations in the abundance and size of oocytes. Gonad growth thus appears to be under different control to oocyte recruitment. Jones & Bromage (1987) found that ration had a significant effect on fecundity of female rainbow trout, above that due to fish size. It had no effect on egg size and therefore influenced egg number. In female Atlantic salmon parr changes in ration during midwinter and summer can respectively reduce and increase relative gonad size, but changes in autumn or spring have no effect. It is probable that changes in fecundity of female salmonids related to rations will be significantly affected by the seasonal timing of the rationing.

CHAPTER 4

The role of fat stores in maturation

4.1 INTRODUCTION

A number of studies have suggested that the size of lipid stores is associated with the initiation of maturation in salmonids. Calow (1981) showed that as fish size increases, the energetic cost of food gathering increases at a faster rate than the energetic cost of maintenance and growth. He argued on theoretical grounds that fish should reproduce when their net energy gain is greatest (ie. at an optimized body size). Fat content is correlated with fish size in many fish (Shu'lman 1974) and the minimum size reported before maturation in many species may reflect an optimal energy content. However, the optimal size model does not account for seasonal differences in the energetic cost of spawning. For example, fish spawning at a time or place, which coincides with optimum feeding conditions, do not require as great a fat store as fish which spawn in winter, or well away from feeding grounds. The latter species require a seasonal cycle of fat storage (Townsend & Calow 1981).

Shu'lman (1974), in an extensive review of the literature on this topic, found evidence to support the concept of a minimum fat content before maturation is initiated in a

number of species. Reshetnikov *et al.* (1970) found that the onset of maturation in whitefish (*Coregonus lavaretus*) was connected with the attainment of a definite level of fat reserves, specific to each population, but did not indicate how or when this level was assessed. Similarly, Barbour (1984) measured seasonal energy contents of Arctic charr and hypothesized that the onset of maturation was triggered by the amount of energy they *will* have at their disposal during maturation. Both studies imply the genetic success of individuals that had high, or increasing fat stores at an appropriate time and the failure of those that didn't. They also imply that there is a mechanism linking the control of maturation with growth in fat reserves at the time maturation is initiated.

Thurow (1966, cited by Saunders & Henderson 1976) proposed that Atlantic salmon, returning from the sea, needed a fat content of 12% in spring if they were likely to spawn during the following autumn. Maturation in male parr of Atlantic salmon is likely to be subject to similar energetic considerations, and Myers *et al.* (1986) suggested that the minimum size required before male parr will mature, may reflect the acquisition of sufficient energy stores for both maturation and overwinter survival. Thorpe (1986) hypothesized that maturation would be initiated in fish whose rate of acquisition of fat was above a genetically determined level in spring.

The studies described in Chapters 2 and 3 have shown that the Atlantic salmon parr which mature in autumn have greater increases in condition factor during April than non-maturing parr. Furthermore, maturation rates were correlated with feeding opportunity in this and the next two months, but not with size, or with specific growth rates. As condition factor is correlated with fat content in Atlantic salmon parr during spring months (Pinder & Eales (1969), it is apparent that growth in fat reserves, rather than growth in body size, is linked with the control of maturation.

This study therefore tested the hypothesis that fat contents increase more quickly in maturing males than non-maturing males during spring months, and are higher than those of non-maturing males before the onset of maturation, indicated by increases in gonadosomatic index (GSI).

A role for fat levels in the control of maturation implies that fat stores are depleted during maturation, and that this depletion compromises gonad size, or, in iteroparous species, lifetime reproductive fitness through an increased risk of mortality. Idler & Bitners (1960) estimated that only 0.5% of fat reserves is transferred to gonads in male salmon, compared to 8% in females. The energetic cost of differentiating gonad material is

therefore lower in male fish. Love (1970) stated that larger fish lost more lipid than smaller fish during spawning, because of their greater metabolic needs, and had to lay down greater reserves than smaller fish. By analogy, he indicated that female fish would lay down more than males. If so, growth in fat reserves may be relatively unimportant to gonad growth in male fish. However, a number of studies have indicated that lipids are depleted in Atlantic salmon of both sexes during the spawning season (Lovern 1942, Love 1970, Saunders & Sreedharan 1977). Lovern (1942) speculated that the depletion in males was due to the upstream migration and their repeated fighting with each other as they ascended the river. However, Love (1980) stated that maturing male salmon in captivity stop feeding for eight weeks prior to spawning and for several weeks after, while females continue to feed. A number of studies have indicated that growth rates of maturing male parr also decrease during maturation (Leyzerovich 1973; Murphy 1980; Dalley et al. 1983; Gjerde 1984; this study section 2.4). This decrease in growth could be due to either allocation of resources away from somatic growth, to gonad growth, or to reduced feeding, or to both. If feeding is reduced by maturation then fat reserves would be depleted mainly to sustain the metabolic costs associated with spawning.

The hypotheses that feeding rates are reduced in maturing males, and that depletion of fat reserves occurs during the spawning season were therefore tested, to establish a functional role for fat in maturing salmon parr.

The effects of growth restriction during different times of year on total lipid and mesenteric fat stores were also investigated. Observations of maturing male parr revealed the deposition of unusually large amounts of fat on the mesenteric tissues surrounding the gut. The liver is the major fat store in many demersal marine fish, whereas muscle fat is of greater importance in pelagic species (Cowey & Sargent 1972). Mesenteric fat, however is a major store in freshwater fish such as the salmonids (Henderson & Sargent 1981). Leyzerovich (1973) indicated that differences in the dynamics of visceral (=mesenteric) fat characterized maturing male parr of Atlantic salmon, and suggested that this fat store would have a functional role in the maturation process. The aim of this particular study was therefore to determine the seasonal pattern of deposition of mesenteric fat relative to total lipid in both maturing and immature fish, and to see whether growth restriction during different months affected this pattern, and could account for variations in maturation rates between the populations subjected to varying seasonal periods of growth restriction.

4.2 MATERIALS AND METHODS

4.2.1 Feeding studies

Meal size and feeding rates in maturing male, non-maturing male and female parr were measured once a month from July to November, 1987. The method used was the iron-labelling technique (see section 3.2.1), in which food is labelled with iron particles, and the number of these present in the fish's digestive tract is related to food consumed.

The individually microtagged fish (See section 2.2) were used for this experiment, as they were X-rayed each month for identification purposes. The iron particles are revealed by radiography, so measurements of meal size could be assigned to individually tagged fish, which were later identified as maturing males, immature males or females. The microtagged fish were fed iron-labelled food over a 5-6 hour period, from before dawn until midmorning. The calibration curve for calculating meal size is the same as for the feeding study in Chapter 3 (Fig. 19), and feeding rates were calculated as mg food consumed per g body weight per hour. A significant proportion of fish were not feeding in autumn months and so mean feeding rates were calculated only for fish with some food present in their digestive tracts.

Differences in the proportions of non-feeding mature males, non-maturing males and females were tested using X^2

analysis, whereas differences in mean feeding rates were tested by analysis of variance, after the data were checked for normality.

4.2.2 Studies of mesenteric fat in maturing and non-maturing parr

Samples of approximately 100 fish were taken from the stock population of sibling PS2 parr (see Section 2.2.1) in July, September and November 1987. Each fish was weighed, measured and killed in anaesthetic (MS 222). The fish was then sexed and its gonads weighed to .0001g. The visceral contents were also removed, and all fatty tissue dissected out and weighed, again to .0001g. The major portion (60-80%) of fatty tissue occurred in 3 distinct lobes, one around the stomach and one on either side of the hind gut. However, a significant amount also occurred on and between the pyloric caeca, as occurs in brown trout (Salmo trutta) (Epple & Schneider 1974). This was readily removed, but it was not possible to distinguish and separate fatty tissue from pancreatic tissue. As a consequence weights of mesenteric fat include pancreatic tissue. Mesenteric fat contains more than 90% lipid (Fig. 42), and so the contribution of pancreatic tissue is minor. Nevertheless, data are expressed in terms of a mesenteric fat index (MFI), rather than as percentages of mesenteric fat.

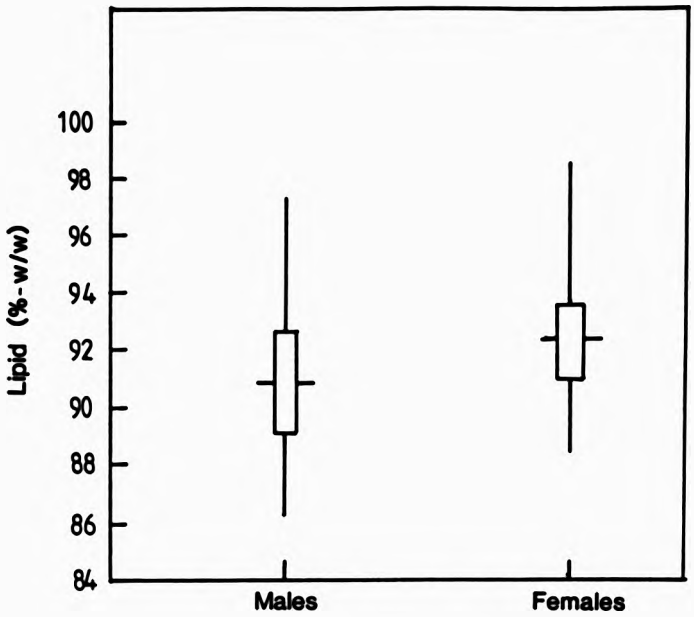


Fig. 42. Lipid content of mesenteric fatty tissue in 1+ male and female parr. (Horizontal bar is mean, box and whisker lines represent ± S.E. and range of values respectively).

Mesenteric fat indices were calculated as follows:

$$MFI = \frac{W_1}{W_2} \times 100$$

where MFI is mesenteric fat index

W_1 is wet weight of mesenteric fatty tissue (g)

W_2 is wet weight of fish (g)

Maturing and non-maturing males were distinguished by determining the maximum GSI for non-maturing males in each of the 3 months, and mean mesenteric fat indices were calculated for maturing male, immature male and female parr in each month. GSI's were plotted against MFI for individual maturing and non-maturing male parr in each of the 3 months, to determine the effects of gonad growth on fat levels.

4.2.3 Studies on the effects of food restriction on lipid stores

Samples of 20 fish were removed for lipid analysis from each of the PS2 populations in the restricted feeding experiment (see Chapter 3), before and after the respective period of food restriction, and from all populations in July (see Appendix 1). Samples were also taken from the control population (no food restriction) in November, February, and April. The samples, taken each month before the periods of food restriction, provided a chronological data set for determining monthly changes in lipid levels.

as well as a seasonal control for distinguishing effects of food restriction on fat stores from the seasonal changes in lipid levels. The post food restriction samples were used to determine the effects of fasting, at different times of the year, on lipid levels. Mean ratios of mesenteric fat to total lipid were calculated for each month, using a conversion rate of 91% lipid content for mesenteric fatty tissue (Fig. 42). The timing of lipid deposition into the mesenteric fat store was determined by comparing changes in the proportion of mesenteric fat to total lipid with changes in total lipid content.

Each fish taken was weighed, measured, and sexed. In addition gonad weight and mesenteric fat weights were determined. The entire fish was then analysed for lipid content using the method described by Hanson and Olley (1963).

4.3 RESULTS

4.3.1 Changes in feeding rates during maturation

Non-feeding fish

All fish were feeding in July, but 7-8% were not feeding in August and September (Table 14). Numbers of non-feeding fish increased as water temperatures declined, reaching 40% by November. Proportions of non-feeding fish were highest in maturing, and lowest in non-maturing male parr, between August and October.

Feeding fish

Food consumption rates of feeding fish were all relatively high in July, but decreased significantly in August (Fig. 43), even though water temperatures were still above 10°C (Fig.2). Thereafter, they decreased more slowly to November. During July and August feeding rates of maturing males were lower than in both non-maturing males and females, but only significantly so in August (Table 15). Females had higher feeding rates than male fish in all months, significantly so compared to maturing males in July, August and September.

TABLE 14. Proportions of non-feeding fish in the microtagged population of salmon parr.

Group	Non-feeders (%)				
	July	August	September	October	November
Maturing males	0.0	9.5	19.1 +++	38.1	42.9 ---
Non-maturing males	0.0	0.0 ---	0.0 ---	16.7	50.0 +++
Females	0.0	9.1 +++	0.0 ---	30.3	36.4
Total	0.0	8.3	6.7	31.7	40.0

Differences in proportions between groups of fish were tested by χ^2 analysis. +++ and --- indicate respectively higher and lower than expected contributions to χ^2 at $p<.001$ level).

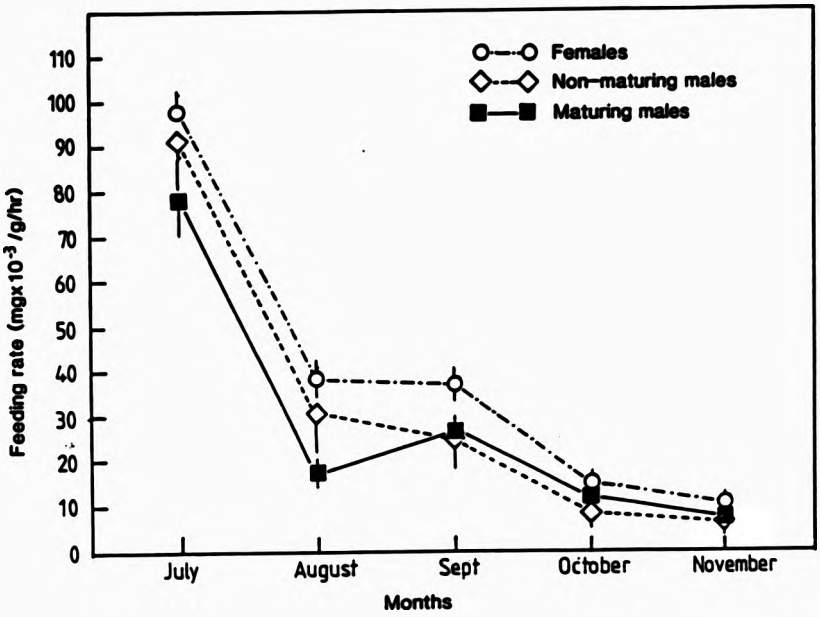


Fig. 43. Differences in mean feeding rates between mature male and non-maturing parr from late summer to autumn 1987. (Vertical bars are ± S.E.).

TABLE 15. Changes in feeding rates for fish in the microtagged population (mean ± S.E).

Period	Mean feeding rate ($mg.10^{-3}.g^{-1}.h^{-1}$)		
	Maturing males	Non-maturing males	Females
Jul	78.2 (8.26) **[1]	91.6 (11.05)	97.2 (5.77)
Aug	16.6 (2.62) **[2]	31.3 (10.86)	37.7 (4.07)
Sep	24.9 (3.37) **[1]	24.0 (5.96)	36.5 (3.29)
Oct	12.1 (1.61)	8.6 (1.12)	14.8 (2.02)
Nov	7.3 (1.47)	7.3 (1.79)	10.2 (2.40)

**[1] Significant difference ($p < 0.005$) between maturing males and females.

**[2] Significant difference ($p < 0.005$) between maturing males and all other fish.

4.3.2 Changes in mesenteric fat indices (MFIs) during maturation

GSI values were used as a basis for relating changes in mesenteric fat to the progress of the maturation process. Despite increases in fish size between July and November (Fig. 44), mean GSI's of female fish remained constant at a modal value of 0.45% (Fig. 45). In comparison, GSI's of male fish varied with time, and were separated into two distinct groups by September, reflecting the difference between maturing and non-maturing fish (Fig. 45).

In January GSI's of male parr in the stock population were all less than 0.05% (Table 1). GSIs of non-maturing males remained at less than 0.05% throughout the year, while maturing parr had GSI values of up to 3.5% in July, 4.5-12.5% (mode 10%) in September, and 6.0% in November (Figs.46 and 48). Maturing males were thus readily identified by GSI values in excess of 0.05%. Maturing males occurred throughout the the range of fish sizes in each month, and their proportions were constant at approximately 84% between July and September, indicating that they had all begun to mature by July.

Mesenteric fatty tissue contained 91% lipid (Fig. 42) and its weight was strongly correlated with total body lipids (Table 16). The MFI was therefore a useful indicator of both total lipid and mesenteric fat. In July, MFI and GSI were positively correlated ($r = +0.70$) (Fig. 46), but by

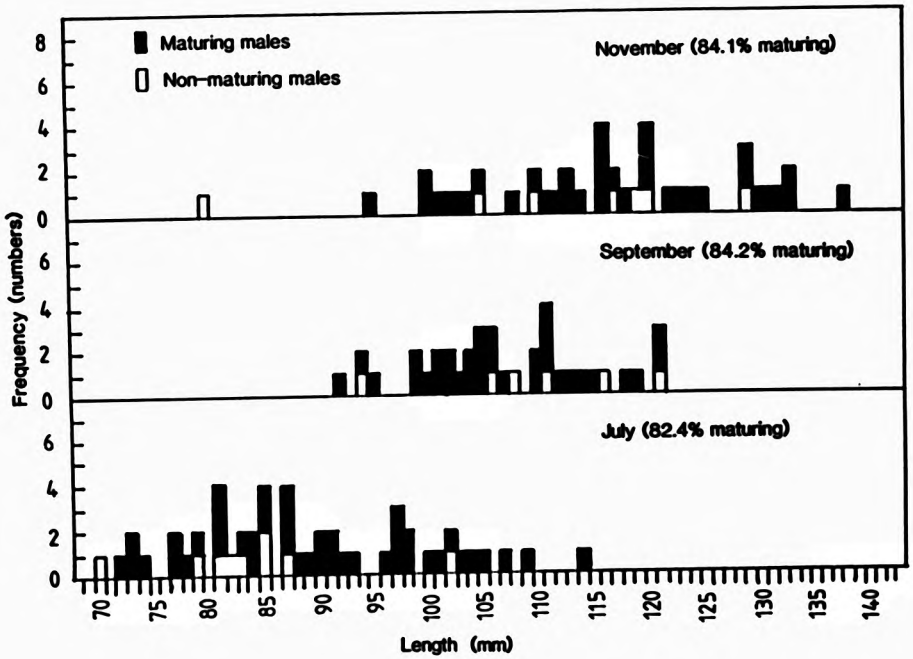


Fig. 44. Size distributions of maturing and non-maturing 1+ male parr in stock population, 1987.

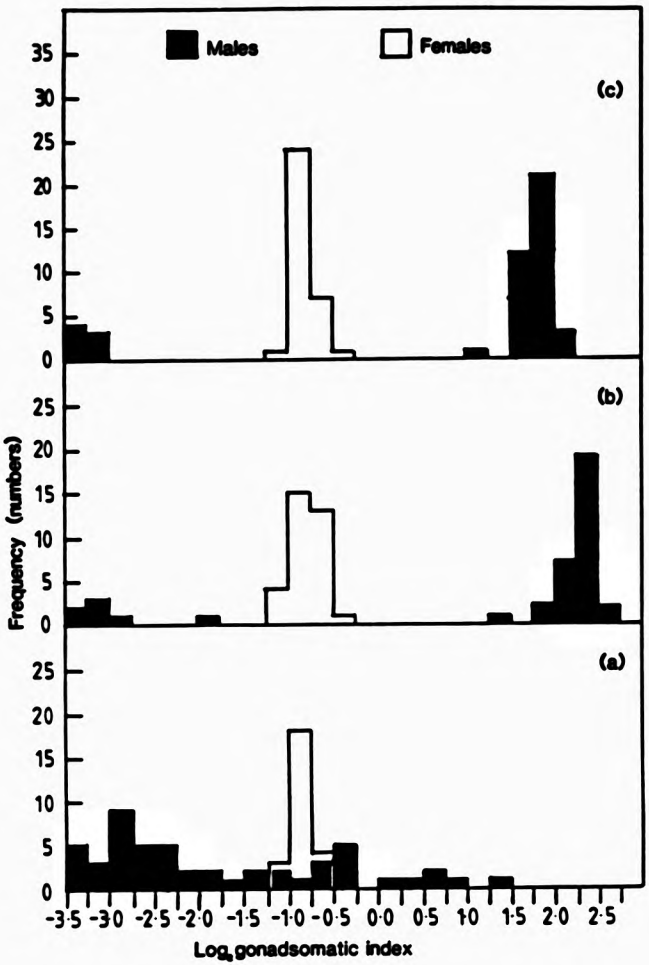


Fig. 45. Changes in GSI of male and female 1+ salmon parr between (a) July, (b) September, and (c) November, 1987.

TABLE 16. Regressions of mesenteric lipid(g) on total lipid(g) for parr from the control populations (no food restriction) and experimental populations (starved during alternate weeks of previous 2 months).

Date	Control populations			Experimental populations		
	Correlation (r)	Intercept (a)	Slope (b)	Correlation (r)	Intercept (a)	Slope (b)
16 Nov	0.92	2.14	0.07	-	-	-
14 Dec	0.96	1.85	0.06	-	-	-
11 Jan	0.98	1.73	0.06	0.94	1.74	0.05
08 Feb	0.98	1.73	0.07	0.97	1.93	0.06
07 Mar	0.81	2.22	0.06	0.81	2.41	0.06
04 Apr	0.98	2.17	0.06	0.94	2.77	0.04
02 May	0.94	2.51	0.08	0.96	2.10	0.07
01 Jun	0.96	2.39	0.13	0.91	2.84	0.12
04 Jul	0.89	1.72	0.50	0.97	2.10	0.20

September they were inversely related (r = -0.61). MFIs for non-maturing fish increased between July and November (Figs. 46 and 48), and there was no difference between mean MFIs of non-maturing males and females during this period (Fig. 47). However, the mean MFI of maturing males declined between July and September, and did not increase between September and November (Fig. 47). Similar seasonal differences in MFI occurred in the maturing male, non-maturing male and female parr in the populations of individually tagged fish (Table 17).

4.3.3 Seasonal variations in lipid stores

In general weights of lipid in salmon parr were proportional to fish weight (Appendix 1). The mean size of male fish did not change significantly between mid-November and the end of March (Figs. 49 and 50), but a small increase in weight occurred in April. In comparison mean size of females increased between mid-November to mid-January, but then remained constant until the beginning of May. All fish increased significantly in size in June and July.

Males were consistently larger than females. Consequently the differences in lipid weights of males and females in any one month were largely due to size, males being larger and fatter. The small sample sizes for each sex precluded

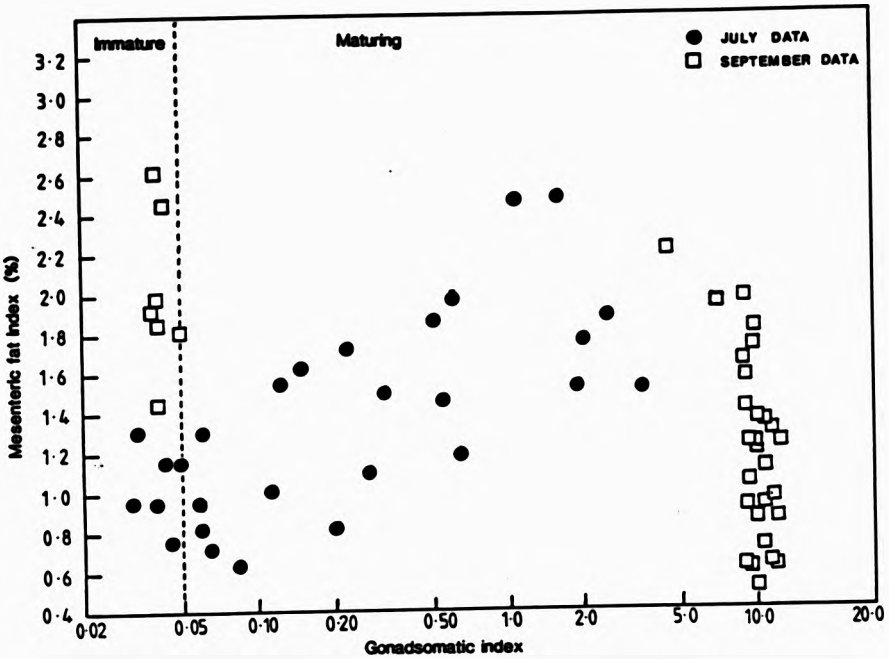


Fig. 46. Relationship between mesentric fat indices and GSI for maturing and non-maturing male parr between July and September, 1987.

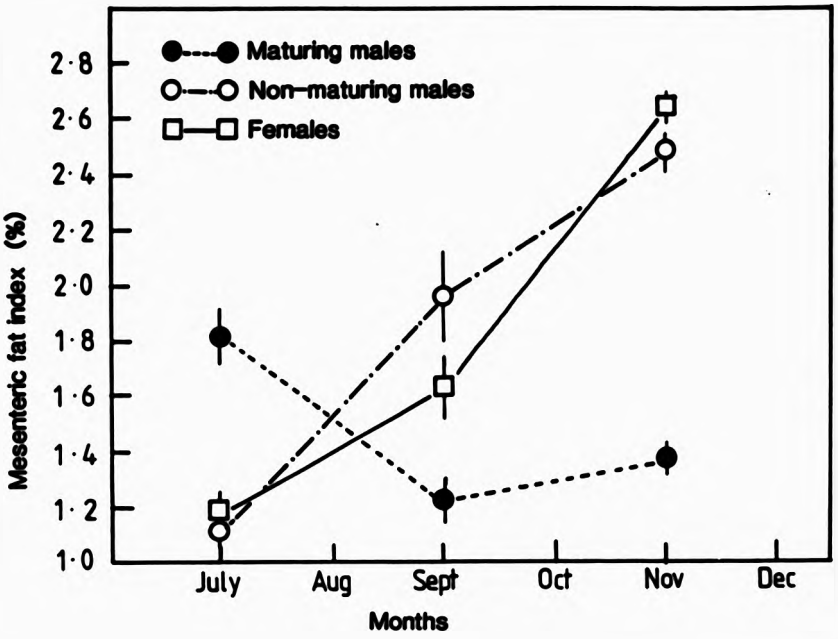


Fig. 47. Changes in MFI of maturing male and non-maturing 1+ salmon parr between July and November, 1987.

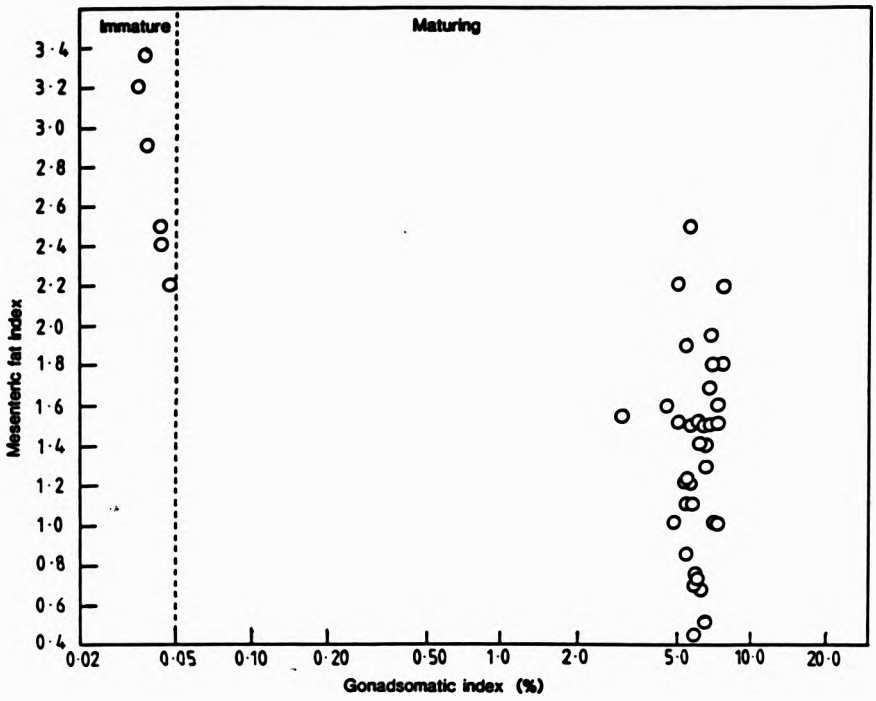


Fig. 48. Relationship between MFI and GSI of maturing and non-maturing male parr in November 1987.

TABLE 17. Changes in mean mesenteric fat indices during autumn for individually tagged salmon parr (± S.E).

Fish group	July	September
Maturing males	2.31 (0.17)	2.01 (0.15)
Non-maturing males	-	2.40 (0.16)
Females	2.21 (0.11)	2.69 (0.14)

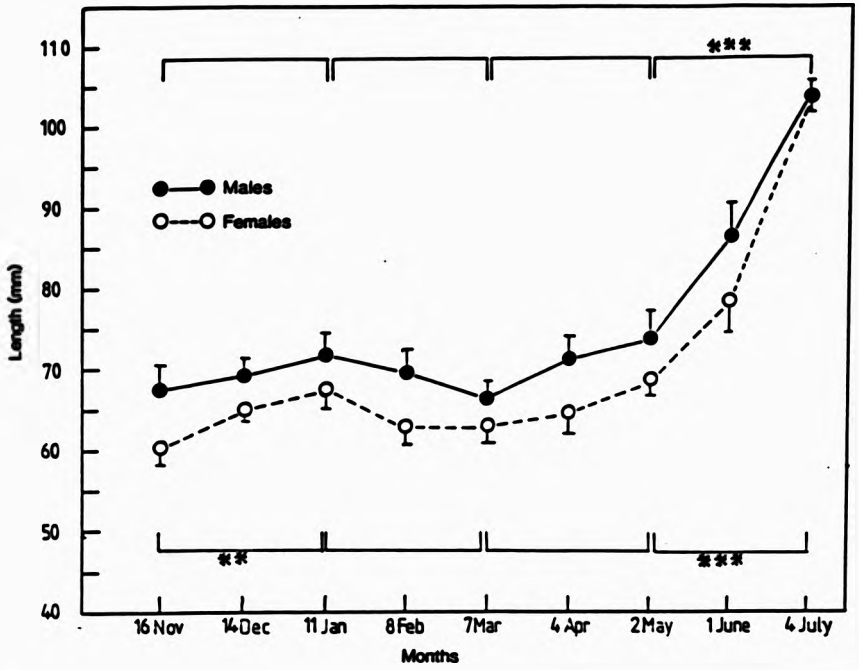


Fig. 49. Monthly differences in mean (± S.E.) length of 1+ parr sampled for lipid analysis during 1988 (Asterisks indicate significant differences between bracketed values; *** $p<0.001$, ** $p<0.05$, * $p<0.10$).

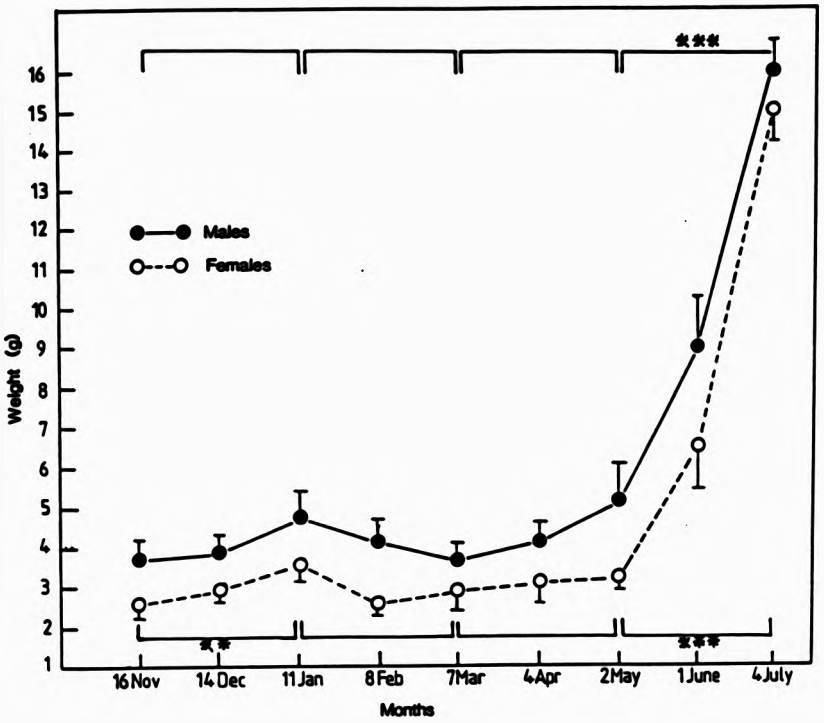


Fig. 50. Monthly differences in mean (± S.E.) weight of 1+ parr sampled for lipid analyses during 1988 (Asterisks indicate significant differences between bracketed values; *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

analysis of covariance to determine whether differences in lipid content between males and females were independent of size. Nevertheless, when lipid content was expressed as a proportion of fish weight, mean percentage values were consistently higher in males than in females indicating a difference between the sexes. Consequently results are expressed as mean percentage values and are reported separately for both males and females.

In the seasonal control fish, lipid contents of both males and females declined from approximately 7% to 5.5%, between 16 November and 11 January, then increased slightly but significantly to 6-7% during the latter part of January (Fig. 51 and Table 18). They then declined again in February to below 6% and remained static over March. During April lipid levels of males increased to 7%, but in females they did not begin to increase until May. During May fat contents in both the sexes increased rapidly, by more than 3% up to 10% in males, and by 2% up to 8% in females. By early July mean lipid levels were 11%, and there was no difference between the sexes.

A similar seasonal pattern of depletion and deposition occurred in the fish subjected to food restriction (Fig. 52 and Table 18). Although monthly levels were lower than for the seasonal controls, they increased noticeably in both sexes in the latter part of January and early February to approximately 6%, then declined to 4.5% by the

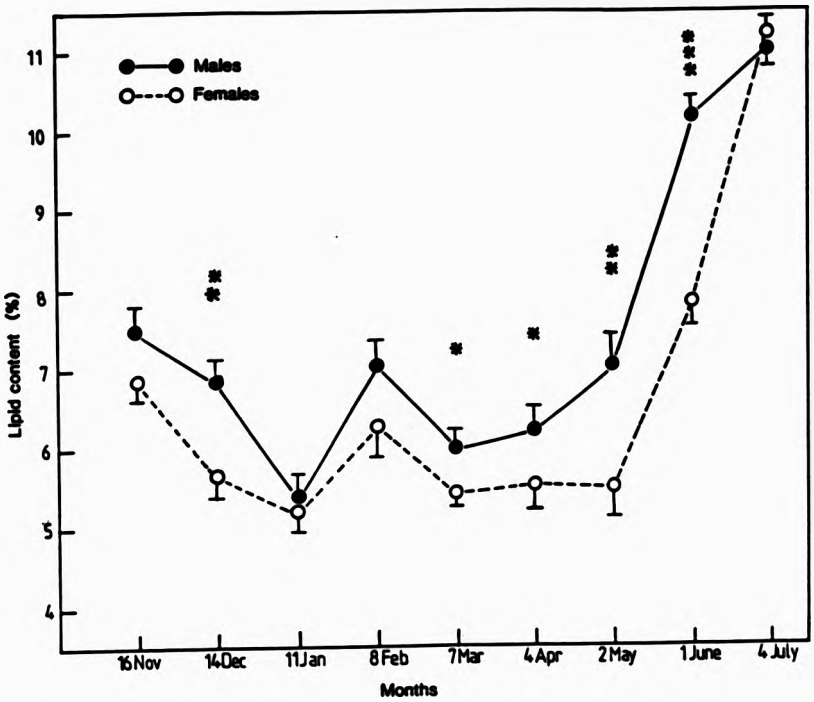


Fig. 51. Monthly changes in mean (± S.E.) lipid content of 1+ salmon parr in the seasonal control populations during 1988. (Asterisks indicate significant differences between males and females *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

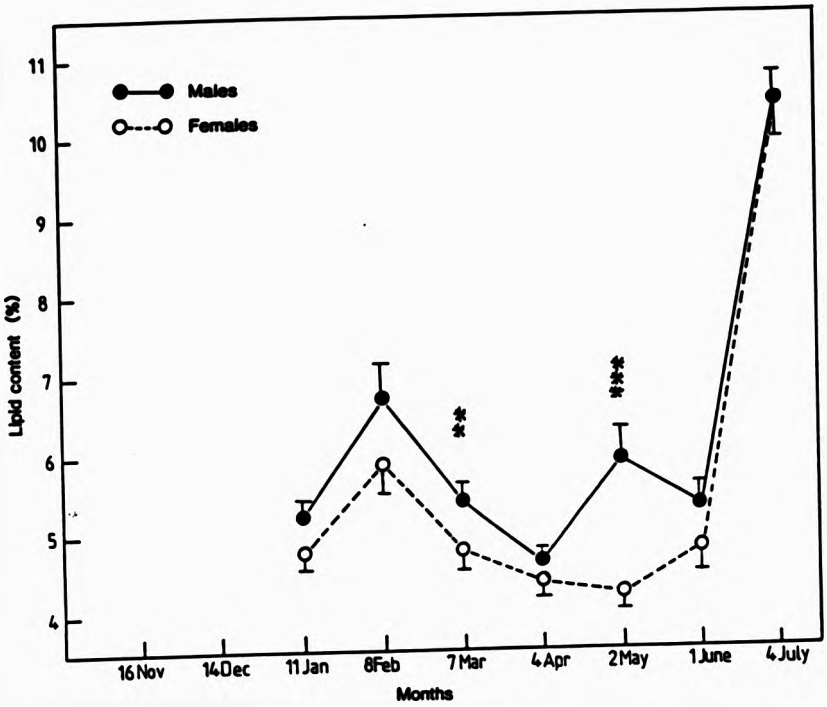


Fig. 52. Monthly changes in mean (± S.E.) lipid content of 1+ salmon parr in the food restricted populations during 1988. (Asterisks indicate significant differences between males and females *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

TABLE 18. Significance of differences in mean total lipid content (%) between months for male and female parr in the seasonal control and food restricted populations. (Results of ANOVA to determine F ratios for equality of means).

Months	Control populations		Food restricted populations	
	Males	Females	Males	Females
Nov-Dec	N.S.	$p<.001$	--	--
Dec-Jan	$p<.001$	N.S.	--	--
Jan-Feb	$p<.001$	$p<.10$	$p<.001$	$p<.05$
Feb-Mar	$p<.05$	N.S.	$p<.05$	$p<.05$
Mar-Apr	N.S.	N.S.	$p<.001$	N.S.
Apr-May	N.S.	N.S.	$p<.001$	N.S.
May-Jun	$p<.001$	$p<.001$	N.S.	N.S.
Jun-Jul	$p<.05$	$p<.001$	$p<.001$	$p<.001$

end of March. In April they increased significantly in males only, while in June they increased substantially to reach 10.5% in both sexes by early July.

These data indicate that fat stores of male fish were generally greater than those of females and, following a winter decline, began to increase in April, one month earlier than in females. As a consequence mean fat contents of males were 30% higher by the end of May. Fat deposition was substantial in all fish during June, when growth was also rapid, and the difference in lipid content between the sexes was eliminated by the beginning of July.

The mesenteric fat store contains 30 to 40% of total lipid, and there was a strong correlation between mesenteric lipid and total lipid for all months (Table 15). MFI's reflect changes in total lipid, but were measured to determine whether the seasonal dynamics of deposition and depletion in this store followed the same pattern as for total lipids.

As with total fat levels, MFIs were higher in male than in female parr (Fig. 53). They were relatively constant for both sexes from mid-November to mid-January, but then began to decline, most noticeably in the fish on restricted rations (Fig. 54 and Table 19). By the end of April the mean MFI of male fish was significantly higher than for females in both control and food restricted

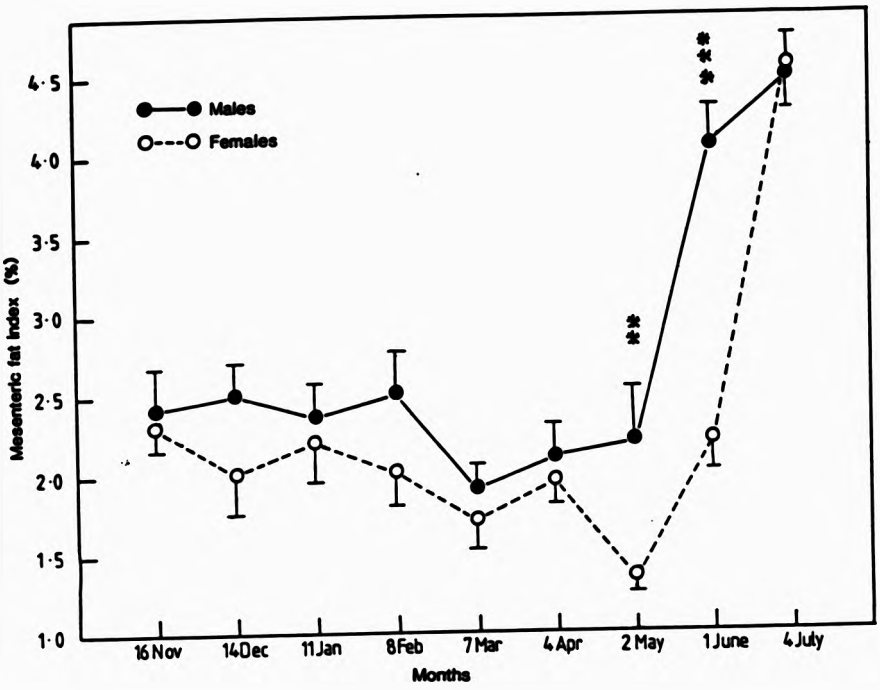


Fig. 53. Monthly changes in mean MFI (± S.E.) of 1+ salmon parr in the seasonal control populations during 1988. (Asterisks indicate significant differences between males and females; *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

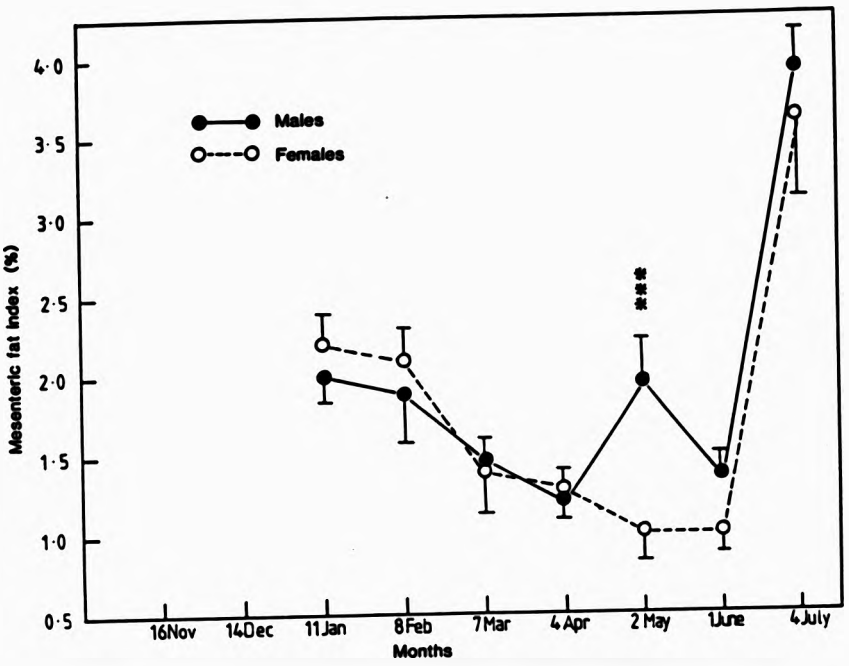


Fig. 54. Monthly changes in mean MFI (± S.E.) for 1+ salmon parr in the food restricted populations during 1988. (Asterisks indicate significant differences between males and females; *** $p < 0.001$, ** $p < 0.05$, * $p < 0.10$).

TABLE 19. Significance of differences in mean mesenteric fat index (%) between months for male and female parr in the seasonal control and food restricted populations. (Results of ANOVA to determine F ratios for equality of means).

Months	Control populations		Food restricted populations	
	Males	Females	Males	Females
Nov-Dec	N.S.	N.S.	--	--
Dec-Jan	N.S.	N.S.	--	--
Jan-Feb	N.S.	N.S.	N.S.	N.S.
Feb-Mar	$p<.10$	N.S.	N.S.	$p<.10$
Mar-Apr	N.S.	N.S.	N.S.	N.S.
Apr-May	N.S.	$p<.001$	$p<.05$	N.S.
May-Jun	$p<.001$	$p<.001$	$p<.10$	N.S.
Jun-Jul	N.S.	$p<.001$	$p<.001$	$p<.001$
Nov-Mar	$p<.05$	$p<.05$	--	--
Feb-Apr	--	--	$p<.05$	$p<.05$

populations. This difference was due mainly to the continuing decline for females, however, the mean MFI for males increased significantly during April to 2% in the food restricted population.

Ratios of mesenteric fat to total body fat for both sexes in both control and food restricted populations varied on a seasonal basis (Figs. 55 and 56). In general, the proportion of mesenteric fat in control fish increased from November to January, decreased during February to April, and increased again in May and June. This pattern of decline and increase differed in timing for the food restricted fish. The proportion of mesenteric fat began declining a month earlier, in January, and started to increase again in June, a month later than in control fish. These seasonal changes in the proportion of fat in the mesenteric store are also reflected by changes in the monthly regression parameters for total lipid on mesenteric lipid (Table 16).

4.3.4 Effect of seasonal food restriction on lipid stores

Fat weight increased with fish weight in a linear fashion for most months, but was occasionally curvilinear (Appendix 1). Over the entire experimental period the relationship was curvilinear. Consequently differences in fat content between populations were tested by analysis of covariance on log transformed fat and fish weights.

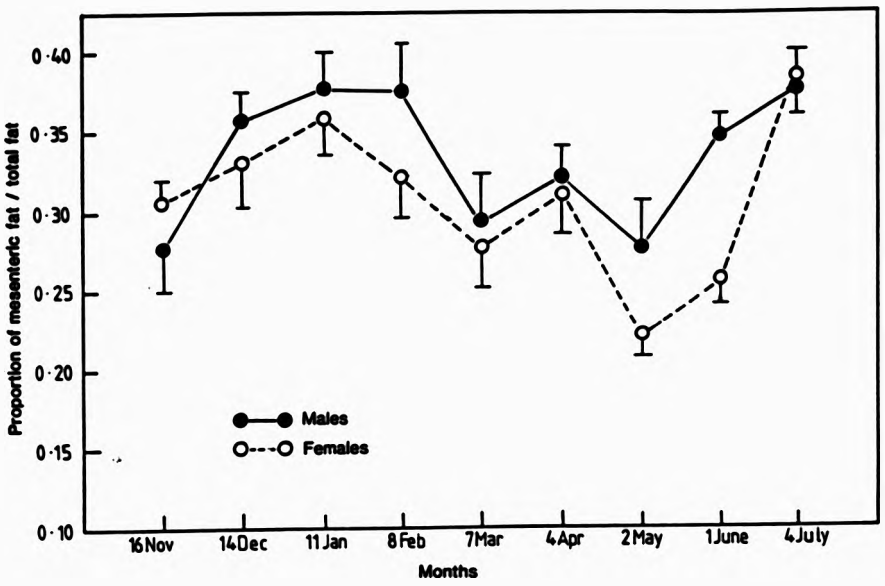


Fig. 55. Monthly changes in the mean (± S.E.) proportion of mesentric fat for salmon parr in the seasonal control population during 1988.

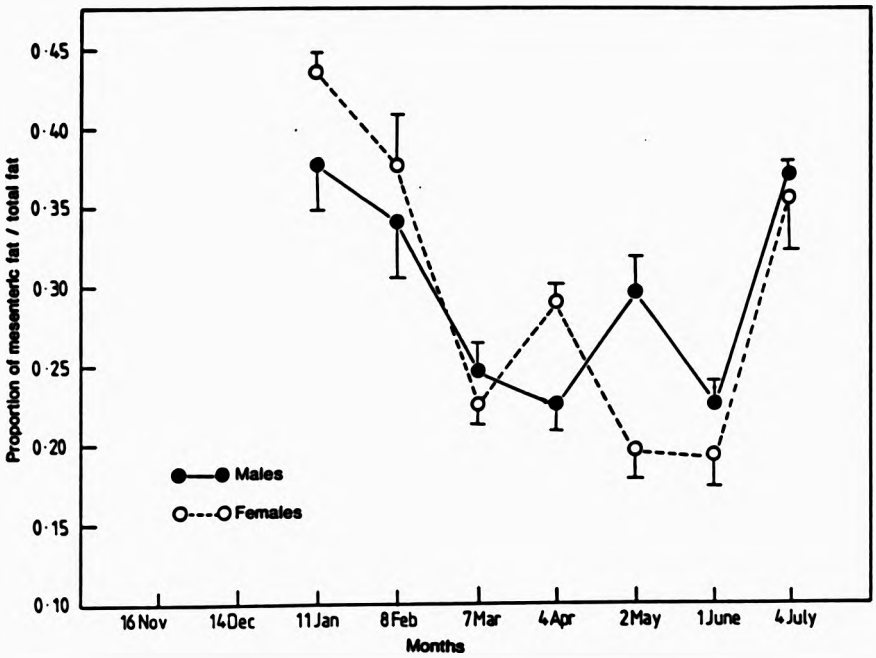


Fig. 56. Monthly changes in the mean (± S.E.) proportion of mesenteric fat for salmon parr in the food restricted populations during 1988.

There were significant differences in fat content between fish in seasonal control populations and fish in the populations whose feeding was restricted (Table 20). However there was a significant interaction component indicating that these differences were not consistent. The overall mean weight of fish sampled was 3.8g, so regressions of fat weight on body weight, calculated using a pooled slope of 1.232 (S.E. = 0.0247), were used to estimate fat contents of this standard sized fish. Differences in the resulting "fitted" fat values between control and food restricted fish reflect differences between populations over time (Fig. 57). The "fitted" population values followed the same seasonal trend as noted for mean percent total lipid in male and female fish. Fat levels of control populations decreased during November and December, increased during January, decreased again until early April, and then increased again from April to July. Values for food restricted populations behave similarly, but did not begin to increase until June. Food restriction between November and December had no effect on fat levels but food restriction during February and March reduced them significantly compared to control populations. Food restriction after March depressed fat levels of all populations, with greatest effect following restriction during April and May, and least effect in May and June, when fat levels were increasing rapidly in all fish.

TABLE 20. ANCOVA for fat contents (% w/w) on fish weights (g) between food restricted and seasonal control populations of salmon parr.

Source of variation	df	Sum of squares	Mean square	Variance ratio
Between restricted and control populations adjusted	1	0.356	0.356	54.69 ***
Between months adjusted	5	0.356	0.071	10.93 ***
Interaction adjusted	5	0.271	0.054	8.34 ***
Residual adjusted	227	1.478	0.007	

*** ($p < 0.001$).

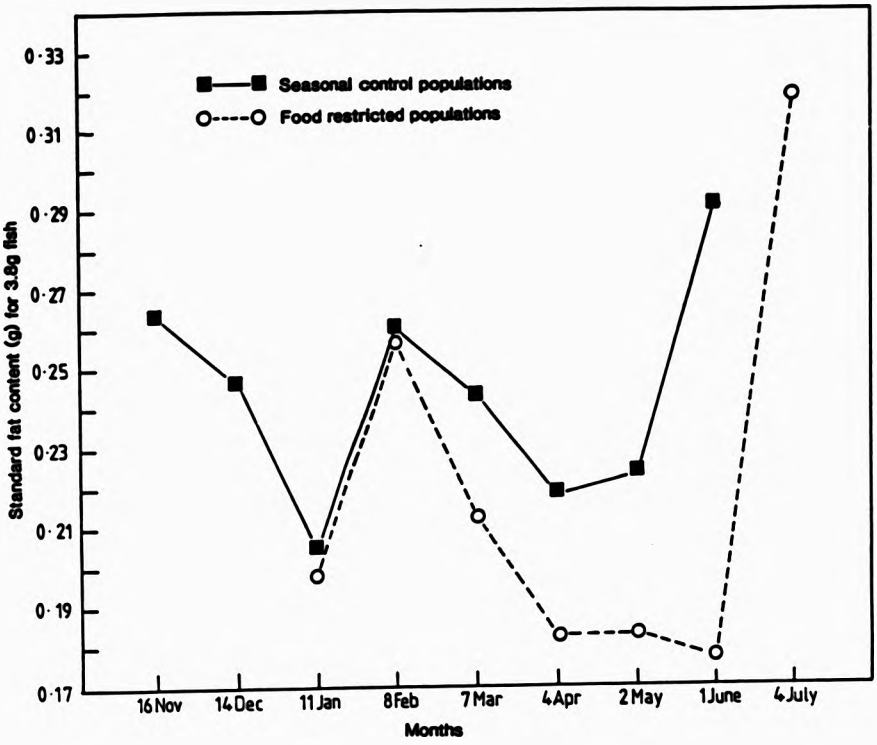


Fig. 57. Effect of food restriction (fasting during alternate weeks for an 8 week period) on fat contents of 1+ salmon parr populations (Values are "fitted" from regressions of fat content on weight for each population using common slope determined during ANCOVA).

4.4 CONCLUSIONS AND DISCUSSION

4.4.1 Effect of maturation on feeding rates of mature parr

Proportions of non-feeding fish were highest in maturing males between July and October, and maturing males which were feeding had lower mean feeding rates in July and August. This reduction in food intake by maturing males during the period when the gonad is differentiated is unlikely to be due to restricted rations as, although rations were limited to 1% of the biomass, this is above the optimal level for salmon parr at the water temperatures prevailing (Farmer et al. 1983). No aggresive behaviour, which could have interfered with feeding, was observed between fish (the design of the radial flow tanks inhibits this), so the reduction in food intake is likely to reflect a relative decrease in appetite of maturing males.

This reduction in appetite may be caused by the high testosterone levels present in maturing parr at this time. Saunders et al. (1977) showed that addition of 17a-methyltestosterone to the food of salmon parr had a dose dependent effect on growth. They indicated that doses large enough to cause extensive changes in the testes interfered with growth and other physiological processes in salmon. Testosterone levels in maturing male parr are likely to have been above 5 ug/100ml and increasing by

July (Murphy 1980), but may have been higher in this stock as GSI's of these fish peaked one month earlier than Murphy's. Androgens are suspected of retarding growth during maturation in the platyfish (Xiphophorus maculatus) (Schreibman & Kallman 1977), and at high levels are reported to induce atrophy of the alimentary canal in the lamprey (Lampetra fluviatilis) (Dockray & Pickering 1972). Atrophy of the stomach, intestine and pyloric caeca has been reported for mature salmon (Gulland 1898; Greene 1926, both cited by Love 1970), but apparently this is reversible (Love 1970), at least after spawning (pers. comm. C. Talbot). The reduced feeding of maturing male parr may therefore be related to their higher levels of serum testosterone, which reduce appetite, and/or gut function.

4.4.2 Effect of maturation on lipid stores of maturing males

Maturing males had higher levels of mesenteric fat than non-maturing males or females in July, but MFIs declined between July and September, while MFIs of non-maturing males and females increased. Saunders & Sreedharan (1977) recorded a similar autumnal pattern of lipid decline in maturing male parr of Atlantic salmon, while lipid levels increased in non-maturing fish. They measured total lipids, and these declined from August to December in maturing male parr, while fat contents of other males and

females increased.

During autumn, and as gonad size increased, feeding and somatic growth of maturing males decreased. The proportionate decline in mesenteric fat at this time could not be accounted for by the small increase in weight of maturing fish, and was due to actual depletion of the mesenteric store. This depletion was in marked contrast to proportionate increases in the mesenteric fat levels of immature males and females which continued to grow. The mobilization of lipid in the mesenteric store of maturing fish is presumably needed to support the energetic costs of gonad differentiation and elaboration of secondary sexual characteristics, such as skin thickening (Murphy 1980), and lower jaw elongation noted for some fish, at a time when feeding is reduced.

MFIs of maturing males did not change between September and November, but total lipids (Saunders & Sreedharan 1977) did. The lack of change in MFI probably indicates the completion of both gonad growth and the elaboration of the secondary sexual characteristics by September. However, the continued decline in total lipids of mature parr, relative to slightly increasing levels in non-maturing fish (Saunders & Sreedharan 1977), indicates that feeding is still reduced in mature males, and that fat depletion of non-mesenteric stores occurs after maturation, but before spawning.

As a result of the depletion of mesenteric fat, total lipid levels of maturing male parr were reduced to 3% prior to winter, compared with 8% in immature males and females (Saunders & Sreedharan 1977). This is likely to adversely affect survival of maturing males, both during the winter and after smolting, as the probability of winter mortality is related to fat content in autumn (Gardiner & Geddes 1980). Furthermore, mesenteric fat is likely to be an important store enabling smolts to adjust to marine feeding. Smolts are characterized by reduced fat levels, but smoltification in freshwater only affects muscle and liver stores. Mesenteric fats remain intact (Sheridan et al. 1983) and are probably "preserved" to support costs associated with entry to the marine environment.

4.4.3 Seasonal timing of fat depletion and deposition

In general lipid contents of both male and female parr declined from November to late February. The rise in % lipid recorded during January is likely to be an artifact, and due to loss of fish weight at this time, rather than to an actual increase in lipid. Parker & Vanstone (1966) showed that when feeding in pink salmon parr (Oncorhynchus gorbuscha) declined to the point where growth in length ceased, the loss in weight due to lipid depletion was compensated for by increasing water content. Fish weight

only declined in the latter stages of starvation, and this was due to a loss of protein, lipid and water. Gardiner & Geddes (1980) recorded a similar phenomenon for Atlantic salmon parr. Weights remained reasonably constant during winter, despite a large decrease in energy content, and a rise in % water. It is apparent that when feeding is reduced below the level needed for maintenance, initial losses of fat are compensated for by increased water. Weight only declines later, after fat reserves have already been substantially depleted. The mean weights of the salmon parr sampled for lipid analysis in this experiment were remarkably constant between November and March. However, these values are from small samples, and different populations. While they reflect major seasonal changes in the size of experimental fish, they are unlikely to reflect the true extent of weight loss during winter months. Nevertheless, a decline in mean size of fish occured after January and the mean weights of fat per fish declined slightly during this month (0.246g to 0.225g). The increase in % lipid during January is therefore due to a loss in weight of these fish, rather than to an increase in fat content.

It has been shown that there is no difference in mesenteric fat levels (this study) and total lipids (Saunders & Sreedharan 1977; Koch & Bergstrom 1978) between non-maturing males and females. As the majority (more than 80%) of male fish in the control and

experimental populations matured, the differences in fat dynamics between the sexes in this study reflects differences between maturing and non-maturing fish.

Lipid contents of maturing male fish, began increasing during April, a month before there was any change in females. As a consequence differences between the sexes were maximal by the end of May. It is apparent that maturation is associated with an earlier start to the replenishment of fat stores, the result of which is a 25% increase in mean weight and a 46% increase in mean lipid content of maturing male fish during April.

The April increase in condition factor noted previously (Chapter 2) for maturing males is therefore associated with comparatively larger increases in fat, and males which mature in autumn are replenishing their fat stores one month earlier than non-maturing males. Koch & Bergstrom (1978) obtained very similar results between maturing and non-maturing Atlantic salmon parr, the only difference being that the fat contents of their maturing males began increasing substantially in both March and April, while fat contents of non-maturing fish (male and female) remained static. The increase in fat content of their maturing males is one month earlier than recorded here, but this may be due to the fact that their fish had already matured previously, and were approaching their

second spawning season.

The seasonal pattern of depletion and replenishment of mesenteric fat differed to that for total lipids. Although total lipid levels declined from November to February, mesenteric fat was maintained at a constant proportion of body weight until the end of January. Mesenteric fat levels then declined during February, and in maturing males didn't increase significantly until May, a month after increases in total lipids were noted. The timing of fat deposition into the mesenteric store occurred a month later in June for females, or non-maturing fish.

These seasonal changes in the proportion of mesenteric fat to total body fat show that non-mesenteric fat stores (ie. liver and muscle) were depleted initially (Nov-Dec), while mesenteric stores remained relatively intact. Water replaced the body fat, compensating for weight loss, but in January, weight also declined. After this, in February, mesenteric fat levels also began to decrease. Weight and total lipids remained relatively constant during March indicating that feeding was maintaining metabolic needs. However, mesenteric fat continued to decline, indicating selective mobilization either for metabolic needs, or for transfer to non-mesenteric fat stores.

In April rising water temperatures and greater feeding

permitted increases in fish size, but total lipids increased significantly only in maturing fish. Despite this April increase in total lipids in males, the decline in mesenteric fat as a proportion of total lipid continued. It is apparent that maturing fish were replenishing other, non-mesenteric stores first. Finally, in May mesenteric fat levels increased, spectacularly so in maturing fish (the mean MFI increased by nearly 100% to reach 4% of body weight, so that mesenteric lipid accounted for nearly 40% of total lipids).

It is apparent that after winter, salmon parr replenish body fat before mesenteric fat, and that this process occurs much earlier in male fish which subsequently mature. Approximately 11% of the weight gains of maturing male fish in April were accounted for by lipid. This is the proportion of fat incorporated into foods for salmon parr (Farmer et al. 1983), and it is apparent that very little dietary lipid is used for metabolism at this time. The April replenishment of body fat is followed by fat deposition into the mesenteric store in May in maturing fish. This occurs in June in non-maturing fish, primarily because replenishment of body fats is delayed until May.

As a consequence of the earlier depostion of fat into the mesenteric store, the size of this is greater in maturing males at the onset of maturation. Murphy (1980) has shown that increases in GSI past 3% are associated with advanced

stages of maturation, notably the formation of primary spermatocytes. Maturing male parr were characterized by comparatively high levels of mesenteric fat compared with non-maturing fish before this stage was reached. In addition, the positive correlation, between mesenteric fat indices and GSI at this time, indicates that maturing males with relatively high fat levels had either started to mature earlier than maturing males with lower fat levels, or were maturing at a faster rate.

Mesenteric fatty tissue contained high levels of lipid (91%) and its weight was highly correlated with total lipids in all months. It is a major fat store in Atlantic salmon parr, accounting for up to 50% of total lipid in some fish by mid-summer. The results of the fat analyses show that maturing male parr store relatively more fat during April than non-maturing males or females. This replenishes fat lost during winter months so that deposition into the mesenteric store is well under way in maturing male fish by May. As a consequence maturing males have higher mesenteric fat stores than non-maturing males by July when the early stages of maturation are underway. Thus the comparatively early replenishment, in April, of fat lost over winter, affects the timing of fat deposition into the mesenteric store, and is ultimately responsible for the higher levels of mesenteric fat in maturing fish by the end of May.

The null hypotheses, that fat contents, and in particular mesenteric fat levels, of maturing males increase at the same rate as those of non-maturing males during spring and early summer months, and are no different when gonad growth beyond the 1° spermatogonia stage begins, were therefore both rejected. However, the conclusion from this, that maturing males have faster rates of fat deposition in spring months, disguises the crucial importance of both replenishment of non-mesenteric fat stores in maturing males, in April, a month before this occurs in non-maturing and female fish, and the continued supply of fat in May, to increase levels in the mesenteric store. The early onset of replenishment, rather than the rate of replenishment, is the significant difference which distinguishes maturing from non-maturing fish.

Unfortunately data on feeding rates of maturing male parr were not obtained in this study during spring months, and so it is not known whether maturing males begin feeding earlier than non-maturing males, or are more efficient at fat storage. However, Johnston et al. (1987) noted that reconditioned Atlantic salmon kelts, which later re-matured, began feeding in January and February, several weeks earlier than non-maturing kelts.

4.4.3 Effect of fasting on fat dynamics

In general food restriction had little effect on total lipid content during the months November to January. However during February and March, fasting reduced fat contents relative to control fish. Feeding was therefore important, even during these months, when water temperatures were below 4°C, and served to minimize depletion of fat stores. During April and May food restriction delayed replenishment of lipid stores until June, two months after replenishment began in maturing males. However fat contents for the population whose food was restricted in May and June were the same as for control fish, suggesting that food restriction had no effect on fat stores during these months. Fat deposition is particularly rapid during June, even in food restricted fish. Even so, this result is likely to have been biased. Fish from this population were sampled for fat analysis 1 week after the resumption of feeding, instead of the normal 2 to 3 days. Given the fast growth rates occurring at this time of year, fat stores could have been readily replenished in one week.

Reduced maturation rates only occurred in the populations whose food was restricted during the periods April to May and May to June. However, the decrease was relatively small, approximately 10%. Most males were therefore unaffected by food restriction and maturation was only

suppressed in a few of them. The basis for the genetic control of maturation rates is not fully understood yet, but it is clear that different families of salmon have different thresholds for growth above which maturation occurs. The fat contents of these parr were in the order of 4-5% during late winter, even in the food restricted populations. These levels are comparable with those of other populations of Atlantic salmon parr (Foda 1974), and so this stock is not characterized by unusually high fat contents. However it was atypical in that approximately 30% age 0+ males matured under natural temperatures and photoperiod. It is clear that the threshold for maturation is low in this stock and so effects of food restriction on maturation rates will be comparatively lower.

May was the time when there were substantial increases in mesenteric fat in maturing male fish. Replenishment of body fat lost during winter had already occured during April, but the corresponding increases for non-maturing parr occured a month later, in May. It is concluded that fasting during April and May reduced maturation rates because it delayed both replenishment of total body fat during April, and the concomitant increase in mesenteric fat during May. Johnston et al. (1987) came to a similar conclusion. They noted that oogenesis and vitellogenesis proceeded in reconditioned kelts which restored fat and protein reserves early in spring, but not in those which were slow to restore energy reserves.

CHAPTER 5
Synthesis and general discussion

5.1 THE ROLE OF FEEDING OPPORTUNITY DURING SPRING IN THE CONTROL OF MATURATION

Maturing male parr of Atlantic salmon were distinguished from non-maturing males by greater increases in condition factor during April. There was no association between maturation and size or specific growth rates during any month, and the predictive relationship with April increases in condition factor is supported by the results of three other independent studies on individually tagged Atlantic salmon (Hunt et al. 1982; Johnston et al. 1987; Herbinger 1987).

Changes in mean condition factor during each month were correlated with maturation rates for the 8 sibling populations used in experiments during 1987, and a highly significant positive correlation was obtained only in April (Table 21). When the April increases in mean condition were plotted against maturation rate for each population, the relationship was found to be curvilinear (Figure 58). Furthermore, it was apparent that the maturation rates of the experimental populations of PS2 parr were all suppressed relative to the stock population, whose growth was not restricted at any time.

TABLE 21. Correlations between maturation rate (arcsine transformed values) and monthly changes in mean condition factor (K) for all sibling populations of salmon parr in the 1987 experiments (Significance levels; *** $p < 0.01$, ** $p < 0.05$, * $p < 0.10$).

	Correlation coefficient
Feb-Mar	-0.11
Mar-Apr	+0.56 *
Apr-Apr	+0.87 ***
Apr-May	-0.12
May-Jun	-0.14
Jun-Jul	+0.26
Jul-Aug	-0.50 *

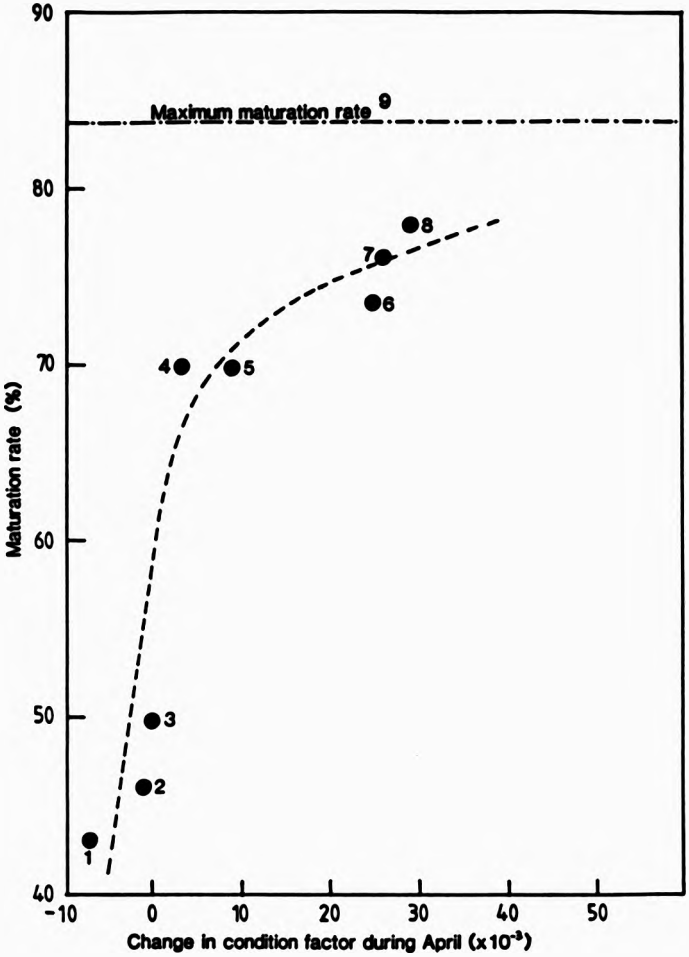


Fig. 58. Relationship between condition factor changes during April and maturation rate for all sibling populations of 1+ parr in 1987.

(1. Optimal growth Feb-Mar, 2. Optimal growth Aug-Sep, 3. Slow-growing control, 4. Fast growing control, 5. Optimal growth Jun-Jul, 6. Microtagged fish, 7. Bandtagged fish, 8. Optimal growth Apr-May, 9. Stock population (growth not suppressed).

Increases in condition factor imply greater gains in weight relative to length, and results reported earlier show that starvation during April decreases condition factor, while feeding raises it. Condition factor is correlated with lipid levels in Atlantic salmon parr (Hoar 1939; Pinder & Eales 1969), and studies on the fat dynamics of salmon parr have shown that April is a critical time for post-winter replenishment of body lipids (ie. non-mesenteric stores such as muscle and liver) in maturing males. Replenishment occurs later, in May, in non-maturing fish.

The increase in condition factor in April therefore reflects replenishment of body lipids, and this determines the timing of lipid deposition into the mesenteric store. Mesenteric fat is mobilized to support energetic costs involved in maturation and spawning, and its size is likely to be important for maturation. Increases in mesenteric fat levels occur earlier in maturing males, and the size of this store is subsequently larger when GSI values begin to increase. The relationship between April increases in condition factor and maturation therefore reflects the earlier replenishment and increase in body lipids of maturing fish, which in turn affects deposition into the mesenteric store in May.

The increases in condition factor and fat in maturing fish during April, and in mesenteric fat during May, may be merely symptomatic of maturation, particularly as maturing

fish are distinguishable before April. For example, maturing salmon are distinguishable on the basis of lower condition factors during late winter (Herbinger 1987), and March (male parr this study), and maturing salmon show significant elevations in plasma testosterone levels during February and March (Abdullah 1981; Hunt et al. 1982; Youngson & McLay 1985). These results indicate that physiological processes leading to maturation have been initiated before April. However it is also clear that maturation can be suppressed by reduced feeding in April and May. Burger (1985) cited by Herbinger (1987), also found that the extent of feeding in April influenced maturation rates of salmonids. The suppression of maturation in fish already programmed to mature can only be explained by the existence of a growth-related, physiological mechanism, which overides the maturation decision, switching it off.

Growth restriction in both April and May suppresses maturation, and can be expected to delay deposition of fat into the mesenteric store, reducing its size and rate of increase in May. The mesenteric store has an important functional role in maturation, and its size by mid-summer is likely to be important for the success of spawning. As growth restriction when it is being filled in May suppresses maturation, the decision to mature or not is likely to be left until May, although it is apparent that

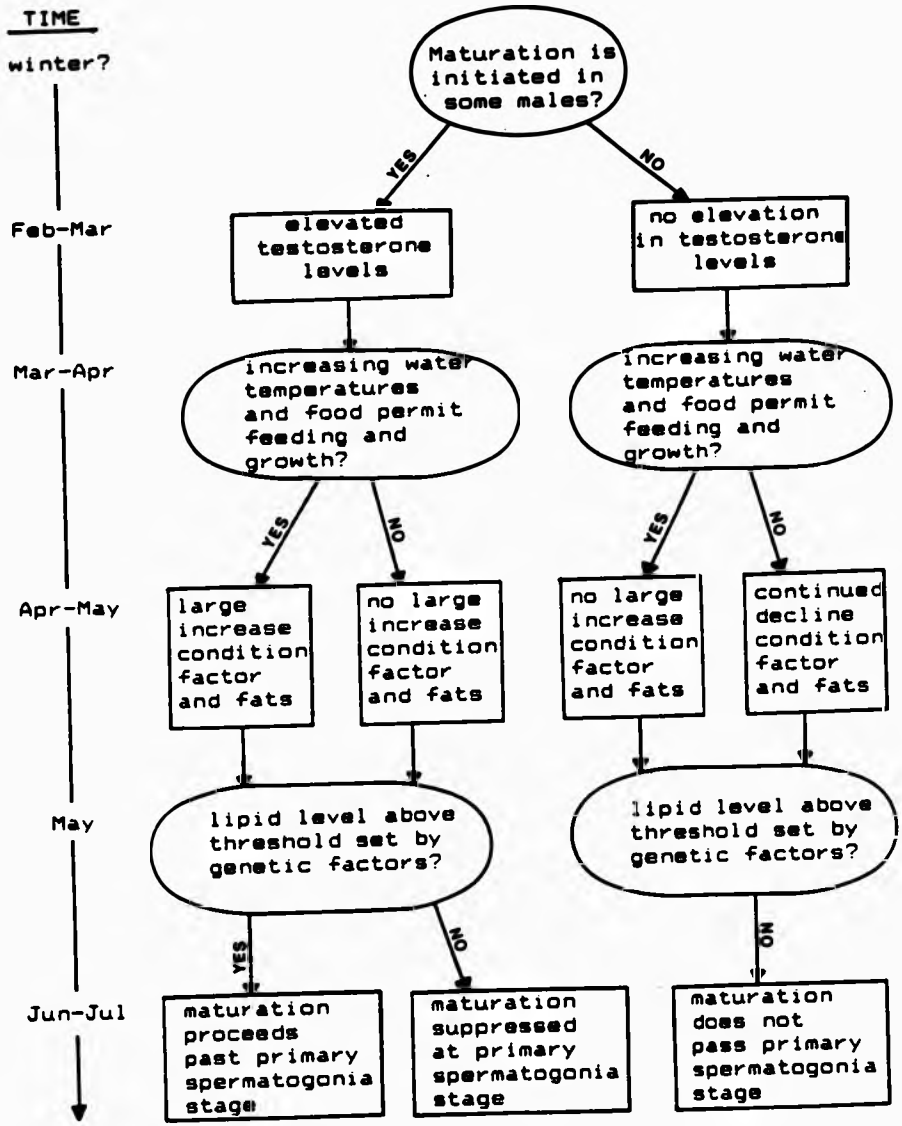
growth in April has a strong bearing on whether or not the mesenteric store will be filling in May.

Food restriction during both winter and spring months suppresses maturation rates of Atlantic salmon grilse, with greatest effect occurring in populations subjected to periods of fasting in late winter to spring (Thorpe et al. 1988). Maturation rates of parr were not suppressed by food restriction during winter, probably because compensation growth by these fish restored fat levels by April and May. A similar effect is believed to account for the failure of food restriction in some winter months to suppress maturation rates of male grilse during 1987/88 (Thorpe et al. 1988). However, this exception aside, winter food restriction suppressed maturation in female grilse in 1987/88, and in males in 1986/87. Suppression was greatest in populations subjected to fasting in February/March and March/April compared with December/January or January/February. The differences in effects of winter fasting on maturation rates of parr and grilse implies either slower replenishment of fat reserves in grilse compared with parr, such that reserves were still below control levels when the maturation decision occurred, or an earlier time for the maturation decision. Support for the latter view is provided by Gwinner (1986) who found that developmental changes occurred earlier in older animals. In this respect Koch & Bergstrom (1978) recorded increases in fat content of re-maturing, and hence older Atlantic salmon

parr, in March. Female salmon require more fat for maturation than males (Idler & Bitners 1960, Randall et al. 1986) and are likely to have a higher threshold for the initiation of maturation. The greater suppression of maturation rates in females by food restriction is consistent with this.

The results obtained in this study indicate that the maturation suppression decision is likely to be left until May, but is sensitive to growth in April through the dynamics of fat deposition. The decision is probably associated with a physiological process linking the level, or rate of increase in fat reserves during May with hormonal events increasing GtH production. Variation in maturation rates between salmon families indicates that there is a strong genetic component to the control of maturation (Thorpe et al. 1983), and it is probable that this sets the threshold for the physiological events, associated with the size or rate of increase in fat stores. This role of spring feeding in the control of maturation in male parr of Atlantic salmon is illustrated in Figure 59. Burton & Idler (1987) reported the existence of a maturation suppression switch, related to nutritional status, in winter flounder (Pseudopleuronectes americanus), and Herbinger (1987) postulated that a maturation "switch" occurs in Atlantic salmon. Results reported here establish its existence in male parr of Atlantic salmon.

Figure 59. A model for the role of growth in the control of maturation in male Atlantic salmon parr.



5.2 PHYSIOLOGICAL MECHANISMS LINKING SPRING FEEDING WITH THE CONTROL OF MATURATION

It is apparent that a decrease in feeding opportunity during April and May suppresses maturation, and it is probable that maturation is "switched off" in many fish because no, or reduced, feeding at this time delays replenishment of body lipids, and the filling of the mesenteric fat store.

Thorpe (1986) proposed that salmon are physiologically aware of their growth rate through acquisition of surplus energy, and the hormone kinetics associated with its storage. He stated that provided this was above a genetically determined threshold in spring months maturation would occur in the following autumn. The conclusions of this study support the underlying principle behind Thorpe's hypothesis, that growth of fat reserves have a controlling effect on maturation. However, it is apparent that the process of maturation is not <u>initiated</u> by growth in fat stores during spring. Rather it is <u>allowed to continue</u>, or is suppressed if growth in fat stores is inadequate.

Further studies of growth and maturation can at best consolidate the predictive link between spring feeding and the control of maturation, and provide more evidence to support the association between reduced lipid deposition in

April-May and suppression of maturation. Despite the potential economic value of such studies to fish farming, acceptance of the hypothesis, and proof of the role of lipid stores in controlling maturation requires an experimental approach based on physiological models for the biochemical mechanism by which fat dynamics affect hormones of the hypothalamic-pituitary-gonadal axis.

McCormick & Naiman (1984) produced such a conceptual model for the effect of growth on maturation of brook trout. However, while attempting to integrate the interplay of genetic effects, growth and photoperiod in determining the initiation of maturation, it is too general to be tested, as it does not indicate how growth might influence hormonal systems controlling maturation, only that it does. A model is outlined here, and shows that a biochemical pathway, linking growth in lipid stores during spring with the hormonal control of maturation, exists in higher vertebrates and could also operate in fish. Such hypothetical models provide the necessary basis for refining and testing hypotheses for the role of fat dynamics during spring on the control of maturation.

Frisch (1988) proposed that levels of estrogens needed for maturation and ovulation in women are in large measure dependent on the size of fat stores. She outlined studies showing a positive correlation between minimum ratios of fat to lean tissue and age of puberty in girls, and

described ovulation problems in anorexic women and women athletes, as well as their resolution following an increase in body fat. On the basis of her studies linking fat levels to maturation and the known ability of human adipose tissue to aromatise testosterone to estrogen, she proposed that the hypothalamic production of gonadotropin releasing hormone (GtRH) is dependent on the levels of estradiol aromatised from testosterone in fatty tissue, and hence on the amount of fatty tissue itself. What is the evidence for the existence of such a mechanism in lower vertebrates, particularly fish such as the Salmonidae in which growth and fat storage also play an important role in maturation?

Siiteri (1987) found extremely high estrogen levels and testosterone aromatisation rates in male squirrel monkeys (Saimiri sciureus) during the breeding season. Males of this species are somewhat unique in that they increase in weight by up to 40% during the breeding season, mainly as a result of the accumulation of upper body fat, giving them their seasonal cushingoid appearance. It is not known what role the high levels of estrogen play in this species' reproduction, but it is clear that aromatisation of testosterones to estrogens occurs in fatty tissue of other vertebrates in which reproduction is linked to storage of fat. This example also shows that high fat contents and aromatisation activity are associated with reproduction in males as well as females.

There are no known reports of aromatisation of testosterone to estrogen in the fatty tissue of male or female fish, however, the aromatase enzyme responsible for the conversion process has been found in the brain tissue of salmonids (Callard 1982; Lambert et al. 1982). Recently Andersson et al. (1988) found it in the brain and pituitary of Atlantic salmon parr, the aromatase activity being greatest in extracts from mature male parr.

In humans most aromatase activity is found in the stromal (interstitial) cells of adipose tissue (Cleland et al. 1983), more occurring in the stromal cells of omental fat than in subcutaneous fat (Ackerman et al. 1981). In female rainbow trout steroidogenesis takes place in both granulosa and stromal cells of the ovary, and aromatase enzymes synthesise estrogens in the ovary from May to August, but not during previtellogenesis in March (Lambert et al. 1978). Thus aromatase activity occurs principally in stromal cells, but the extent of activity is likely to vary depending on the tissue in which the stromal cells occur. It is also apparent that aromatase activity is not constant but varies with the time of year, or season, and with the maturation status of the fish.

Callard (1982) indicated that aromatisation needs to be site specific in fish, and present in the brain or pituitary, as the gills remove most circulating estrogen.

Lambert & van Bohemen (1979) therefore suggested that the brain may be the major source of oestrogen in rainbow trout as preliminary, but unreported results showed that other tissues, including fatty tissue, did not contain aromatising enzymes. Although it is clear that aromatase activity is present in the brain, there is little direct evidence for its absence in other tissues, including fatty tissue. It is apparent that future studies on aromatisation activity in fish need to take into account the type of stromal cells, the time of year, the sex of the fish and its maturational status, before aromatisation in other tissues is excluded as a significant source of estrogen.

This has not been done yet and aromatase activity in the stromal cells of fatty tissue from prepuberal male salmon parr in spring cannot be ruled out. There is however a weight of circumstantial evidence indicating that it is a distinct possibility. The time of maximum aromatase activity is likely to be when seasonal cortisol levels are highest. Simpson et al. (1981) found that aromatase activity was stimulated 20 to 100 fold by glucocorticosteroids including cortisol. Extraordinarily high levels of cortisol (50 to 100 times higher than in humans and old world primates) are found in the squirrel monkey during the breeding season (Klosterman et al. 1986, cited in Siiteri 1987), and Siiteri (1987) reported high

testosterone and estrogen levels at this time. He concluded that the high cortisol and testosterone levels would account for the high plasma estrogen levels in the males during the breeding season. Thorpe et al. (1987) measured seasonal cortisol levels in Atlantic salmon parr and found highest levels in spring months, with a peak in March.

Testosterone is also available as a substrate for aromatisation in Atlantic salmon at this time. Abdullah (1981), Hunt et al. (1982) and Youngson & McLay (1985) have all recorded small peaks in testosterone and 11-ketotestosterone in the blood plasma of Atlantic salmon during February to March. Fish with elevated androgens during this period all matured, whereas most fish not showing such spring peaks did not mature. Yamada et al. (1988) indicated that the head kidney was the major source of testosterone during spring months, with production being relatively high in March, and declining after April. In comparison testosterone production by the gonad was low during spring, and only began to increase in June.

Prematuration peaks in plasma estrogen levels have also been recorded in salmonids during spring months (Billard et al. 1978; Elliot et al. 1984; Sower et al. 1984b; Yamada et al. 1988). There is therefore evidence for spring elevations in plasma cortisol, testosterone and estrogen in prepuberal fish. The testosterone is of extragonadal origin, coming principally from the head kidney, and the

estrogen is likely to be aromatised from this testosterone (Sower et al. 1984b; Yamada et al. 1988).

Peter (1982) and Goos (1987) reviewed evidence for both positive and negative sex steroid feedback on pituitary gonadotropin (GtH) levels. They noted that positive feedback by testosterone was of special importance during the prepuberal period of development. Testosterone implants result in both pituitary synthesis and release of GtH in a number of salmonids, and in male parr of Atlantic salmon stimulate precocious development (Crim & Evans 1982). Furthermore testosterone treatment stimulates GtRH activity in the hypothalamus (Goos et al. 1986). These and other studies show that above normal levels of testosterone influence the hypothalamus and stimulate gonadal development via production and release of GtH by the pituitary. However, testosterone may not be the active hormone, and may in fact provide higher substrate levels for aromatisation to estrogen, which is itself the active hormone. Evidence for this is provided by Crim et al. (1981) who found that only estrogens and aromatizable testosterones stimulated GtH production. An aromatase inhibitor significantly reduced GtH production in response to elevated testosterone, establishing the importance of estrogen in initiating gonad growth. Testosterone is now believed to act primarily as a precursor for estrogen production in female fish (Greeley et al. 1988), and has

also been implicated in the initiation of maturation in males (Peter 1982).

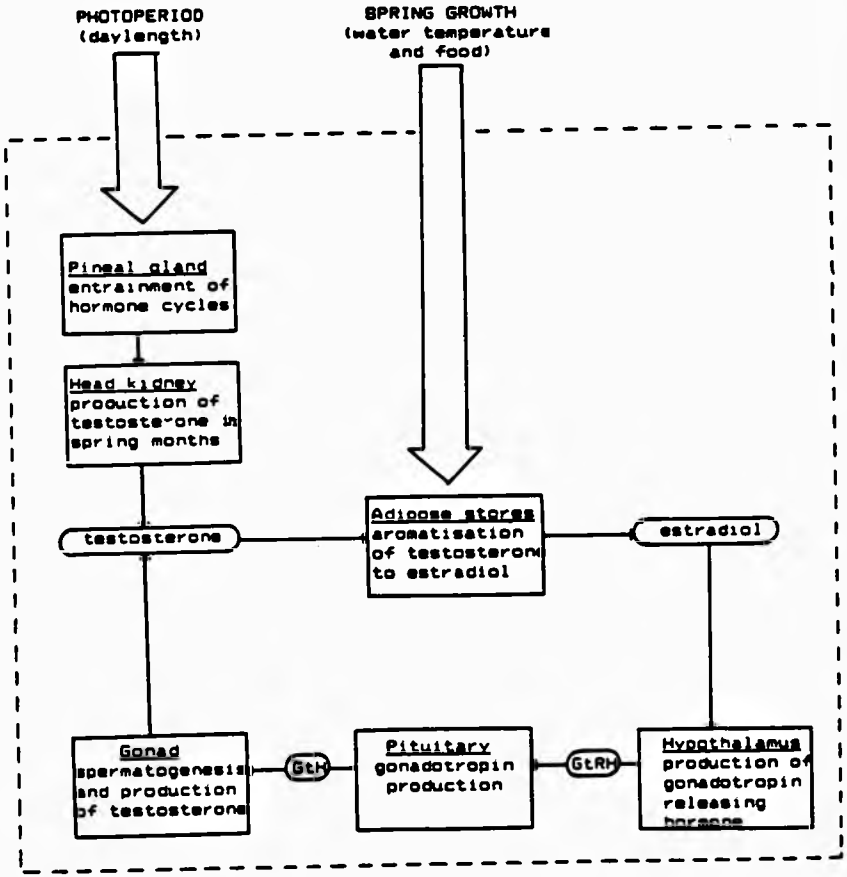
It is clear that aromatisation of testosterone to estrogen is involved in the control (if not initiation) of maturation in Atlantic salmon parr. The seasonal timing of prematuration peaks in testosterone, estrogen, cortisol, aromatase activity and growth in fat reserves in Atlantic salmon parr are all consistent with Frisch's hypothesis, as is the role of estrogen in priming the hypothalamic-pituitary-gonadal axis. What is now at issue is the principal source of aromatised estrogen. A number of workers believe this to be the brain and provide evidence that such activity occurs in parts of this organ. However, other sources of estrogen have not been satisfactorily excluded yet. It is apparent that reduced spring feeding and failure to replenish fat reserves by April to May can suppress maturation. This is likely to occur because the size of fat reserves limits the extent of aromatase activity and hence estrogen production.

It is doubtful that estrogen production is limited by levels of testosterone, or cholesterol. Cholesterol is the precursor for the sex hormones and poor growth during spring may exert control over maturation by limiting concentrations of cholesterol available for synthesis of testosterone in the head kidney. Koch & Bergstrom (1978)

found that cholesterol levels of mature males of Atlantic salmon increase by over 25% from 400mg/100ml to 550mg/100ml in March and April. In comparison cholesterol levels of immature parr remained low at 350mg/100ml. Furthermore, Magri et al. (1985) found that testosterone implants only stimulated maturation in a proportion of male parr of rainbow trout. No control fish matured, indicating that testosterone stimulated maturation in some fish, but not all. It is apparent that lack of testosterone was not a factor in the failure of the rest to mature.

The hypothetical mechanisms by which spring feeding opportunity and hence fat dynamics could exert control over the hormone systems involved in gonad growth are depicted in Figure 60. This model attemps to unify current thinking on the control of maturation in salmonids, developed independently in the respective fields of reproductive endocrinology, genetics, and growth studies. As a theoretical exercise it shows that the hormonal control of maturation is probably influenced by some aspect of fat dynamics. Results reported here show that this influence is restricted in time, occurring primarily in the period April to May.

Figure 60. Hypothetical model for the environmental regulation and control of maturation in Atlantic salmon



5.3 ADAPTIVE SIGNIFICANCE OF A MATURATION SUPPRESSION SWITCH IN SPRING

It is not known what initiates maturation in fish. Fast growth during a particular time of year may well be involved, but it is apparent that much of the general correlation between growth and maturation in salmonids can be explained by the existence of a maturation suppression mechanism, linked to reduced feeding and growth in spring months. The existence of such a switch requires some justification on evolutionary grounds. What is its value to the organism and specifically to its reproductive success, and what selective pressures could have produced it?

Many authors have dealt with the phenomenon of parr maturation and developed theoretical models to explain its evolutionary stability on the basis of size dependent variations in reproductive fitness (Myers 1984; Gross 1985; Leonardsson & Lundberg 1986). Myers (1986) extended some of these arguments to the control of age at maturation in Atlantic salmon, and concluded that in males, age of maturation is linked to its effect on sex ratios and the number of matings possible for anadromous males.

These arguments help to explain the selective forces maintaining the variation in age at maturation, but do not answer the question of how age of maturation is controlled at the level of individual fish. Thorpe (1986) pointed out

that fish cannot make decisions based on the probability of their survival, fecundity or lifetime reproductive fitness. Such judgements are made by population demographers post hoc. and Thorpe(1986) indicated that the motivation for change in metabolic strategy (eg. somatic growth to gonad growth) must arise from proximate cues such as rate of growth in energy reserves.

Policansky (1983) concluded that fish with access to abundant food and stable conditions for growth would mature as soon as they are developmentally able to do so. In some stocks of Atlantic salmon, male parr can mature in their first (0+) year (Sower et al. 1984a; Saunders et al. 1982; results of this study), and it is apparent that maturation can occur at a very early age in many stocks of Atlantic salmon, provided early growth is fast enough. The proximate cue for change is therefore more likely to be one which results in suppression, or delay, of maturation rather than one which initiates it. In this respect the existence of a maturation suppression switch linked to poor growth is likely to be an important adaptive component of the maturation process. The significance of spring as a time for this switch to operate is also likely to be adaptive, but the precise nature of the survival value of the switch and its timing is speculative, and is best considered from the perspective of life history theory.

A central assumption of life history theory is that selective forces will operate to maximize lifetime reproductive fitness. There is therefore a trade-off between present and future reproduction, which is mediated by the risk of mortality (Wootton 1985, Calow 1985). It is therefore reasonable to expect the control of maturation to be influenced by factors which affect the probability of mortality. In autumn-spawning fish, stored fat is depleted during maturation, but is also required for over-winter survival. Fat is likely to be the most important factor controlling maturation in such fish.

Atlantic salmon spawn in early winter months (October-December) when water temperatures in rivers are decreasing. Feeding is minimal (Love 1970) and spawning fish require the prior storage of sufficient fat reserves to support the metabolic demands of migration upriver, gonad differentiation, the elaboration of secondary sexual characteristics and spawning behaviour. Atlantic salmon are iteroparous, compared with the semelparous Pacific salmon species, and post-spawning survival is possible if spent fish are able to feed (Johnston et al. 1987). However, spawning stocks contain few (10%) kelts (Ducharme 1969), indicating a high mortality of post spawned fish, and for most fish a precarious balance between stored energy and that expended on reproduction. Maturation without sufficient lipid stores reduces gonad size (see Chapter 3), and is likely to increase the probability of

post spawning mortality in parr, so reducing total reproductive fitness. A maturation suppression switch would prevent salmon from maturing when the cost of doing so will increase their probability of mortality, above a level set by the compromise to attain optimum reproductive fitness.

In salmon a decision to commit resources to maturation occurs well before the spawning season, and current evidence points to this being in winter, before the growing season during which fat stores, required for maturation and overwintering, must be accumulated. However control over maturation also occurs in spring, when it is suppressed by inadequated feeding and reduced fat deposition. The question arises as to why the decision to mature is made in spring. What is the significance of spring growth for the summer accumulation of fat stores required to complete spawning without a high risk of mortality?

Cohen (1967) examined the theoretical reproductive strategies for optimizing reproduction in variable environments. He concluded that, when correlations exist between reproductive success and conditions at the time the decision to mature is made, an organism's decision to mature or not will be made by using available information, about forthcoming environmental conditions, as a predictor of reproductive success (Giesel 1976). This implies that selection processes in variable environments will lead to a

switching mechanism, based on the variable(s) which best predict future environmental conditions important for successful reproduction. Such a switch has been found for Atlantic salmon, and fat stores are likely to be the most important variable affecting reproductive fitness. I propose that this switch operates in spring months because future growing conditions, affecting the size of fat stores, are influenced primarily by the length of the growing season, and this would be reduced by a late spring. In this context growth in spring months would be indicative of an average growing season, and a high probability of acquiring the resources required for maturation. Lack of growth in spring would indicate a shorter than average growing season and a low probability of accumulating the necessary resources. Fat is likely to be the variable used to assess growth at this time.

The growth of fish is influenced by many physical and biotic variables such as water temperature, light levels, fish density, prey density, and interference by predators, all of which can quickly change in both space and time. Nevertheless, fish have a remarkable ability to compensate for short term variations in their food supply. Salmon may avoid regions of low water temperature in the sea (Saunders et al. 1983), and move from regions of low to high food density (Thorpe 1988). Compensation growth also occurs in Atlantic salmon parr and grilse, and serves to even out

fine scale variations in the environmental and biotic factors affecting growth. Thus salmon can recover from short term restrictions on growth, caused by a variety of physical and biotic variables. However, Thorpe et al. (in press) found growth of salmon parr was more accurately predicted when they incorporated a time component (hours of light during which salmon feed), into estimates of degree-days, previously used as a measure of the quality of a growing season. An important variable influencing fish growth over the entire growing season, and over which they have little control, is thus likely to be time, or the length of the growing season, determined by seasonal changes in water temperatures and light levels.

A short growing season, produced by the low water temperatures of a long winter and late spring, would limit the time for growth and may restrict the growth of fat reserves needed for maturation. Conversely an average or early start to the growing season would not limit growth in fat reserves and would not compromise reproductive success.

Ivanova & Volodin (1981) found that the rate of sexual maturation in populations of the smelt (Osmerus eperlanus) was subject to phenotypic variation, and was largely influenced by physico-geographical features. They concluded that water temperature during the period of larval development when maturation is initiated was the most important factor affecting early growth and hence

maturation. Dempson et al. (1986) examined the relationship between water temperature and age of 1st maturation for Atlantic salmon. They concluded that ocean temperatures had no effect on age of 1st maturation, but they only considered temperatures for July and August. A number of other reports indicate the importance of water temperatures in winter or spring for the maturation of this species. Saunders et al. (1983) suggested that low winter temperatures could explain reduced maturation rates of cage-reared grilse, compared with sea ranched salmon of the same stock, but acknowledged that the different foods and feeding regimes could also explain this difference. Scarnecchia (1983) found that maturation rates of grilse were directly related to ocean temperatures, and concomitant data on oceanographic conditions indicated that poor yields of salmon followed cold springs (Thorpe 1983). Naevdal (1984) also reported low incidences of mature salmon in breeding seasons following cold winters. Saunders (1986b) reviewed the effects of low water temperatures on salmonid culture, and suggested that low winter temperatures may inhibit maturation, and account for the increasing proportion of grilse to multi-sea-winter (MSW) salmon from northern to southern latitudes. A non-grilse strain of Norwegian salmon produced 50-70% grilse when grown in sea cages in Brittany, and the grilse/MSW ratio in Norwegian sea farms was reported to vary from year to year, with fewer grilse being produced following colder winters.

These reports show that there is a correlation between low water temperatures in winter or spring and suppression of maturation. This correlation is consistent with a spring maturation switch, which serves as an adaptation to prevent maturation in short growing seasons, when the probability of acquiring resources for reproduction and some chance of continued survival is low.

In an evolutionary sense the switch provides salmon with the means to estimate the length of the growing season. A long winter, and colder than normal water temperatures during spring, will reduce time for growth, and are likely to provide the best basis for assessing the length of the growing season, and hence the size of energy reserves by mid-summer, when GSIs increase. However, this does not imply that grilse percentages in all wild fisheries will be decreased following cold sea temperatures during spring. Martin & Mitchell (1985) found grilse numbers actually increased in years when water temperatures were lower than average. However, low annual average temperatures may be more indicative of a poor summer, rather than a colder winter and later spring. Martin & Mitchell (1985) indicated that their finding could also be explained by the colder northerly waters restricting salmon to more southerly and warmer waters, where growth would be better than average.

At a physiological level the presence or absence of spring growth is likely to be assessed by endogenously regulated elevations in plasma testosterone, in fish already primed to mature. The spring elevations in testosterone levels noted for prepuberal Atlantic salmon (Abdullah 1981; Hunt et al. 1982; Youngson & McLay 1985), masu salmon (Yamada et al. 1988) and rainbow trout (Elliot et al. 1984) are likely to be produced by the head kidney (Yamada et al. 1988), and are believed to have a functional significance (Elliot et al. 1984). They may prime steroid receptors (Lam & Munro 1987), or they may act anabolically. Low pharmacological doses of methyltestosterone increase growth in weight (Saunders et al. 1977), and diethylstilbestrol raises plasma lipid levels in salmonids (Takashima et al. 1972). Estrogens increased lipid deposition in fish at low temperatures and at long or intermediate photoperiods (12L:12D) (de Vlaming et al. 1977), and it is conceivable that the spring elevations in testosterone stimulate appetite and growth in maturing fish, while estrogens, aromatised from the testosterone, increase fat levels. If aromatisation activity occurs in fatty tissues of salmonids, then a positive feedback loop between growth in lipid stores and hormone levels would be created, the higher estrogen levels leading to stimulation of the hypothalamic-pituitary-gonadal axis and gonad growth (See Fig. 60). However, if feeding and growth are minimal or delayed until April, then the spring elevations in testosterone levels will be of little use. Testosterone

production from the head kidney declines in April (Yamada et al. 1988), and its priming effect on growth and fat stores will be too late. Estrogen levels will remain low and maturation will not proceed. The April decline in testosterone production is likely to be set by endogenous rhythms entrained by photoperiod and should be independent of temperature. It probably sets the time base against which the onset of growth is measured.

Spring elevations in testosterone production could therefore provide a way by which fish assess whether growth is occuring, and therefore the length of the growing season, and the probability of acquiring sufficient fat reserves for spawning, and some chance of post spawning survival. In this context, the growth response in spring is primed to ensure increased lipid deposition, higher estrogen levels, and through these changes, maturation. If growth is not possible, then the positive feedback relationship between fat reserves and hormones will not begin, and maturation is effectively suppressed.

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APPENDIX 1.

FOOD RESTRICTION EXPERIMENT
FAT CONTENT ANALYSIS

An experiment was conducted to suppress maturation of PS2 parr by subjecting them to periods of fasting at various times of the year. The fish in each of seven tanks of 189 parr, were fasted during alternate weeks over an eight week period, marked with an "S" in Table 1. Fish in an eighth tank were not starved and served as a control.

Table 1 Design of experiment to control seasonal growth by fasting (numbers are parr sampled for lipid analysis).

		Tank No.							
Date	Week	1	2	3	4	9	10	11	12
16 Nov	1	24	22						
	2		S						
	3								
7 Dec	4		S						
	5			20					
	6		S	S					
	7								
4 Jan	8		S	S					
	9		21		20*				
	10			S	S				
	11								
1 Feb	12			S	S				
	13			20		20*			
	14	20			S	S			
	15								
	16				S	S			
7 Mar	17				20		20*		
	18					S	S		
	19								
	20					S	S		
4 Apr	21					20		20*	
	22						S	S	
	23	20							
	24						S	S	
2 May	25						20		20[a]
	26							S	S
	27								
	28							S	S
	29		20*					22	
6 Jun	30								S
	31								
	32								S
	33								
4 Jul	34	21[a]	20	20	20	20	20	20	20

While the fish were being fed they received more than 4% body weight of food per day by being fed every fifteen minutes.

Approximately 20 parr were sampled for fat analysis at the beginning and end of treatment periods (Table 1). As the parr grew, fat content increased, particularly in early summer (Table 2).

Table 2 Number of observations and range of fish weight (g) and fat content (g) in each data set.

Tank	Date	Number	Weight range	Range of fat content
1	16/11/87	24	1.39 - 6.19	0.0662 - 0.5572
2	16/11/87	22	1.34 - 4.95	0.0652 - 0.3650
3	14/12/87	20	1.60 - 6.90	0.0817 - 0.5390
2	11/1/88	21	1.23 - 7.74	0.0478 - 0.4743
4	11/1/88	20	1.65 - 9.71	0.0617 - 0.6990
3	8/2/88	20	1.75 - 9.26	0.0780 - 0.8783
9	8/2/88	20	1.27 - 9.63	0.0590 - 0.8308
1	19/2/88	20	1.57 - 5.42	0.0660 - 0.3957
4	7/3/88	20	1.54 - 5.82	0.0863 - 0.4655
10	7/3/88	20	1.33 - 6.61	0.0792 - 0.4078
9	4/4/88	20	1.77 - 6.90	0.0788 - 0.4607
11	4/4/88	20	1.44 - 11.34	0.0575 - 0.7745
1	18/4/88	20	2.14 - 10.35	0.1177 - 0.7878
10	2/5/88	20	1.58 - 7.61	0.0747 - 0.6073
12	2/5/88	20	2.12 - 8.14	0.0907 - 0.6855
2	1/6/88	20	1.71 - 14.93	0.0652 - 1.6535
11	1/6/88	22	2.77 - 12.96	0.0892 - 0.7597
1	4/7/88	21	6.93 - 21.87	0.5490 - 2.4692
2	4/7/88	20*	12.49 - 24.00	1.1029 - 2.5576
3	4/7/88	20	6.94 - 18.59	0.5993 - 2.2985
4	4/7/88	20	9.52 - 22.19	0.8910 - 2.6577
9	4/7/88	20	7.66 - 25.25	0.7123 - 2.8338
10	4/7/88	20*	7.72 - 24.26	0.7033 - 3.0799
11	4/7/88	20	5.73 - 16.20	0.5138 - 2.1440
12	4/7/88	20	4.33 - 15.46	0.3247 - 1.8025

* twenty parr were sampled but fat content was obtained for only nineteen.

Fat content also increased with fish size on any given date (Figs. 1-5), but the exact form of the relationship was less clearly defined.

Consideration was given to the best way of analysing fat content. Knowing that weight of fat was some proportion of total fish weight, a number of options were possible, depending on whether the relationship between fish weight and fat content was linear or curvilinear:

(1) divide fat weight(y) by total fish weight(x), and analyse y/x
(2) divide fat weight by fish weight minus fat weight, and analyse y/(x-y)
(3) divide fat weight by fish weight raised to a power (b), and analyse y/x^b
(4) divide fat weight by fish weight minus fat weight raised to a power, and analyse $y/(x-y)^b$

The first option above implies that weight of fat is proportional to fish weight (ie. that the relationship between y and x is linear, and passes through the origin), while the second implies that fat weight is proportional to all other tissues. Neither of these were borne out by the data. The third and fourth options imply that fat weight is proportional to some power of fish weight and to some power of non-fatty tissue respectively.

For many of the data sets a linear relationship would be adequate, but for a few, particularly those which include heavier parr, a curvilinear relationship would provide a closer fit (Figs. 1-5). It is not uncommon to find this when samples are taken over a growth period. The relationship over the entire experimental period was therefore examined for the control fish, in Tank 1. The slope of the regression of log fat content on log fish weight was significantly greater than unity, indicating that the relationship between weight of fat and fish weight is curvilinear. This corressponds to option 3 above, and so analyses were made on log transformed data for each of the 25 data sets (Table 3).

Slopes of the regressions for the experimental populations (ie. Tanks 2-12) did not differ significantly, although their intercepts did ($p<0.001$). An analysis of covariance on the results from these experimental tanks was carried out to test for differences in fat contents pre- and post-starvation, and for the effects of fasting, during different months, on fat content by mid-summer (ie. July).

Fat content was expected to decline during winter months, and to increase with increasing water temperatures during spring. Any differences in fat content between pre- and post-starvation would reflect this seasonal variation, so another analysis compared the post-starvation data from each experimental population with a seasonal control data set. This control set was taken at the same time from another tank in which the parr had not been subjected to periods of fasting (see * in Table 1). The post-starvation results from tank 11 taken on 1 June, were compared with those from tank 2, also taken at the same time, as it was felt that effects of fasting on parr in tank 2 would be least apparent by this time.

Table 3 estimates of slope and intercept, and correlation coefficient from the logarithmic regressions of fat weight on fish weight.

Tank	Date	Number	Intercept	Slope	Correlation
1	16/11/87	24	-1.2663	1.2575	0.986
2	16/11/87	22	-1.2902	1.2122	0.968
3	14/12/87	20	-1.3801	1.3300	0.975
2	11/1/88	21	-1.4098	1.2233	0.977
4	11/1/88	20	-1.4486	1.2996	0.971
3	8/2/88	20	-1.3023	1.1945	0.939
9	8/2/88	20	-1.3021	1.2239	0.976
1	19/2/88	20	-1.3697	1.2584	0.963
4	7/3/88	20	-1.3543	1.1554	0.916
10	7/3/88	20	-1.2321	1.0220	0.893
9	4/4/88	20	-1.4959	1.2981	0.933
11	4/4/88	20	-1.3824	1.2364	0.952
1	18/4/88	20	-1.3317	1.1381	0.901
10	2/5/88	20	-1.3907	1.1081	0.894
12	2/5/88	20	-1.4855	1.4205	0.967
2	1/6/88	20	-1.3143	1.3020	0.973
11	1/6/88	22	-1.6128	1.4040	0.979
1	4/7/88	21	-1.3167	1.2981	0.972
2	4/7/88	19	-1.1401	1.1541	0.924
3	4/7/88	20	-1.1979	1.2152	0.967
4	4/7/88	20	-1.1407	1.1614	0.967
9	4/7/88	20	-1.1118	1.1128	0.972
10	4/7/88	19	-1.3601	1.3163	0.981
11	4/7/88	20	-1.1920	1.1848	0.980
12	4/7/88	20	-1.2423	1.2582	0.979

Differences in fat content pre- and post-starvation

On most occasions samples of twenty parr were examined both pre- and post-starvation from the seven experimental tanks. However, on three occasions more than twenty individuals were examined and so to achieve balance in the the analysis of covariance, and to make computations considerably easier, these data sets were reduced to twenty by the random exclusion of fish.

There were significant differences in the average fat content, between both pre- and post-starvation samples, and between tanks. However, the interaction was also highly significant, indicating that the differences were not consistent (Table 4). This lack of consistency is likely to be due to the effects of seasonal variations on fat contents, and there is therefore no simple way of summarizing these comparisons.

The pooled slope of 1.2323 (s.e.$\pm$ 0.0247) was used to calculate a revised intercept for each sample (Table 5). As the overall mean

weight of fish sampled was approximately 3.8g, the fitted fat content corressponding to this weight was calculated for both pre- and post-starvation samples to highlight differences in fat content between populations (Table 6).

Clearly the effect of four week-long periods of starvation is quite different from tank to tank. Tank 12 stands out from all others, probably because it was sampled 1 week, rather than 2-3 days, after the last period of fasting, allowing greater recovery from starvation. As this may invalidate any comparison between tank 12 and any other tank, the analysis of covariance was repeated excluding tank 12. (Table 7). The conclusions drawn from the previous analysis remain unchanged.

Table 4 ANCOVA for relationships between log fat weight and log fish weight for the experimental tanks

	df	$S(y^2)$	$S(xy)$	$S(x^2)$
Between pre- and post-starvation	1	0.38051	0.49427	0.64205
Between tanks	6	6.05508	4.11809	3.03296
Interaction	6	4.01768	2.26215	1.49763
Residual	266	15.62087	11.44675	9.28888
Total	279	26.07414	18.32126	14.46152

	df	S of S	ms	vr
Between pre- and post- adjusted	1	0.12845	0.12845	22.47***
Between tanks adjusted	6	0.49962	0.08327	14.57***
Interaction adjusted	6	0.70054	0.11676	20.42***
Residual adjusted	265	1.51496	0.005717	

Table 5 Calculated intercepts using a common slope of 1.2323 for each experimental sample.

Tank	Fasting period	Pre-starvation	Post-starvation
2	Nov-Dec	-1.2996	-1.4205
3	Dec-Jan	-1.3280	-1.3215
4	Jan-Feb	-1.4091	-1.3935
9	Feb-Mar	-1.3063	-1.4602
10	Mar-Apr	-1.3363	-1.4586
11	Apr-May	-1.3801	-1.4699
12	May-Jun	-1.3716	-1.2172

Table 6 Fitted fat contents pre- and post-starvation for each experimental tank

Tank	Fasting period	Pre	Post	Percentage change
2	Nov-Dec	0.2639	0.1997	-24%
3	Dec-Jan	0.2471	0.2509	2%
4	Jan-Feb	0.2050	0.2125	4%
9	Feb-Mar	0.2598	0.1823	-30%
10	Mar-Apr	0.2425	0.1830	-25%
11	Apr-May	0.2192	0.1783	-19%
12	May-Jun	0.2235	0.3190	43%

Table 7 ANCOVA as in Table 4 but excluding Tank 12

	df	$S(y^2)$	$S(xy)$	$S(x^2)$
Between pre- and post-starvation	1	0.01199	-0.04355	0.15821
Between tanks	5	0.97176	0.93065	1.03430
Interaction	5	0.77540	0.62290	0.66886
Residual	228	13.46971	9.94580	8.17519
Total	239	15.22886	11.45580	10.03656

	df	S of S	ms	vr
Between pre- and post- adjusted	1	0.34543	0.34543	57.24***
Between tanks adjusted	5	0.22651	0.04530	7.51***
Interaction adjusted	5	0.24562	0.04912	8.14***
Residual adjusted	227	1.36982	0.006034	

The pooled slope from the analysis excluding tank 12 was 1.2166 (s.e. $\pm$ 0.0272). The intercepts from this pooled slope, and the percentage changes in fat content post-starvation in a 3.8g parr differed only slightly (by less than 1%) from those in the first analysis.

Fat contents of experimental populations in mid-summer

Fat content on the final sampling occasion (4 Jul 1988) was also examined by analysis of covariance. All eight tanks were compared and significant differences ($p<0.05$) were found between tanks (Table 8).

Table 8 ANCOVA of fat weight and body weight for all populations on 4 July 1988

	df	S(y^2)	S(xy)	S(x^2)
Between tanks	7	1.79375	1.54475	1.34742
Within tanks	151	3.71531	2.88647	2.37735
Total	158	5.50906	4.43122	3.72477

	df	S of S	ms	vr
between tanks adjusted	7	0.02671	0.00382	2.72 *
Within tanks adjusted	150	0.21069	0.001405	
Total adjusted	157	0.23740		

Mean fat content for each tank was adjusted using the pooled slope of 1.2142 derived from the analysis of covariance (Table 9). A range of means is displayed with the largest being judged to be significantly different from the smallest. Fat content of fish subjected to fasting in the months February to May were lower than the control value, whereas fasting during December to February resuled in higher values.

Table 9 Adjusted mean fat content of parr in each tank by mid-summer(July).

Tank	Fasting period	Adjusted weight of fat (g)
1	Control	1.5035
2	Nov-Dec	1.5192
3	Dec-Jan	1.5798
4	Jan-Feb	1.5478
9	Feb-Mar	1.4495
10	Mar-Apr	1.4488
11	Apr-May	1.4880
12	May-Jun	1.5690

Comparisons between post-starvation fat content and seasonal controls

There were significant differences between fat contents for the post-starvation samples and the seasonal control, and the interaction component was highly significant, indicating that these differences were not constant in time (Table 10).

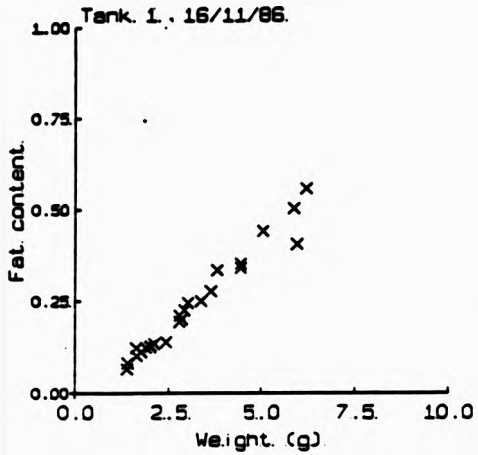
Table 10 ANCOVA for fat contents of post-starvation and seasonal control parr

	df	S(y²)	S(xy)	S(x²)	
Between seas. control and post	1	0.69208	0.15766	0.03592	
Between times	5	4.73704	3.52541	2.83659	
Interaction	5	0.30671	0.05924	0.07281	
Residual	228	14.96383	10.92036	8.84278	
Total	239	20.69966	14.66267	11.78810	
	df	**S of S**	**ms**	**vr**	
Between seas. control and post	1	0.35601	0.35601	54.69	***
Between times adjusted	5	0.35567	0.07113	10.93	***
Interaction adjusted	5	0.27133	0.05427	8.34	***
Residual adjusted	227	1.47777	0.00651		

The pooled slope from the above analysis was 1.2350 (S.E. ±0.027) and the average fat content of a 3.8g parr was calculated for both the post-starvation and seasonal control samples, using this value (Table 11). The standard error of the difference between two fitted means is 0.0255.

Table 11 Fitted fat contents for seasonal controls and post starvation parr.

Time	seasonal control		post-starvation		difference
	tank	fat(g)	tank	fat(g)	(%)
11/1/88	4	0.2054	2	0.2003	-2%
8/2/88	9	0.2604	3	0.2515	-3%
7/3/88	10	0.2431	4	0.2131	-12%
4/4/88	11	0.2197	9	0.1827	-17%
2/5/88	12	0.2240	10	0.1834	-18%
1/6/88	2	0.2913	11	0.1784	-39%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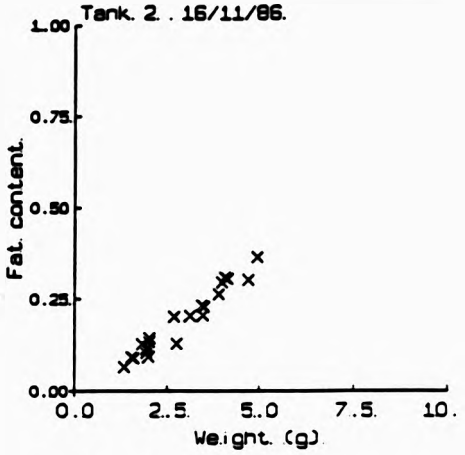
Weight. (g).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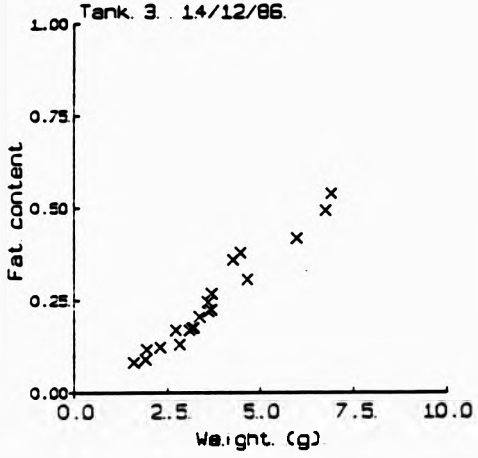
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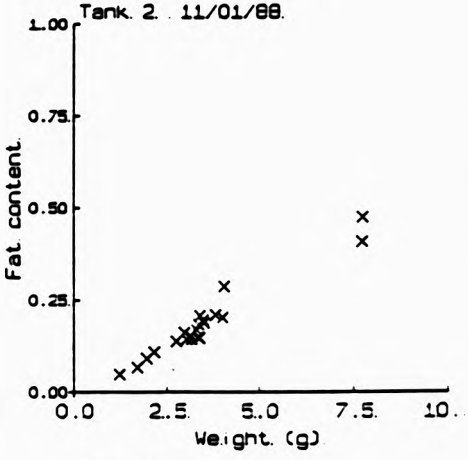
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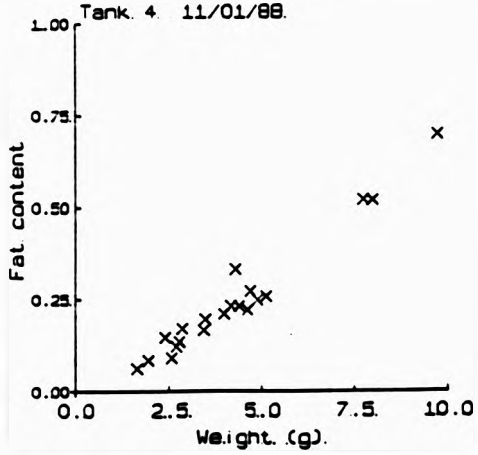
Weight. (g)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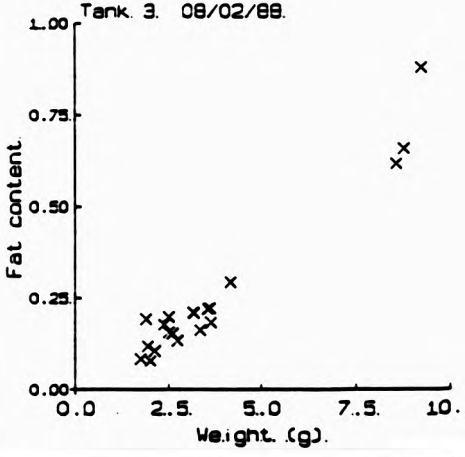
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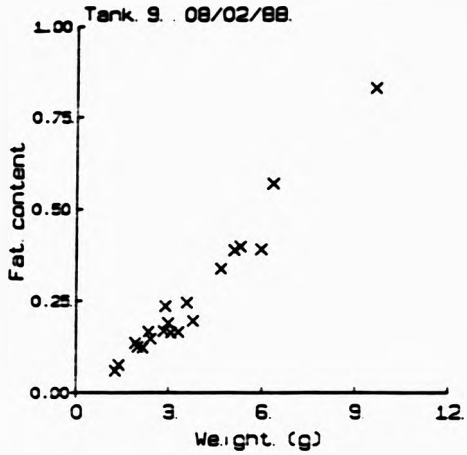
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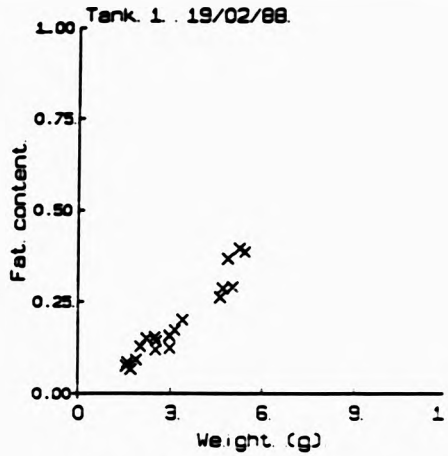
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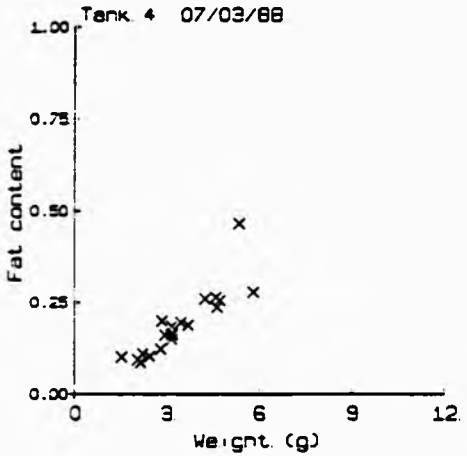
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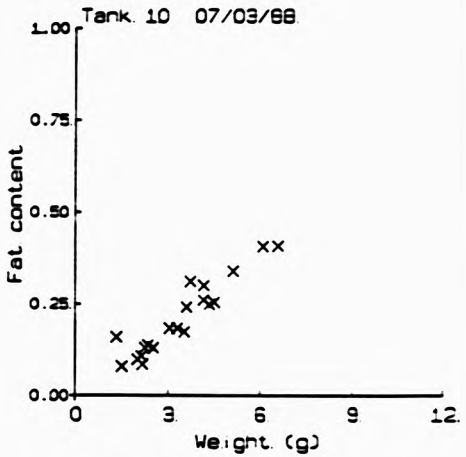
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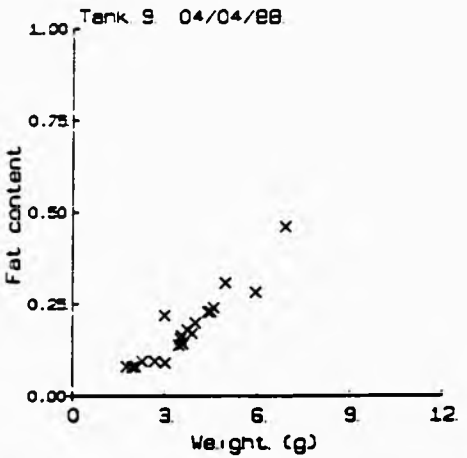
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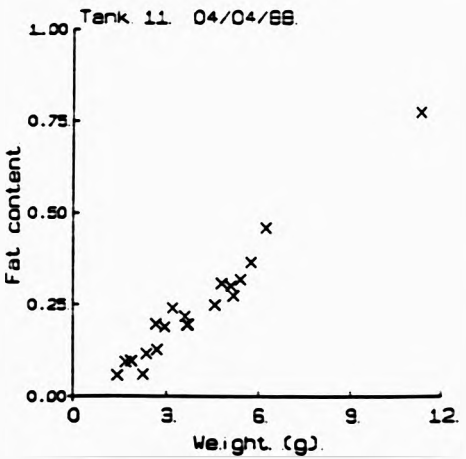
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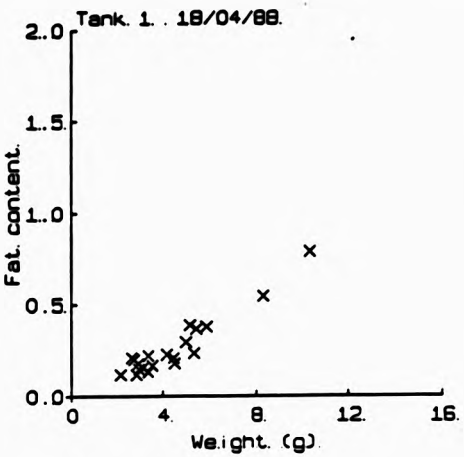
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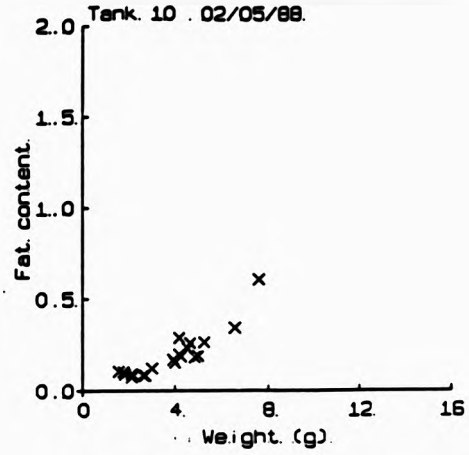
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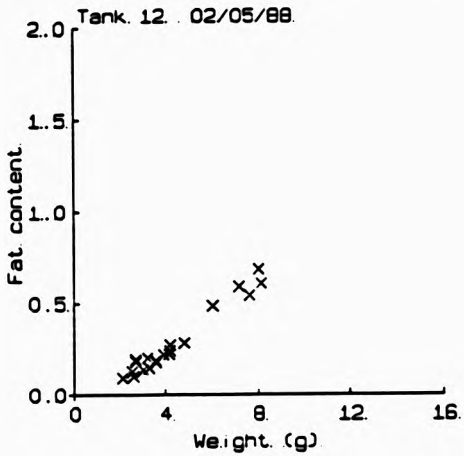
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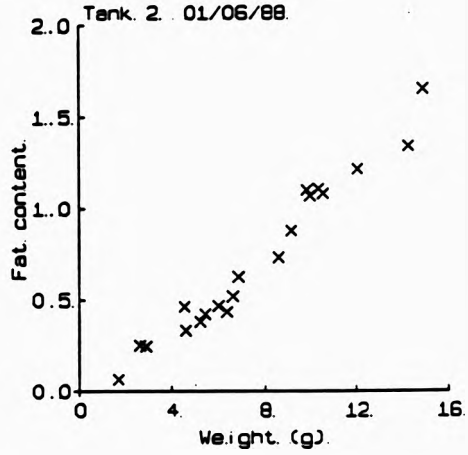
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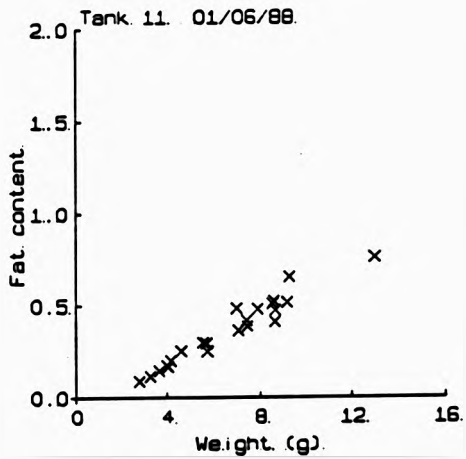
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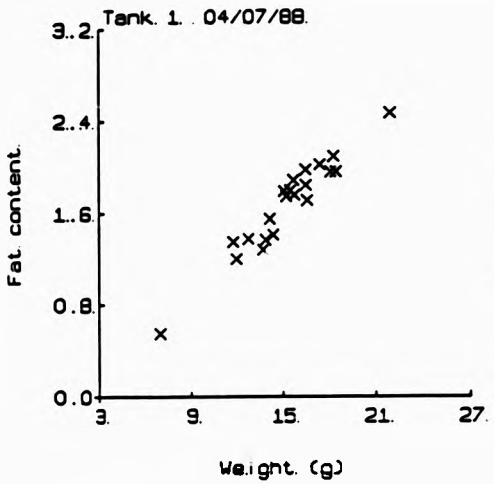
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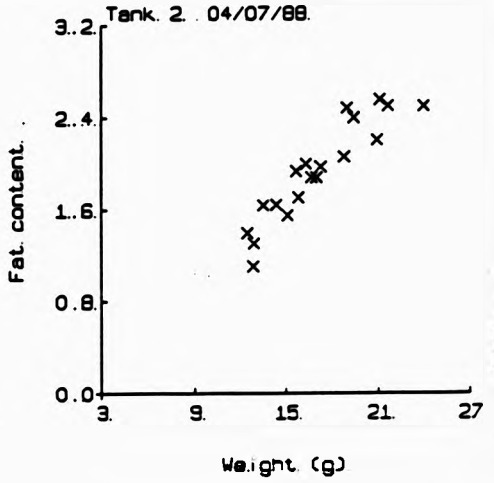
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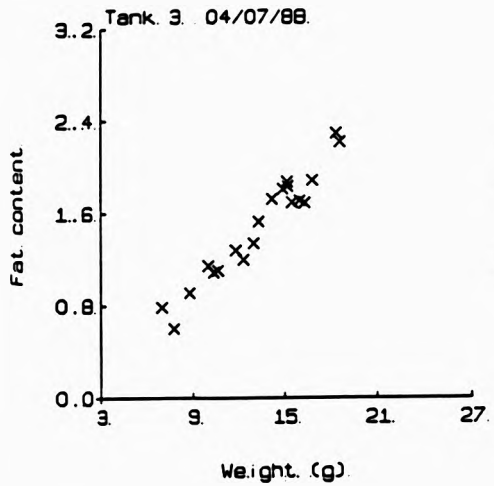
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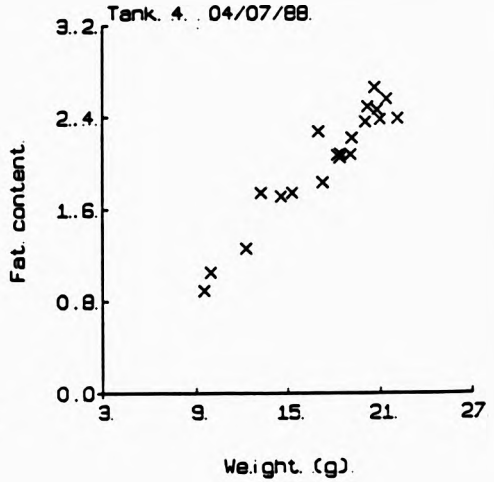
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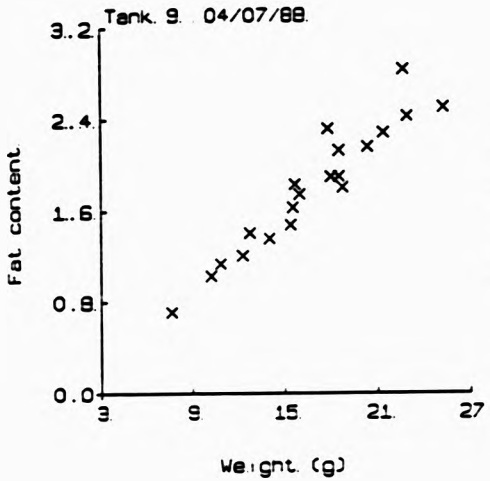
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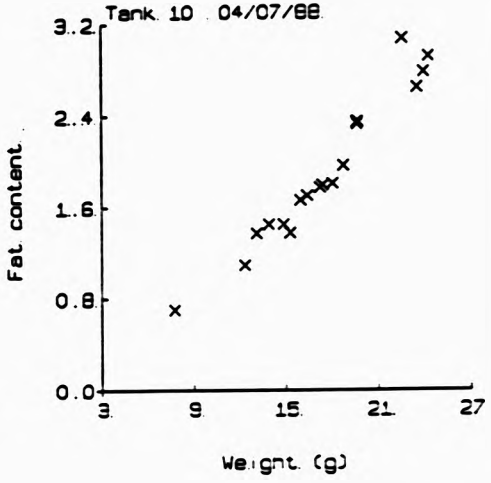
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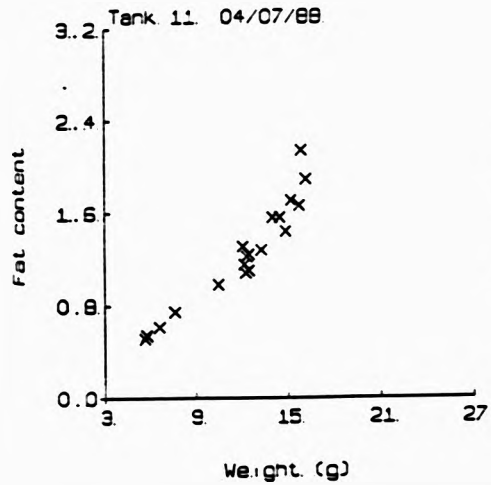
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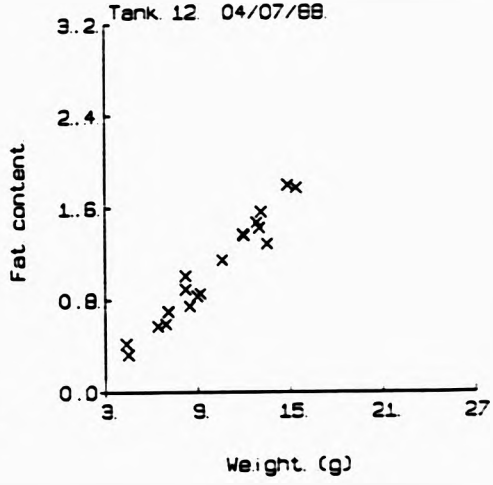
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