Investigation of the factors influencing maturation in Atlantic salmon, *Salmo salar* L., parr.

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Declaration.

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

Anna L. Simpson
Sept. 1993

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Summary

This thesis investigates the factors influencing maturation in Atlantic salmon (*salmo salar* L.) parr. Experiments were designed to investigate the relative effects of size and lipid levels as determinants of maturation within sibling groups. The possibility of using these factors to predict which fish would mature and of using them to control maturation rates was also considered.

Chapter 2 - Previous work had implicated lipid levels in the control of maturation. Therefore it was necessary to develop a non-destructive method for estimating the fat content of live salmon parr. Two non-destructive methods were used to predict fat content. Total body electrical conductivity (TOBEC) gave a good prediction of lean mass in fish, of wet weights 13.7-151.8g, but could only be used together with wet weight and fork length to accurately predict fat in fish of above 30g. However, the increase in accuracy produced by using TOBEC was small and it was concluded that this technique was not suitable for use in subsequent experiments involving small salmon parr.

The biometric method, based on simple body measurements, accurately predicted the fat content of different sized fish (wet weights 1.8-151.8g) at two times of year (November/December and July). Multiple regression equations for fat prediction were based on a combination of the following measurements: wet weight, fork length, opercular height, dorsal height, opercular width, dorsal width, anal width, adipose fin length and condition factor. In every case predictions based on multiple regression equations were more accurate than condition factor alone. This biometric method is easily employed and can potentially be used both in biological studies and on fish farms to assess body condition of individual fish. Since it is non-destructive, it has the additional advantage of allowing repeated monitoring of fat levels in the same fish, and was therefore used in subsequent experiments in this thesis.

Chapter 3 - Previous work had indicated that growth rates during late winter / early spring were influential in determining maturation rates. This chapter comprises three experiments in which monthly measurements of body size and lipid levels of a group of sibling 0+ salmon parr were made. In the first experiment, the variables were monitored from January to October 1990. In the second, the lipid level of the diet was manipulated over the early spring period (from January to April 1991) at three treatment levels (Low fat = 7-10%; Medium fat = 12-15%; High fat = 17-22%) to try and influence maturation rates. From May to September (1991) all fish were given a commercial fish food (15-17% fat). In the third experiment a starvation regime was used to try and influence maturation rates. One group of fish (sibling 0+ parr) was fed a reduced ration every fourth week during November and December 1991. Rations were increased gradually from January to March 1992 and from April to September all fish were fed the same ration.

Neither of the above treatments were successful in reducing the maturation rates of male parr. Those males that subsequently matured as parr were larger and had a higher fat content than non-maturing males in November of their first year. Neither specific growth rate nor rate of change in fat over the experimental period seemed to be important for maturation. Fat levels were positively correlated with fork length in most months with maturing males tending to have a higher percent fat for a given length than non-maturing males. This relationship became negative towards the breeding season. Logistic regressions based on fat reserves and body size could be used during the winter to predict the probability that a male parr would mature the following autumn.

From these experiments it was concluded that maturation must begin prior to the first autumn and therefore experiments designed to reduce maturation rates must target the late summer/early autumn period.

Chapter 4 - Previous work had shown that maturing parr tended to be larger than their non-maturing siblings. Since one way of growing larger is by consuming more food, it seemed sensible to look at the appetite of these fish. Therefore, the appetite of parr from the three experiments in Chapter 3 was

monitored by two different techniques; behavioural observation and radiography. In all experiments there were no differences in appetite between maturing and non-maturing fish. All results showed a similar peak in appetite in May which did not correspond to a peak in temperature. May was the first month that the temperature rose above the lower critical level for activity in juvenile salmon. It was suggested that appetite peaks in May due to the rise in temperature allowing greater activity and therefore feeding. The decline after May was unexplained since temperatures remained suitable for feeding. The hypothesis that this decline has evolved to enable the fish to survive a period of low prey abundance in the natural stream habitat was tested in Chapter 6.

Chapter 5 - Recent evidence had shown that the gonads of salmon in their first year in sea cages began growing a year before the time of spawning. Therefore, the gonad growth of salmon parr was investigated in the year preceding maturation. The size and gonadosomatic index (G.S.I.) were measured from 100 0+ parr each month from October to May. Testis were examined histologically for signs of spermatogenetic activity. The gonad growth of male parr seemed to begin between October and November, a year before any spawning activity would take place. It was difficult to tell with any certainty, from either G.S.I. or histological evidence which fish would have matured the following autumn. Male G.S.I. was positively correlated with fork length in all months. However, G.S.I. was not a good indicator of stage of maturity until April and May. March was the first month in which almost half of the slides examined had initiated spermatogenesis. This experiment confirmed the conclusion from Chapter 3 that the maturation process must begin prior to the first autumn.

Chapter 6 - Appetite results (Chapter 4) had shown an unexplained decline through the summer months. This chapter looks at fluctuations in the availability of natural prey by investigating changes in invertebrate drift (a main source of prey items for wild juvenile salmonids) during the summer months.

Samples of invertebrate drift were collected each month from May to August. Organisms were identified and their lengths and widths measured. There were about four times as many organisms

drifting during twilight than during the day. Those drifting during twilight were larger (both by length and width) than those drifting during the day. There was a significant decrease in both the total number and number of optimally sized prey items from June to August. It was suggested that this decrease over the summer months, when temperatures are increasing would increase feeding costs and reduce returns, thereby making the reduction in appetite found in Chapter 4 adaptive.

Chapter 7 - Throughout this thesis the sex of parr had to be determined and the only simple method available was dissection. This chapter investigates the possibility of using various head measurements as a non-destructive method of sex determination.

Head morphometrics taken from photographs of 1+ parr were used to predict sex and maturity. Sex, irrespective of maturity could be predicted with 73% accuracy. Greater accuracy (93%) was obtained in identifying maturing male parr. Within immature fish, males could be distinguished from females with an increase of only 27% from that gained by chance. Discriminating characteristics of males were their shorter, wider and deeper heads and a wider upper mandibular width than females. It was concluded that sexual differences in head morphology exist in Atlantic salmon parr but that these cannot be used with great accuracy to predict sex.

Chapter 8; conclusions - This work supports suggestions (cited in Chapter 8) that the maturation process has no starting point and is present from hatching.

Lipid levels have been shown to be important for maturation but not for gonad production itself. It is suggested (Chapter 8) that fat reserves are needed by maturing parr for increased activity sustained over the breeding season and for subsequent overwinter survival.

It is proposed that the peak in appetite in May is a compensatory response to the fat deficit that has arisen over the winter and the subsequent decline in appetite over the summer months is due to a combination of the controlling effect of negative feedback and an adaptive appetite response to the reduction of natural prey at this time.

Contents

Chapter 1 - General Introduction

1.1 Introduction

For the majority of individuals in the animal kingdom, sexual maturation is a fact of life. The age at which this first occurs varies from species to species and is usually within a few years of a species-specific fixed minimum age. For endothermic animals, such as birds and mammals, which show determinate growth, age and size are initially very closely related, with younger animals having faster growth rates and adults usually reaching a fixed maximum size after which growth does not occur. In contrast, the growth of ectothermic animals, such as reptiles and fish, is indeterminate, growth rates and final body size depending more heavily on temperature and food supply. Since ectotherms may continue growing throughout their lives and growth rates are so variable, there is no fixed age for first reproduction.

Teleost fish are ectothermic, and as a consequence, exhibit wide variation in the age at first maturity (Wootton, 1990; Roff, 1992). This ranges from a few weeks for cyprinodonts in temporary tropical pools (Miller, 1979; Simpson, 1979) to fifteen years for *Hippoglossus* flatfish (Roff, 1981). Policansky (1983) suggested that under stable conditions with abundant food supply, fish should grow rapidly and mature as soon as they are developmentally able to do so. However, maximising lifetime reproductive success involves an ultimate trade-off between maturing as soon as possible and delaying maturation until a greater reproductive output can be produced. Moreover, the optimum age of first maturity may differ between males and females due to their different reproductive roles. In females, fecundity is generally positively related to body size (e.g. Thorpe *et al.*, 1984) therefore delaying maturation until a greater size is attained will lead to an increase in initial fecundity. This may outweigh the advantages of earlier maturation, especially in semelparous species such as Pacific salmon (*Oncorhynchus* spp.) (Roff, 1992; Stearns, 1992). In contrast, male size is not a physiological constraint on maturation since sperm production is relatively cheap. However, the success of obtaining a mate is frequently associated with

large size. Therefore, where competition for mates occurs, selection would favour a delay in male maturity. The outcome of these contests among males may be density dependent, which can lead to the maintenance of more than one maturation phenotype in the male population. For example, both in bluegill sunfish, *Lepomis macrochirus* (Gross and Charnov, 1980) and blue-headed wrasse, *Thalassoma bifasciatum* (Warner, 1984), two types of males can be present: large dominant males that defend nests or mating territories and display to attract females and small, cryptic males that sneak into the spawnings of the large, dominant males to achieve their reproductive success. This leads to the evolutionary maintenance of alternative reproductive strategies and therefore alternative life histories.

Salmonids exemplify such variation in life history strategies, with fish from the same population becoming sexually mature before one or after as many as seven years. Large dominant males compete for access to females, whilst smaller satellite males (e.g. jacks in Pacific salmon or parr in Atlantic salmon, *Salmo salar*) use the sneaking tactic to successfully fertilise eggs (Schroder, 1981; Gross, 1985; Myers and Hutchings, 1986). Although part of this variation can be attributed to environmental effects on growth rates, different life history strategies can be adopted by sibling individuals living within the same stretch of river and experiencing more-or-less the same conditions and food supply. This thesis deals with the factors determining age at first reproduction in the Atlantic salmon.

1.2 Salmonid life history strategies

The life cycle of the Atlantic salmon demonstrates alternative developmental pathways towards the attainment of reproductive maturity. Like all salmon, this species spawns in fresh water. This occurs in the autumn in the northern part of its geographical range, but later into the winter in the south where incubation times for the eggs are shorter. The eggs remain in the gravel over winter and the fry hatch and emerge in spring. From this point onwards there are various life-history strategies which can be adopted by the juveniles (parr), some involving the structural and physiological adaptation to life in seawater, i.e. the smolting process. This process becomes apparent the following spring when the fish lose their parr markings and take on a silvery colour (Jones, 1959; Hoar, 1976). Their bodies also become more stream-

lined and in the wild this is accompanied by a downstream migration from fresh water out to the more productive environment of the sea. Therefore smolting involves the abandonment of freshwater adaptations. In contrast, reproduction takes place in autumn in the freshwater environment, thereby requiring the retention of such adaptations.

Maturation can take place before or after smolting, but these two processes conflict to some extent. Fish that migrate to sea spend at least one winter there before returning to freshwater to spawn. Therefore in the wild, smolting in the spring precludes maturation the following autumn (Thorpe, 1986), although smolts that are retained in fresh water over the summer, recover their freshwater adaptations and are able to mature by autumn (Lundqvist and Fridberg, 1982). Fish that mature in the autumn are able to smolt the following spring (Österdahl, 1969; Eriksson *et al.*, 1979; Leyzerovich and Melnikova, 1979; Hansen, 1980; Thorpe and Morgan, 1980; Saunders *et al.*, 1982; Thorpe, 1982; Naevdal, 1983; Bagliniere and Maisse, 1985; Lundqvist *et al.*, 1986), though they may suffer higher mortality than immature fish (Saunders, 1972; Leyzerovich, 1973; Mitans, 1973; Langdon and Thorpe, 1985; McCormick and Naiman, 1985; Lundqvist *et al.*, 1988) and there is evidence that only those in the best condition are able to undergo the transition successfully (Malikova, 1957; Saunders and Sreedharan, 1978). In addition, if a fish matures before smolting, it is more likely to remain in freshwater, maturing in successive years, than to smolt and emigrate to sea (Lundqvist, 1980; Hansen *et al.*, 1989). Therefore to some extent maturation inhibits smolting the following spring.

In the wild, smolting can occur in fish from 1-8 years of age and first maturation from the autumn of the first year up to 9 years of age, depending partly on genotype (Donaldson, 1970; Ricker, 1972; Thorpe, 1975; Thorpe and Morgan, 1978; Piggins, 1979; Bailey *et al.*, 1980; Naevdal, 1983; Thorpe *et al.*, 1983) and partly on environmental conditions (Metcalfe and Thorpe, 1990). With optimal growth conditions, groups of sibling parr develop a bimodal size distribution by the end of their first summer (Simpson and Thorpe, 1976; Thorpe, 1977; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985). The larger parr, forming the upper modal group (UMG), continue feeding and growing throughout the autumn and winter. In the spring all these fish will smolt. The smaller parr, forming the lower modal group (LMG),

reduce feeding and therefore growth during the autumn, eat very little over the winter and resume feeding and growing again when the temperature begins to increase in late winter / early spring. The majority of these fish will smolt one year later than their siblings in the UMG, the remainder taking yet another year. Some will become sexually mature by their second autumn. These are generally males since sperm production is less energetically costly than egg production (Wootton, 1990), but some mature females have been recorded (Bagliniere *et al.*, 1981; Prouzet, 1981; Bagliniere and Maisse, 1985; Shirahata, 1985; Hindar and Nordland, 1989). The early maturation of male salmonid parr has been widely reported, both in the hatchery and the wild (Thorpe 1975; Naevdal *et al.*, 1978; Thorpe & Morgan 1980; Saunders *et al.* 1982; Dalley *et al.* 1983; Bagliniere and Maisse, 1985; Myers *et al.* 1986; Riley and Power 1987; Thorpe 1987; Adams and Thorpe 1989; Garcia de Leániz 1990), with the percentage of males maturing at 1+ ranging from 0-100% over different rivers and varying between years (e.g. in the Little Codroy river, Canada, male parr maturation rates ranged between 66 and 81% over a seven year period, Myers *et al.*, 1986).

This thesis concentrates on the factors influencing maturity in parr. This is important since maturation influences the number and sex ratio in the smolt run (Forsythe, 1967), due to differing mortality rates for previously mature fish (Leyzerovich, 1973; Mitans, 1973; Lundqvist *et al.*, 1988) and therefore it will have major repercussions for the commercial management of wild salmon populations. In cultured salmon, early maturation is undesirable since it is also associated with a reduction in growth rates (Eriksson *et al.* 1979; Hunt *et al.* 1982; Lundqvist & Fridberg 1982; Dalley *et al.* 1983; Myers *et al.* 1986; Skilbrei 1989) and impaired smolting (Sato *et al.* 1985; Thorpe, 1986, 1987; Hansen *et al.* 1989; Berglund 1991). Genetic selection for both fast growth and low incidence of parr maturity is improbable, since rapid development implies early maturity (Alm, 1959; Saunders *et al.* 1982). Environmental manipulation of energy intake may be the key to reducing early maturation rates (Rowe and Thorpe 1990a; Thorpe *et al.* 1990), but a full understanding of the mechanisms influencing salmonid maturation is needed to improve the efficiency of this as a production stratagem.

1.3 Aims and objectives

The overall aim of this thesis was therefore to investigate the factors influencing maturation in male Atlantic salmon parr. Experiments were designed to investigate the relative effects of size and lipid levels as determinants of maturation within sibling groups. The possibility of using these factors to predict which fish would mature and of using them to control maturation rates was also considered.

Since lipid levels have been implicated in the control of maturation (see Chapter 3), it was first necessary to develop a non-destructive method for estimating the fat content of live salmon. This aspect is covered in Chapter 2. Chapters 3 and 4 deal with the identification of factors important for maturation, their manipulation and effects on eventual maturation rates.

During the course of this study two results came to light which needed investigation. Firstly, Thorpe (1994b) showed that the gonads of salmon in their first year in sea cages began growing a year before the time of spawning. Therefore gonad growth of parr was investigated in the year preceding maturation; this is described in Chapter 5. Secondly, results from the first year's work showed that appetite in all parr peaked dramatically in early summer (Chapter 4). It was thought that the reason for this might lie in seasonal fluctuations in the availability of natural prey, therefore changes in prey availability in the wild are examined in Chapter 6.

Throughout this thesis the sex of parr had to be determined and the only simple method available was dissection. Chapter 7 introduces a possible technique for non-destructive sex determination.

Chapter 2: Non-destructive methods for estimating lipid levels in Atlantic salmon.

2.1 Introduction

As part of a series of studies on the determinants of age at maturation in salmon (Thorpe, 1986), Rowe and Thorpe (1990) suggested that the reproductive state of an individual is related to its fat content earlier in the year. In order to test this hypothesis, non-destructive screening of fat levels is essential. In the past, several methods have been developed to determine the fat content of a variety of animals (e.g. mice (*Mus* spp.), Bailey *et al.*, 1960; pigs, Wood and Groves, 1965; pink salmon (*Oncorhynchus gorbuscha* (Walbaum)), Parker and Vanstone, 1966). Most of these have entailed the slaughter of the animals involved, but more recently studies have concentrated on non-destructive methods. These offer the potential for repeated sampling from the same individual and so allow greater flexibility in research design. In addition they also offer ethical advantages since the number of animals killed is reduced. However, each of these methods has its limitations.

Durnin and Womersley (1974) developed a technique of skinfold measurement to estimate body fat in humans, but this obviously cannot be applied to fish. Groves (1970) determined a set of equations based on wet weight and fork length for sockeye salmon, *O.nerka* (Walbaum), allowing fat to be estimated from these measurements alone. The equations however covered a large size range of fish (0.5-300g) and were not accurate for predicting individual variation in fat within one size-class. Talbot *et al.* (1986) used neutron activation analysis (which can be used on live animals) to predict, among other components, the fat content in Atlantic salmon, *Salmo salar* L. This relied on an accurate estimate of fat from one of its constituent elements, oxygen. Since there were no specific stoichiometric data on fish tissues, those for humans were used, potentially leading to compounding errors. Gjerde (1987) used computerised tomography (in which X-ray transmission data is combined with computer calculations to analyse cross-

sections of an object) to estimate fat levels in rainbow trout, *O.mykiss* (Walbaum). The same method has also been used on lambs (Sehested, 1986) and chickens (Bentsen *et al.*, 1986). Although this method was successful, the expense of obtaining and maintaining the equipment would make it impractical for use on a fish farm or in most research laboratories. Measurements of total body electrical conductivity (TOBEC) have been used successfully by several researchers to estimate the total lean mass and therefore the fat content of live birds and mammals (Fiorotto *et al.*, 1987; Walsberg, 1988; Castro *et al.*, 1990; Scott *et al.*, 1991). This technique has not, however, previously been tested on fish.

This chapter presents two studies designed to evaluate different non-destructive methods of fat estimation in individual Atlantic salmon parr; the first uses the TOBEC technique, while the second uses simple body measurements (biometrics).

2.2 Use of Total Body Electrical Conductivity (TOBEC) for Non-destructive Fat Estimation In Atlantic Salmon Parr

2.2.1 Introduction

TOBEC (total body electrical conductivity) relies on the principle that, when placed inside a solenoidal coil producing an oscillating magnetic field, a conductor, such as the body of an animal, will change the resulting impedance (Harker, 1973). The degree of change is related to the total electrical conductivity of the conductor. When considering animal tissue, TOBEC gives an index of total lean mass, since the conductivity of lipids is only about 5% that of lean tissue (Pethig, 1979). Walsberg (1988) used this technique to determine calibration equations (relating TOBEC readings to lean mass) for small rodents and birds. Since then Castro *et al.* (1990) and Scott *et al.* (1991) have developed intraspecific equations for several bird species.

The main aim of the present study was to investigate the suitability of this technique for predicting lean

mass and fat content in Atlantic salmon (*Salmo salar* L.). All three studies quoted above noted that changes in temperature of the animal tissue (from 20 - 45°C) had significant effects on the TOBEC reading. A second aim was therefore to determine the effects of temperature changes below 30°C in this poikilothermic species. Finally, as it is often convenient to freeze material, the effect of freezing and defrosting on the TOBEC reading was investigated.

What follows is a series of small scale investigations which gave me a preliminary view of the suitability of this method for my project.

2.2.2 Materials and Methods

Animals -

All fish used in the following experiments were hatched and reared at the SOAFD Salmon Research Unit at Almondbank, Perthshire and were offspring of sea-run salmon caught in the river Almond. The fish were of mixed age classes (1-3 yrs, wet weights 13.7 - 151.8g).

Equipment -

A Small Animal Body Composition Analyser (SA-1) (manufactured by EM-SCAN Inc.) was used to measure the TOBEC of the fish. The live fish were placed inside an open-ended transparent perspex cylinder (36.7cm long, 7cm internal diameter) and slid into the machine. The cylinder had one red 'centralising' ring and two black 'limit' rings on its surface to mark the region where the sample would have the greatest influence on the machine's readings (10 and 33 cm from the outermost end). The fish was positioned inside the cylinder in such a way that its centre of mass (estimated by eye) lined up with the red centralising ring, subject to the constraint that the animal should not extend past the black limit rings (one towards each end of the cylinder).

Basic procedure -

1. A reference reading was taken (R) (without the cylinder or animal in place).
2. A reading was taken with the empty cylinder in place (E).
3. Each fish was first anaesthetised with Benzocaine and weighed (to 0.01g). The anaesthetised fish was

then blotted to remove excess water by rolling once in a paper towel, placed on its side on an appropriately-sized piece of perspex so that the vertical centre of the animal was as close as possible to the vertical centre of the cylinder in which it was placed. Its head was pointed into the machine and the red centralising line of the cylinder was 2-3mm behind the outer-edge of the operculum. This standard was used for fish up to approx. 15cm fork length. Those larger than this were moved forward (since their tails extended out of the machine) so that the operculum was about 10mm in front of the red centralising line.

4. A reading was taken with the fish in place (S).

5. Steps 2 and 4 were repeated several times depending on the experiment so that any variation in the position or movement of the animal was taken into account.

6. A final reference reading was taken.

The TOBEC Index was calculated from the machine readings as follows:

$$\text{TOBEC Index} = \text{Mean}(E-S) / \text{Mean } R \qquad (1)$$

where E is a reading with the cylinder empty, S is a reading with a fish in the cylinder and R is the reference reading.

Lipid extraction method -

Individual dead fish were placed on two pieces of labelled, circular filter paper and scored on both flanks with a scalpel. These were then packaged-up and closed with staples. The packages were weighed and placed in a drying oven at 60-65°C until the weight stabilised (about 3 or 4 days, depending on fish size). Several packages were then placed in the Soxhelet apparatus and chloroform was passed through them 3 or 4 times until the liquid ran clear, showing that all the fat had been removed (Schifferli, 1976; Perdeck, 1985). They were then returned to the drying oven until their weight stabilised (1 or 2 days). Fat content was determined as the change in weight after extraction and expressed as a percentage of the wet weight of the fish.

2.2.2.1 Preliminary test

Readings from 11 large fish (wet weights 46.8-151.8g, 2+yrs) were taken as follows: Steps 1-4 of the basic procedure were carried out as above. The fish was then killed by an overdose of benzocaine. Steps 2 and 4 were then repeated four times with the dead fish, making a total of five sample readings for each fish. Then Step 6 was carried out. Six of the eleven fish were frozen at -20°C overnight. The fat content of the remaining five fish was determined by the lipid extraction method above. The following day the six frozen fish were defrosted, five TOBEC readings per fish taken as before and the fat content determined by lipid extraction.

2.2.2.2 Effect of sample temperature

Using dead fish

Twelve small fish (wet weights 13.7-31.8g, 1+yrs) were used to determine the effect of sample temperature on the TOBEC readings. Five TOBEC readings per fish were taken as above at ambient temperature (14°C). The fish were killed by an overdose of benzocaine and were placed in water at 22°C and left for 2 hours to equilibrate to that temperature, then another five TOBEC readings were taken. This method was repeated at 4°C. The fat content of the first five fish was determined as before. The remaining seven fish were frozen at -20°C overnight. The following day they were defrosted, brought up to 14°C and five TOBEC readings were taken. Fat content was determined as before.

Using live fish

To avoid the effect of water uptake of dead fish this experiment used 12 fish (wet weights 16.83 - 117.8g, 1-3yrs) that were alive throughout the experiment. Each fish was anaesthetised and four TOBEC readings taken at each of several temperatures between 4.4 and 18.5°C. The temperature was adjusted slowly by moving the fish in their container first to a cold (6°C) room and then into a warmer, 24°C, room. To take the fish below 6°C a plastic bag of ice was added to the water. The fish were fed every other day.

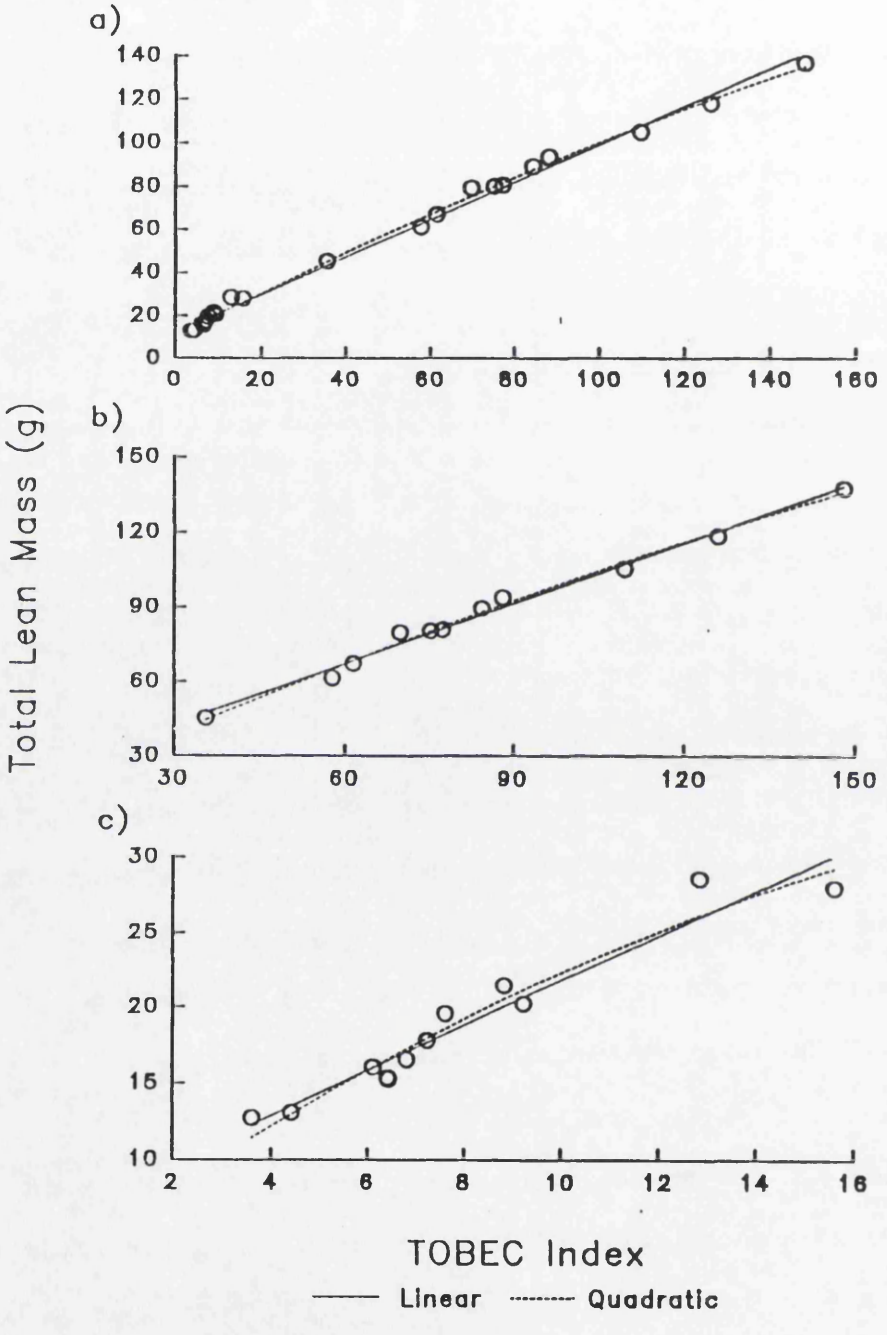


Fig. 2.1. The linear and quadratic relationships between Total Lean Mass and TOBEC Index for Atlantic salmon (wet weights 13.7-151.8g). a) All fish, b) Large fish (46.8-151.8g), c) Small fish (13.7-31.8g).

2.2.3 Results

The results from the large and small fish were analysed both together and separately. Total lean mass (TLM) (g) was positively related to TOBEC Index (TI) for all fish groupings (Fig. 2.1, Eqs. 2-4).

All fish:	TLM = 12.5 + 0.873 TI	r = 0.997, n = 23, P < 0.001	(2)
Large (46.8-151.8g):	TLM = 18.9 + 0.806 TI	r = 0.994, n = 11, P < 0.001	(3)
Small (13.7-31.8g):	TLM = 6.95 + 1.49 TI	r = 0.969, n = 12, P < 0.001	(4)

Walsberg (1988) had found that the most appropriate equivalent equation for birds and rodents was in the form of a quadratic. Quadratic equations were calculated for the present study's data and are given in Equations 5-7 (Fig. 2.1):

All fish:	$TLM = 10.6 + 1.02(TI) - 0.00117(TI)^2$	r = 0.998, n = 23, P < 0.001	(5)
Large (46.8-151.8g):	$TLM = 9.54 + 1.03(TI) - 0.00121(TI)^2$	r = 0.996, n = 11, P < 0.001	(6)
Small (13.7-31.8g):	$TLM = 3.95 + 2.21(TI) - 0.0377(TI)^2$	r = 0.973, n = 12, P < 0.001	(7)

The quadratic equations explain more of the variation in TLM than do the linear equations but the improvement is minimal.

Predictions of lean mass from all the above equations were used to predict percentage fat from the TOBEC readings (fat = wet weight - lean mass). The only significant correlations with actual percentage fat as measured by lipid extraction were found using the quadratic equation for all fish (Eq. 5) (r = 0.449, n = 23, P = 0.032) and both equations for large fish (Eqs. 3 and 6) (linear, r = 0.633, n = 11, P = 0.036; quadratic, r = 0.609, n = 11, P = 0.047). However, these correlations were not good enough for an accurate estimation of fat content and did not explain more of the variation than when using a combination of wet weight and fork length (all fish, r = 0.598; large fish, r = 0.901) (Table 2.1). A

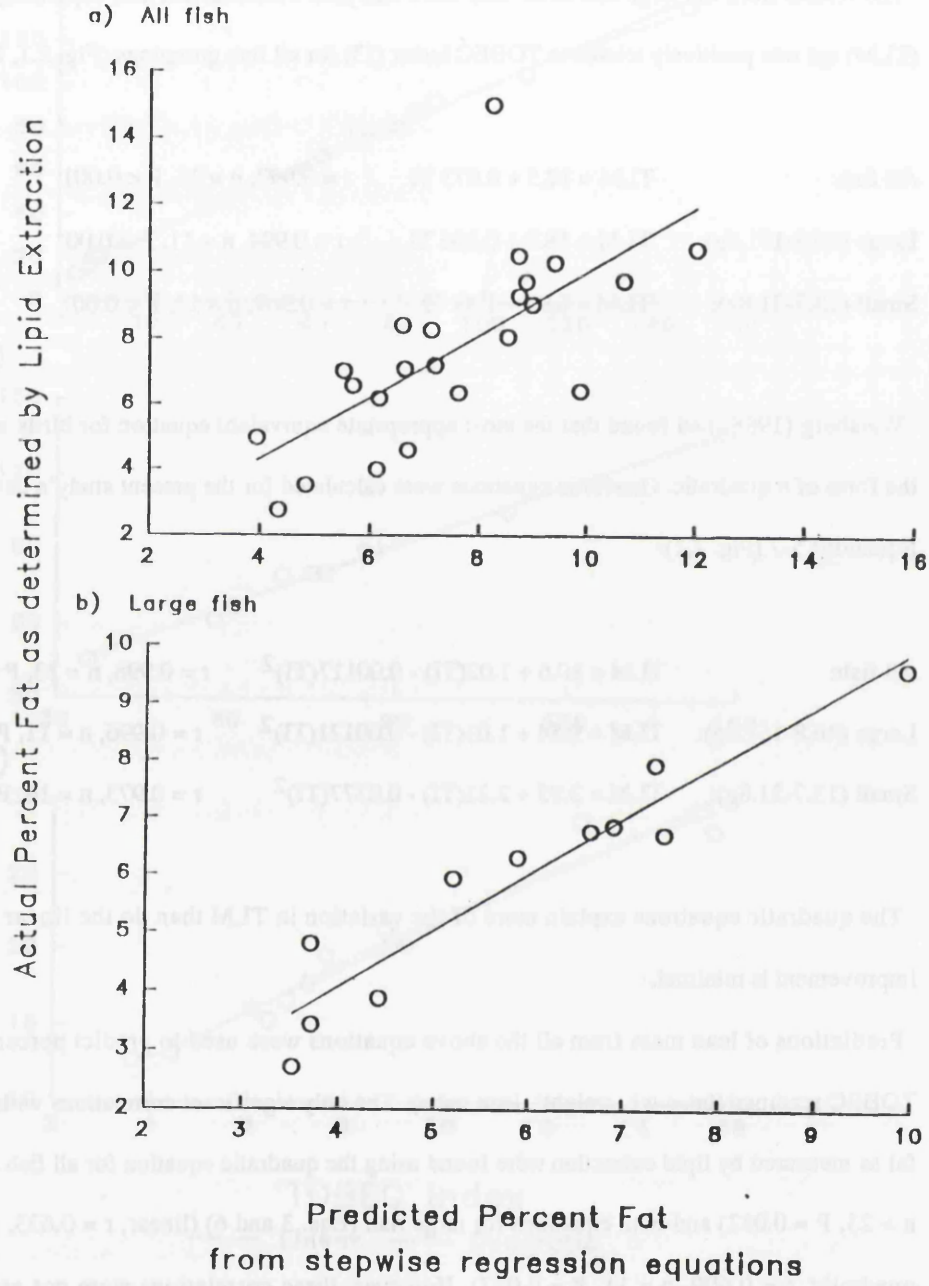


Fig. 2.2. The relationship between actual percentage fat as determined from lipid extraction and percentage fat predicted from multiple regression equations based on fork length, wet weight and %fat estimated using the linear and quadratic TOBEC/lean mass equations. a) All fish (13.7-151.8g) (predicted fat based on Eq. 8), $r = 0.738$, $P < 0.001$. b) Large fish (46.8-151.8g) (Predicted fat based on Eq. 9), $r = 0.943$, $P = 0.001$.

stepwise multiple regression was used to find the combination of factors that gave the best prediction of percent fat. This differed between size groups (Eqs. 8-9, Fig. 2.2). For the small batch of fish (13.7-31.8g) no variables reached the required entry level at step one.

All fish:

$$\%FATsox = 0.231 - 0.00704\ \%FAT_1 + 0.0110\ \%FAT_2 \qquad r = 0.738,\ n = 23,\ P < 0.001 \quad (8)$$

Large (46.8-151.8g):

$$\%FATsox = 0.619 + 0.00273\ W - 0.00324\ FL + 0.00366\ \%FAT_1 \qquad r = 0.943,\ n = 11,\ P = 0.001 \quad (9)$$

where %FATsox is the arcsine value of the percentage fat as determined by lipid extraction, $\%FAT_1$ is percentage fat estimated from the TOBEC Index using the linear equation, $\%FAT_2$ is that estimated from the TOBEC Index using the quadratic equation, W is wet weight (g) and FL is fork length (mm) ($\%Fat_{1,2}$ were not arcsine transformed since being estimates, they are not constrained to lie between 0 and 100%).

Table 2.1. Correlations between actual percent fat as determined by lipid extraction and percent fat predicted from TOBEC readings ($\%Fat_1$ uses linear equations (2-4), $\%Fat_2$ uses quadratic equations (5-7)) and from wet weight and fork length ($\%Fat_3$) for all fish (13.7-151.8g), large (46.8-151.8g) and small fish (13.7-31.8g).

Fish Group	All (n = 23)		Large (n = 11)		Small (n = 12)	
	r	P	r	P	r	P
$\%Fat_1$	0.160	0.466	0.633	0.036	0.279	0.380
$\%Fat_2$	0.449	0.032	0.609	0.047	0.148	0.647
$\%Fat_3$	0.598	0.012	0.901	0.001	0.615	0.118

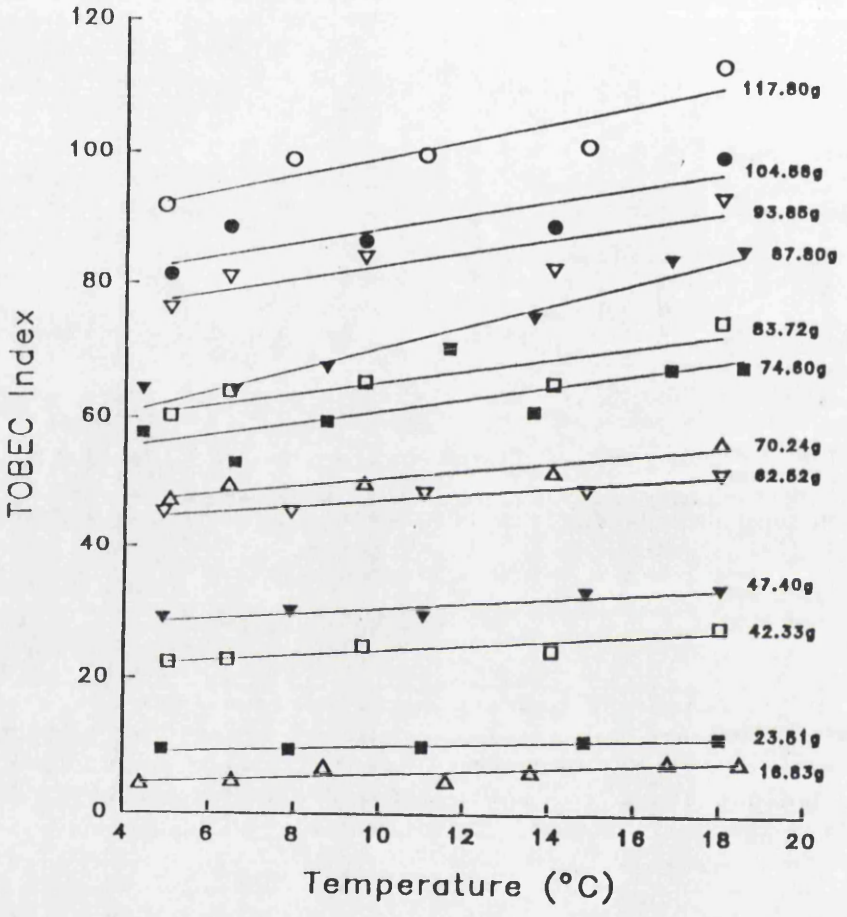


Fig. 2.3. The effect of temperature on the TOBEC Index for different fish sizes (given next to each regression line).

2.2.3.1 Comparing live and dead fish readings

A paired t-test showed that TOBEC readings from live fish were significantly higher than those from dead fish ($t = 2.73$, $n = 23$, $P = 0.012$). Since there was only one live fish reading per fish, subsequent analysis used the mean of the four dead fish readings per fish.

2.2.3.2 Effects of using defrosted material

A paired t-test showed that the readings from defrosted fish were not significantly different from those taken with the same fish prior to freezing ($t = -1.75$, $n = 7$, $P = 0.13$).

2.2.3.3 Effects of sample temperature

Using dead fish

The TOBEC readings of fish at 4°C were slightly, but not significantly, lower than those at 14°C (paired t-test; $t = -1.76$, $n = 12$, $P = 0.11$). However, readings of fish at 22°C were significantly higher than those at 14°C (paired t-test; $t = -4.74$, $n = 12$, $P = 0.0006$).

Using live fish

There was a positive relationship between TOBEC Index and temperature which varied with the weight of the fish (Fig. 2.3). The slope of the regression lines increased with increasing weight, (Fig. 2.4, Eq. 10).

$$SL = -0.130 + 0.0129W \qquad r = 0.881,\ n = 12,\ P < 0.001 \qquad (10)$$

where SL is the slope and W is wet weight (g). However, there was no correlation between the percentage change in TOBEC Index for each 1°C change from 10°C and the wet weight of the fish ($r = -0.467$, $n = 12$, $P = 0.1$; Fig. 2.5). The percentage change for the smallest fish (16.8g) is much larger than the rest and forms an outlying point on the graph. It has a strong influence on the mean percentage change (1.63% including the smallest fish, 1.41% without). This indicates that in relative terms temperature had the same effect on TOBEC indices for all sizes of fish with the exception of the smallest fish.

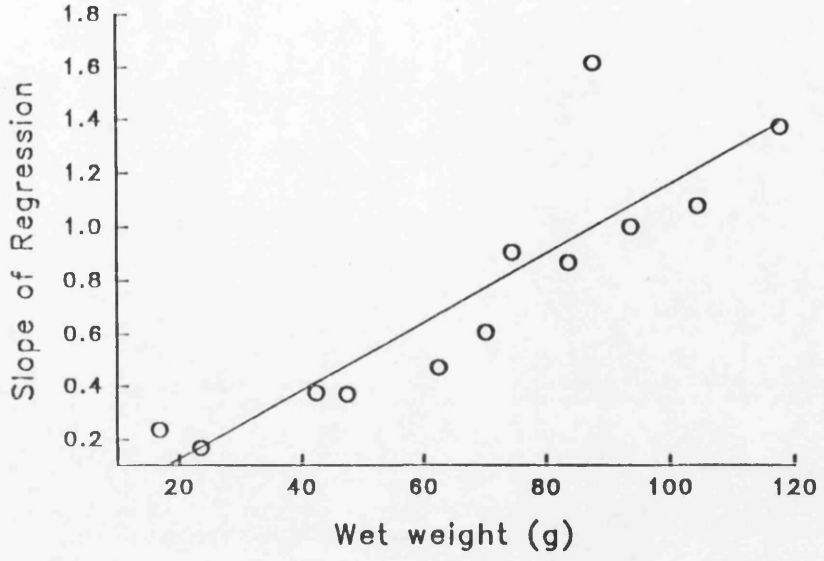


Fig. 2.4. The relationship between the slope of the regression line (TOBEC Index against temperature) for each fish and the wet weight (g) of that fish (Eq. 11; r = 0.881, n = 12, P < 0.001).

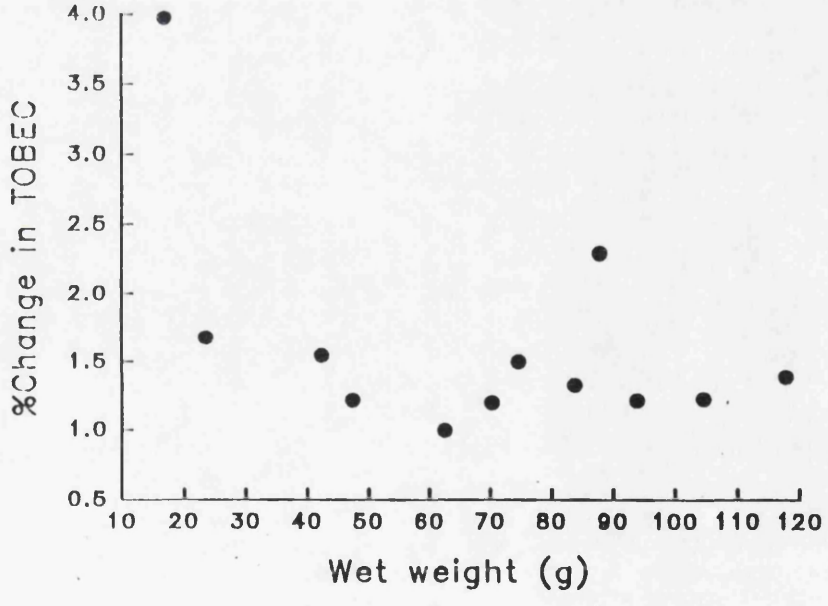


Fig. 2.5. The relationship between percentage change in TOBEC Index per 1°C change from 10°C and the wet weight of the fish (g).

2.2.4 Discussion

Predicting total lean mass - These experiments have shown that TOBEC can be used to give a reasonably accurate estimate of lean mass in juvenile Atlantic salmon in the size range 13.7 - 151.8g. However, the change in reading produced by placing a 14g fish in the machine was only 1 or 2 points (a 0.006% or 0.013% change from the empty reading, respectively). This contrasts with the change produced by a 150g fish which was 74 points (a 0.473% change from the empty reading). Therefore for accurate lean mass predictions from fish at the small end of the size range, the machine sensitivity would have to be increased. The quadratic equation was marginally better than the linear one for predicting total lean mass from the TOBEC Index. In other animal groups (e.g. small rodents (Walsberg, 1988) and birds (Walsberg, 1988; Scott *et al.*, 1991)), a quadratic equation has also been a better predictor, with similar correlation coefficients to those obtained in the present study.

Predicting lipid levels - Walsberg (1988) stated that the comparison of total body mass (determined by weighing) with lean body mass (determined from TOBEC readings) yields a useful measure of fat content. However he did not actually show that TOBEC readings and total body mass gave an accurate prediction of fat content in the rodents and birds he was using. In the present study these two measures could not be used to predict fat content accurately in these size groups of Atlantic salmon. This may be due to the fact that salmon have a naturally low fat content (2-10% of wet weight). A 1% error in the predicted lean mass becomes a 10-50% error when this prediction is used to determine percentage fat. However, when used in conjunction with wet weight and fork length in a multiple regression it was found that the TOBEC machine could be useful in predicting fat in the large fish, but this only explained 7.8% more of the variation compared to the use of wet weight and fork length alone. When the data from all fish were used both predictions of percent fat from the TOBEC readings, combined in a multiple regression, gave an 18.6% increase in explained variation ($r = 0.738$) from that by using wet weight and fork length ($r = 0.598$). No combination of variables was able to explain a significant amount of the variation in the fat content of the smaller fish, again probably due to the insensitivity of the machine at the smaller end of the size range. Thus TOBEC can be used (together with wet weight and fork length)

to give an accurate prediction of percentage fat in a restricted size range of Atlantic salmon: small fish (ww < 20g) do not give a big enough reading, while fish larger than about 150g will not fit into this particular machine. However, the increase in the percentage of the variation in fat content explained by use of the TOBEC machine is small and may not justify the time involved in calibrating and using it. Another point to bear in mind is that this experiment was carried out in October, a time when fat stores are low and being depleted. Several of the larger fish used in this study were producing milt and the sex and maturation state of the remainder was not determined. Using TOBEC at a different time of year when fat stores are high (e.g. June) may, perhaps give better results.

Water uptake problems - The results of the preliminary temperature trials using dead fish were probably confounded by water uptake by the fish as they were equilibrating to a new temperature. This needs to be considered if planning an experiment where dead fish will be left in water for any time.

Body temperature effects - A clearer picture of the relationship between TOBEC Index and sample temperature was gained by using live fish, but this is complicated by fish weight. The readings from larger fish increase more than those of smaller fish for a given temperature change. However, as in previous TOBEC studies (Walsberg, 1988; Scott *et al.*, 1991), this was resolved by calculating the percentage change in TOBEC Index for each 1°C change in temperature, using 10°C as standard. Since there was no correlation between this change and wet weight, the mean percentage change (1.63%) could be used to standardise results for temperature in future experiments (again the insensitivity of the machine for the smaller fish would have to be considered).

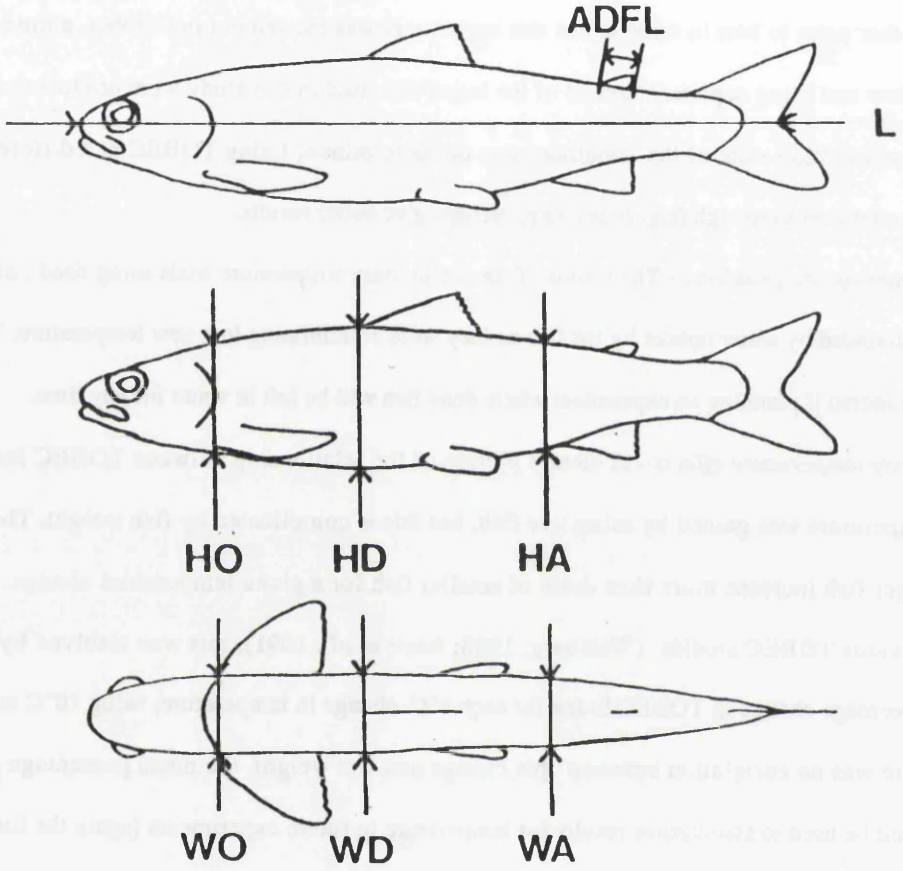


Fig. 2.6. The position of the eight body measurements. ADFL is adipose fin length, L is fork length, HO is opercular height, WO is opercular width (taken just behind the operculum), HD is dorsal height, WD is dorsal width (taken at the point where the dorsal fin arises from the body), HA is anal height, and WA is anal width (taken at the point where the anal fin arises from the body).

2.3 Use of Simple Body Measurements (Biometrics) for Non-destructive Fat Estimation in Atlantic Salmon Parr.

2.3.1 Introduction

The previous preliminary experiments showed that, with the available equipment, TOBEC could not be used to accurately estimate the fat content of small salmon. Since I would be dealing with juvenile salmon weighing from 1-40g another non-destructive method for fat estimation was needed. Salmon store fat in two main locations, the mesenteric store where fat is accumulated around the gut and the non-mesenteric store where fat is deposited in between the muscle fibres. A change in the size of these deposits, especially in the mesenteric store is likely to change the external dimensions of the fish in some way. It seemed likely that an estimate of fat content could be derived from measurements of these dimensions at strategic points along the body of the fish. The following experiments test this hypothesis and also examine the robustness of the technique at different times of year.

2.3.2 Materials and methods

The first part of this study took place in November and December 1989 (winter sample) and used two groups of Atlantic salmon parr with wet weights 1.8-9.8g (0+ yrs) and 13.7-151.8g (1-3 yrs). Both groups came from the SOAFD Salmon Research Unit at Almondbank, Perthshire and each consisted of sibling offspring of one pair of sea-run salmon. Prior to the experiment all fish had been fed to excess on commercial fish food pellets (manufactured by B.P.Nutrition U.K. Ltd.).

The fish were killed by an overdose of benzocaine. The following measurements were then taken from each individual: wet weight (to 0.01g) and eight body measurements (to 0.05mm using vernier callipers; mean discrepancy of repeated measures was 0.12mm). The body measurements were fork length, adipose fin length, and heights and widths at three positions along the body (Fig. 2.6).

Condition factor was calculated using Ricker's formula:

$$K = W / L^b \quad (11)$$

where K is the condition factor, W is wet weight, L is fork length and b is the slope parameter of the regression of $\log_{10}$(wet weight) on $\log_{10}$(fork length) (Bolger and Connolly, 1989) (Table 2.2). The height, width and adipose fin measurements were standardised for length by using a variation of Ricker's formula:

$$X' = X / L^b \quad (12)$$

where X' is the standardised measurement, X is the body measurement in question, L is fork length and b is the slope parameter of the regression of $\log_{10}(X)$ on $\log_{10}$(fork length) (Table 2.2). The fish were then dried to a constant weight in an oven (max. 60°C, two to five days). Fat content was determined by lipid extraction using chloroform as described earlier and expressed as a percentage of wet weight.

The second part of this study took place in July 1990 (summer sample) and used a group of age 1+ parr (wet weights 4.4-20.7g). These fish were siblings of smaller fish used previously and had been kept under similar conditions of excess food. Exactly the same method was used as above.

Table 2.2. The slope parameters of the regression of $\log_{10}(X)$ on $\log_{10}$(fork length), where X is the body measurement in question, for different samples of salmon parr.

	Size category of fish			
Body measurement	1.8-9.8g winter	13.7-151.8g winter	< 32.0g winter	4.4-20.7g summer
Wet weight	3.0800	2.9750	3.1312	3.4073
Heights:				
Opercular	0.9701	1.0273	0.9687	1.2018
Dorsal	1.1518	1.1769	1.0477	1.3372
Anal	1.1305	1.0248	1.0869	1.3321
Widths:				
Opercular	0.9378	0.9692	1.0608	1.1652
Dorsal	1.1109	1.0312	1.1388	1.3990
Anal	1.1457	1.0089	1.1379	1.6069
Adipose fin length	0.6080	0.6636	0.4836	0.8249

2.3.3 Results

A. Winter sample -

Multiple regression analysis was carried out on all data obtained from the November and December sample to find the combination of measurements that gave the most accurate prediction of the percentage fat content of the fish (%Fat). When data from the smaller and the larger fish were taken separately, the most accurate predictions of percent fat were given by equations (13) for small fish and (14) for large. When both the data sets were combined the best prediction was given by equation (15).

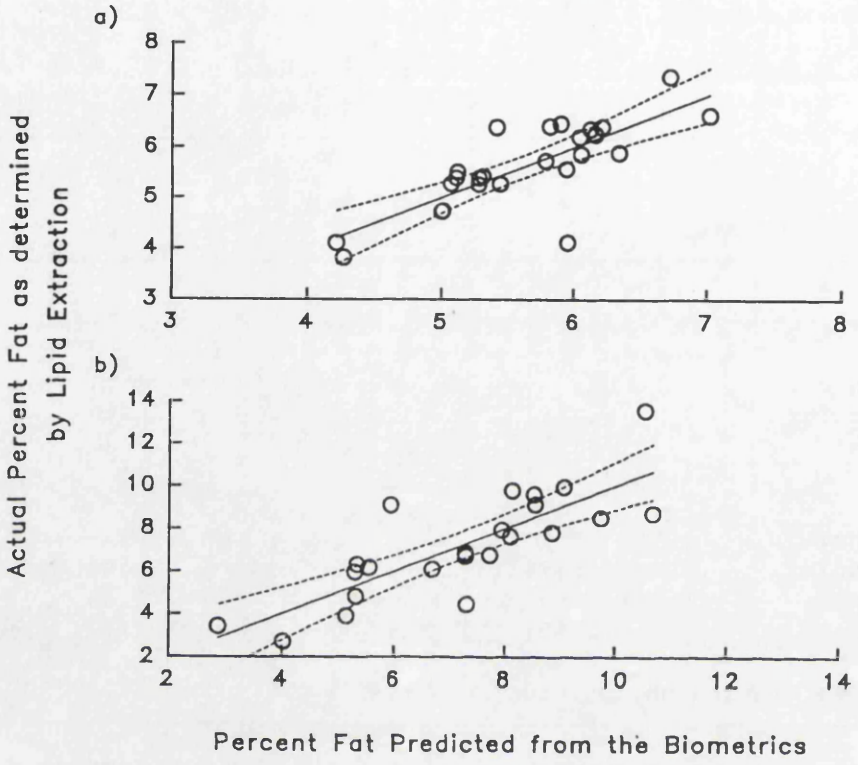


Fig. 2.7. The correlation between actual and predicted percentage body fat for the winter fish (dashed lines indicate 95% confidence limits). (a) Fish wet weights 1.8-9.8g ($r = 0.789$, $P < 0.001$), and (b) Fish wet weights 13.7-151.8g ($r = 0.813$, $P < 0.001$).

Fish 1.8-9.8g, winter:

%Fat = -27.2 - 0.457W + 0.128L + 63.5HO' + 178WD' - 108WA' + 10.6ADFL'

r = 0.789, n = 25, P < 0.001 (13)

Fish 13.7-151.8g, winter:

%Fat = 33.0 + 0.108W - 0.121L - 157HO' + 481WD' - 201WA' + 31.5ADFL'

r = 0.813, n = 23, P < 0.001 (14)

Both groups of fish, winter:

%Fat = 7.6 - 0.020W - 0.022L + 23.6K - 96.4HO' - 169WO' + 155WD'

r = 0.630, n = 48, P < 0.001 (15)

where W is wet weight, L is fork length, HO' is standardised opercular height, WD' is standardised dorsal width, WA' is standardised anal width, ADFL' is standardised adipose fin length, WO' is standardised opercular width and K is Ricker's condition factor. For each size group, the estimated fat content as predicted by the body measurements was closely correlated with the actual fat content determined by lipid extraction (Fig. 2.7).

B. Summer sample -

A multiple regression analysis was carried out as before to determine the combination of biometric measurements that best predicted the percentage fat content of the July sample of fish as determined by lipid extraction. This is given in equation (16):

Fish 4.4-20.7g, Summer:

%Fat = -46.5 - 0.707W + 0.368L + 735K + 199HD' + 103WO' - 21.4ADFL'

r = 0.972, n = 19, P < 0.001 (16)

In order to test how robust equations derived from fish sampled at one time of year were in predicting fat levels in fish measured at another, we derived a new equation for fish from the winter sample in

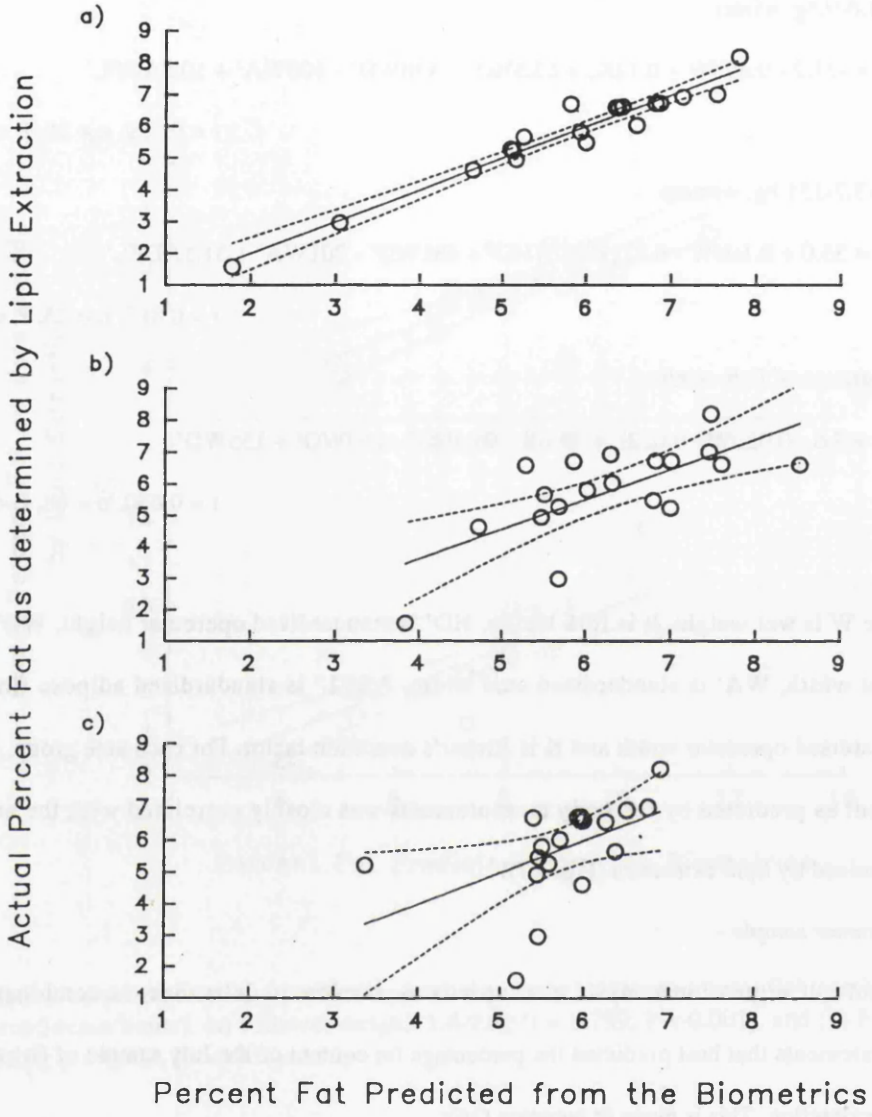


Fig. 2.8. The correlation between actual and predicted percentage body fat for the summer fish (wet weights 4.4-20.7g; dashed lines indicate 95% confidence limits). (a) Prediction equation based on summer data (Eq.16; $r = 0.972$, $P < 0.001$). (b) Prediction equation based on winter data (Eq.17; $r = 0.810$, $P < 0.001$). (c) Prediction equation based on condition factor (summer) alone ($r = 0.495$, $P < 0.05$).

approximately the same size range (i.e. less than 32 g) as those sampled in July (equation (17)):

Fish 1.8-32.0g, Winter:

$$\%Fat = 4.13 + 0.183W - 141WO' + 128WD' + 7.26ADFL' \qquad r = 0.810,\ n = 37,\ P < 0.001 \quad (17)$$

This equation was then tested as a predictor of percentage fat levels for the summer fish. The best predictor of fat levels was, not surprisingly, the equation based on fish at the same time of year (Eq. 16; Fig. 2.8(a)), but the winter equation (Eq. 17) was still a good predictor (Fig. 2.8(b)), and both were substantially better at predicting fat levels than the simple condition factor (Fig. 2.8(c)).

2.3.4 Discussion

From equations 13 and 14 it can be seen that for both size groups of winter fish the same body measurements were found to be important in determining fat content (i.e. wet weight, fork length, opercular height, dorsal width, anal width and adipose fin length). This is in partial agreement with Currens *et al.* (1989) who found that differences in feeding regime (which presumably affected lipid levels) had the greatest effect on measurements in the trunk region of chinook salmon, *O.tshawytscha* (Walbaum). However when both these data sets are combined as in equation 15 a different set of measurements is important, one of which is the condition factor. The condition factor again becomes important in predicting fat content in data from the summer fish (equation 16). It would therefore appear that condition factor is related to fat content only when analysing a large size range of fish or at certain times of year. Even then, when used alone it only explained 11.2% of the variation in fat levels of the large winter fish, 18.3% in the small winter fish, 17.6% when large and small winter fish were combined and 24.5% in the summer fish. A reasonable estimate of the fat content of fish in July was obtained by using equation 17, derived from data obtained in November and December. So if scientific requirements (such as the need for long term monitoring of the same individual) preclude the killing of fish, biometric equations derived from earlier lipid assays can be used and give a more accurate estimate than condition

factor alone (Fig. 2.8b,c).

The close correlation between actual and predicted percentage fat in the different groups of fish demonstrates that simple non-destructive body measurements can be used to estimate fat levels accurately in salmon of the weight range used in this study. The technique is reasonably robust and has potential both in biological studies and on fish farms when it is necessary to repeatedly assess body condition in individual fish. However, I would suggest that the most accurate results will be obtained if equations are derived for each particular application, since fish from different stocks or size ranges and at different times of year may vary in their basic body shape.

2.4 Conclusions

In the previous experiments I set out to find a non-destructive method for estimating the fat content of Atlantic salmon. I needed a method that could be used on fish from 1-40g.

The TOBEC machine was useful at predicting total lean mass but not fat content. It also could not be used reliably on fish smaller than about 20g. Therefore this method was rejected as unsuitable for my project.

The biometric method, however, could be used to reliably estimate the fat content of salmon over a large size range (1.8-151.8g) with the potential of wider applications. Therefore it was this method that was used in subsequent experiments for fat estimation.

Chapter 3 - The influence of growth and lipid levels on maturation

3.1 Introduction

Maturation is a costly process for salmonids. Energy reserves (in the form of fat) need to be built up prior to maturation, since reproduction uses energy and, as the hormones released may have an inhibitory effect on appetite late in the process (Rowe and Thorpe 1990b), less energy would be coming into the system at this time. It has been proposed that a minimum critical size must be achieved before maturation can take place (Utoh 1976; Myers *et al.* 1986). An alternative hypothesis suggests that (in Atlantic salmon) the rate of acquisition of surplus energy or nutritional state, specifically the fat content of the fish, during the early spring and summer may be more influential than absolute size in determining the life history path taken. Those individuals that fail to exceed a threshold level of stored fat switch off the maturation process (Thorpe 1986, 1989; Rowe and Thorpe 1990a and b). However, work in this area has so far been based on indirect evidence. Firstly, condition index has been used to give an indication of fat content, but this index only shows the relative weight for a given length, so that a high condition index could be due to an increase in water content or gonad production, not necessarily fat (see Chapter 2). Condition index has, however, been shown to correlate with fat content during some months of the year (Pinder and Eales, 1969). Secondly, fat content has been determined by dissection and such destructive techniques require assumptions about whether fish would have matured had they not been killed and so prevent long-term studies of the relationship between fat dynamics and maturation at the individual level.

The aim of the following experiments is to extend existing information on factors controlling age at maturation in Atlantic salmon, by examining changes in the fat content, growth and appetite of individual salmon parr in the year preceding maturation and relating this to subsequent reproductive state. This is made possible by the biometric technique described in Chapter 2, which allows accurate

estimation of fat levels in live fish. It enables exploration of the hypothesis that lipid levels are important in determining whether the maturation path is taken and the possibility that they can be used early in the year as a means of separating maturing from non-maturing fish. The first experiment involved simple monitoring of the factors thought to affect maturation. The second was designed to influence maturation rates by using low fat diets in the early part of the year. In the third, fish were starved over the winter to try and reduce fat levels at an earlier stage in order to tease apart the importance of size and lipid levels in the maturation process. The parallel changes in appetite occurring during these experiments are described in Chapter 4.

3.2 Materials and Methods

3.2.1. Experiment 1, 1990; Evaluating factors affecting maturation.

Supply and husbandry of fish -

The study fish were sibling offspring of a pair of sea-run Atlantic salmon from the river Almond, Perthshire, and were aged 0+ at the start of this experiment (January 1990). Sibling offspring were chosen so that any genetic differences in growth between maturing and non-maturing fish or any inherited effect on maturation *per se* would be minimised. A total of 143 fish (ranging in size from 41.9 to 69.9mm), none of which had been mature the previous autumn, were selected from the lower modal group of the bimodal size distribution. Such fish would not smolt until at least two years old (Thorpe 1977) and so, if male, could potentially mature in the autumn of 1990, aged 1+. These fish were transferred to the University Field Station at Rowardennan, Glasgow, prior to the start of the experiment. When their appetite was not being monitored the fish were kept in two radial flow tanks (1m diameter) (described by Wankowski and Thorpe 1979) from January to March and a 1m^2 tangential flow tank from April to October. Whilst in the holding tanks they were fed to excess on commercial salmon pellets (manufactured by B.P.Nutrition (U.K.) Ltd.) of a diameter that produced optimum feeding responses and

maximal growth for the size of fish (Wankowski and Thorpe 1979).

Body measurements -

All fish were given an individual combination of alcian blue dye spots by injection on their ventral surface. They were re-marked, weighed (to 0.01g) and measured (fork length, to 0.05mm) every month from January to October. Body measurements (biometrics) were taken each month to enable the fat reserves of the fish to be estimated (see Chapter 2 for further details). Equations (1) and (2) (from Chapter 2, Eqs. 13 and 17 respectively) were used to estimate fat content. Equation (1) was used from January to June and (2) from July to October, the change of equation allowing a more precise matching of fish sizes to those calibrated in Chapter 2.

$$\%Fat = -27.2 - 0.457W + 0.128L + 63.5HO' + 178WD' - 108WA' + 10.6ADFL' \quad (1)$$

$$\%Fat = 4.13 + 0.183W - 141WO' + 128WD' + 7.26ADFL' \quad (2)$$

where %Fat is fat as a percentage of wet weight, W is wet weight, L is fork length, HO' is opercular height standardised for fish length, WD' is standardised dorsal width, WA' is standardised anal width, ADFL' is standardised adipose fin length and WO' is standardised opercular width.

Since all fish were subjected to the same level of handling, the possible stress induced by marking and measuring every month would not influence any differences between maturing and non-maturing fish.

Maturation -

In November 1990 mature males were identified by the presence of running milt and immature fish were killed and then sexed by dissection.

Growth estimates -

Specific growth rates based on weight (SGR_W) and length (SGR_L) were calculated according to Equation (3) (Ricker 1979).

$$SGR_v = \frac{(\ln(v_2) - \ln(v_1)) \times 100}{t} \quad (3)$$

where v is the variable being measured (weight or length), v_n is the variable at time n (wet weight (g) or fork length (mm)) and t is the time interval in days between measuring v_1 and v_2. Rate of change in %fat was determined using equation (4).

$$\text{Rate of change in \%Fat} = \frac{(\%Fat_2 - \%Fat_1)}{t} \quad (4)$$

where $\%Fat_n$ is the %fat at time n and t is the time interval in days between measuring $\%Fat_1$ and $\%Fat_2$.

3.2.2 Experiment 2, 1991; Dietary fat manipulation.

Supply and husbandry of fish -

The fish used in this study were also sibling offspring of a pair of sea-run Atlantic salmon from the river Almond. They were hatched and reared at the SOAFD Salmon Research Unit at Almondbank, Perthshire, and were aged 0+ at the start of this experiment (January 1991). A total of 900 fish were selected, again from the lower modal group of the bimodal size distribution. In January 100 fish were placed in each of nine 1m diameter radial flow holding tanks. From January to April (i.e. during the proposed period of the maturation trigger) the fish were fed to excess on one of three diets differing in fat levels. Two different fish meals were used as the basis of these diets. One was used from January to March and produced diets of low, medium and high fat content as follows : 7.0%, 11.8%, 16.6%. The other, used during the month of April, had a slightly higher fat content and produced diets of 9.9%, 14.6% and 21.7% fat respectively (see *Diet preparation* below and Table 3.1). The tanks and treatments were arranged in a latin square design so that any variance from systematic outside influences could be

controlled for in the analysis. From May to October all the fish were fed to excess on the same standard commercial fish food (15-17% fat) (manufactured by B.P.Nutrition (U.K.) Ltd.).

Diet preparation -

Ingredients- Fish meal (of the lowest fat content available), resembling a slightly moist powder, and fish oil were obtained from B.P.Nutrition in Cheshire. Alpha-cellulose was used as a bulking agent and varied in proportion with the fish oil (see Table 3.1). Carboxymethyl-cellulose was used in small amounts as a binder to hold the mixture together.

Table 3.1. The constituents and nutritional analysis of the three diets (all expressed as percentages).

	Diet 1	Diet 2	Diet 3
Constituents			
Fish meal	80	80	80
Alpha-cellulose	18	13.5	9
Carboxymethyl-cellulose	2	2	2
Fish oil	0	4.5	9
Analysis*			
January - March			
Crude fibre	14.43	11.62	7.21
Crude protein	60.50	59.60	59.50
Oil content	7.03	11.83	16.64
April			
Crude fibre	12.34	9.48	5.66
Crude protein	53.0	53.0	54.0
Oil content	9.86	14.63	21.70

*Analysis carried out by B.P.Nutrition (U.K.) Ltd.

Method - Food was made, 500g at a time, using a Kenwood Chef food mixer with mincer attachment. The fish meal was placed in the bowl and the alpha-cellulose was added gradually to avoid excessive dust formation. Once these had been combined, the carboxymethyl-cellulose was added and mixed in

thoroughly. Oil was added at this stage (where applicable) and then warm water while the mixture was being stirred until it would just hold together. The mincer attachment was then fitted with the appropriate mincer plate for the required food size. Two mincer plates were made out of perspex with drilled holes of 1.5 and 2.0mm diameter to simulate commercial size 02 and 03 fry foods respectively. The food was then pushed through the mincer and collected on a drying oven tray that had been covered in tin-foil. At this stage the spaghetti-like food was broken up by gently rubbing it between the fingers and spread evenly over the tray. This was then dried at about 40°C for 1-2 days (depending on how much water had been added). The dried food was then passed through sieves of the correct size range for the required food (size 02, 1.1 - 1.5mm; size 03, 1.5 - 2.3mm). Food particles that were too large were ground through the sieves to create pellets of acceptable size. Samples of each diet were sent to B.P.Nutrition for analysis (Table 3.1)

Body measurements -

A third of the fish in each tank were individually marked on their ventral surface, as before, with a combination of alcian blue dye spots. Each marked fish was re-marked, weighed (to 0.01g) and measured (to 1mm) monthly from January to September. This year the biometrics (for fat estimation) were only measured from January to May (Equation 1).

Maturation and growth estimates -

At the end of September the sex and maturation state of each fish was determined. Specific growth rates and changes in fat were calculated as above (equations 3 and 4).

3.2.3. Experiment 3, 1991-1992; Winter starvation.

Supply and husbandry of fish -

These study fish were again sibling offspring of a pair of sea-run Atlantic salmon from the river Almond. They were hatched and reared at the SOAFD Salmon Research Unit at Almondbank, Perthshire, and were aged 0+ at the start of the experiment (November 1991). A total of 400 fish were

selected from the lower modal group of the bimodal size distribution. In November 100 fish were placed in each of four radial flow holding tanks (described by Wankowski & Thorpe, 1979). All fish were individually marked on their ventral surface with a combination of alcian blue dye spots. From November to January (a period of anorexia and general loss of weight in lower modal group fish even when fed to excess (Metcalfe & Thorpe, 1992)) the fish in two of the four tanks were placed on a starvation regime. This regime consisted of a cycle of three weeks without food and one week on a reduced ration (20% of the food intake of the two control tanks as determined by X-radiography - see Chapter 4). These fish will subsequently be referred to as "starved" even though they received food for 1 week in every four. The fish in the two remaining tanks acted as controls and were fed to excess. The food used throughout the study was a commercial fish food (manufactured by B.P.Nutrition (U.K.) Ltd.) and was of an optimum diameter for the size of fish (Wankowski & Thorpe, 1979).

From January to April (a period when the fish should begin to grow) the starved fish were fed on a reduced ration such that their growth rate was as close as possible to that of the control fish i.e. to try and avoid compensatory growth. This reduced ration ranged from about 5-13% (by weight) of the food given to the control tanks. From April to September the starved fish were placed on the same feeding regime as the control fish i.e. to excess.

Body measurements -

Throughout the treatment period (November to March) a marked subgroup of the starved fish (approx. 30 per tank) were monitored weekly to gauge the effect of the feeding regime (by measurement of wet weight (to 0.01g) and fork length (to the nearest mm)).

All fish were re-marked, weighed (to 0.01g) and measured (to the nearest mm) monthly from November to July. Biometric measurements were also taken each month to enable the fat reserves of the fish to be estimated (Equation 1).

Maturation and growth estimates -

At the end of the experiment (September) the sex and maturation state of each fish was determined by dissection. Specific growth rates and changes in fat were calculated as above (Equations 3 and 4).

Statistical analyses -

In analyses which involved more than eight similar statistical tests, the probability level at which a test was regarded as statistically significant was taken as 0.01, to reduce the risk of Type I errors.

3.3 Results

3.3.1 Maturation rates

Expt. 1: Evaluation of maturation factors - Of the 93 fish that survived until November, 8 were mature males, 48 were immature males and 37 were immature females; therefore only 14% of the males matured.

Expt. 2: Dietary fat manipulation - Of the 300 fish per diet that began the experiment in January 1991, 275, 258 and 263 remained in September for diet 1 (low fat), diet 2 (medium) and diet 3 (high fat) respectively. The percentage of males maturing did not differ significantly between the three diets and was extremely high (95%, d1; 94%, d2; 95%, d3) (see Table 3.2 for percentages of mature males, immature males and females in each diet group). Because of the low numbers of immature males in each diet, analysis was confined to differences between maturing males and females except where data from all diets could be combined, in which case immature males were included in the analysis.

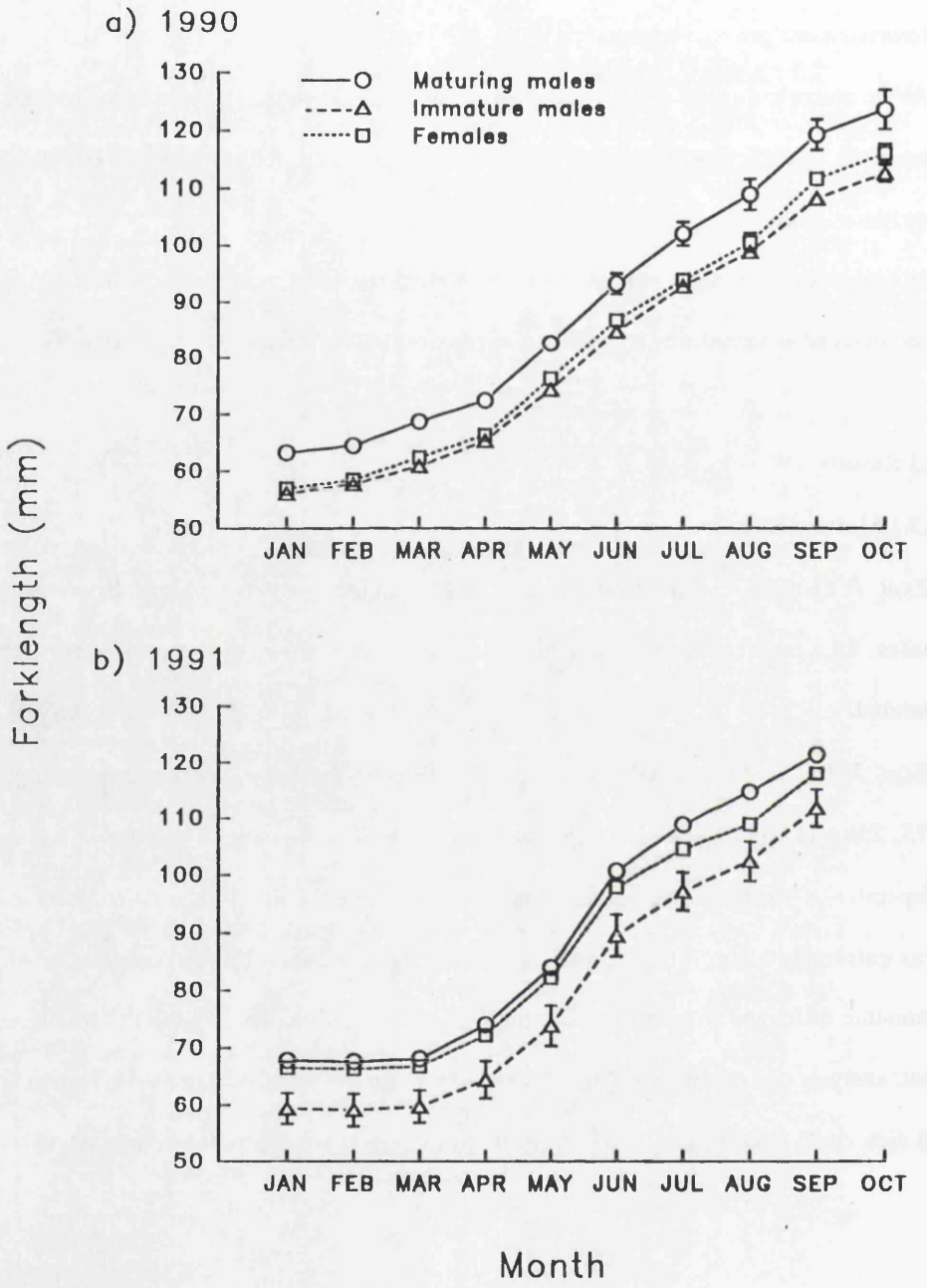


Fig. 3.1. Mean fork length (± S.E.) from a) Expt. 1, January to October 1990 and b) Expt. 2, January to September 1991 of maturing males, immature males and immature females.

Table 3.2. The percentages of mature males (Mm), immature males (Im) and immature females (If) for marked, unmarked and all fish in each diet group (Experiment 2, 1991).

	Diet 1			Diet 2			Diet 3		
	Mm	Im	If	Mm	Im	If	Mm	Im	If
Marked	53	1	46	38	5	57	38	2	60
Unmarked	48	4	48	53	2	45	55	3	42
All fish	50	3	47	48	3	49	49	3	48
n		275			258			263	

Expt. 3: Winter starvation - The percentage of males maturing in the starved tanks was not significantly different from that in the control tanks (chi-squared = 1.9035, 1 d.f., N.S.). The average maturation rate in males was 46.25% (Table 3.3).

Table 3.3. The percentages of mature males, immature males and immature females in each treatment group (Experiment 3, 1991-1992).

	Control	Starved
Mature males	26	34
Immature males	36	32
Immature females	38	34
n	190	187

3.3.2. Length

Expt. 1: Evaluation of maturation factors - The maturing fish were significantly larger (ANOVA $F_{2,92}$ = 5.28, $P < 0.01$) in January than the non-maturing fish and maintained this size difference throughout the period of study (ANOVA $F > 3.50$, $P < 0.05$ in all months) (Fig. 3.1a, Appendix I - Table I.1). There were no significant differences in the lengths of immature males and females throughout the study.

Expt. 2: Dietary fat manipulation - There were no significant differences in size between fish that had

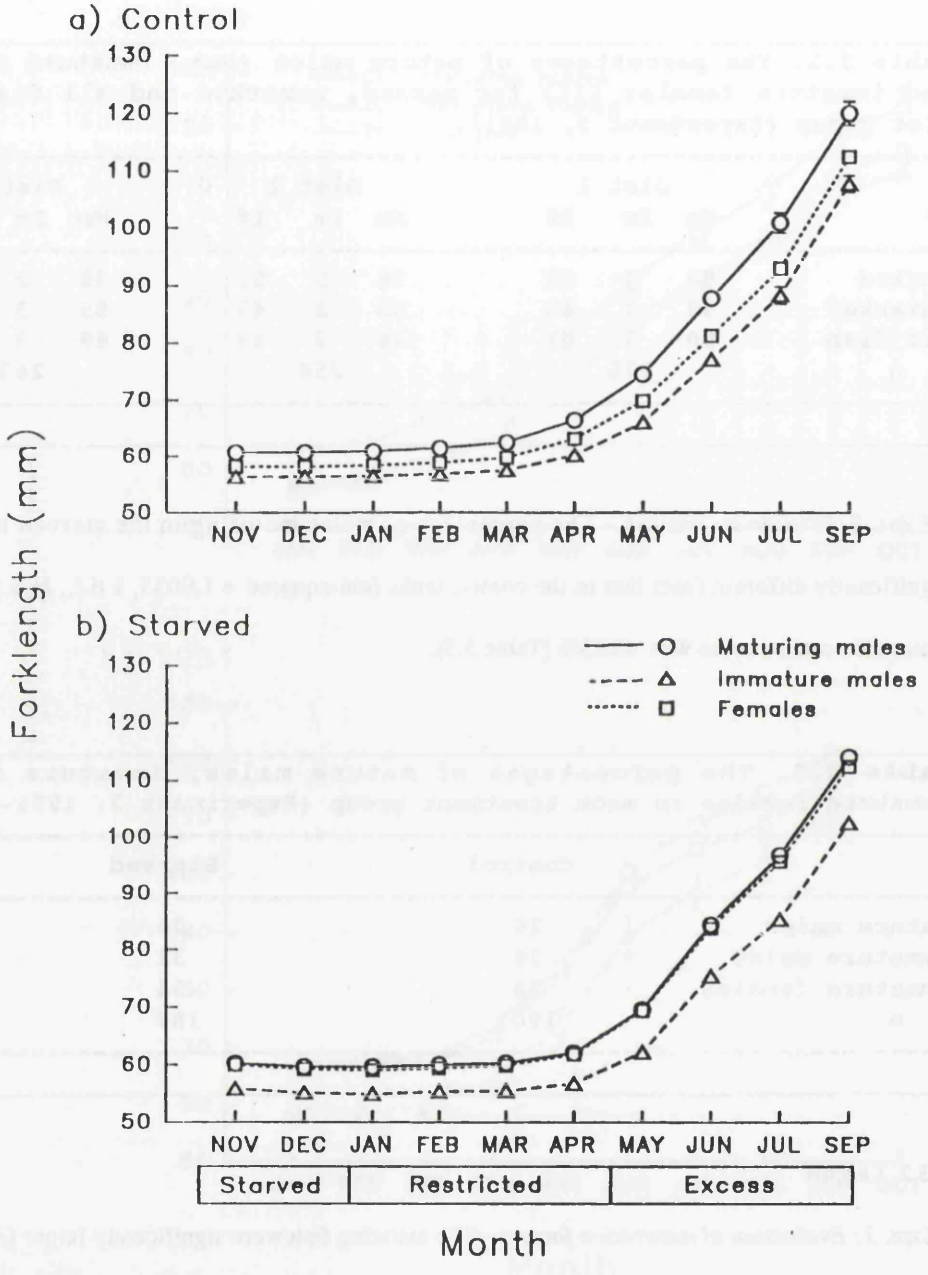


Fig. 3.2. Mean fork length (± S.E.) from Expt. 3, November to September 1991-1992 of maturing males, immature males and immature females from a) Control tanks and b) Starved tanks.

been on different diets (Appendix II - Table II.1). Therefore diet results were combined for analysis of effect of maturity group. Again maturing males were already larger than non-maturing males by January (ANOVA $F_{2,258} = 7.07$, $P = 0.001$; Fig. 3.1b, Appendix II - Table II.2). This size difference was maintained throughout the study. This year however, there were no significant differences in size between females and maturing males.

Expt. 3: Winter starvation - In both treatments males that were largest at the start of the experiment in November were more likely to mature the following autumn (ANOVA: Controls, $F_{2,189} = 13.34$, $P < 0.01$; Starved, $F_{2,186} = 22.22$, $P < 0.01$) (Fig. 3.2, Appendix III - Table III.1). Maturing males in the starved group were generally (and usually significantly) smaller than those from control tanks from March to September (t-tests, least significant, $t = 2.05$, $P < 0.05$) (Appendix III - Table III.2). In the control group there were no significant differences between immature males and females in any month. However, in the starved group the females were significantly larger than the immature males in all months and there were no differences between females and maturing males.

3.3.3 Fat levels

Expt. 1: Evaluation of maturation factors - The maturing fish had a significantly higher total fat content than the non-maturing fish in February (ANOVA $F_{2,91} = 4.83$, $P < 0.01$) and this difference was maintained up until October, when milt was running in the mature fish and their total fat content dropped to that of the immatures (Fig. 3.3, Appendix I - Table I.2). There was no significant difference at any point between the fat content of immature males and females.

Expt. 2: Dietary fat manipulation - There were no significant differences in fat levels between fish that had been on different diets (Appendix II - Table II.3), nor were there differences between the fat levels of maturing males and immature females on the same diet (Appendix II - Table II.4). Maturing males had higher mean fat contents than non-maturing males throughout the study but this could not be analysed

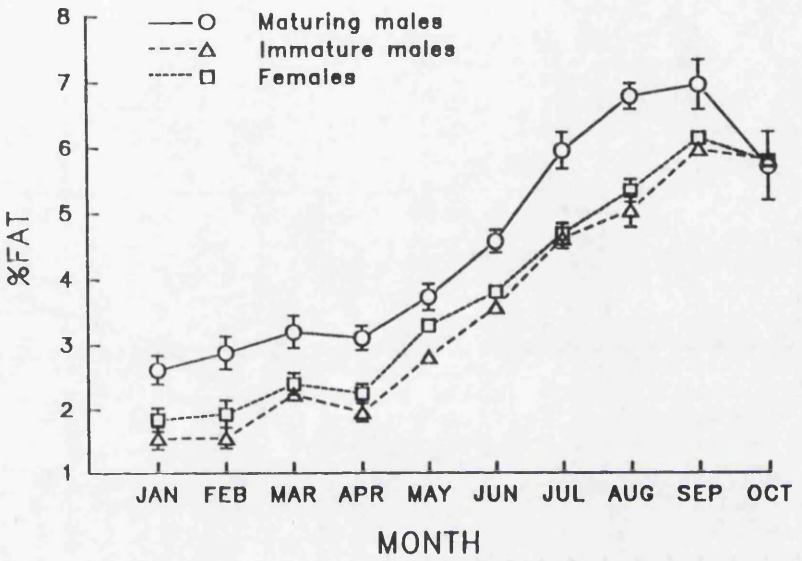


Fig. 3.3. Mean percentage fat (± S.E.) for maturing males, immature males and immature females from Expt. 1, January to October 1990.

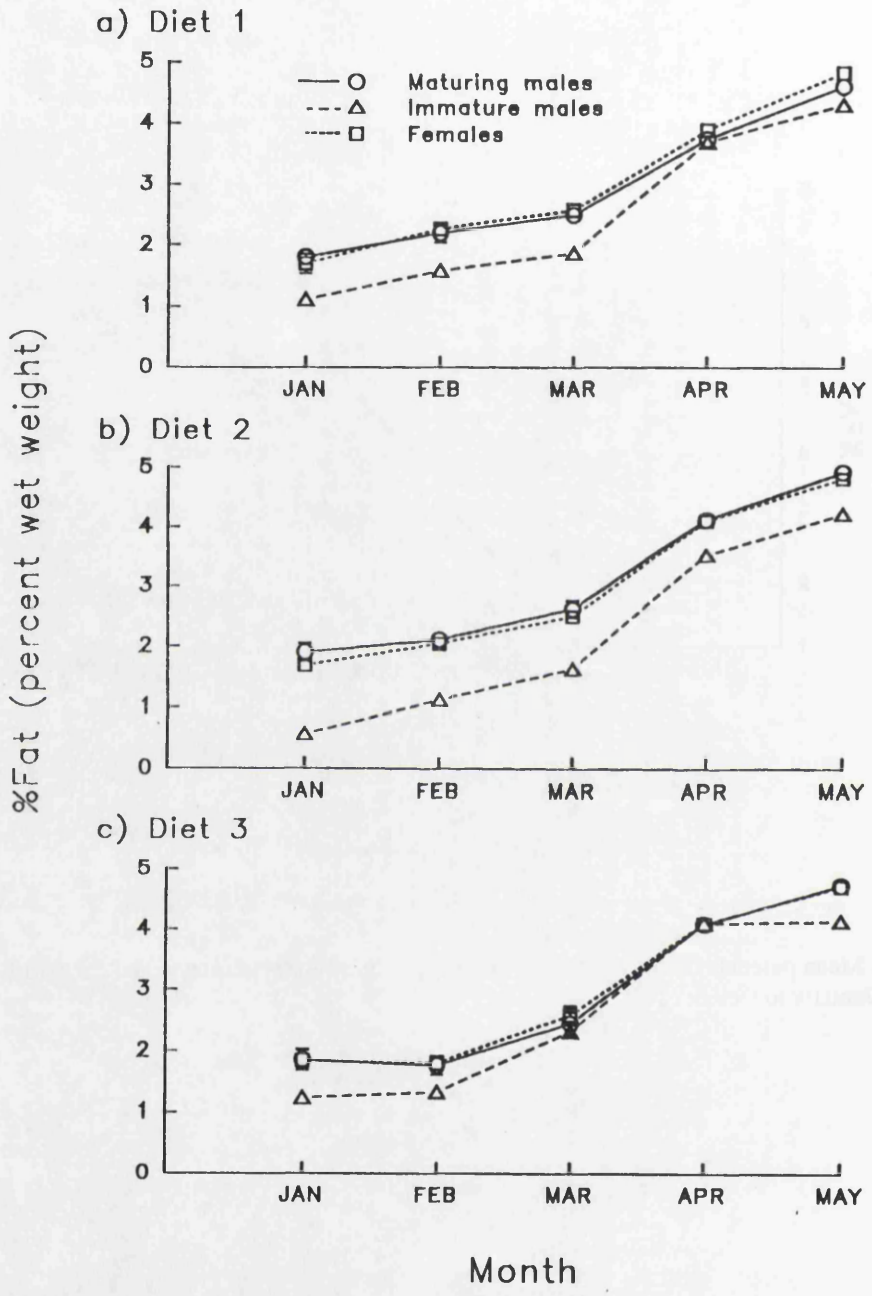


Fig. 3.4. Mean percentage fat (± S.E.) for maturing males , immature males and immature females from Expt. 2, January to May 1991 from a) Diet 1 (low fat), b) Diet 2 (medium fat) and c) Diet 3 (high fat).

statistically due to the low numbers of the latter (Fig. 3.4).

Expt. 3: Winter starvation - Males that had the highest fat levels in November were more likely to mature the following autumn (ANOVA, Controls, $F_{2,189} = 6.24$, $P < 0.01$; Starved, $F_{2,186} = 5.75$, $P < 0.01$) and this difference was maintained until July (Appendix III - Table III.3) (Fig. 3.5). The starved fish had generally (and usually significantly) lower fat levels than the controls from December to April (ANOVAS least significant $F = 2.00$, $P < 0.05$; Appendix III - Table III.4) but by May there was no difference and in June and July the starved fish had slightly greater fat levels than the controls, although this was only significant for immature males in June (ANOVA, $F_{1,125} = 3.70$, $P < 0.001$) (Appendix III - Table III.4). Within the starved group the females carried more fat than the immature males from November to May but in June there was no significant difference. Within the control group differences between females and immature males were only significant in December, February, March, May and June.

3.3.4 Specific growth rates

SGR_W, changes in weight -

Expt. 1: Evaluation of maturation factors - The specific growth rates (based on weight) for the maturing fish were not significantly different from those of the immatures at any point over the 10-month study period (Fig. 3.6, Appendix I - Table I.3).

Expt. 2: Dietary fat manipulation - Specific growth rates differed between diet groups but there was no consistent tendency for fish on a particular diet to show better growth (Fig. 3.7a,b,c, Appendix II - Table II.5). When looking at the differences between maturity groups, maturing males on all three diets had a higher growth rate than immature females from June to August (t-test, lowest $t = 2.05$, D.F. = 72, $P = 0.044$), but lower rates from August to September (t-test, lowest $t = 4.17$, D.F. = 78, $P = 0.0001$) (Appendix II, Table II.6).

Expt. 3: Winter starvation - Throughout the period of starvation (November to January) specific growth

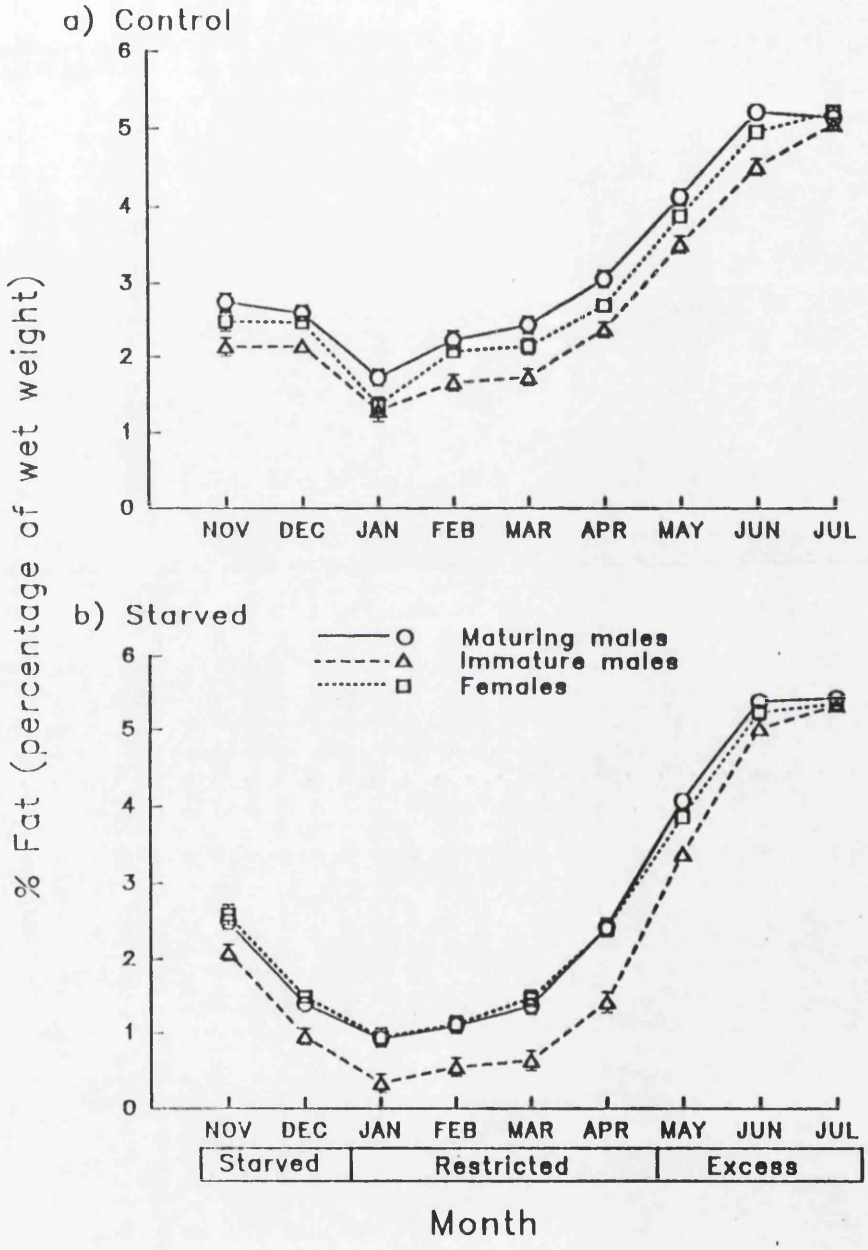


Fig. 3.5. Mean percentage fat (± S.E.) from Expt. 3, November to June 1991-1992 for maturing males, immature males and immature females from different feeding regimes a) Controls and b) Starved.

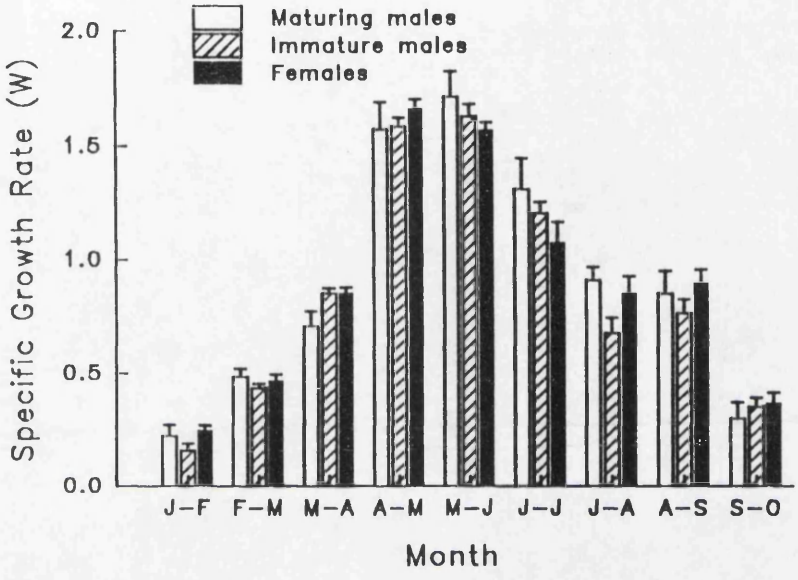


Fig. 3.6. The mean specific growth rates (± S.E.) based on wet weight for maturing males, immature males and immature females from Expt. 1, January to October 1990.

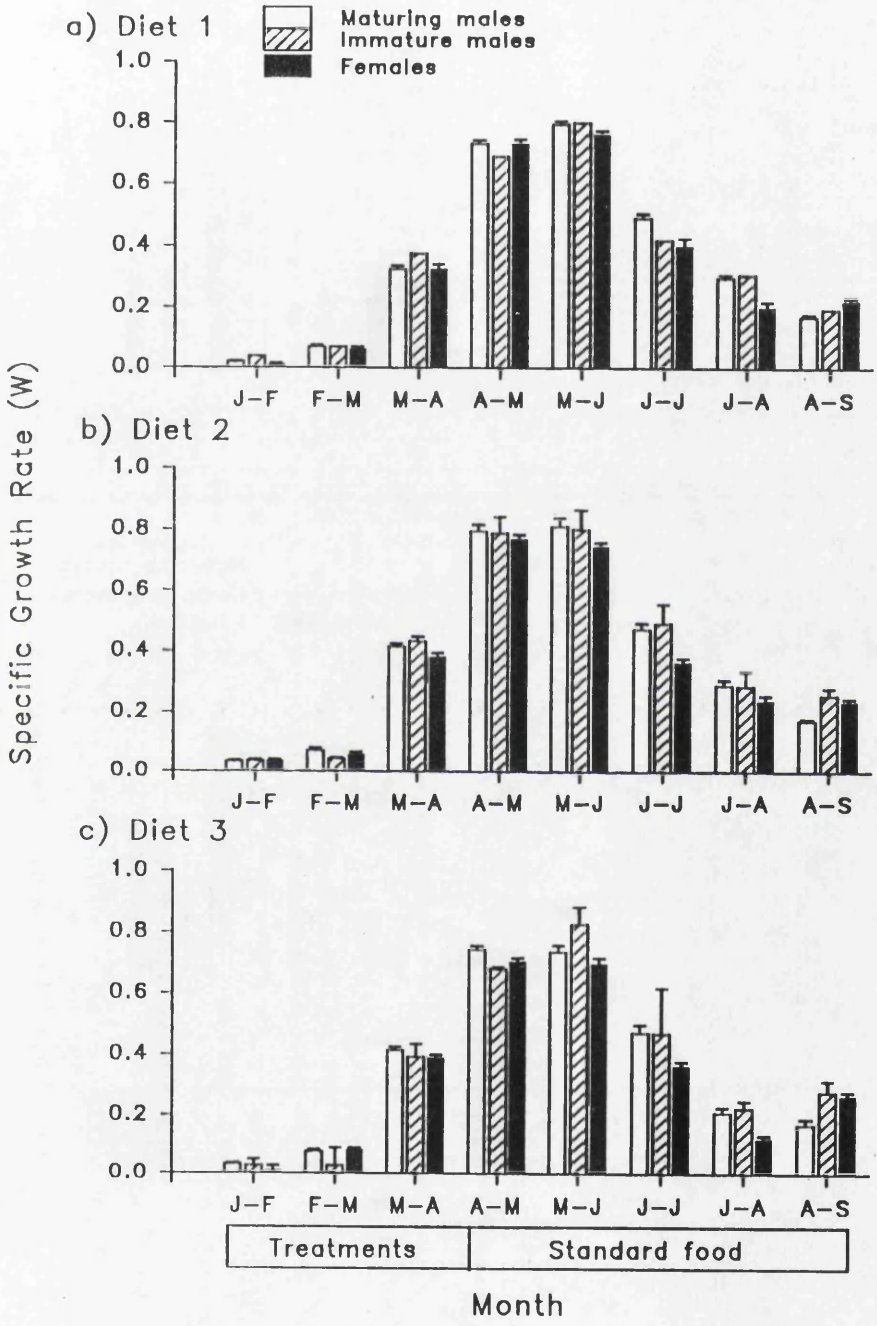


Fig. 3.7. The mean specific growth rates (± S.E.) based on weight for the maturing males, immature males and immature females from Expt. 2. a) Diet 1 (low fat), b) Diet 2 (medium), c) Diet 3 (high fat).

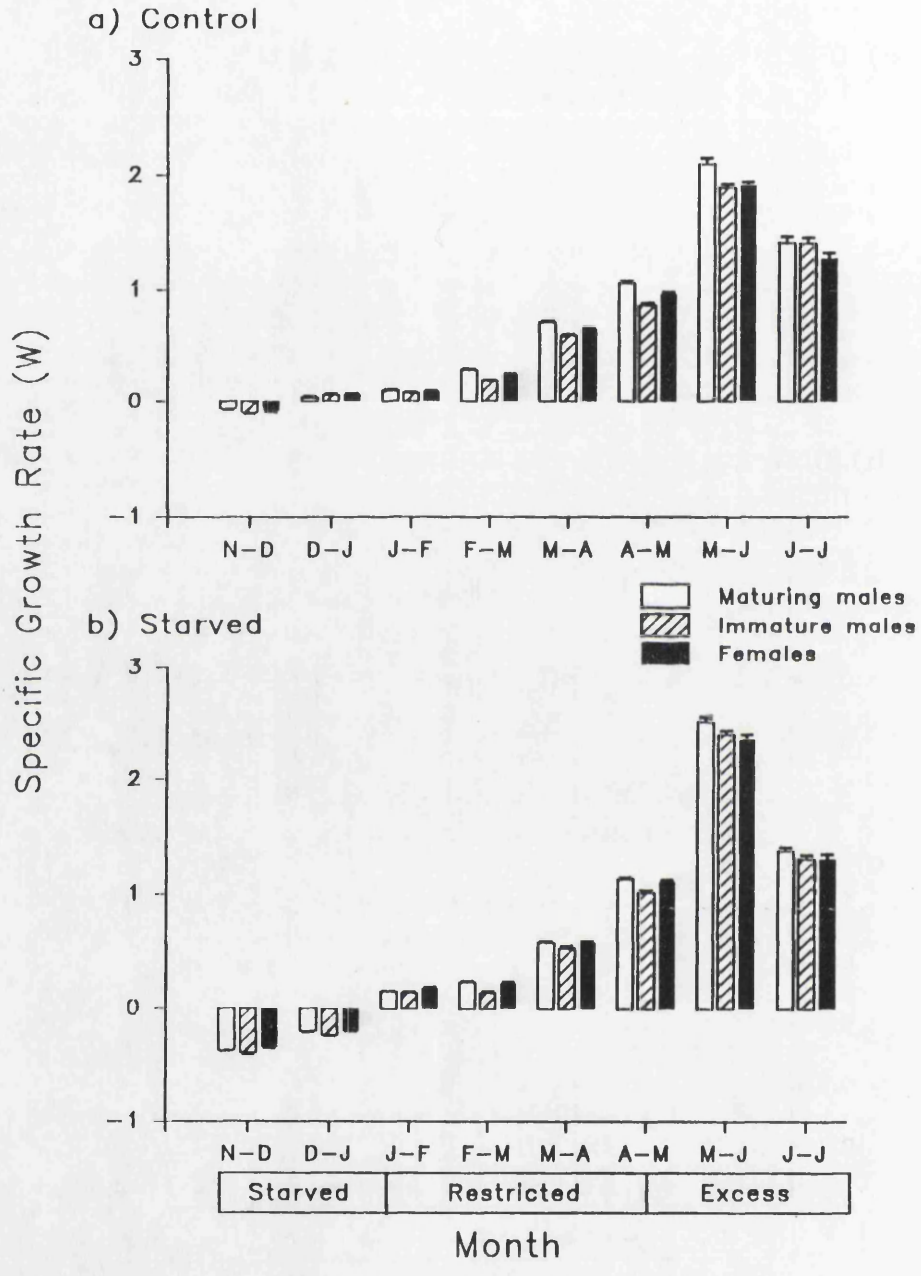


Fig. 3.8. The mean specific growth rates (± S.E.) based on weight for maturing males, immature males and immature females in each feeding regime from Expt. 3. a) Controls, b) Starved.

rates of the controls were (not surprisingly) significantly higher than those of the starved fish (ANOVAS, $F > 10.00$, $P < 0.01$) (Appendix III- Table III.6, Fig. 3.8). From January to April it was not possible to keep the growth rates of controls and starved fish equal. The starved fish had significantly higher growth rates during January (t-tests, $t > 3.00$, $P < 0.001$) and lower rates during February and March. In April and May, when all fish were being fed to excess, the starved group compensated for the earlier period of deprivation and had higher growth rates than controls (t-tests, $t > 2.50$, $P < 0.01$), but during June their growth rates had slowed and there were no significant differences between the two groups.

Maturing males in the control group had higher growth rates than the non-maturing males from February to June (ANOVAS, $F > 6.00$, $P < 0.01$). However those in the starved group showed a different pattern, with maturing males putting on significantly more weight than non-maturing males only during February ($F_{2,186} = 12.74$, $P < 0.001$) and April ($F_{2,186} = 10.60$, $P < 0.001$) (Appendix III - Table III.5).

SGR_L, changes in length -

Expt. 1: Evaluation of maturation factors - There were no significant differences in specific growth rates (based on length) between maturity groups (ANOVA, highest $F_{2,92} = 2.22$, $P = 0.114$) (Appendix I, Table I.4).

Expt. 2: Dietary fat manipulation - SGR_L differed significantly between diets in several months, but there was no consistent tendency for fish on a particular diet to do better. For both maturing males and females (immature males were excluded from the analysis due to the small sample size), those on the low fat diet showed a smaller change in length from March to April than those on the other two diets (ANOVA, maturing males $F_{2,107} = 24.65$, $P < 0.001$; females $F_{2,134} = 6.14$, $P = 0.003$). This difference reappeared in July for the females only (ANOVA, $F_{2,139} = 6.67$, $P = 0.002$) (Appendix II - Table II.7). There were differences between maturing males and females within diets that showed some consistency. Maturing males tended to have a greater SGR_L than females during June in diet 1 (t-test, $t = 2.70$, d.f. = 75, $P = 0.0087$) and a lower SGR_L during August in all diets (t-tests, D1, $t = 4.05$, d.f. = 77, $P = 0.0001$;

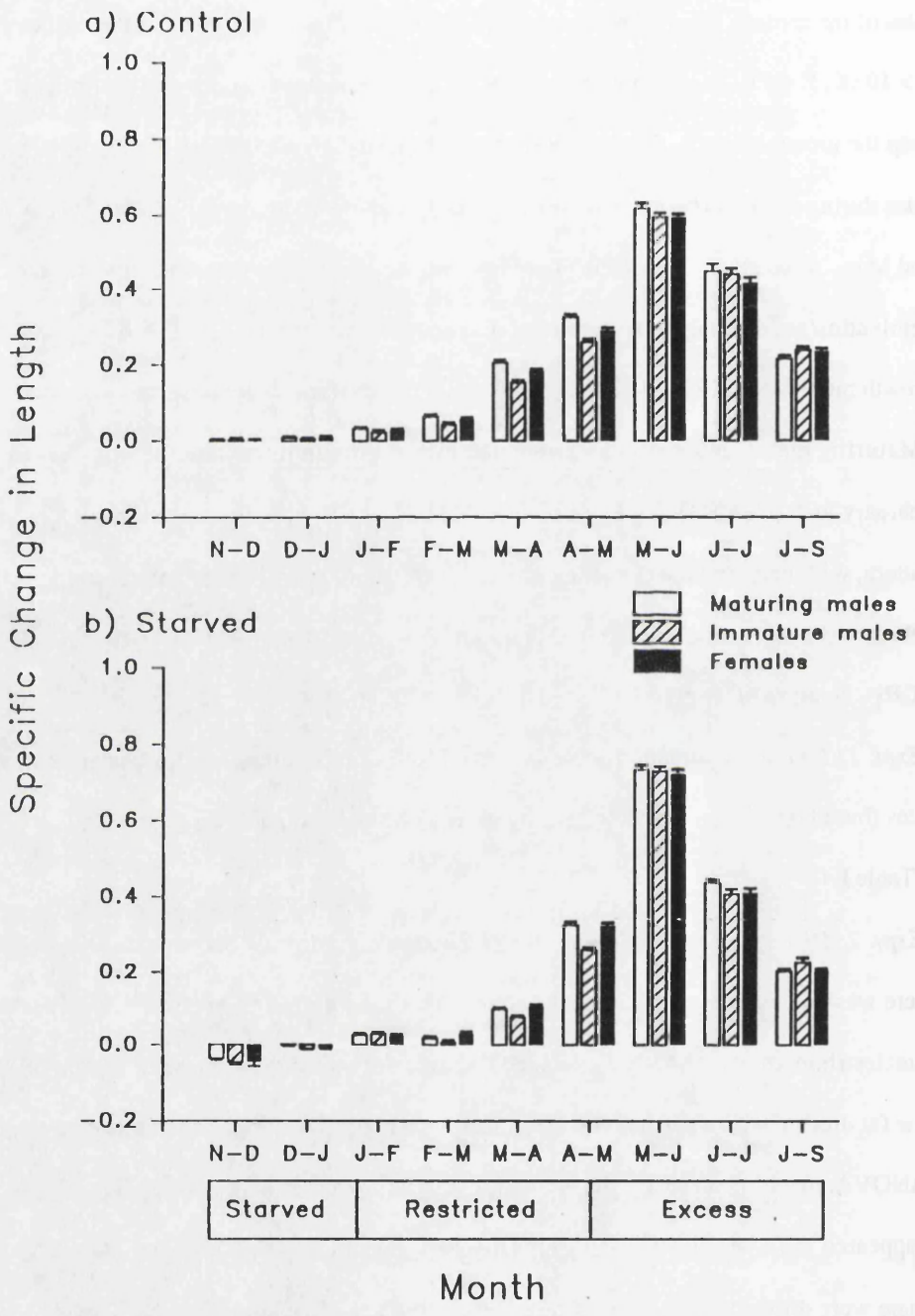


Fig. 3.9. The mean specific change in length (± S.E.) for maturing males, immature males and immature females from Expt. 3. a) Controls and b) Starved.

D2, t = 5.84, d.f. = 78, P < 0.0001; D3, t = 5.56, d.f. = 73, P < 0.0001) (Appendix II - Table II.8).

Expt. 3: Winter starvation - Control fish tended to have greater SGR_Ls than starved fish with the starved fish having greater SGR_Ls only in May, (t-tests, t > 5.50, P < 0.001) (Fig. 3.9, Appendix III - Table III.8). Differences between the maturity groups were only significant from March to May in both treatment groups, with maturing males having greater SGR_Ls than non-maturing males (ANOVAS, F > 7.00, P < 0.001) (Appendix III - Table III.7).

3.3.5. Rates of change in fat levels

Expt. 1: Evaluation of maturation factors - The rate of change in total fat content (Fig. 3.10, Appendix I - Table I.5) differed between maturing and immature fish in only one period, with maturing fish showing a significantly greater fat loss between September and October (ANOVA, $F_{2,66} = 30.48$, P < 0.001).

Expt. 2: Dietary fat manipulation - Results for rates of change in percent fat the following year were similar. There were no significant differences between the rates of change percent fat of mature males and immature females on different diets over the period of measurement (Jan to May) (t-test, highest t = 1.16, d.f. = 63, P = 0.25) (Appendix II - Table II.10). There were no consistent significant differences between the diets. The greatest increases in percent fat occurred for all fish between March and April (Fig. 3.11a,b,c, Appendix II - Table II.9).

Expt. 3: Winter starvation - All fish lost fat during November but this loss was greater for the starved fish (Fig. 3.12, Appendix III - Table III.10). Starved fish (especially maturing males) had greater increases in fat content than controls during March and April. For the control group the only significant differences between maturing and non-maturing fish were during June, when non-maturing males had greater increases in percent fat than did maturing males ($F_{2,150} = 7.71$, P = 0.0007). In the starved group however, a similar result was obtained in April ($F_{2,185} = 7.96$, P = 0.0005) (Appendix III - Table III.9).

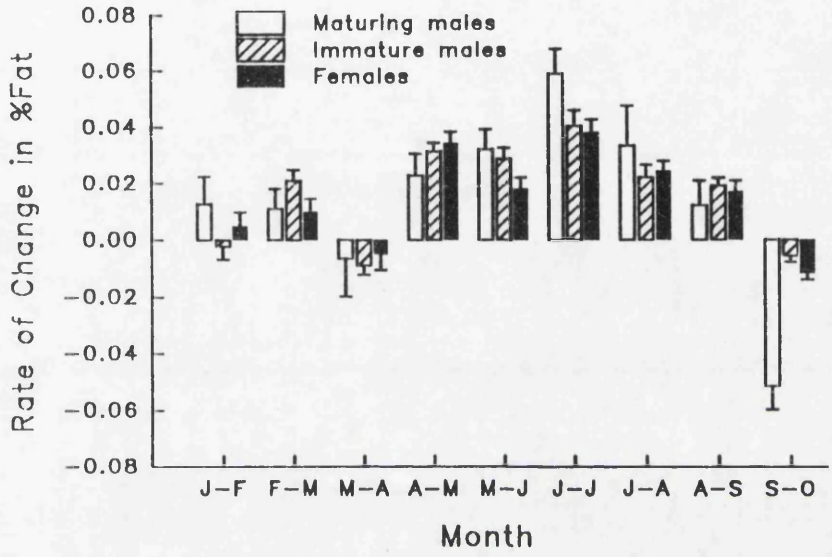


Fig. 3.10. The mean rate of change in %fat (± S.E.) for maturing males, immature males and immature females from Expt. 1, January to October 1990.

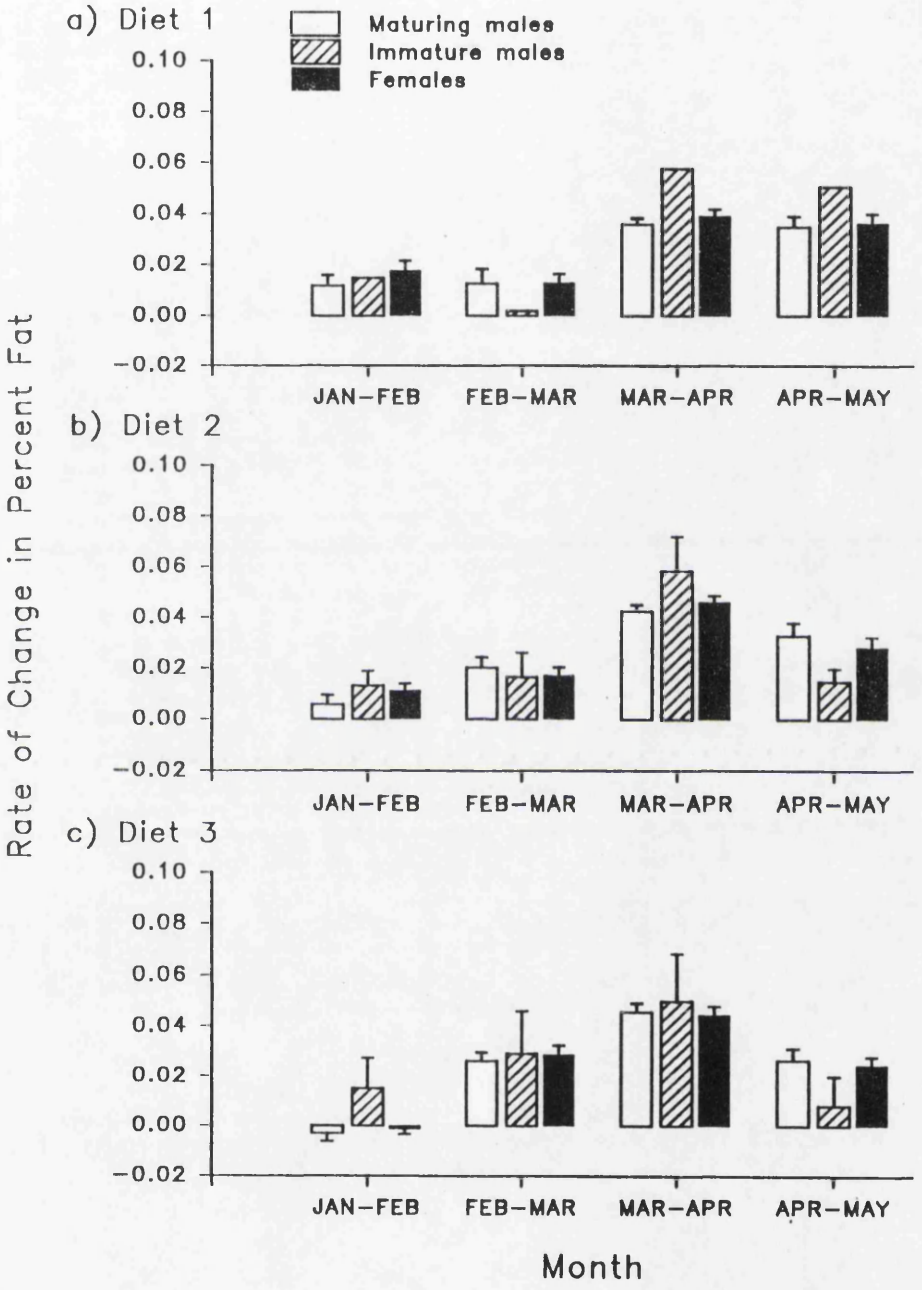


Fig. 3.11. The mean rate of change in %fat (± S.E.) for maturing males, immature males and immature females from Expt. 2. a) Diet 1, b) Diet 2, c) Diet 3.

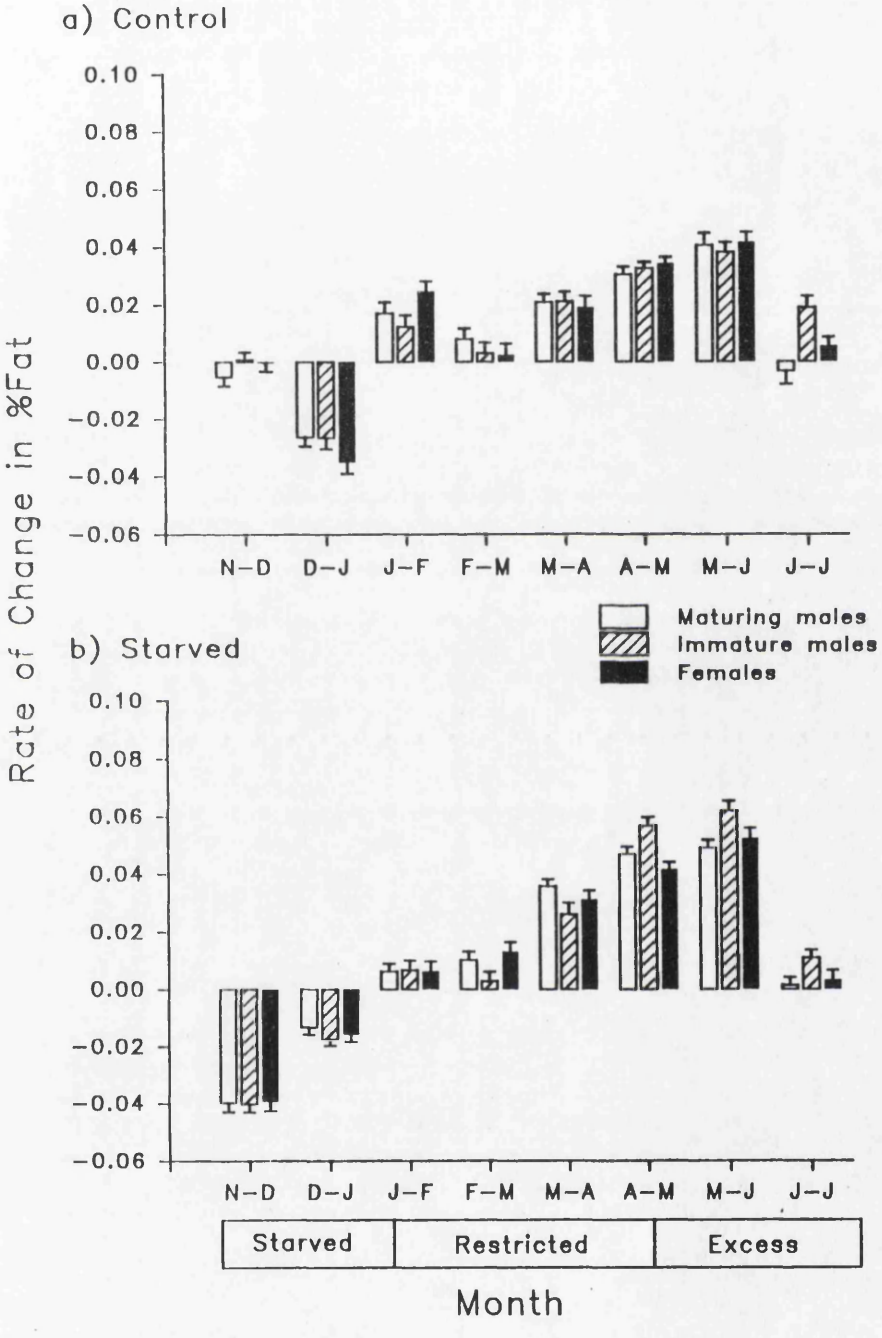


Fig. 3.12. The mean rate of change in %fat (± S.E.) for maturing males, immature males and immature females from Expt. 3. a) Controls, b) Starved.

3.3.6. Fat/Length relationships

Percent fat and fork length were correlated in most months in each experiment with the larger fish tending to have a higher percentage fat content. Covariance analysis was used to see if this relationship differed between maturing and non-maturing males.

Expt. 1: Evaluation of maturation factors - From January to July there were no differences between maturing and immature males in the fat/length relationship. However, the slopes of the regression lines of percent fat on fork length were significantly different between maturing and non-maturing males in August, September and October (Appendix I - Table I.6). In August and September the larger immature males had a higher fat content, but in October there was no relationship between fat and length. In contrast, in all three months the larger maturing males had the lowest percentage fat content (Fig. 3.13a,b,c).

Expt. 2: Dietary fat manipulation - In 1991 the regression lines for maturing versus non-maturing males had significantly different slopes in January, March and May (Appendix II - Table II.11, Fig. 3.14a,b,c). In all months the line for the immature males had a greater slope than that for the maturing males indicating that there was more variation in fat content relative to length among the immature males.

Expt. 3: Winter starvation - Over the period of starvation the regression line for the starved fish was significantly lower than that of the control fish (Fig. 3.15). With the increase in ration from January to April (but still reduced compared to controls), the larger fish in the starved group were able to regain lost fat reserves before the smaller fish. By May (all fish fed to excess) and June all the starved fish had increased their fat reserves to such an extent that for a given length the starved fish had a higher fat content than the controls (Appendix III - Table III.11). Comparing maturing and non-maturing males within a regime showed that there were no significant differences in the slope of the fat/length relationship from November to May. This continued to be the case for the starved fish in June and July. However, in the controls, the larger non-maturing males had higher fat levels than similar-sized maturing

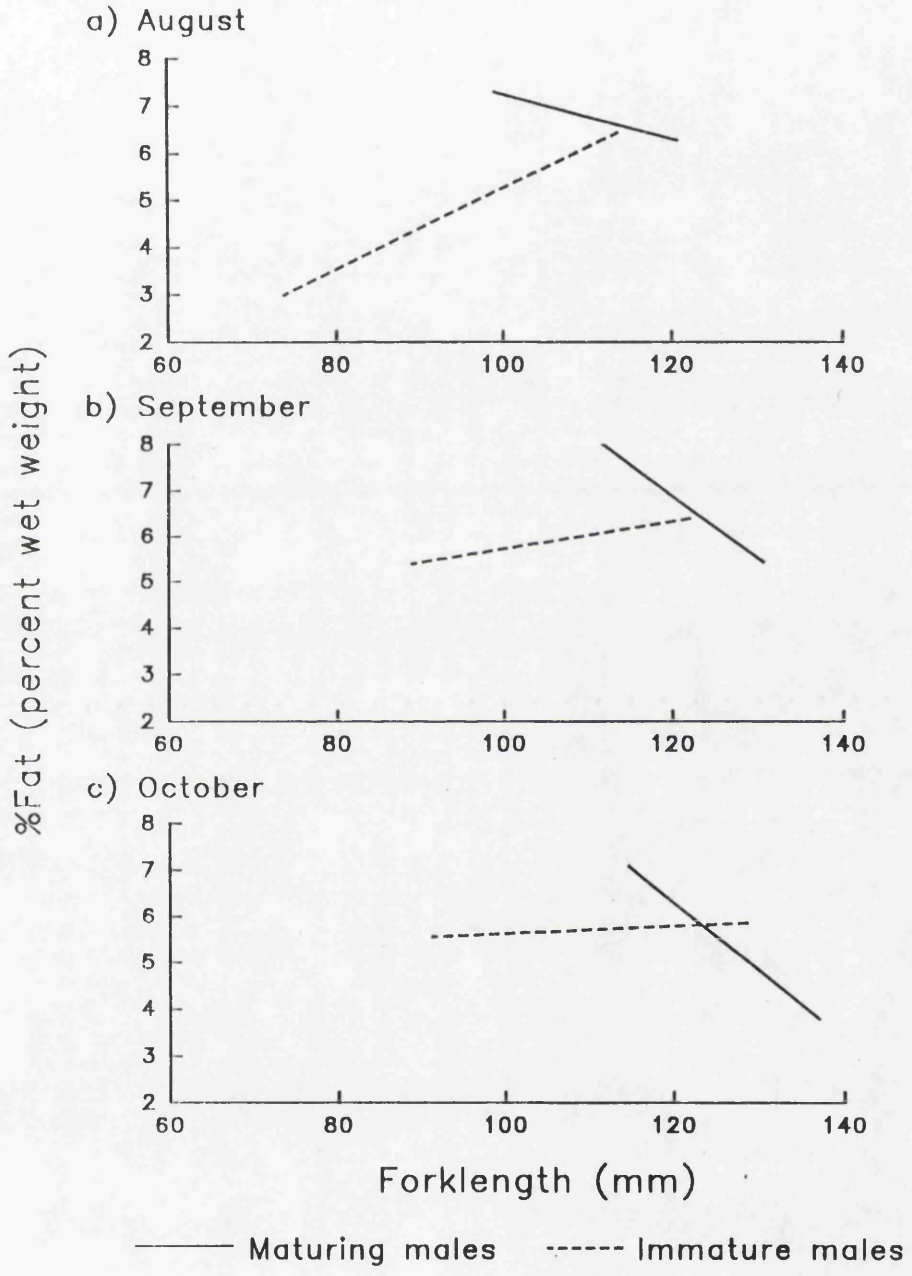


Fig. 3.13. The regression lines giving the relationships between percent fat and fork length for maturing and non-maturing males from Expt. 1. a) August, b) September, c) October 1990.

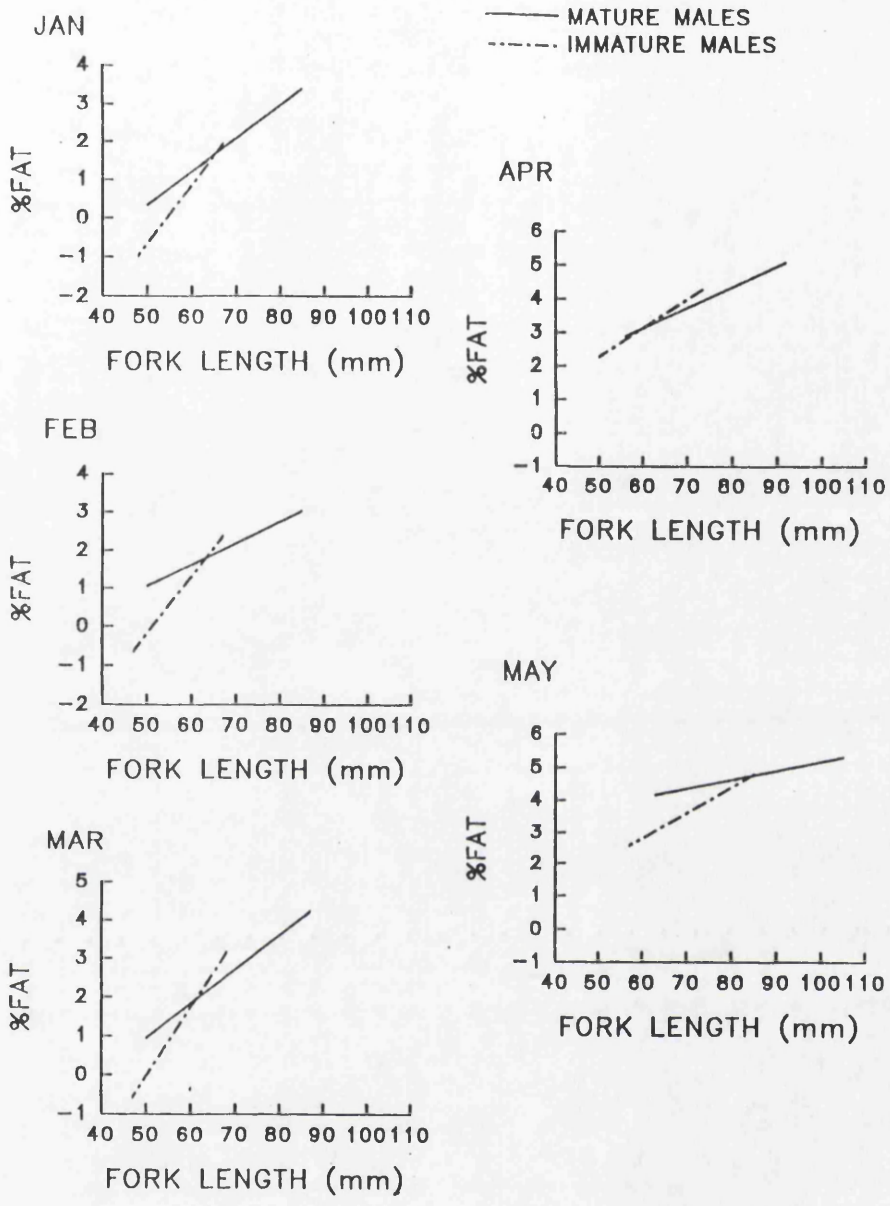


Fig. 3.14. The regression lines giving the relationships between percent fat and fork length for maturing and non-maturing males from Expt. 2, January to May 1991.

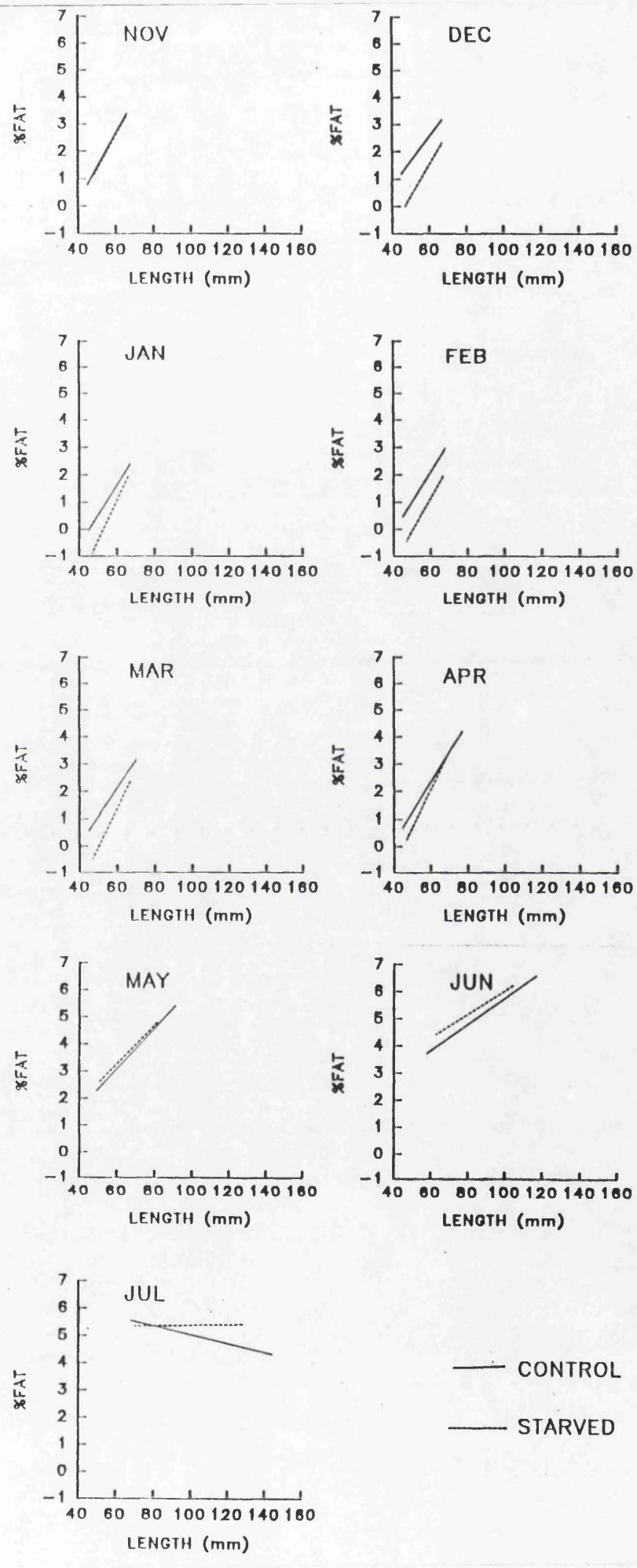


Fig. 3.15. The regression lines giving the relationships between percent fat and fork length for control and starved fish from Expt. 3, November 1991 to July 1992.

males in both these months (Fig. 3.16; Appendix III - Tables III.12, III.13).

3.3.7. Maturation predictors

Expt. 1: Evaluation of maturation factors - Stepwise logistic regressions (Dixon, 1985) were used to test whether percent fat and (or) fork length could be used early in the year to predict whether fish would subsequently mature. A separate analysis was conducted for measurements obtained in each month between January and March. Initially, only males were included, since the females were not going to mature. The results showed that in January fork length was a significant predictor of maturity ($F_{1,53}$ = 10.05, $P < 0.01$), males larger than about 66mm having a greater than 50% chance of maturing the following autumn while the likelihood for those smaller than about 53mm was less than 1% (Fig. 3.17a, Appendix I - Table I.7). The fat content of the fish did not explain any of the residual variation ($F_{1,53}$ = 0.08, N.S.). In February fat content was the most significant variable in predicting maturation ($F_{1,52}$ = 8.73, $P < 0.01$) (Fig. 3.17b). Thus males with higher than 3.6% fat in February had a greater than 50% chance of maturing while those with lower than 1% fat had a less than 1% chance. At this time fork length failed to explain a significant portion of the residual variation ($F_{1,52}$ = 1.98, N.S.). The graphs (not shown) for March and April, were similar with length being the best predictor in March and fat the best predictor in April (Appendix I - Table I.7).

To see if this technique could be applied in a practical situation where sex is not known, data from the females were added. In this case, length was the best predictor of maturation in January, February and March and percent fat was the best predictor in April, but in all months the logistic regressions failed to produce a greater then 50% probability of maturation for any value of length or fat content (Appendix I - Table I.8). For example, in January the probability of a 66mm fish maturing was now only 30% compared to 50% when only the males were included.

Expt. 2: Dietary fat manipulation - Results were very similar to the previous year, with the best predictor for maturation in any given month switching between length and percent fat (Appendix II -

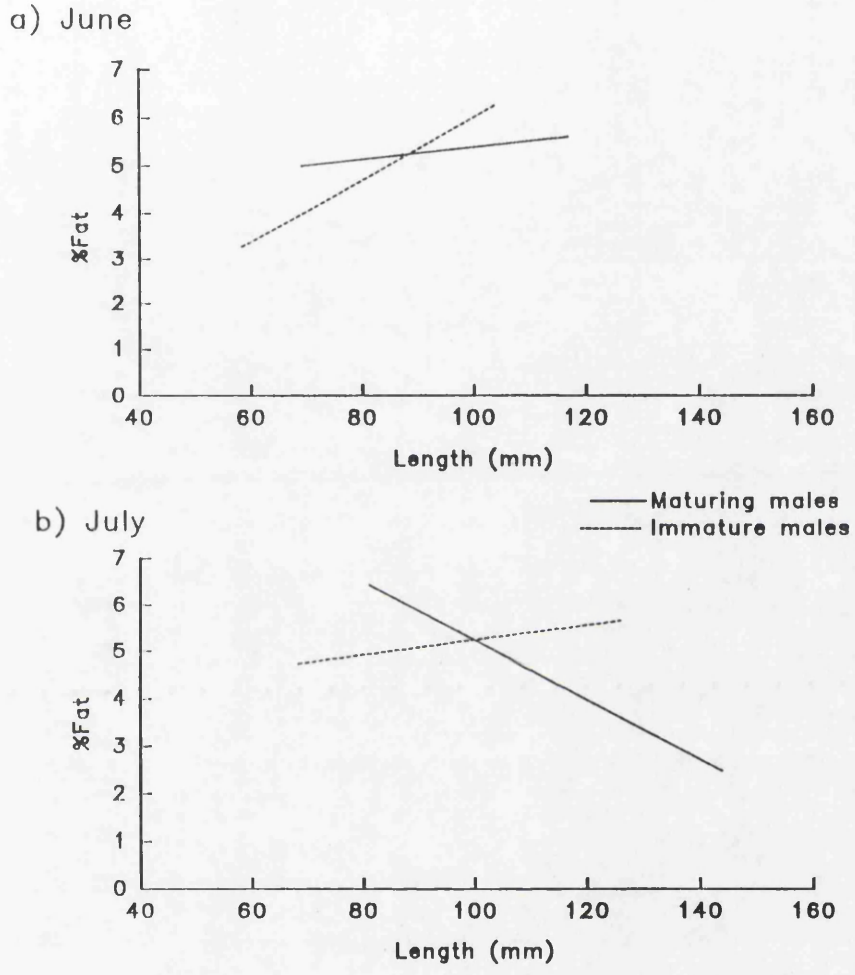


Fig. 3.16. The regression lines giving the relationships between percent fat and fork length for maturing and immature males in the control tanks from Expt. 3. a) June, b) July.

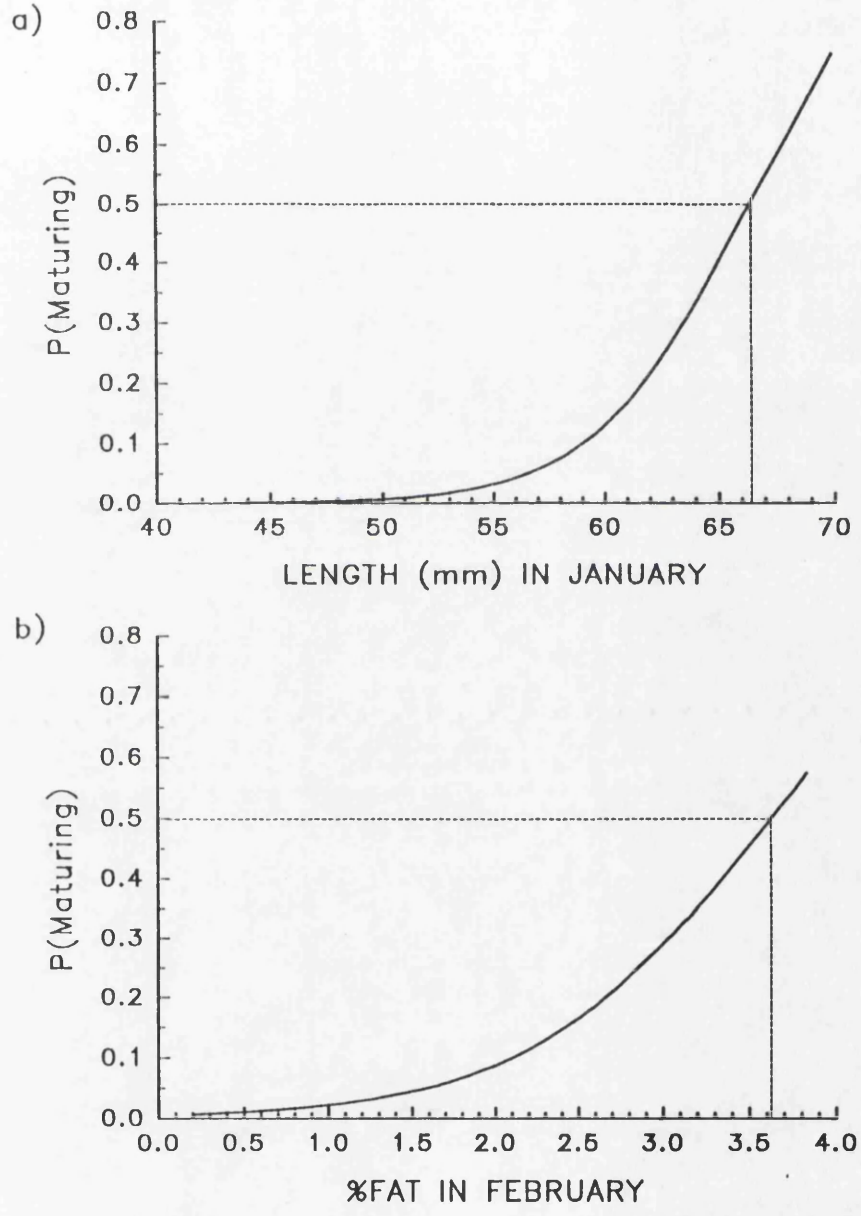


Fig. 3.17. Logistic regressions from Expt. 1. a) The probability that a male will mature in the autumn based on its fork length in January. b) The probability that a male will mature in the autumn based on its %fat in February.

Table II.12). This year %fat was the important variable in January and length became important in February.

The reason that the best predictor switched between percent fat and fork length (with the other variable failing to explain any additional variation in the probability of maturing) was probably due to the high correlation between fat and fork length. Thus the alternation of the best predictor between these two variables depends on which measure is slightly more variable and is of no biological significance.

Expt. 3: Winter starvation - The aim of this experiment was partly to reduce the correlation between length and fat and so see which was most important. In this experiment data from males in both treatment groups were combined. Length was the most important predictor of maturation in all months (November - June). In December, January and June percent fat was also entered into the regression equation. However, the P-values for entry were greater than the usual critical P = 0.05 level (Dec, P = 0.0866; Jan, P = 0.0577; Jun, P = 0.078) (Appendix III - Table III.14). Therefore the improvement in prediction using the additional information from fat levels was minimal.

3.4 Discussion

3.4.1. Differences between years

In 1990 there were no significant differences in mean length or fat content between immature males and females. However, in 1991 the mean for the females was not significantly different from that of maturing males. This is simply a function of the different maturation rates in each year. By plotting histograms of percent fat and length for each maturity group in March of both years (Figs. 3.18, 3.19) it is possible to see how this occurs. In 1990 only a small percentage of the males matured. These tended to be the larger and fatter fish. In 1991 only a small percentage of the males did not mature and these were the smaller, less fat fish. Therefore in both years there was a difference between maturing and non-maturing males. However, the females have a normal distribution over a similar range in both years, therefore in years

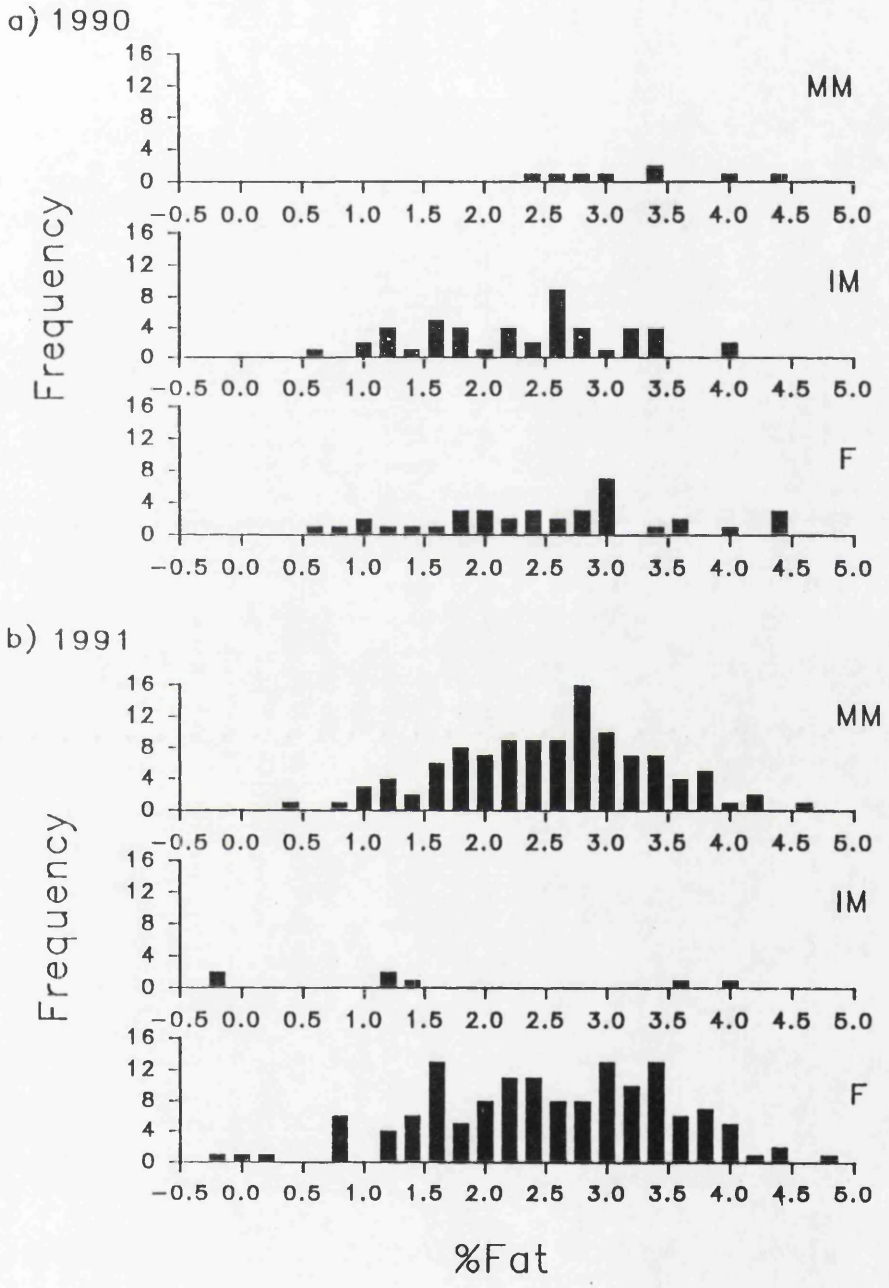


Fig. 3.18. Histograms of percent fat of each maturity group in March from Expt. 1 and 2. MM = maturing males, IM = immature males, F = immature females.

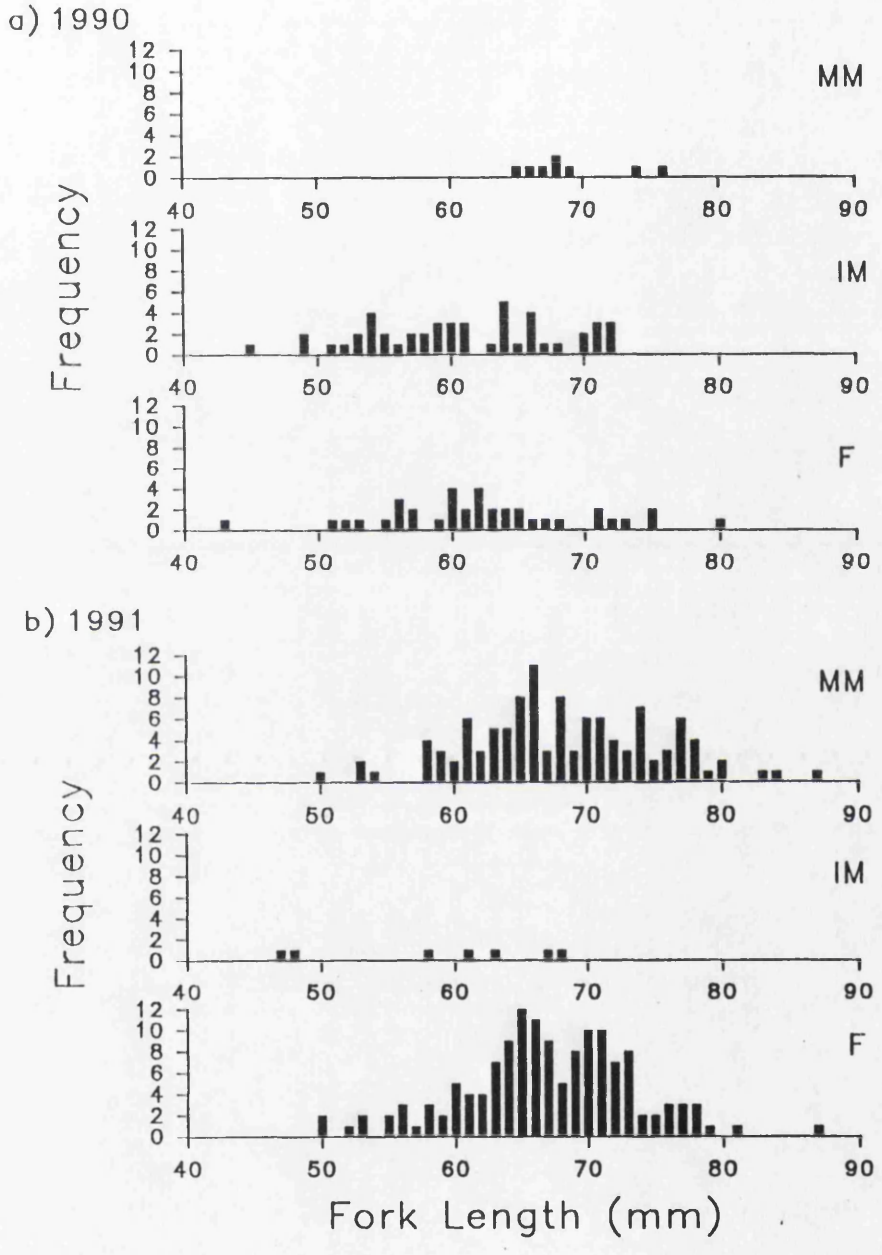


Fig. 3.19. Histograms of fork length of each maturity group in March from Expt. 1 and 2. MM = maturing males, IM = immature males, F = immature females.

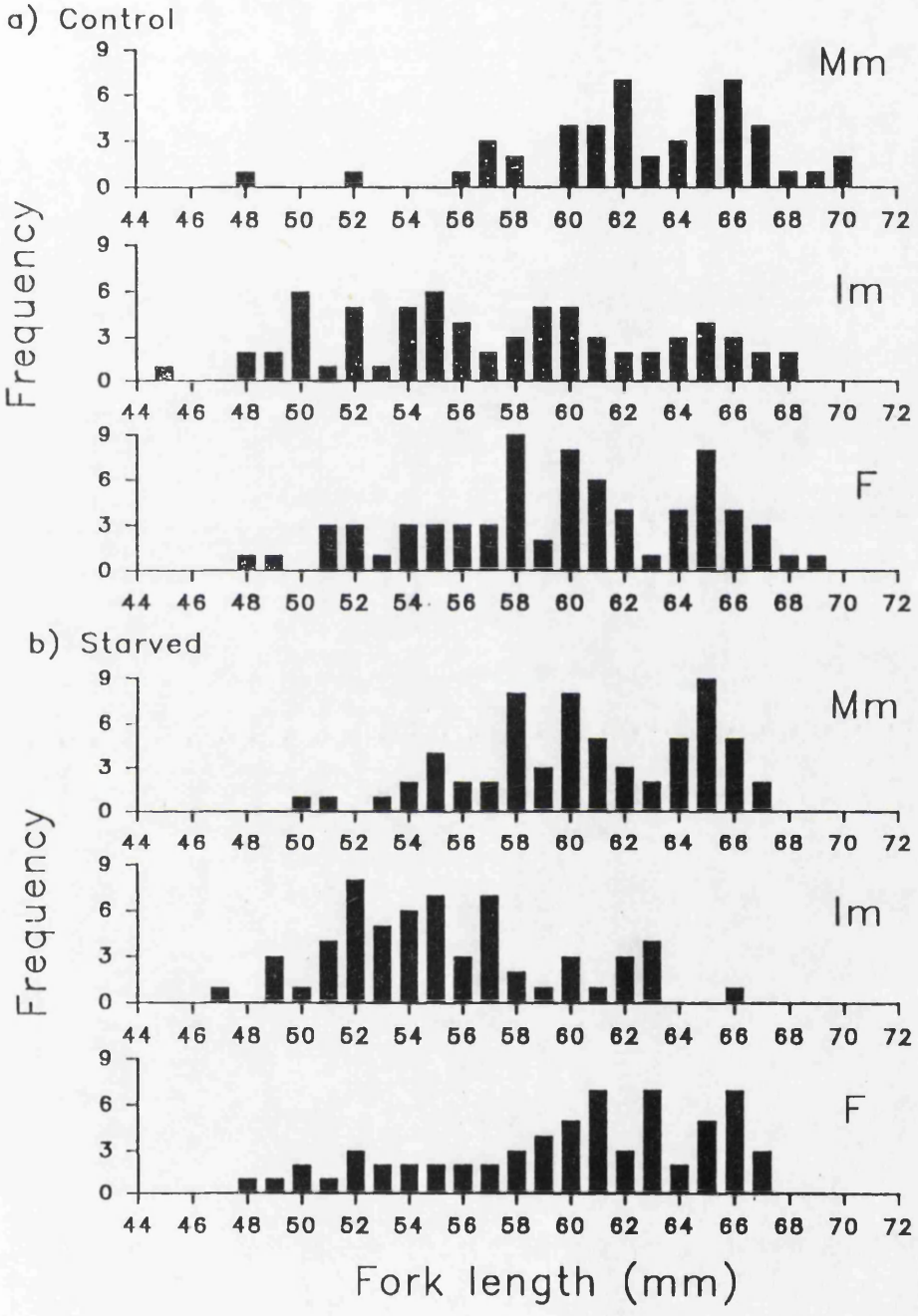


Fig. 3.20. Histograms of fork length of each maturity group in March from Expt. 3 a) Control, b) Starved. Mm = maturing males, Im = immature males, F = immature females.

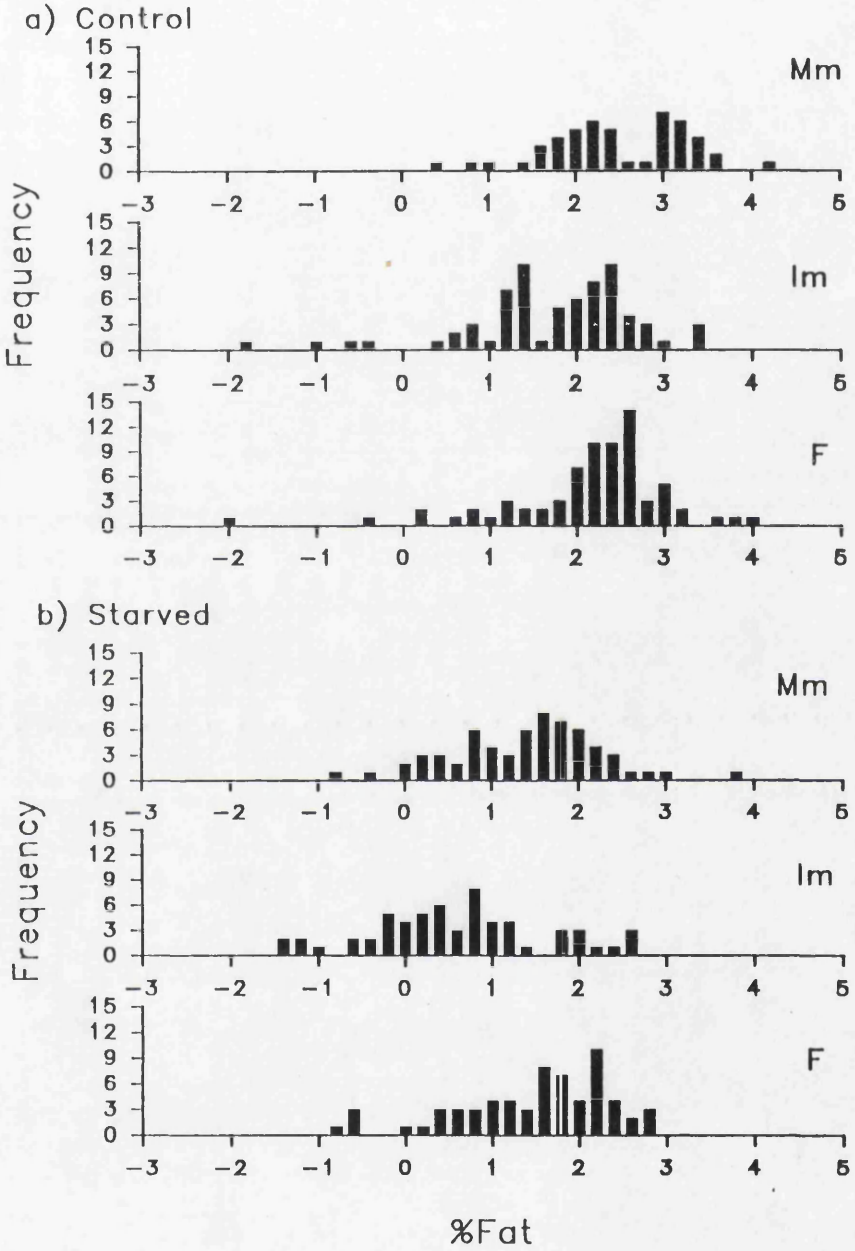


Fig. 3.21. Histograms of percent fat of each maturity group in March from Expt. 3 a) Control, b) Starved. Mm = maturing males, Im = immature males, F = immature females.

where only a small number of males manage to mature the mean of the females will be closer to that of the non-maturing males, and vice versa. A similar pattern is seen in the data from Experiment 3 (Figs. 3.20, 3.21).

3.4.2. Fat, length and maturation

Length - Maturing fish were significantly larger (from November to the following October) than those that failed to mature. Although similar results were found by Berglund (1992), results from other researchers in this field have not been so clear-cut, with significant size differences between maturing and non-maturing fish developing only a few months prior to spawning (Atlantic salmon, Rowe and Thorpe, 1990b; Rainbow trout (*Oncorhynchus mykiss*), Tveranger, 1985; Masu salmon (*Oncorhynchus masou*), Utoh, 1976; Chinook salmon (*Oncorhynchus tshawytscha*), Taylor, 1989). While Hunt *et al.* (1982) found that male Atlantic salmon grilse were larger and heavier than non-maturing fish of the same age, Jobling and Baardvik (1991) found no such differences in a mixed parentage group of Arctic charr (*Salvelinus alpinus*) and suggested that the size effects may be masked when data from several families are pooled. However, this cannot be a complete explanation, since Rowe and Thorpe (1990b) did not find long-term significant size differences despite using full-sib populations.

Fat - Changes in the fat content of individual fish with time have not been measured previously, although several researchers have looked at other methods of following individual changes in the condition of fish. The most common has been the use of the condition index, which has been found to correlate with percent fat in some studies of Atlantic salmon (Hoar 1939; Pinder and Eales 1969). However, Chapter 2 shows that condition index gave a very poor estimate of fat content. Given this limitation, differences in condition index have been found between maturing and non-maturing rainbow trout from 3-6 months prior to spawning (Tveranger, 1985), and in Atlantic salmon the condition index increased faster over the spring period for maturing than for non-maturing males (Rowe and Thorpe, 1990(b)). In a later paper Rowe *et al.* (1991), having assumed that all males would mature, found that the

total fat content (by dissection) of the presumed maturing fish (males) was higher than that of the presumed immatures (females) in January. However this difference had disappeared by July. In the current experiments I was able to look at the fat content of both the immature as well as the mature males using the non-destructive biometric technique (Chapter 2); the difference in fat reserves between the two categories of male indicates that the differences found by Rowe *et al.* (1991) were not just due to gender but are indeed related to maturity. The drop in percent fat found in the maturing fish at the beginning of the breeding season could possibly be attributed to the extra cost of milt production combining with a loss of appetite (Rowe and Thorpe, 1990b) to create an energy deficit which was balanced by the metabolism of fat reserves.

These experiments also brought to light the strong correlation between fork length and percent fat in salmon parr, which lends some credence to the existence of both a critical size and nutritional state threshold. If a fish needs a given amount of fat to be able to survive maturation, and fat and length are so closely linked, then it will inevitably need to attain a certain size before maturation becomes a viable option. Baglinière and Maisse (1985) found that mature (1+) male parr had been intermediate in length between upper and lower modal group fish in their first autumn and maturing male Baltic salmon parr were larger than their non-maturing male siblings in their first November (Berglund, 1992). Both these findings support the hypothesis that the split of maturity groups occurs before the parr enter their first winter. J.E. Thorpe, I.P. Smith, M.S. Miles, D.S. Keay and J.S. Muir (unpubl.) have recently found that gonad growth in seawater salmon begins to accelerate in November, leading to the segregation of these groups from that time. It has already been established that lower modal group parr enter a state of anorexia during their first winter (Metcalfe and Thorpe, 1992), when they lose weight and deplete their fat reserves. It would seem likely therefore that those fish that enter the winter in the best condition (i.e. with the higher fat reserves) are likely to have a high fat content at the end of the winter and therefore are more able to mature the following autumn. This is supported by the present results. The naturally high

correlation between percent fat and size initially made it difficult to establish which was most important. However, the starvation period imposed on one group of fish in Expt. 3 caused a separation of the two factors, since it produced a decrease in fat content in all fish (irrespective of length) over the period from December to March. In these conditions length was found to be more important than fat as a predictor of maturation.

3.4.3 Effect of manipulations

Neither of the manipulations carried out in Expt. 2 and 3 were successful in reducing maturation rates. Low fat diets (7-9% fat) in Expt. 2 did not affect the lipid levels of fish. Therefore either the fish are able to utilise their food more efficiently on a diet of this quality or the dietary fat level was well above the nutritional requirement of the fish at this time of year. Expt. 3 showed that maturation rates could not be suppressed by poor food conditions in mid-winter. Fat levels had been reduced over the winter in the treatment group and length became more important in predicting maturation. These results require expansion of the idea put forward above. They reinforce the suggestion that fat levels at the end of the summer are important and that the maturation pathway had already been taken before November. However, they indicate that loss of fat reserves over the winter period is not, in itself, enough to suppress maturation. After the period of food deprivation, fish in the starved group replenished their fat reserves first, before they began to increase in length. Therefore it seems that replenishing fat is more important than fast growth at this time of year (early spring). This response to good spring conditions might then enable the maturation process to continue in fish that had experienced a harsh winter.

3.4.4 Maturation predictors

The logistic regressions allow the prediction of the probability of male salmon parr maturing in the autumn based on their length or fat content earlier in the year. Unfortunately, as yet, there is no non-destructive method of sexing such small immature fish reliably. This limits the application of these

regression techniques since there is a complete overlap in both size and fat content between males (which may mature) and females (which will not), and so potentially maturing males will be inseparable from large, fat females. The hypothesis that immature males and females may be separated on the basis of dimensions of their head or jaw (so that predictions of maturation can be done on males alone) is tested in Chapter 7.

In this study the probability of maturation was greater than 50% in males with a length greater than 60mm in their first November. Berglund (1992) used a probit regression model on length data from Baltic salmon parr to gain similar results. However, the size threshold for maturity of males in that population was larger. They had to be greater than 70mm in November to have >50% chance of maturing. Perhaps the short growing season in the Baltic, compared to Scotland necessitates this larger size and therefore condition threshold before maturation can occur. This implies that there will be stock-specific threshold levels for maturation and therefore stock-specific predictors will be needed.

3.4.5. Growth rates

It has already been noted that it is the fastest growing fish that are more likely to mature. For Atlantic salmon, the critical time for growth is thought to be in the late winter / early spring. Populations having fast growth during this period had a higher incidence of parr maturation the following autumn than those that had fast growth over the winter or summer (Rowe and Thorpe, 1990a). This is in agreement with several other studies of both parr and later stages (Hunt *et al.*, 1982; Tveranger, 1985; Myers *et al.*, 1986; Skilbrei, 1989). It has also been found previously that the growth of the maturing fish slows down from July as they put energy into gonad production (Lundqvist and Fridberg, 1982; Skilbrei, 1989; Rowe and Thorpe, 1990b).

In contrast, there was no such clear split between maturing and non-maturing fish in this study, with all fish increasing their growth rates during the spring to a peak in May-June, then showing a gradual

decline in growth into the autumn. Nor did relative changes in fat hold the key to the maturation process. The third experiment allowed the effect of starvation on subsequent growth rates to be studied. In a review on compensatory growth, Russell (1991) showed that fish have a great ability to survive periods of low food availability and regain lost growth once the food level increases. To avoid compensatory growth in this third experiment the ration level was increased very slowly and growth rates were monitored to estimate the effect of the increased food supply. Unfortunately even with this cautionary step the fish's ability to compensate was underestimated and compensatory growth was seen in the previously starved fish from April to June.

3.4.6. Conclusions

The first two experiments were designed to take place over the period when differences would first start to appear between maturing and non-maturing fish. The results show that by January there was already a difference in mean size and percent fat between subsequently maturing and non-maturing males. These differences did not subsequently become any larger, since there were no differences in growth rates or fat changes between maturing and immature fish until late autumn. It was therefore clear that the months leading up to mid-winter needed to be investigated if this early maturation process was to be understood completely, hence the third experiment began in November. But as we have seen the difference between maturity groups was already present at this time. Therefore it seems that maturation is already switched on in some male parr in their first November, one year before they will actually produce sperm and become sexually active. Moreover, a deprivation period over the winter or low fat diet during the spring is not sufficient to switch this off.

Chapter 4 - Effects of season and sexual maturation on appetite

4.1 Introduction

Several lines of evidence indicate that appetite may be an important indicator of the maturation process. Firstly, fish, like mammals, seem to regulate food intake to meet energetic needs (Rozin and Mayer, 1961; Lee and Putnam, 1973; Grove *et al.*, 1978; Flowerdew and Grove, 1979). Secondly, maturing male Atlantic salmon parr are larger (Rowe and Thorpe, 1990b) and increase their fat stores earlier than non-maturing fish (Rowe *et al.*, 1991). And, indeed, food restriction during this period of fat deposition had been shown to reduce subsequent maturation rates (Rowe and Thorpe, 1990a). Therefore, it seemed likely that maturing male parr would increase their food consumption to enable fat deposition to occur.

Various factors control appetite in fish:

The rate of food consumption is dependent on the rate at which food is evacuated from the stomach (Brett, 1971; Windell *et al.*, 1972; Grove and Crawford, 1980). Appetite at any point will then be related to stomach fullness at that time.

Temperature affects appetite through its influence on both metabolic requirements and the gastric evacuation rate. With increasing temperature food is evacuated from the stomach faster, producing an earlier return of appetite (Brett and Higgs, 1970; Gerald, 1973; Jobling and Davies, 1979; Persson, 1979; Grove *et al.*, 1985). However, each species and developmental stage (Hokanson *et al.*, 1976) has its own maximum temperature threshold after which further increases will produce a rapid decrease in consumption. For instance, in brown trout this threshold is about 18°C (Elliott, 1975).

Body weight also influences appetite. As fish grow, the weight of food they consume relative to their body weight decreases, although the absolute amount consumed increases (Wootton, 1990). Therefore, after consuming the same percentage of their body weight of food a smaller fish will have a quicker

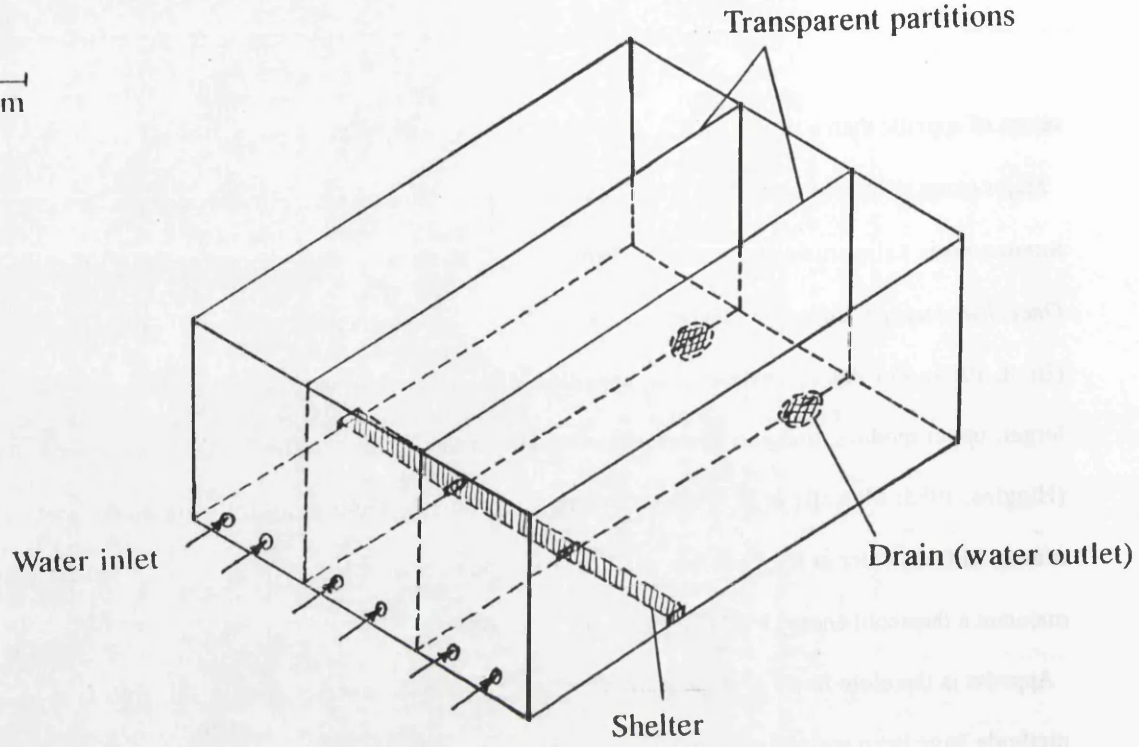
return of appetite than a larger fish (Pandian, 1967; Jobling *et al.*, 1977; Persson, 1981).

Major changes in food consumption can occur as the physiological state of a fish changes. For example, anadromous salmonids cease to feed during their spawning migration and the gut of several *Oncorhynchus* spp. undergoes degenerative changes which actually prevent any normal food processing (Brett, 1983). For Atlantic salmon parr, appetite has been shown to vary with life history strategy. The larger, upper modal group parr (those that will smolt in the Spring) continue to feed during the winter (Higgins, 1985; Metcalfe *et al.*, 1988). However, their smaller siblings in the lower modal group (that remain in freshwater at least another year) enter a state of anorexia during this period and eat only to maintain a threshold energy level (Metcalfe and Thorpe, 1992).

Appetite is therefore likely to show seasonal variation due to the interaction of all these factors. Several methods have been widely used to determine food consumption. Destructive methods relying on the direct examination of stomach contents, preclude the opportunity to follow changes in individual fish with time. This can be overcome in the laboratory by incorporating radio-opaque markers into the food and measuring the food intake of live fish by X-raying them (Talbot and Higgins, 1983). Direct observation of feeding behaviour is also a useful measure of appetite allowing individual feeding motivation to be determined (Metcalfe *et al.*, 1986).

In this chapter experiments in which appetite is monitored using both direct observations and the X-ray radiography method are described. The experiments were designed to measure appetite over different seasons and under varying feeding regimes with the aim of relating the results to the subsequent maturity group of the fish. Experiment 1 monitors seasonal appetite changes in maturing and non-maturing parr, Experiment 2 looks at how appetite was influenced by the lipid content of the food during the spring, and Experiment 3 looks at the influence of ration restriction over the winter period.

a) Behavioural observation tank



b) Side view of observation set-up

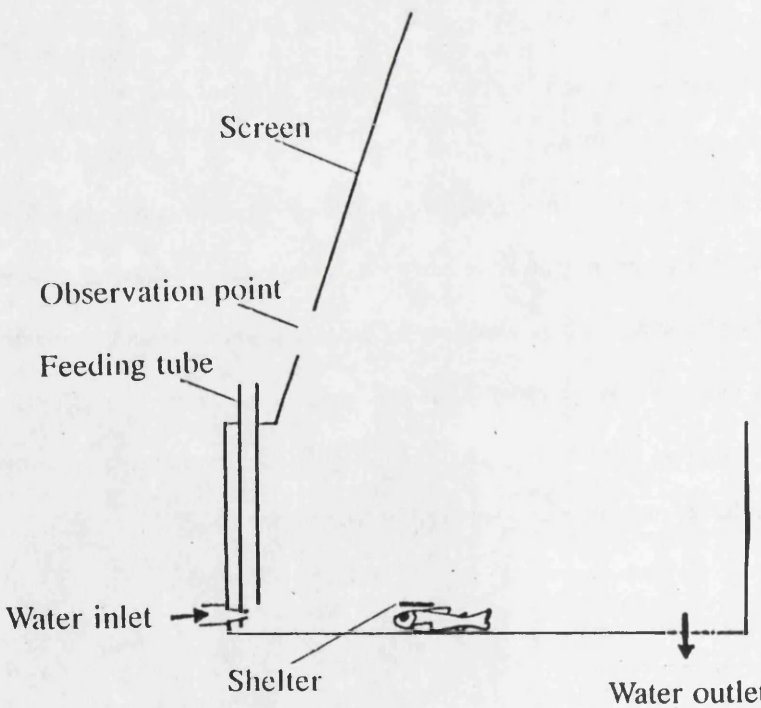


Fig. 4.1. a) The observation tank and b) a side view of the observation set-up for recording feeding behaviour used in Expt. 1, 1990.

4.2 Materials and methods

The fish and husbandry details of each experiment are the same as in the experiments described in Chapter 3.

4.2.1 Experiment 1, 1990: Direct observation of feeding behaviour

Appetite - Appetite was measured each month from January to September by observations of feeding behaviour. For this, fish were transferred to individual compartments of tanks that allowed controlled presentation of food. Each observation tank was made of opaque perspex (30x40x16cm) and divided into three compartments by transparent perspex (Fig. 4.1a). Two water inlet nozzles were positioned low down on one wall of each compartment, the water flowing along the tank and out of drains at the opposite end. The water depth was maintained at about 5cm by standpipes. Each compartment contained a shelter (2x10cm) about 2cm off the floor of the tank under which the fish would rest (Fig. 4.1b). Observations were made through a small slit in an opaque screen that otherwise visually isolated the observer from the tank. Each month one fish was placed in each compartment of twelve observation tanks and left under simulated natural photoperiod and without food for 48hrs to settle. Appetite was then monitored on the following day by presenting each fish with ten food pellets throughout the daylight hours, with a minimum of 20mins between pellets. Food pellets were introduced to the tank through feeding pipes (on the observer's side of the screen) that terminated directly above one of the inlet nozzles in each compartment. The pellet would be carried by the water current directly past the fish resting under the shelter.

The fish's response to the pellet was defined and scored as follows (after Metcalfe *et al.*, 1986) :

0 = No response.

1 = Orientation only - the fish orientated towards the passing pellet but did not move towards it.

2 = Turnback - the fish orientated and moved partway towards the pellet but turned back to its resting position before reaching it.

3 = Miss - the fish orientated and moved to the pellet but appeared to miss it.

3 = Reject - the fish orientated, moved to the pellet and intercepted it but then spat it out.

6 = Consume - the fish orientated, moved to the pellet, intercepted it and ate it.

It was not always possible to make the pellet flow past the fish due to either the fish's position, the water current or a combination of both. In these cases a maximum of three pellets were sent down the feeding tube. If the third pellet would still not flow past the fish then the outcome was discounted for that pellet. All fish could be screened for appetite within a two-week period each month. A mean appetite score was calculated for each fish from the scored outcome of each pellet. Individual results were only included in the analysis if more than four of the ten pellets flowed passed the fish.

4.2.2 Experiment 2, 1991: Food intake in fish with dietary fat manipulation

Appetite - The large number of fish involved in this experiment precluded the use of direct observations of feeding behaviour to determine appetite. Instead appetite was measured by monitoring food intake monthly (from January to September) using an X-ray method similar to that described by Talbot and Higgins (1983).

A labelled food was prepared in a similar way to the different diets (described in Chapter 3) but a radio-opaque marker, 7% (by weight), size 9 ballotini balls (small glass beads) was added to the dry mix before combining with water. The labelled food was then processed in the same way as the other diets (food preparation method Chapter 3) to obtain dried pellets of suitable sizes. These were fed to the fish in the radial flow tanks in place of their usual food (at the same rate and in the same manner i.e to excess) so that the fish would not have been aware of any change in their normal feeding regime. From January to May the labelled food was used for six hours in the morning. This was reduced to four hours from June to August because the digestion rate of the fish had increased due to the higher water temperatures at this time of year causing some of the labelled food to pass through the digestive tract completely in six hours. All the dye-marked fish were then X-rayed (75kV, 100amps, 0.5sec). From the developed X-ray

plates the number of ballotini marker balls in each fish was counted. This was converted into weight of food ingested by the following calibration equations (1-7) derived from X-ray plates of weights of food alone and was then expressed as a percentage of fish wet weight per hour.

January - March -

Diet 1: $W = 0.000313 + 0.00181(B)$ $r = 0.993, n = 22, P < 0.001$ (1)

Diet 2: $W = 0.000688 + 0.00201(B)$ $r = 0.970, n = 22, P < 0.001$ (2)

Diet 3: $W = -0.00195 + 0.00208(B)$ $r = 0.990, n = 22, P < 0.001$ (3)

April -

Diet 1: $W = -0.00187 + 0.00158(B)$ $r = 0.990, n = 26, P < 0.001$ (4)

Diet 2: $W = -0.00024 + 0.00162(B)$ $r = 0.985, n = 25, P < 0.001$ (5)

Diet 3: $W = 0.00250 + 0.00164(B)$ $r = 0.989, n = 23, P < 0.001$ (6)

May - September -

Standard: $W = 0.001940 + 0.00166(B)$ $r = 0.990, n = 22, P < 0.001$ (7)

where W is weight of labelled food (g) and B is number of ballotini.

4.2.3 Experiment 3, 1991-1992: Food intake in fish in winter starvation experiment

Appetite - Appetite was measured by the same X-ray method as Experiment 2. The labelled food (with 7%, No.9 Ballotini balls) was prepared in a similar way as before but since the fish were feeding on a standard commercial fish food this year, the basic dry mix used was the smallest size of this food available (fry 00 - B.P.Nutrition). The usual food fed to the fish was replaced by this labelled food as before for six hours in the morning from November to May and again reduced to four hours in June and

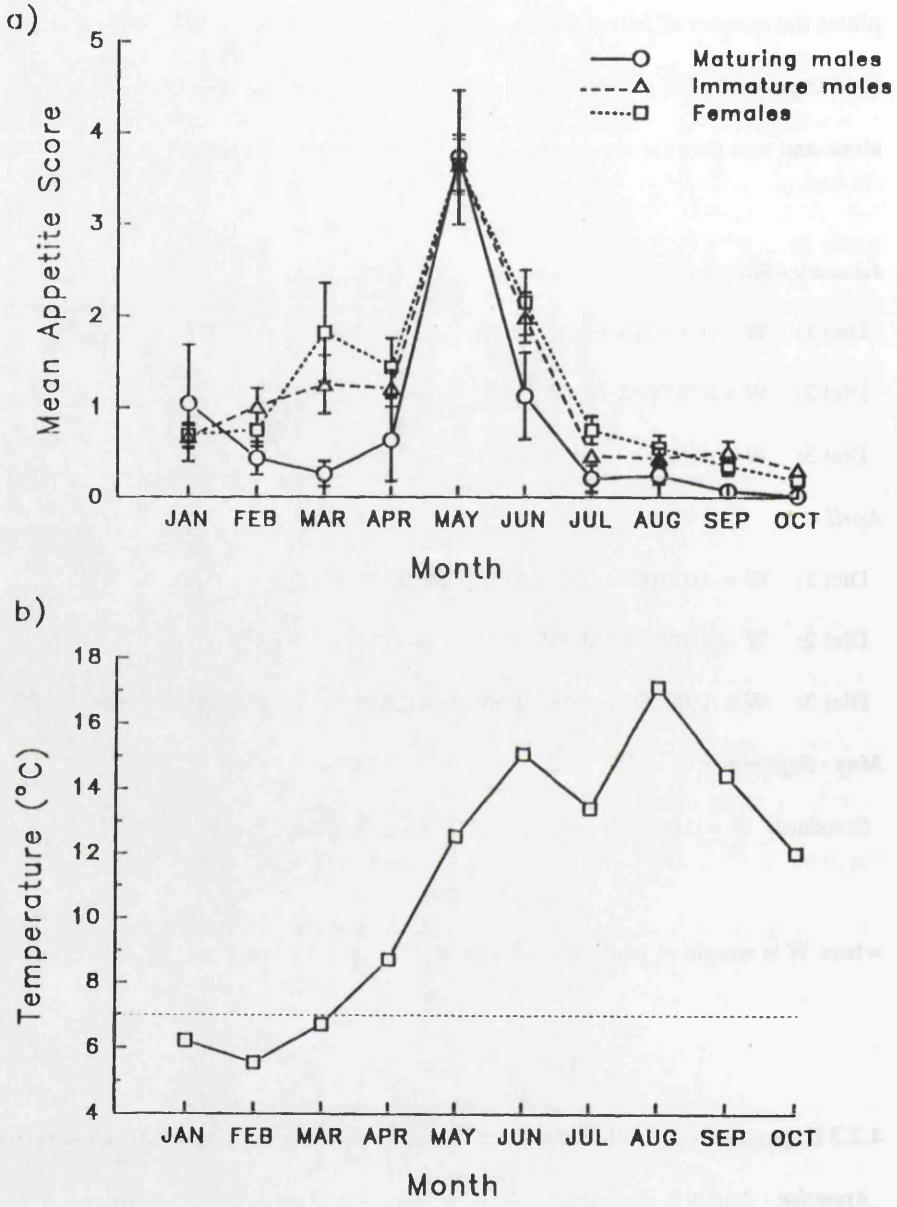


Fig. 4.2. Expt. 1, 1990. a) The mean appetite scores (±S.E.) of maturing males, immature males and immature females for each month. b) The corresponding mean temperatures at the time of the monthly appetite observations.

July (Equations 8-10). In these last two months two tanks were fed on the labelled food in the morning and two in the afternoon to see if there were any differences in appetite at these times. Due to the starvation regime imposed on two of the tanks the food intake of these starved fish was only monitored from May to July. The calibration equations relating number of ballotini (B) to weight of food (W) in g were as follows:

November - February -	$W = 0.00764 + 0.00161(B)$	$r = 0.971, n = 23, P < 0.001$	(8)
March - June -	$W = 0.00479 + 0.00174(B)$	$r = 0.980, n = 23, P < 0.001$	(9)
July -	$W = 0.01000 + 0.00179(B)$	$r = 0.980, n =$, $P < 0.001$	(10)

At the end of each experiment (October 1990, September 1991, and September 1992 respectively) the sex and maturation status of all fish was determined.

4.3. Results

As in Chapter 3, in cases where more than eight similar statistical tests were carried out in the same analysis, the results were only regarded as significant if the probability was below 0.01, to reduce the risk of Type I errors.

Expt. 1: Direct observation of feeding behaviour -

All three categories of fish showed a large increase in May which did not correspond to a peak in temperature (Spearman rank correlation between mean monthly appetite score and mean monthly temperature, $r_S = -0.164$, $n = 10$, $P > 0.05$, N.S.) (Fig. 4.2b). Thereafter there was a sharp decline for all fish even though the temperature remained suitable for feeding. There were no significant differences in appetite scores between the three maturity groups (Fig. 4.2a, Appendix I - Table I.9).

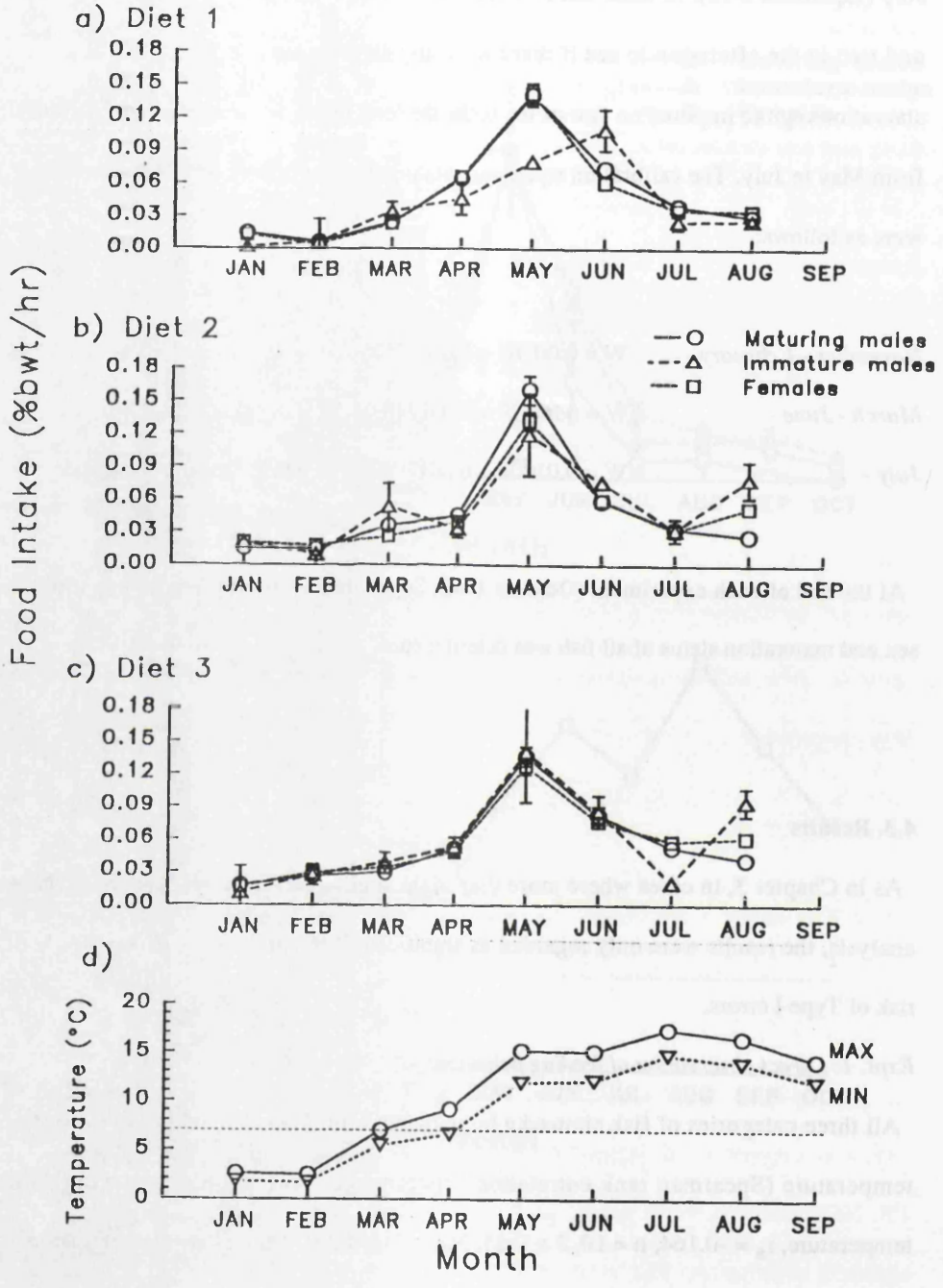


Fig. 4.3. Food intake (mean percentage of body weight per hour, ±S.E.) against month for maturing males, immature males and immature females in Expt. 2, 1991. a) Low fat diet (7%fat), b) Medium fat diet (11%), c) High fat diet (18%). d) Mean daily minimum and maximum temperature for each month.

Expt. 2: Food intake in fish in dietary fat manipulation -

Again all fish showed a large increase in appetite in May which did not correspond to a peak in temperature (correlation between mean monthly appetite and mean monthly temperature, $r = 0.569$, $n = 8$, $P = 0.141$) and a similar sharp decline thereafter even though the temperature remained suitable for feeding (Fig. 4.3).

Data from immature males have been excluded from the following analyses due to the small sample size (only seven fish in total). Mature males and females showed similar differences in appetite between diets at several points during the study. In February, those on the low fat diet (Diet 1) were eating significantly less than those on the high fat diet (Diet 3) (ANOVA mature males, $F_{2,111} = 8.80$, $P < 0.001$; females, $F_{2,136} = 16.41$, $P < 0.001$). However, in April, the maturing males on Diet 1 were eating significantly more than those on Diet 2 (the medium fat diet) (ANOVA $F_{2,109} = 5.78$, $P = 0.004$) and the females on Diet 1 had significantly greater appetites than those on either of the two higher fat diets (ANOVA $F_{2,139} = 16.88$, $P < 0.001$). In June, there was some evidence of differences both in maturing males and females between Diet 2 and Diet 3, those on Diet 3 having a slightly greater appetite (ANOVA, maturing males, $F_{2,111} = 3.95$, $P = 0.022$; females, $F_{2,139} = 4.37$, $P = 0.014$). Females showed further differences in July and August with those on the high fat diet having greater appetites than those on the low fat diet (ANOVA July, $F_{2,136} = 10.70$, $P < 0.001$; August, $F_{2,136} = 6.10$, $P = 0.003$) (Appendix II - Table II.13).

Given these differences, the diets were analysed separately with respect to maturity. There were no significant differences in food intake between maturing males and females on the low fat diet, Diet 1 (the greatest difference occurring in June, t-test, $t = 1.92$, d.f. = 81, $P = 0.058$). Significant differences occurred on Diet 2 in August when the females had the greater appetites (t-test, $t = -4.14$, d.f. = 75, $P = 0.0001$). This last difference in August reoccurred in fish on the high fat diet, Diet 3, females > maturing males (t-test, $t = -2.97$, d.f. = 74, $P = 0.0041$) (Appendix II - Table II.14).

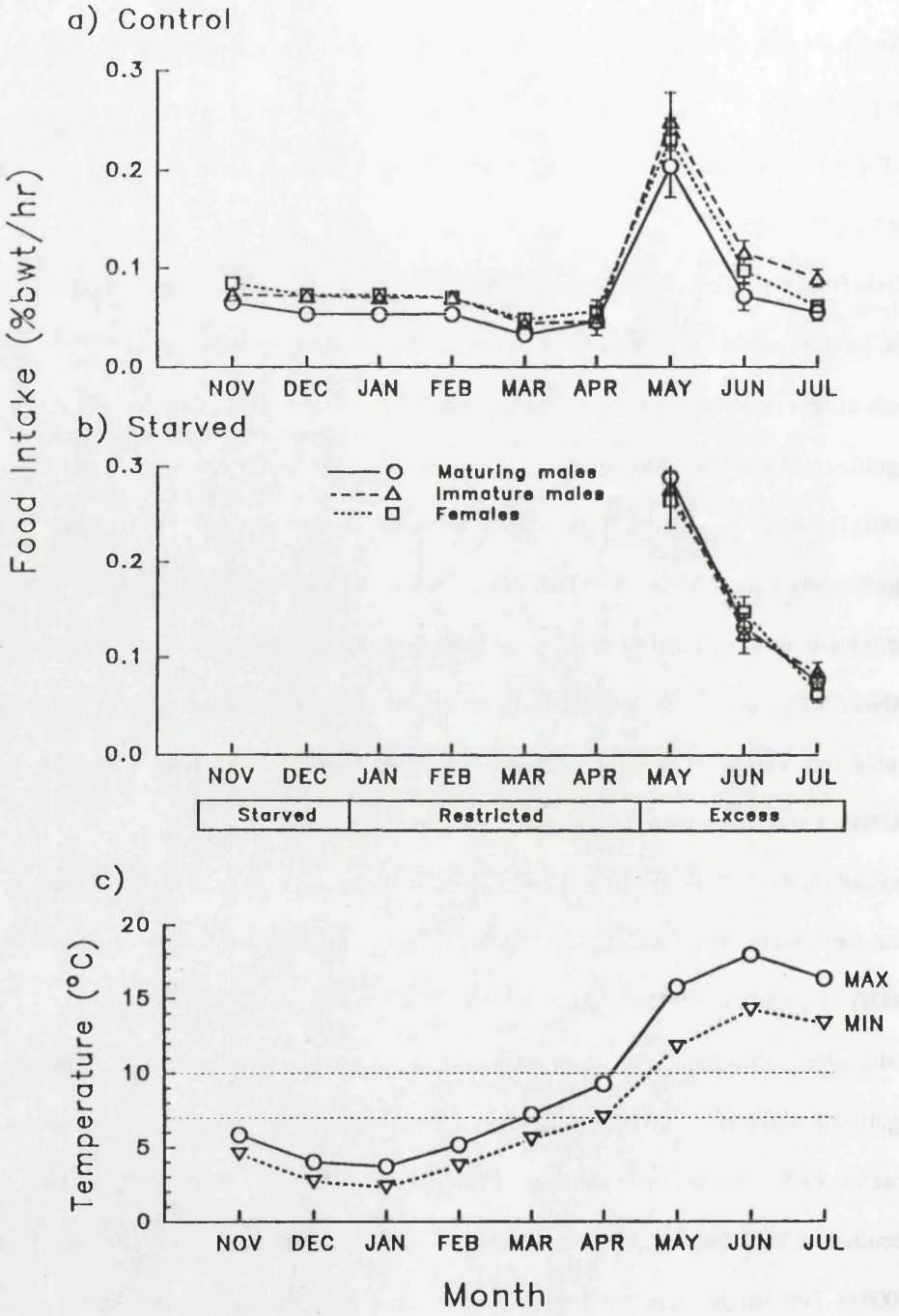


Fig. 4.4. Food intake (mean percentage of body weight per hour, ±S.E.) against month for maturing males, immature males and immature females in Expt. 3. a) Control fish, b) Starved fish. c) Mean daily minimum and maximum temperature for each month.

Expt. 3: Food intake in fish in winter starvation experiment -

Again there was a similar pattern to previous years with food intake peaking sharply in May and declining thereafter with no correlation with temperature ($r = 0.407$, $n = 9$, $P = 0.277$) (Fig. 4.4). In the controls, the only occasion when the fish differed in appetite was in July, when the immature males had a higher food intake than both maturing males and immature females (ANOVA, $F = 7.01$, $P = 0.0019$) (Appendix III - Table III.15). There were no significant differences in food intake between maturing and non-maturing fish in the starved tanks (May to July) (ANOVA, highest $F = 1.46$, $P = 0.24$) (Appendix III - Table III.15). Differences between the food intake of control and previously starved fish were only significant in May and June (i.e. following the lifting of the restricted ration) where fish from the starved tanks had higher food intakes than those in control groups (Two-way ANOVA of food intake against maturity group and treatment, May, $F_{1,84} = 4.91$, $P = 0.030$; June, $F_{1,103} = 14.11$, $P < 0.001$). Results from each category of fish are given in Appendix III - Table III.16. In July there were no treatment effects but there were differences between maturity groups, immature males having greater appetites than both females and maturing males (Two-way ANOVA, $F_{2,102} = 5.34$, $P = 0.006$).

Correlates of appetite and fat content -

In the first two experiments (direct observation of feeding behaviour and food intake in fish in dietary fat manipulation) appetite was not correlated with an individual's fat content in any month (Appendix I - Table I.10; Appendix II - Table II.15). However, in the third experiment there was a significant negative correlation between the two variables in the control fish from November to March (least significant $r = 0.457$, $n = 42$, $P = 0.001$; see Appendix III - Table III.17). Thus, fish with lower lipid levels had a greater appetite.

Correlates of appetite and specific growth rate -

Measures of appetite were significantly correlated with specific growth rate in only a few months each year with no consistent pattern (only 3 out of 48 correlations were significant; Appendix tables I.11, II.16 and III.18). The strongest correlation was found in Expt. 2 in August ($r = 0.438$, $n = 248$, $P < 0.001$;

see Appendix II - Table II.16); in all cases of a significant relationship, a high appetite coincides with a high specific growth rate.

Correlates of appetite and rate of change in %fat -

The relationship between appetite and rate of change in percent fat was only significant in two months throughout all the experiments (Appendix tables I.12, II.17 and III.19). In the second experiment the appetite in April was correlated with the rate of change in fat from April to May ($r = 0.161$, $n = 248$, $P = 0.01$)(Appendix II - Table II.17). In the third experiment the appetite in November was correlated with the rates of change in fat from November to December ($r = 0.431$, $n = 44$, $P = 0.004$) (Appendix III - Table III.19). Therefore a high appetite coincided with a large increase in fat content; however, these statistically significant relationships should be treated with caution due to the large number ($n = 41$) of correlations performed.

4.4. Discussion

Appetite and compensatory responses -

Fish that had been on low fat diets (Experiment 2) or that had been starved (Experiment 3) all showed compensatory appetite responses when they were once again fed to excess on a standard commercial fish food. This increased appetite in relation to controls allowed them to regain some of the lost growth and fat reserves (see Chapter 3). In a review on compensatory growth, Russell (1991) showed that this is not an unusual phenomenon in fish. This compensatory response allows them to regain lost growth time, in some cases almost as if there had not been a food shortage. This ability to compensate is an important consideration in any treatment designed to reduce growth or fat levels via food intake.

A potential mechanism by which appetite is regulated was investigated by looking at the correlations between appetite and fat levels, specific growth rates and rates of change in fat. Metcalfe and Thorpe (1992) had shown that lower modal group fish would defend an energy level if starved during the winter

i.e. after a period of starvation, when their fat levels had been reduced, the fish only increased their food intake relative to controls until fat stores had been replenished, thereafter their appetites returned to control levels. A similar pattern was seen from November to March in the winter starvation experiment with fish that had a low fat level having a higher appetite. It is not clear why this relationship only occurred in the third experiment, since the range of fat levels was similar in each experiment. It is unlikely that fish have a direct estimate of how fat or how large they are, but it may be that rates of change of these variables give a physiological signal that may influence appetite. However, specific growth rates and rates of change in fat were not consistently related to appetite, so the exact mechanism by which fish compensate for lost growth with increased appetite is not clear.

Appetite and maturation -

None of the results showed the expected greater appetite in maturing males. Since these maturing males are larger and have higher fat levels than non-maturing fish in November of their first year (Chapter 3), it is clear that something has happened during the first summer to create this size and fat difference. Either the maturing males have greater appetites during this summer period, or alternatively, they must utilising their food more efficiently. Food conversion efficiency has been shown to increase when androgens such as 17-α-methyltestosterone or ethylestrenol have been added to the food of coho salmon, rainbow trout and carp (Fagerlund *et al.*, 1979; Ince *et al.*, 1982; Lone and Matty, 1983, respectively). Therefore it is conceivable that the hormones associated with maturation may increase the digestive efficiency of the maturing fish allowing them to grow larger and fatter than immature fish on the same ration. However, since maturing males were already larger and fatter in November, perhaps it is the more efficient fish that are able to mature rather than the maturation process increasing efficiency. There has been no work on the metabolic rate or digestive efficiency of the different categories of fish, so there is no direct evidence to back up this hypothesis.

Appetite and season -

In this chapter similar results were obtained when appetite was measured by two completely different techniques over three different years. Therefore it is unlikely that the sharp peak observed in May is a quirk of the methodology or husbandry conditions. Peaks at this time of year have been observed in the stomach contents of wild juvenile salmon (Allen, 1940, 1941; Eriksson and Mortensen, 1977) and can possibly be explained by increases in prey numbers at this time (Hynes, 1970; Muller, 1978; Eriksson and Alanara, 1990). However, the proximate mechanism is not food availability since the same pattern has now been shown to occur under conditions of excess food.

The peak in appetite in May did not correlate with temperature. However, it is interesting to note that May was the first month in which the temperature rose above the lower critical level for activity in juvenile salmon (7°C, Allen, 1940). Elliott (1976) found that the maximum gross growth efficiency was obtained with temperatures from 8-11 °C in trout. It may be that appetite peaks in May due to the rise in temperature allowing greater activity and therefore feeding; the fish might thus be taking advantage of the first good opportunity after the winter to replenish lost fat reserves. However, this would not account for the subsequent decline in appetite through the summer months. This decline cannot be attributed to the maturation process since it occurs in all fish, maturing and non-maturing. Since it occurs in the presence of excess food, there must be some pre-programmed mechanism that suppresses appetite after May. Perhaps it has evolved to enable the fish to survive a period of low prey abundance in the natural stream habitat, in particular a scarcity of food of an optimum size. This hypothesis is explored and tested in Chapter 6.

Chapter 5 - Changes in gonad tissue in the year preceding maturation.

5.1 Introduction

Throughout this thesis experiments have been designed to pinpoint the time at which life history paths for maturing and non-maturing male parr first separate. Results from these experiments (Chapter 3) indicate that this divergence occurs much earlier than previously thought, i.e. November of the first year. Maturation is unavoidably associated with gonad growth. Therefore measuring this growth may allow the start of the maturation process to be identified.

In most fish species gonad growth begins many months prior to maturation, although a large increase in growth occurs close to the breeding season. After the breeding season absorption of the gonads may occur to some extent. These two facts lead to a cycling of gonad growth related to the timing of the breeding season (Wootton, 1990). Atlantic salmon are autumn spawners; gametogenesis takes place over the summer and ripe eggs and sperm are available at spawning time. This involves a large increase in the gonadosomatic index (G.S.I.) of maturing fish from about July onwards. The G.S.I. decreases after the spawning period to a low winter level and begins to increase again in early spring. During their first year in sea-cages, the gonad growth spurt in both male and female maturing salmon occurred in November of the year prior to spawning (Thorpe, 1994b).

Little is known about the timing of gonadal development in maturing parr and in particular the point of divergence between maturing and non-maturing fish. The main aim of the experiment described in this chapter is to provide such information and to see if G.S.I. can be used as an accurate measure of gonadal development at this time of year.

5.2 Materials and Methods

Every month from October 1991 to May 1992, a sample of 100 0+ Atlantic salmon parr were removed from a $2m^2$ stock tank in which the fish had been fed to excess on an optimally-sized commercial fish

Table 5.1. Definition of the spermatogenetic stages found in salmon parr testes, 7-13 months after first feeding in April (adapted from Billard, 1992). Sp.gonia - spermatogonia, A - germ cell, B - germ cells in cysts; Sp.cytes - spermatocytes; Sp.tids - spermatids; Sp.zoa - spermatozoa, L - in lobules, D - in sperm duct. 0 - absent, + - present, ++ - present in large numbers, (+) - maybe present.

Stage	Definition	Activity					
		Sp.gonia		Sp.cytes	Sp.tids	Sp.zoa	
		A	B			L	D
Io	Prepubertal	+	0	0	0	0	0
Ii	Previously mature	+	0	0	0	(+)r	(+)r
II	Initiation of spermatogenesis	++	+	0	0	0	0
III	Many cysts of germ cells	+	++	+	(+)	0	0
IV	Many spermatocytes	+	++	++	+	(+)	0
V	Many spermatids	+	+	++	++	+	0
VI	Lobules filled with Sp.zoa	+	+	+	++	++	(+)
IX	End of spermiation resorption of sp.zoa	+	0	0	0	+	+

r - remnants from previous cycle.

food. Each fish from the sample was weighed (to 0.01g) and a measurement of fork length taken (to 1mm). The fish were killed by an overdose of Benzocaine and the gonads were removed by dissection and weighed separately (wet weight to 0.0001g). They were then fixed in Bouins solution for up to 24hrs before being transferred to formal saline. Stained slides were made of gonad sections from 20 males and 20 females chosen to give a representative sample of the G.S.I. distribution that month.

The gonadosomatic index (G.S.I.) of each fish was calculated as follows:

$$G.S.I. = (G \times 100) / W \quad (1)$$

where G is the wet weight of the gonads (g) and W is the wet weight of the fish (g, before dissection).

In September 1992 another sample of 100 fish was taken from the same tank as before. The fish were killed as above and wet weight, fork length and sex were recorded. Mature males were distinguished by visual observation of the testes. Those whose testes were large and solid white in colour, indicating that they contained sperm, were classed as maturing. Gonads of immature males and all females were removed, weighed and G.S.I. was calculated as above.

Slides from testes were examined to determine the stage of maturity. Stages were defined according to how much spermatogenetic activity was present, using a scheme similar to that described by Billard (1992) (Table 5.1, Figs. 5.1-5.7).

5.3 Results

Distribution of G.S.I. - A Kolmogorov-Smirnov test was used to determine whether the monthly frequency distributions of G.S.I.s of females or males differed significantly from a normal distribution (Figs. 5.8, 5.9, respectively). The distribution of female G.S.I.s was unimodal in all months (highest K-S $Z = 1.09$, $P = 0.186$), and all G.S.Is were small indicating that none were maturing. There was no correlation between mean G.S.I. and month (Spearman rank correlation, $r_s = 0.350$, $n = 9$, $P > 0.05$; Table 5.2, Fig. 5.10a). The frequency distribution for the males was significantly different from normal

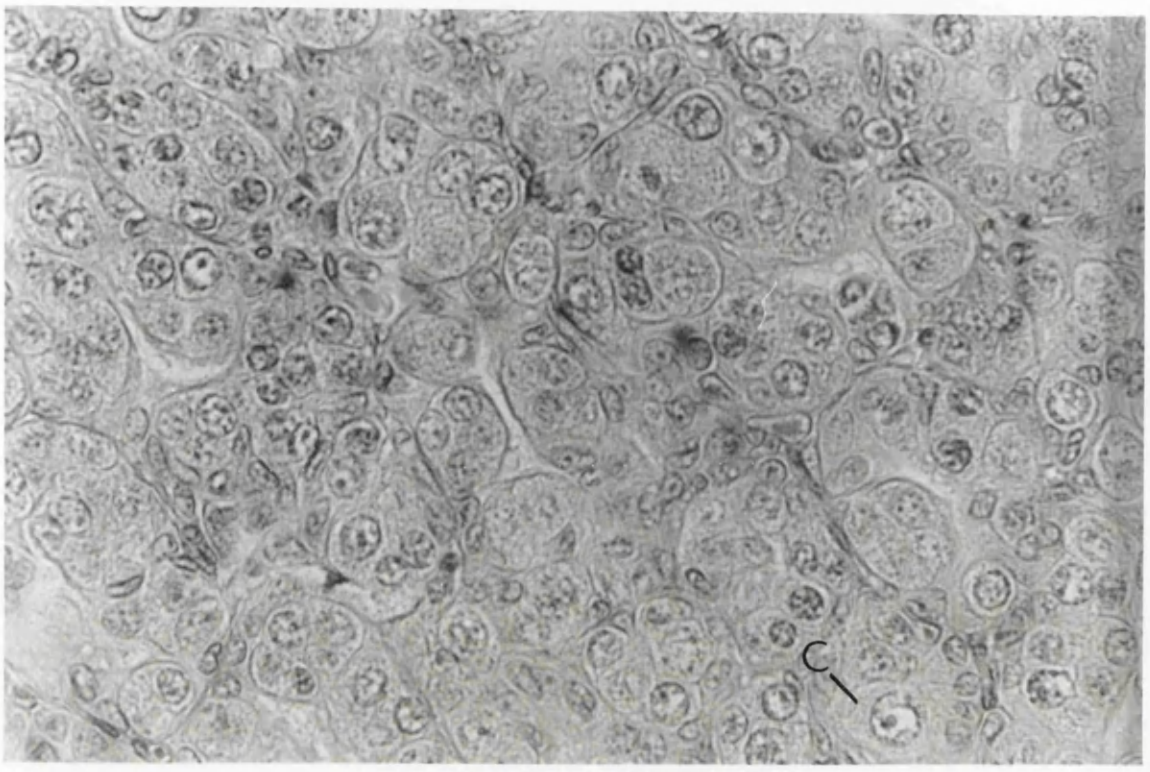


Fig. 5.1. Longitudinal section of a testis in February showing the prepubertal stage, Io, with large germ cells (C).

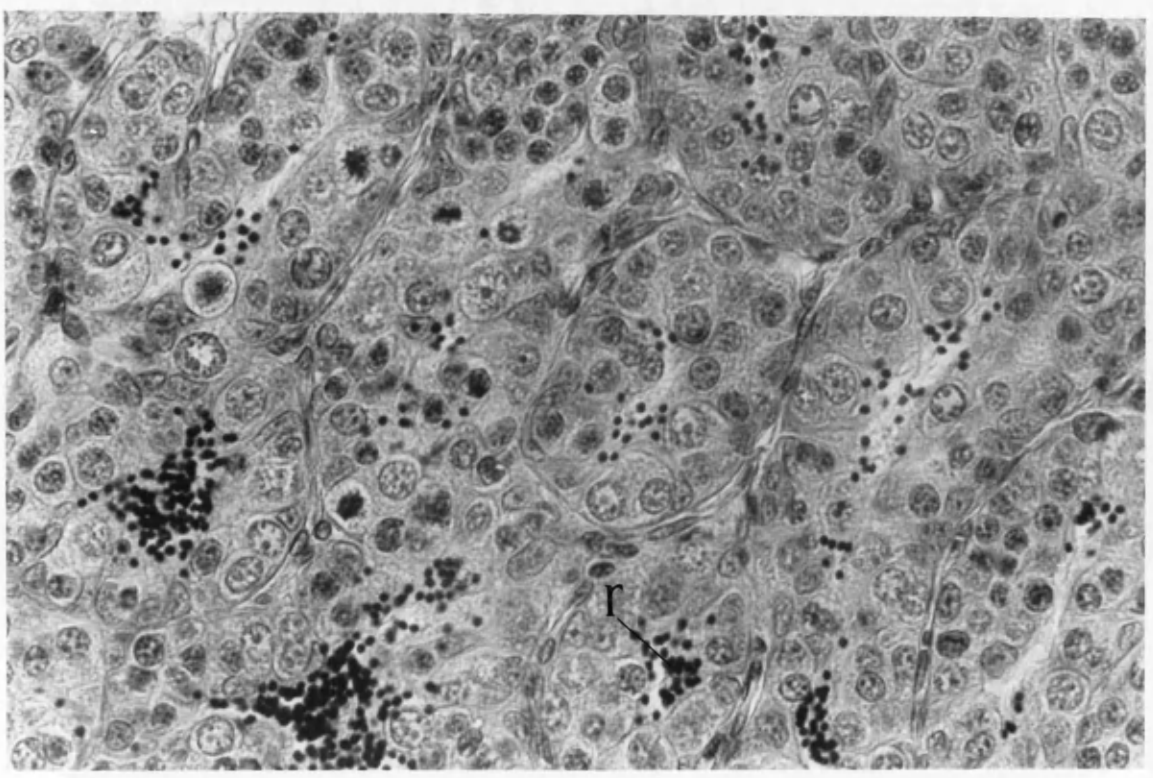


Fig. 5.2. Longitudinal section of a testis in March showing previous maturation, stage Ii. r = remnants of spermatozoa.

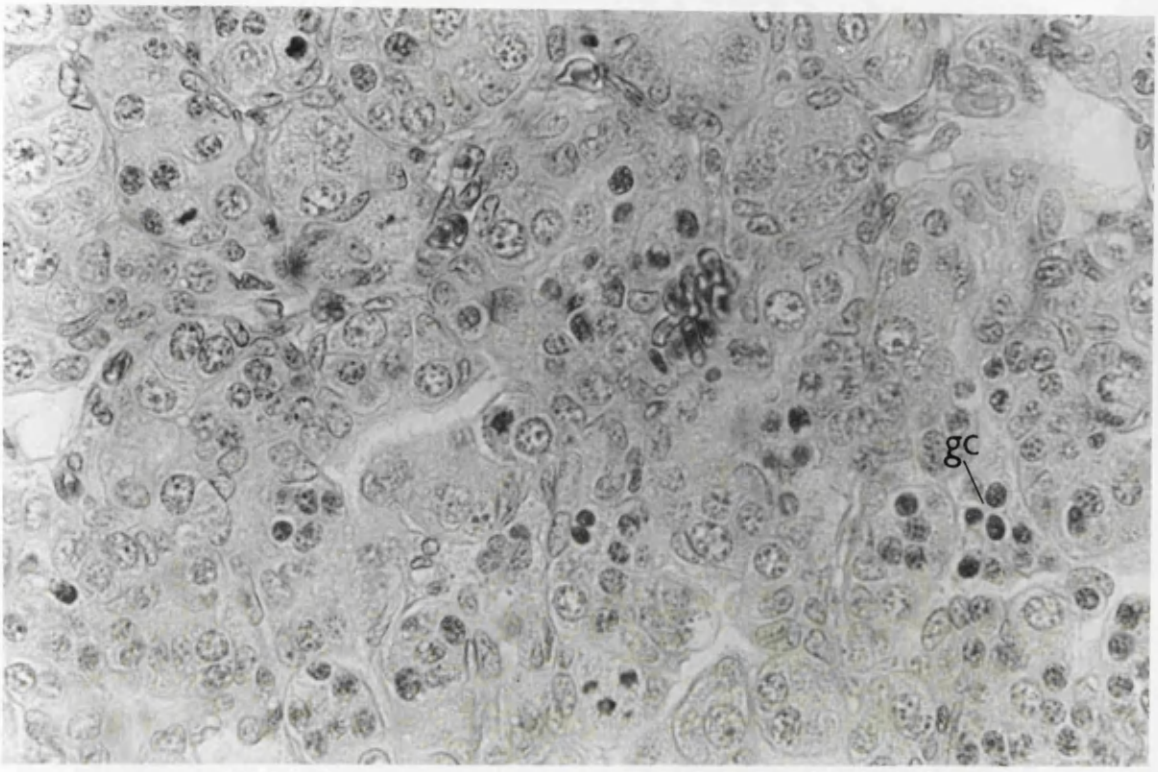


Fig. 5.3. Longitudinal section of a testis in April showing the initiation of spermatogenesis, stage II. gc = germ cell cyst.

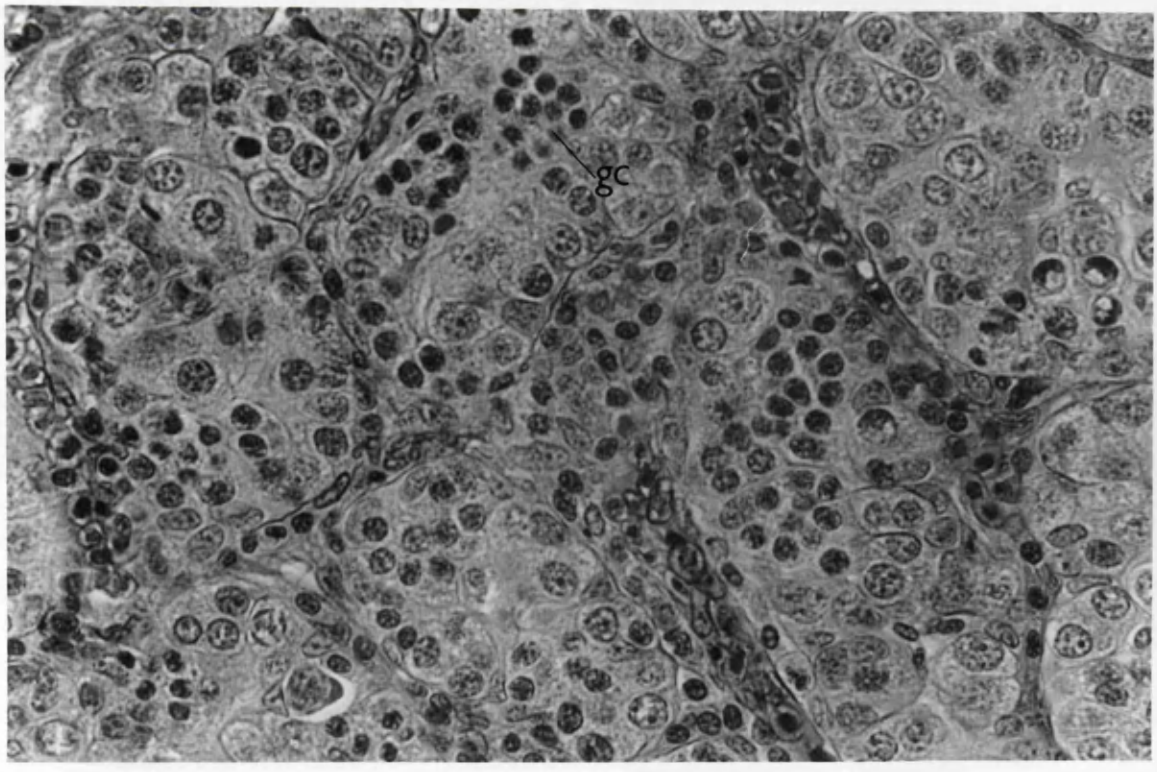


Fig. 5.4. Longitudinal section of a testis in May showing stage II-III with a few germ cell cysts present. gc = germ cell cyst.

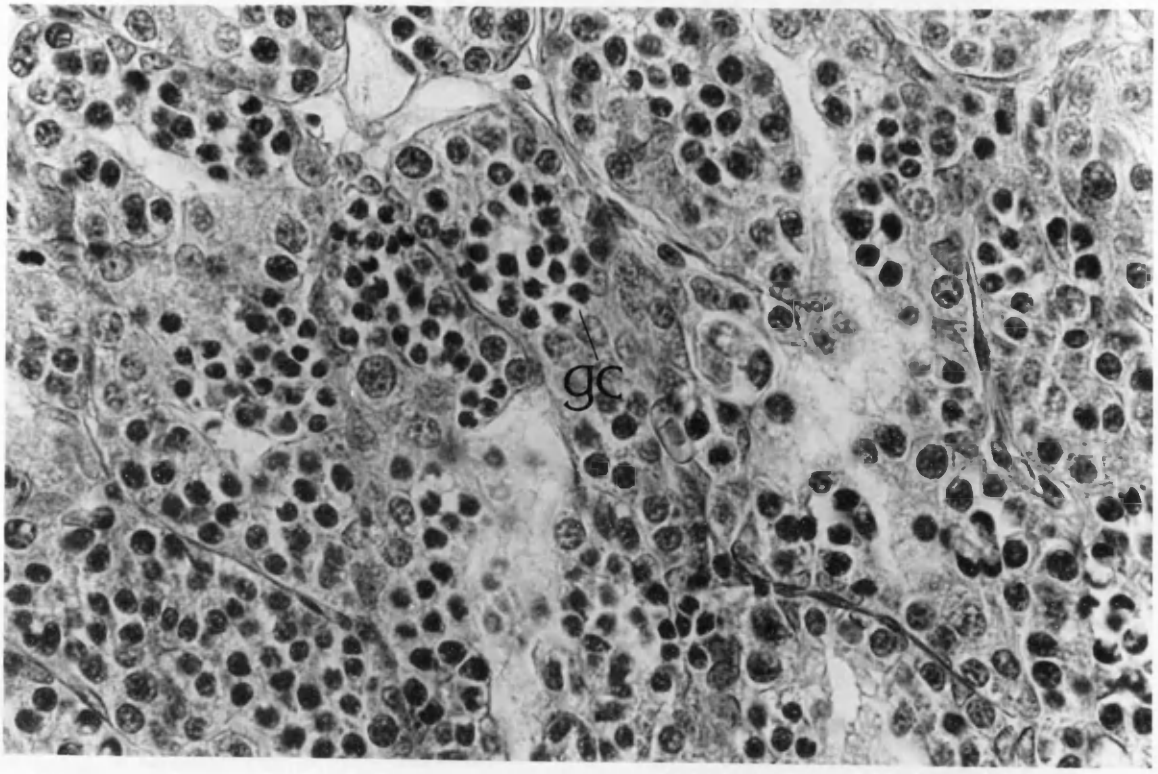


Fig. 5.5. Longitudinal section of a testis in May showing stage III with many cysts of germ cells present. gc = germ cell cyst.

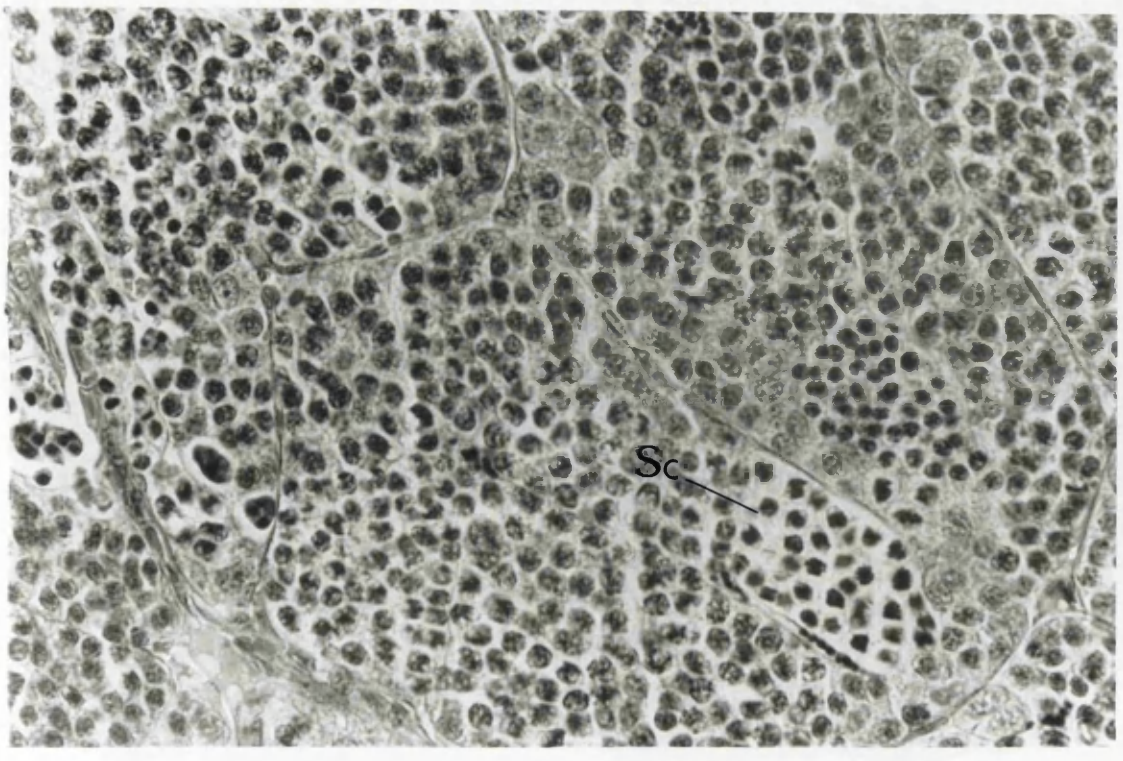


Fig. 5.6. Longitudinal section of a testis in November showing stage III-IV with several spermatocytes present, but no spermatids. Sc = cyst of spermatocytes.

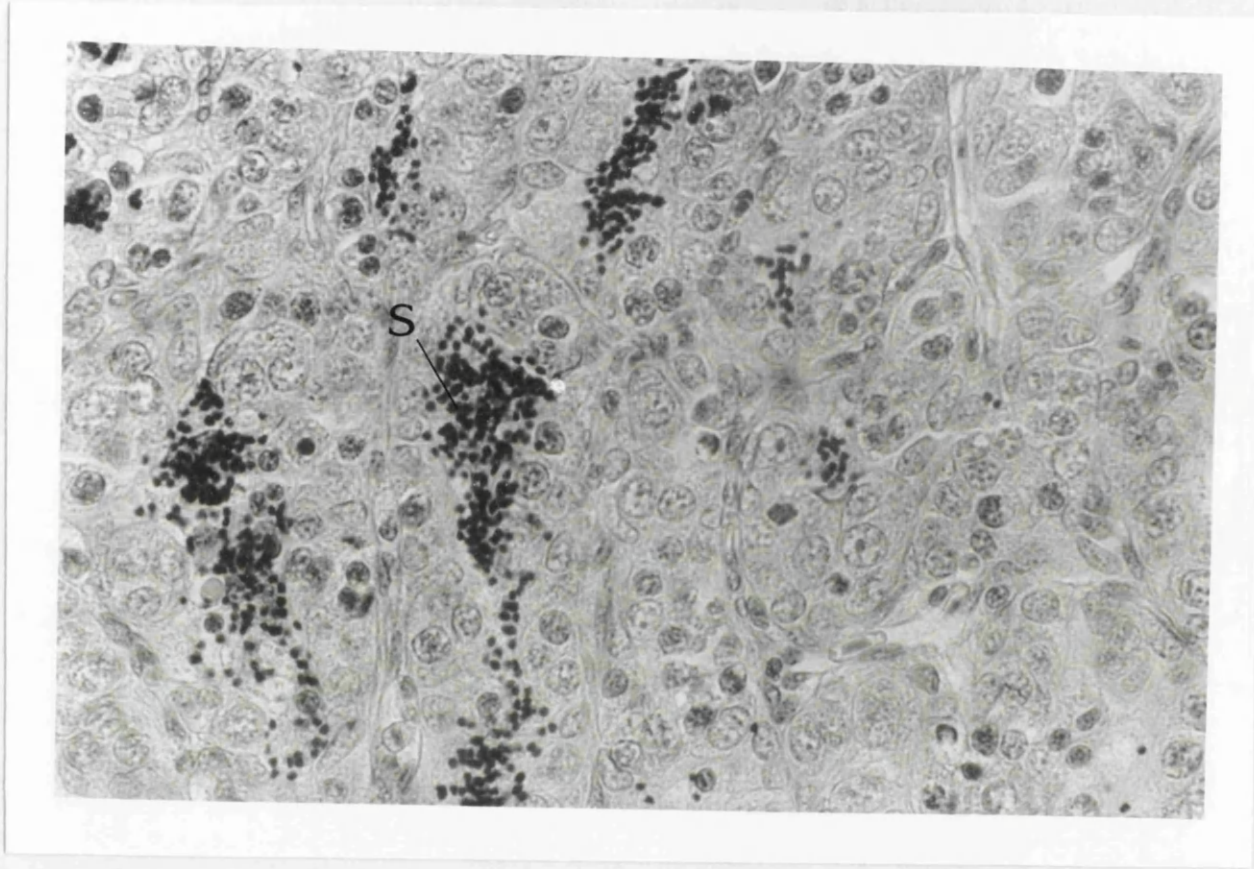


Fig. 5.7. Longitudinal section of a testis in February showing stage IX, the end of spermiation and resorption of spermatozoa (S).

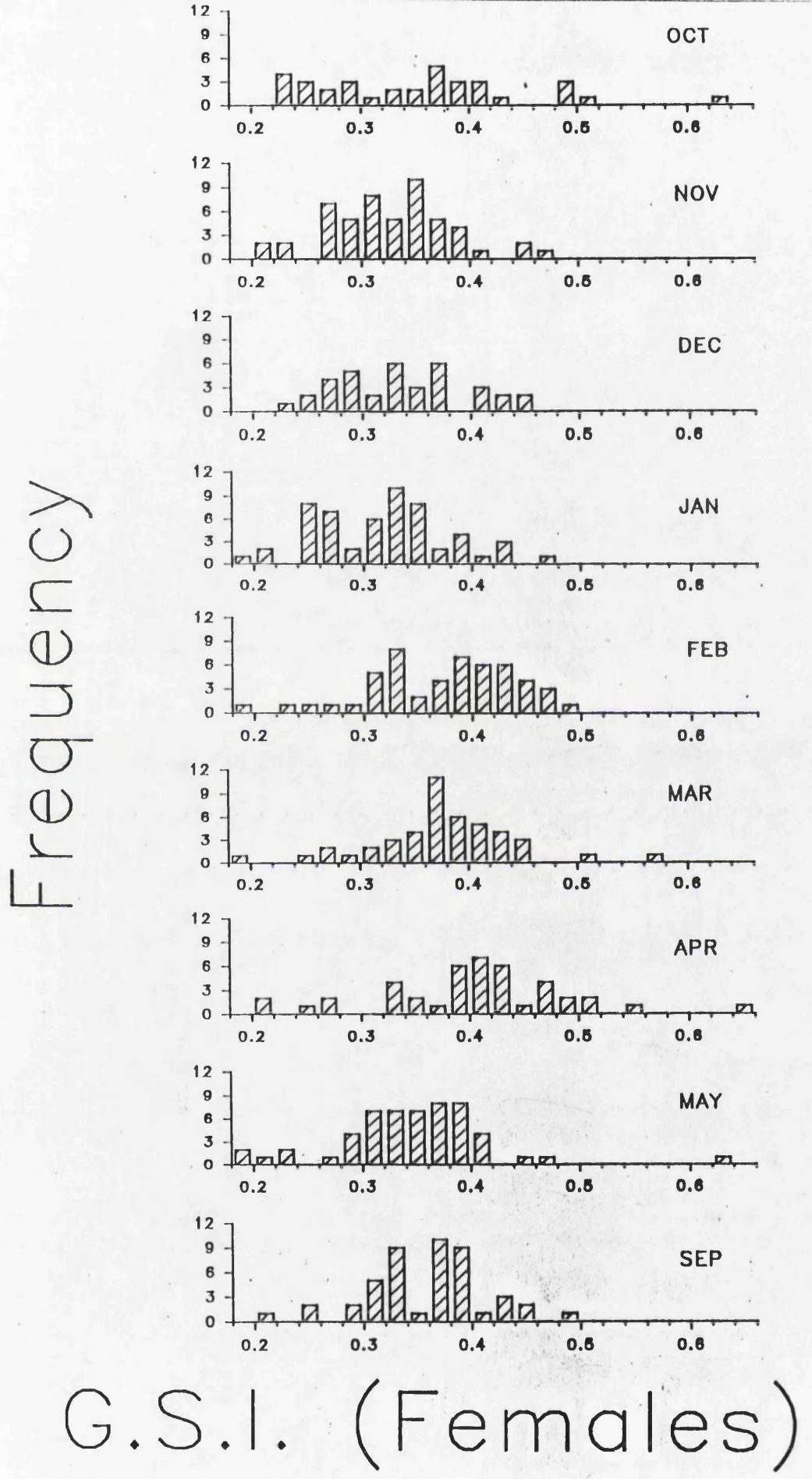


Fig. 5.8. Distribution of female G.S.I.s from October to September, all immature.

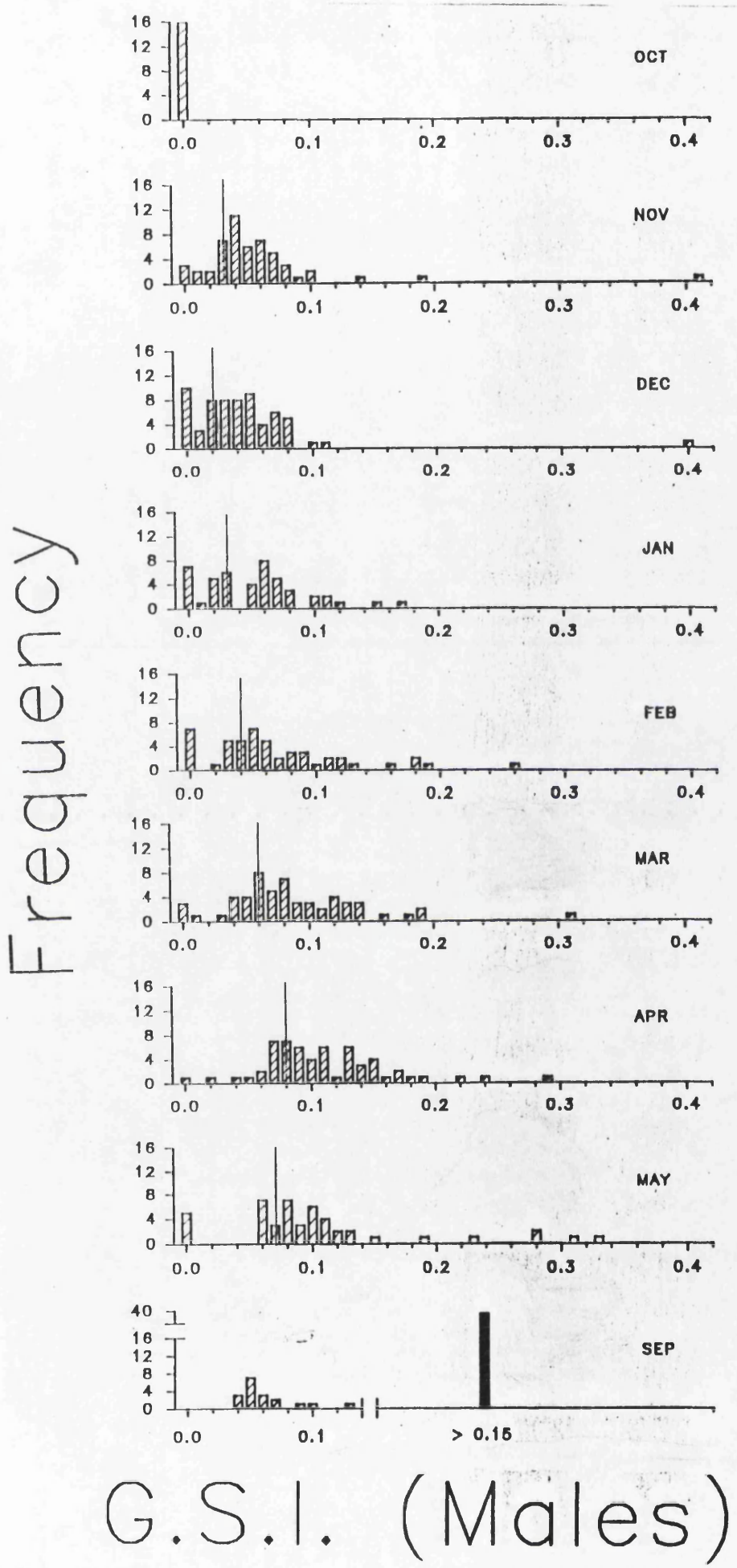


Fig. 5.9. Distribution of male G.S.I.s from October to September; vertical line in graphs from November to May indicates G.S.I. threshold based on September result of 68% of the males maturing.

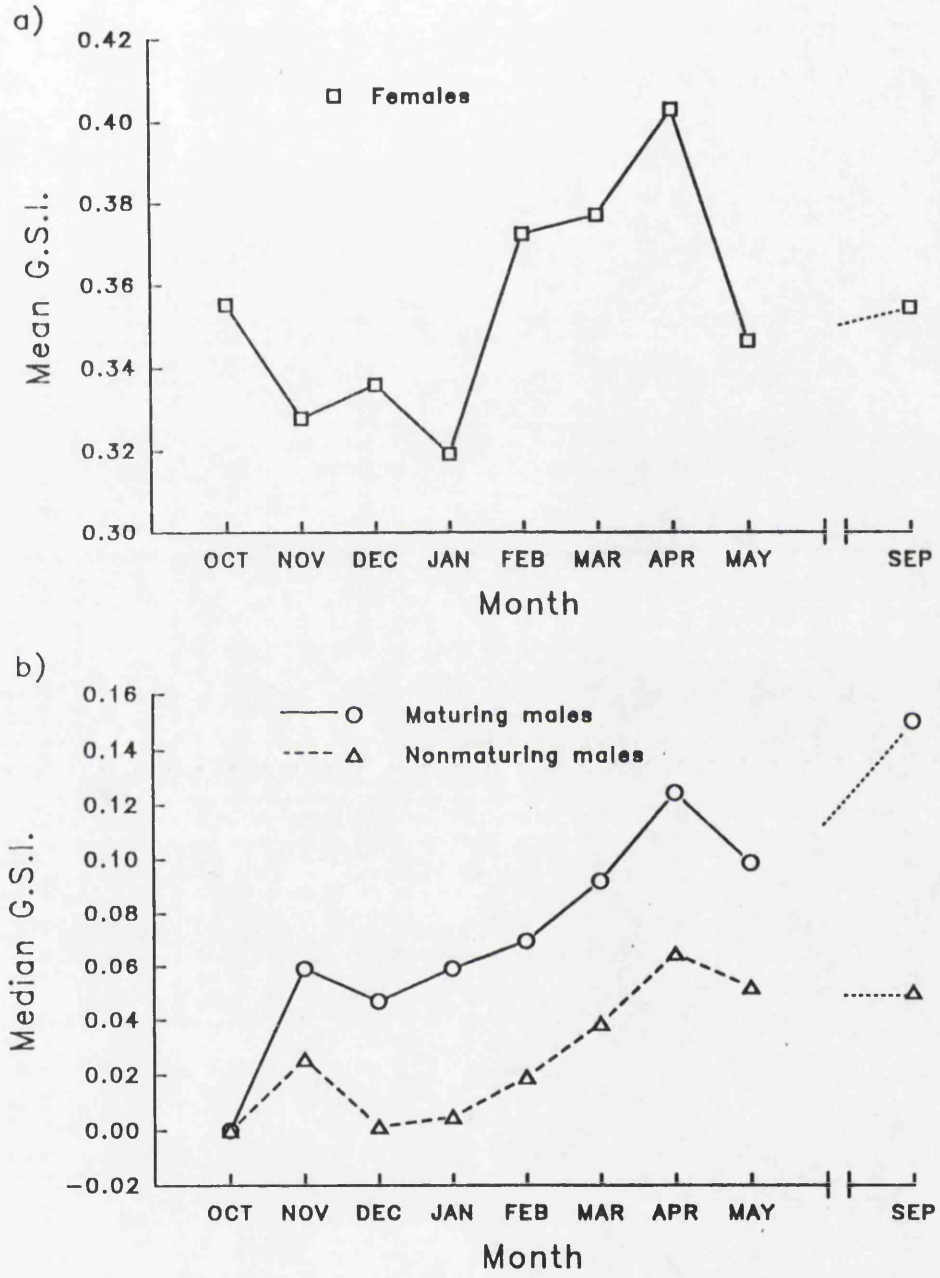


Fig. 5.10. a) Mean female G.S.I. against month, b) Median G.S.I. against month for maturing and non-maturing males; classification of males into these two categories based on the assumption that the 68% of males with the highest G.S.I.s would mature.

in November (K-S Z = 1.683, n = 52, P = 0.007) and December (K-S Z = 1.743, n = 64, P = 0.005), and marginally so in May (K-S Z = 1.509, n = 46, P = 0.021). November and December differences were caused mainly by a few individuals which, on histological examination, were in the mid-stages of maturation (see *Histology* below). Differences in May may show the earliest split in G.S.I. between maturing and non-maturing males. Male G.S.I. was positively correlated with month (Spearman rank correlation, $r_s = 0.952$, n = 8, $P < 0.01$; Table 5.2, Fig. 5.10b).

Table 5.2. Mean female G.S.I. and median male G.S.I. with month.

	Females		Males	
Month	Mean G.S.I.	n	Median G.S.I.	n
October	0.355	34	*	16
November	0.328	52	0.043	52
December	0.336	36	0.035	64
January	0.319	55	0.049	46
February	0.372	51	0.050	49
March	0.377	45	0.075	56
April	0.403	42	0.095	58
May	0.346	54	0.081	46
September	0.354	47	*	*

Percentage maturing - In October testes were so small that it was almost impossible to find them let alone remove and weigh them. From November to May it was assumed that any male whose testis was big enough to allow weighing (i.e. 0.0001g or more) had initiated gonadal development and therefore could be maturing. The percentage of males maturing by this definition is shown for each monthly sample in Table 5.3. There was no significant difference in the percentage of males undergoing gonadal development in the samples taken from November to May (Chi-squared = 10.204, d.f. = 6, $P > 0.05$). However, these predicted maturation rates were significantly higher than the actual rate of 68% found in September (Chi-squared = 34.078, d.f. = 1, $P < 0.001$).

Table 5.3. The percentage of males maturing in each month. Maturity from October to May is defined as testis weighing 0.0001g or more and in September as milt running or testes visibly large and white, almost to their full length.

Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Sep
n	16	52	64	46	49	56	58	46	57
%Maturing	0	94	84	85	86	95	98	89	68

G.S.I. and fork length - Spearman rank correlations were used to examine the relationship between G.S.I. and fork length. Female G.S.I. was negatively correlated with length over the whole study period (least significant r_s = -0.353, n = 47, P < 0.02; Table 5.4). From December to February and from April to May, male G.S.I. was positively correlated with length (least significant r_s = 0.339, n = 46, P < 0.02; Table 5.4). However, the amount of variation in G.S.I. explained by fork length was never greater than 38% for females and 33% for males.

Table 5.4. Spearman Rank correlations between G.S.I. and fork length for males and females.

	Males			Females		
Month	r_s	n	P	r_s	n	P
Oct	*	*	*	-0.542	34	< 0.01
Nov	0.232	52	N.S.	-0.442	52	< 0.01
Dec	0.339	64	< 0.01	-0.503	36	< 0.01
Jan	0.339	46	< 0.02	-0.354	55	< 0.01
Feb	0.345	49	< 0.02	-0.445	51	< 0.01
Mar	0.192	56	N.S.	-0.620	45	< 0.001
Apr	0.359	58	< 0.01	-0.393	42	0.01
May	0.575	46	< 0.001	-0.601	54	< 0.001
Sep	-0.336	18	N.S.**	-0.353	47	< 0.02

(** Excludes all mature males, in which G.S.I. was not measured)

Histology - Histological examination of the testes showed that the majority of males in any month were at a similar stage of maturity. However, several individuals in each month showed an advanced state of development, indicating that gonad growth was not completely synchronous within the population. In November, one male showed signs of maturing as a 0+ fish; the whole testis was almost at the stage of producing spermatids (Fig. 5.6). In February and March there was evidence that two individuals (one from each month) had previously matured in their first autumn. The testes contained remnants of spermatozoa, but the remaining gonadal material was at an early stage of development with cysts of germ cells present (Fig. 5.7, 5.2). G.S.I. was not a good indicator of the stage of gamete development until late spring (April and May) when Spearman rank correlations between G.S.I. and stage of maturity became significant (least significant $r_s = 0.567$, $n = 15$, $P < 0.05$; Table 5.5).

Table 5.5. Spearman rank correlations between G.S.I. and stage of maturity for males ('*' indicates all stage values identical after removal of 0+ mature males therefore correlation is impossible to perform).

Month	r_s	n	P
Nov	*	*	N.S.
Dec	0.348	10	N.S.
Jan	0.480	12	N.S.
Feb	*	*	N.S.
Mar	0.124	13	N.S.
Apr	0.567	15	< 0.05
May	0.747	12	< 0.01

5.4 Discussion

The main aim of this experiment was to characterise the pattern of gonad growth in 0+ parr and to identify the point at which divergence between maturing and non-maturing males became apparent.

The G.S.I.s of female parr did not increase over the period of study, October to September (Fig. 5.10a) and they were negatively correlated with length, suggesting that females did not invest in gonadal tissue during this time. In contrast, the G.S.I.s of male parr increased during this period and were positively correlated with length. The gonad growth of the males seemed to begin between October and November, a year before any spawning would take place (Fig. 5.10b). Similar results have been found in adult sea-cage salmon where a spurt in gonad growth occurs in the autumn preceding maturation (Thorpe, 1994b). However, in contrast to sea-salmon, it was not possible to identify two clear categories of male on the basis of G.S.I. that might correspond to maturing and non-maturing parr. Therefore at this time it was difficult to tell with any certainty, from either G.S.I. or histological evidence, which males were maturing or would have matured had they not been killed. Evropeizeva (1958) showed that although obvious visual signs of maturation in 1+ salmon parr occurred in June, histological changes were present as early as February-March. In the present study, although initiation of spermatogenesis (Stage II, Table 5.1) occurred in a few individuals as early as December, March was the first month in which almost half of the testes examined were at this stage, thereby supporting the findings of Evropeizeva (1958). Early stages of maturation in 0+ fry, accelerated with heated water, have been observed as early as mid-June (Saunders *et al.*, 1982), leading to a relatively high incidence of 0+ maturation. At ambient temperatures, Villareal and Thorpe (1985) found spermatogonial proliferation in both upper and lower modal group parr in late September. This indicates that all males put some energy into gonad growth at this time of year. It is likely that maturing fish will continue to invest in gonad tissue whereas those in which the maturation process has been switched off will reduce investment. However, since measurements of gonadal cells were not taken, it is not possible to provide such evidence from this study. Furthermore, evidence of this kind would have to be based on assumptions of subsequent maturation.

The percentage of males undergoing gonadal development estimated from G.S.I.s in the early part of the year was considerably higher than the actual maturation rate determined in September. This suggests that some fish begin maturing, but the process is switched off at a later date. However, in past experiments G.S.I has not been a good indicator of stage of maturity (De Vlaming, 1975). Similarly, in the present experiment, G.S.I. was not correlated with maturity stage until April and May at which time the assumed criteria for maturation (G.S.I. > 0.002) may not be valid. It is not possible to say what the criteria should be unless we assume that from May the maturation process cannot be switched off. In this case a G.S.I > 0.074 in May would indicate maturation. Thorpe *et al.* (1990) found that a G.S.I. > 0.1 in June indicated maturation in one-seawinter salmon, therefore the assumption that maturation cannot be switched off from the May prior to spawning might be correct. From all this evidence it is still not possible to pinpoint the beginning of the maturation process. Discussion in this area will continue in Chapter 8.

Chapter 6 - Changes in invertebrate drift during the summer months

6.1 Introduction

The results from Chapter 4 showed that the food intake of 1+ salmon parr peaked in May and declined thereafter, even though the temperature remained suitable for feeding. Salmon have lived in the stream environment for millions of years, but have been subjected to intense farming conditions for only a few decades. Therefore we cannot expect their feeding behaviour and their physiology to be adapted to farming practices, which tend to be designed for human convenience rather than that of the fish (Eriksson & Alanärä, 1992). So, in order to find an explanation for these appetite changes, we must direct our attention to the natural environment of salmon parr, namely the stream. Here, food intake is likely to depend on food availability and appetite changes, in addition to the physical conditions, such as temperature, already discussed in Chapter 4.

In streams, salmon parr adopt a sit and wait tactic for catching prey (Wankowski & Thorpe, 1979a; Stradmeyer & Thorpe, 1987a). They feed predominately on invertebrates drifting in the water, but will also take food directly from the substrate, picking off benthic organisms (Egglishaw, 1967; Symons & Heland, 1978; Wankowski & Thorpe, 1979a). Food availability will depend on factors such as current velocity and amount of organic and surface drift. Previous studies indicate that parr will eat almost all species in the drift (Egglishaw, 1967; Elliott, 1965). Therefore food availability could be measured as total number of drifting invertebrates. However, since salmonids ingest whole prey animals, not all of this drift will be suitable as food to the waiting salmon. Factors such as size, shape, texture, taste and behaviour of the prey will all influence which organisms are taken (Allen, 1941a; Sutterlin & Sutterlin, 1970; Stradmeyer *et al.*, 1988). Both Allen (1941a) and Egglishaw (1967) showed that larger fish tended to have larger prey items in their stomachs. Wankowski & Thorpe (1979b) went on to show that the diameter of a food particle that produces maximal feeding and growth responses is between 2.2-2.6% of fish length. Items larger than this are likely to be rejected since they are too big to handle and smaller items do not give enough energy return to be profitable and so tend to be ignored. When this

measurement was applied to prey data from fish stomachs (from Egglishaw, 1967), the width of the prey proved most important, peaking at 2.2-2.6% of fork length (Wankowski, 1979). Salmon also prefer long, thin pellets over round, and soft, gelatinous pellets over hard (Stradmeyer *et al.*, 1988). These are properties of several key prey types that consistently occur in large numbers in the stomachs of salmon throughout the world. All are long and thin in shape; the soft Simulid and Chironomid larvae, and the harder Ephemeroptera nymphs and Trichoptera larvae (Allen, 1940, 1941a; Egglishaw, 1967).

Salmon experience various cyclic changes in their natural environment which may well influence feeding efficiency. The most obvious of these being the light/dark cycle. Since salmon are visual predators, feeding behaviour and therefore food intake will be affected by this cycle. These cyclic changes provide information on time of day and season. The latter has been shown to be important since salmon on an increasing photoperiod regime (spring) had a faster gastric evacuation rate and therefore consumed more food than those on a decreasing photoperiod (autumn) at the same temperature (Higgins & Talbot, 1985). The light/dark cycle also directly affects the amount of drifting prey. Aquatic invertebrate drift tends to occur at night, often peaking at dawn and dusk during the spring and early summer (Elliott, 1965; Eriksson & Alanara, 1992).

Until recently the activity of juvenile Atlantic salmon was thought to be basically crepuscular during late autumn, winter and spring but diurnal during late summer and early autumn (Eriksson *et al.*, 1982). Fresh evidence shows that salmon become nocturnal during winter which is facilitated by temperature related retinal adjustment to low light levels so that the fish are still able to see their prey (Fraser *et al.*, 1993). There is an increasing amount of literature showing that the feeding and locomotor activity of laboratory salmonids reflects the patterns of drift of their natural prey even though they are fed on commercially produced food pellets. Demand feeding patterns of rainbow trout corresponded well with the changing drifting patterns of prey with season (Landless, 1976). In a northern Swedish stream activity of salmon parr in March was concentrated at dawn and dusk which reflected the drift patterns of their main prey (mayfly larvae) at the time (Eriksson & Alanara, 1992).

Further evidence of the presence of biological clocks in salmonids, where seasonal and diel feeding

patterns occur even under fixed light and temperature regimes (Eriksson, 1978; Eriksson & Lundqvist, 1982; Jobling, 1987; Pálsson *et al.*, 1992), suggests that they are pre-programmed to match their feeding to predictable diel and seasonal changes in food supply. This might then explain the reduction in appetite after May in a laboratory situation where food availability is constant.

Previous studies have looked at total drift abundance with season (Allen, 1941a; Egglishaw, 1967; Elliott, 1965), but not the abundance of optimally-sized prey. The aims of the following experiment were, therefore, to examine the natural fluctuations in prey availability from May onwards in a stream from which the fish used in Chapter 4 originated, and more importantly, to look at fluctuations in prey of optimal size in an attempt to find an explanation for the seasonal drop in appetite during the summer.

6.2 Materials and Methods

Samples of invertebrate drift were collected each month from May to August from a lade taking water from the River Almond, Perthshire. Samples were taken in May as follows: A Surber sampler (mouth dimensions = 28 x 32cm, length = 55cm, mesh size = 7/cm) was attached between two poles and lowered into the lade from a low footbridge. The water velocity was measured, using a Nixon Streamflo 422 meter, just after the net was placed in the water each time so that an average velocity for each sampling period could be calculated. Every three hours for 24hrs the net was removed, rinsed into a bucket of water, and replaced in the lade. The bucket of water containing the sample was then sieved and the resulting material was fixed in alcohol. Due to the large amount of material gathered by this method in May, in subsequent months a revised protocol was followed. For samples collected from June to August the net was placed in the water for 1/2hr every three hours over a 24hr period. Water velocity readings were taken as before.

All organisms were identified to Order or Genus level (depending on the taxonomic group) and their lengths and widths were measured (mm $x10^{-1}$). The sampling times were classified as Day, Twilight or Night using data for a latitude of 56°N, extracted from Whitakers Almanac (1985). There was only one sampling time that was classed as 'Night', which occurred in August. It could not be analysed by itself

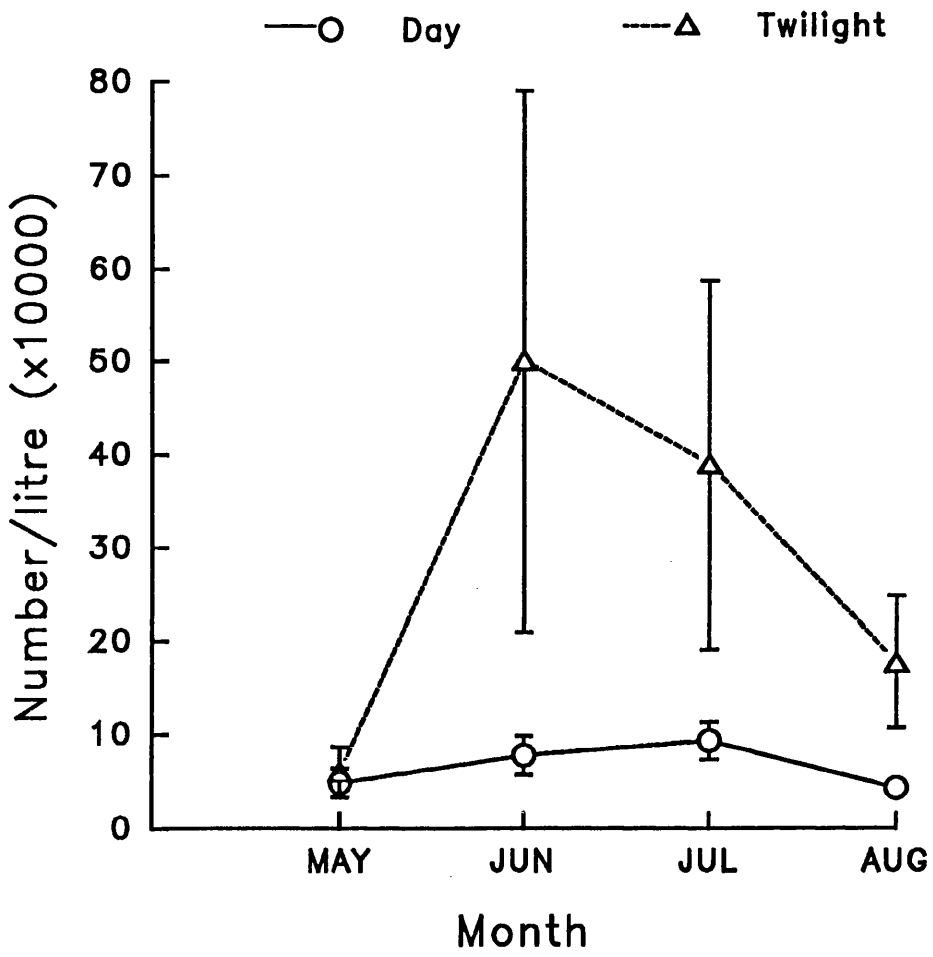


Fig. 6.1. Diel differences in the total number (mean ± S.E.) of organisms caught in the drift with month.

so for the following analyses it was combined with the twilight samples. Fork length data from the three years work described in Chapter 3 was used to calculate an optimum size of prey (based on Wankowski & Thorpe, 1979b) for each month of this study. Hatchery reared fish will clearly be larger than wild fish of the same age but since the aim of this experiment was to determine food availability for fish in which the appetite peak had been shown, it was considered that these fork length data would be valid. The range of optimally sized prey was taken as 2.2% of the smallest fish length and 2.6% of the largest. Prey whose length or width fell within this range were considered optimal.

6.3 Results

Data were analysed to look at the effects of both time of day and season.

Total numbers - There were more organisms drifting during twilight than during the day (Two-way ANOVA of total numbers against time of day (classified as twilight or day) and month, $F_{1,31} = 12.444$, $P = 0.002$) (Fig. 6.1). Numbers also varied significantly between months ($F_{3,31} = 3.336$, $P = 0.036$) with the peak occurring in June but there was no significant interaction between month and time of day ($F_{3,31} = 1.121$, $P = 0.360$). On average there were about three and a half times as many prey items drifting by twilight than by day.

Prey size (all species) - Two-way ANOVAs on both prey length and prey width by time of day (Day or Twilight) and month showed that when all species were combined there were significant time effects only. Thus larger organisms drifted at twilight than by day (measuring organisms in terms of their length $F_{1,31} = 11.37$, $P = 0.003$; measured by width $F_{1,31} = 29.83$, $P < 0.001$). There were no significant month effects but the largest mean size (both in length and width) was in May during twilight (Table 6.2).

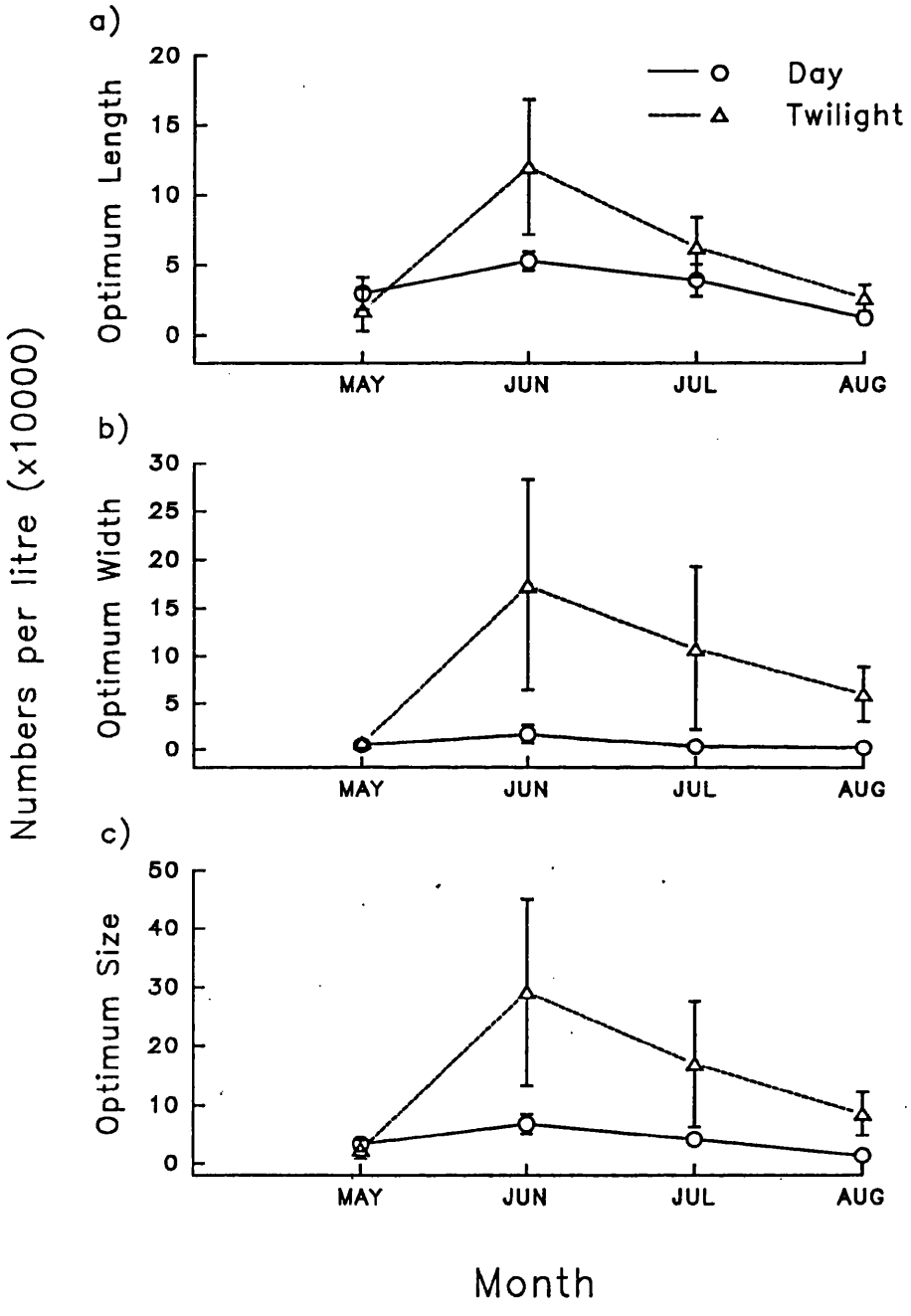


Fig. 6.2. Diel differences in the mean number (± S.E.) of optimal organisms caught in drift samples with month. a) Organisms of optimum length, b) Organisms of optimal width, c) Organisms optimal in either dimension (length or width).

Table 6.2. Mean size (mm x 10^{-1}) of all drift data (species combined) for each month and time of day.

	Mean length		Mean width	
Month	Day	Twilight	Day	Twilight
May	24.58	36.82	6.88	15.26
June	22.71	29.58	6.55	11.08
July	21.63	26.60	5.36	9.41
August	24.85	31.47	5.66	10.96

Numbers of optimally sized organisms - These were calculated in three ways; firstly, numbers of prey items whose length was optimal, secondly, those whose width was optimal and thirdly, those that were optimal in either dimension (width or length).

Optimal length - there were more organisms of optimal length drifting during twilight than during the day (Two-way ANOVA of number of prey against time of day and month, $F_{1,31} = 4.653$, $P = 0.041$). Significant month effects were found, with a peak in June (Two-way ANOVA $F_{3,31} = 7.206$, $P = 0.001$); there were no significant interaction effects between time of day and month (Fig. 6.2a).

Optimal width - there were more organisms of optimal width drifting during twilight (Two-way ANOVA of number of prey against time of day and month, $F_{1,31} = 13.109$, $P = 0.001$), but no differences between months (Fig. 6.2b).

Total numbers of optimal organisms - here again, there were significantly more optimally-sized organisms drifting during twilight than during the day (Two-way ANOVA of number of prey against time of day and month, $F_{1,31} = 11.29$, $P = 0.003$). There were significant month effects ($F_{3,31} = 3.172$, $P = 0.043$), but a Scheffé test was not able to pinpoint the differences, the peak occurring in June. There were no significant interaction effects (Fig. 6.2c).

Key species - Key species that salmon are known to take frequently (Allen, 1940; Egglishaw, 1967) were analysed separately. The Ephemeroptera (Baetidae, Ephemerellidae, Ecdyonuridae) and the

Dipteran larvae (Simulidae and Chironomidae) were analysed using two-way ANOVAs of length and width against time of day and month.

Ephemeroptera - larger Ephemeroptera drifted during twilight than during the day (least significant $F_{1,1941} = 633.596$, $P < 0.001$). There were significant month effects for both length and width measurements, with the smallest organisms being found in May (least significant $F_{1,1941} = 5.763$, $P = 0.001$; Table 6.6).

Table 6.6. Mean size (mm x 10^{-1}) of Ephemeroptera nymphs (Baetidae, Ephemerellidae and Ecdyonuridae) for each month and time of day.

	Mean length		Mean width	
Month	Day	Twilight	Day	Twilight
May	15.64	33.12	8.41	18.92
June	24.29	30.27	14.73	16.61
July	18.92	31.82	10.38	16.28
August	23.81	35.87	12.30	18.36

Diptera - larger Diptera drifted during twilight (lowest $F_{1,604} = 26.058$, $P < 0.001$). Month effects were only significant when looking at the length of the organisms ($F_{3,604} = 39.808$, $P < 0.001$), the peak being in May (Table 6.7).

Table 6.7. Mean size (mm x 10^{-1}) of Diptera larvae (Simulidae and Chironomidae) for each month and time of day.

	Mean length		Mean width	
Month	Day	Twilight	Day	Twilight
May	32.14	41.26	3.06	5.16
June	23.81	25.28	3.78	4.48
July	25.53	28.79	3.67	4.57
August	26.31	30.57	3.42	4.30

6.4 Discussion

The aim of this study was to see whether availability of suitable food varies with season in a manner that could explain the obvious drop in appetite over the summer months that was found in Chapter 4. A whole PhD thesis could be devoted to examining invertebrate drift and this study should be seen as preliminary.

Diel variation -

In all months organisms drifting during the twilight hours were larger (both by length and width), more numerous and approximated more closely to the identified prey size for optimum growth in juvenile salmon than those drifting during the day; this was true within taxonomic groups as well as overall. Similar diel changes have been found by many other authors (e.g. Elliott, 1967; Allan, 1984).

Variation over the summer months -

There was a significant decrease in both the total number of prey and number of optimally sized prey items from June to August (Figs. 6.1, 6.2). This decrease over the summer months, when temperatures were increasing, would increase feeding costs and reduce returns, thereby making a reduction in appetite adaptive. However, in the present study, the levels for May were not higher than those for June. It may be that the different collection method used in May underestimated the amount of drift. In this month the net was left in for 3 hours (as opposed to 1/2 hr in subsequent months) in which time it gradually became layered with material. It is possible that the more active organisms managed to crawl out of the net in this time thereby reducing the number caught.

Although this study was preliminary and the methodology needs to be refined in future, the data generally agree with results from other studies. Schlosser (1982a) found that numbers of drifting invertebrates peaked in late spring / early summer (May-June) and declined during July and August. This summer depression in invertebrate abundance has also been found in several other studies (Angermeier, 1982; Schlosser and Toth, 1984; Angermeier and Carlson, 1985; Schlosser and Angermeier, 1990) and is probably responsible for the summer reduction in growth rates of several fish species (Brown, 1960; Gerking, 1966; Carlander, 1969, 1977; Mason, 1976; Schlosser, 1982b). Egglishaw (1967) found that

the composition of the bottom fauna changed from large organisms in May (43% being 5mm or more in length) to smaller organisms in June (11% being 5mm or more). The dry weight of bottom fauna has been found to peak in May and so has the proportion of organisms larger than 7mm and between 4-6mm, whilst organisms between 1-3mm, peaked in July (Egglishaw & Mackay, 1967). Therefore it seems that the decline in appetite during the summer months does indeed coincide with a decline in availability of good quality prey.

Both Allen (1940) and Egglishaw (1967) saw the importance of quantifying prey abundance in terms of value to the fish. Dry weights were used to give a better indication of the nutritional value of individual prey species. Perhaps if dry weights or even calorific values had been used in this study, monthly differences might have become clearer. A study looking at the relationships between length, width, dry weight and calorific value for the main invertebrate groups would be of immense value to anyone interested in the diets of freshwater fish. It would enable those looking at invertebrate availability to gauge the nutritional value of a particular sample and those looking at fish stomach contents to estimate energy intake.

Chapter 7 - Use of head morphometrics to sex parr

7.1 Introduction

Previous experiments had necessitated the killing of immature parr at the end of the experiment simply to determine their sex. Few methods already exist for determining the sex of an immature fish. Biochemical analyses for the detection of vitellogenin (Idler *et al.*, 1979; Le Bail and Breton, 1981) require a blood sample which is not feasible from this size of parr (< 35g). Using an optic fibre to take a direct look at the gonads requires an operation that would be very traumatic to the fish and also time consuming to the surgeon (Moccia *et al.*, 1984). Although Martin and Myers (1983) concluded that ultrasound sexing techniques could not be used on salmon due to the presence of the swimbladder, Mattsson (1991) was successful in using ultrasound to determine the sex of two-sea-winter Atlantic salmon. This technique has not been tested on parr and is likely to be unsuccessful due to their small size. None of these techniques could therefore be used throughout in this study.

In mature adult salmonids there is obvious sexual dimorphism not only in overall size (Jones, 1959; Naevdal *et al.*, 1981), but also in adipose fin length (Beacham and Murray, 1983; Naesje *et al.*, 1988) and head and jaw morphology (Bodington, 1987; Maisse *et al.*, 1988). Since male parr have the possibility of becoming adult sea-run salmon with large hooked-jaws, whereas female parr will never develop this jaw structure, it is conceivable that their head dimensions may differ at an early age. The aim of the present experiment was to take a preliminary look at the possibility of using head measurements of parr to determine their sex.

7.2 Materials and methods

In September 1991 slide photographs were taken of the top and side view of a group of mature and immature parr (aged 1+) which had previously been used in the diet manipulation experiment described in Chapter 3. The sex and maturity of the parr was then determined by dissection. The following head

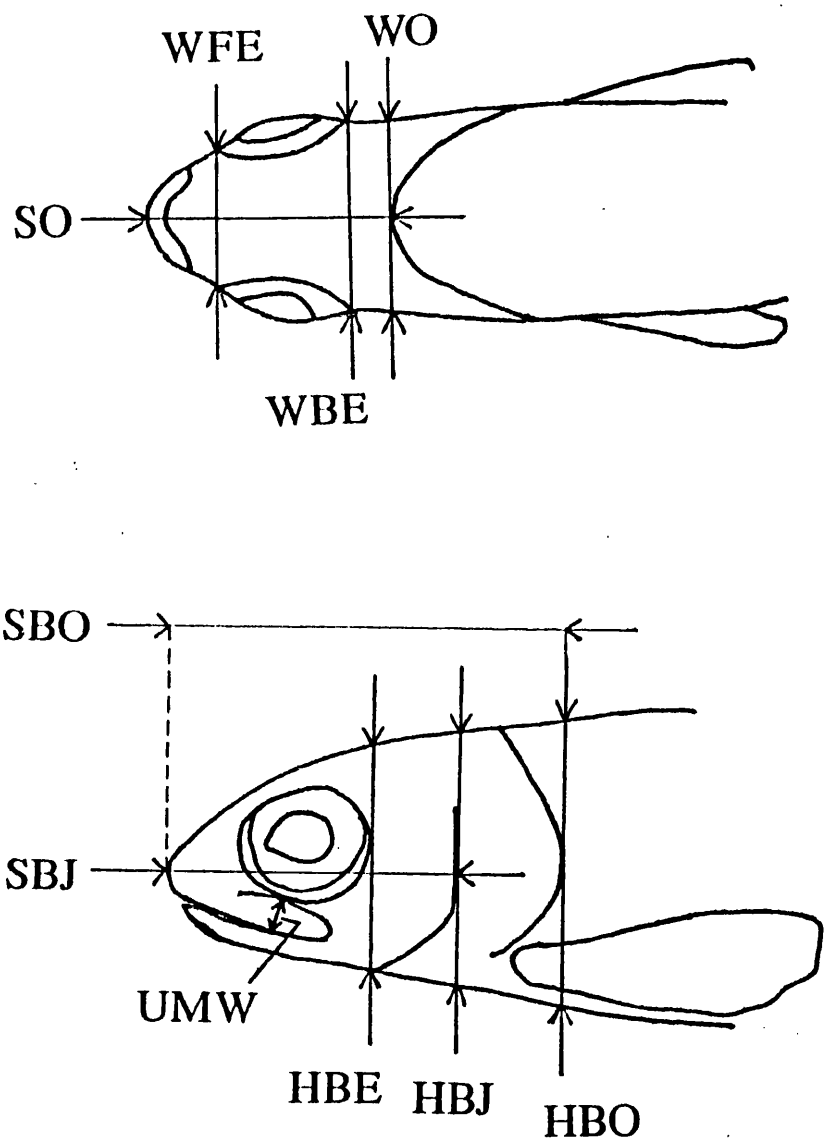


Fig. 7.1. The positions of the head morphometrics. SO, length from snout to top of operculum; WFE, width at front of eyes; WBE, width behind eyes; WO, width at operculum; SBO, length from snout to back of operculum; SBJ, length from snout to back of jaw; HBE, height behind eye; HBJ, height at back of jaw; HBO, height at back of operculum; UMW, upper mandibular width.

dimensions were measured from the projected slide (Fig. 7.1):

Top view:

SO - length from the snout to the top edge of the operculum.

WFE - width at the front of the eyes.

WBE - width behind the eyes.

WO - width at the top edge of the operculum.

Side view:

SBO - length from the snout to the back edge of the operculum.

SBJ - length from the snout to the back of the jaw.

HBE - height behind the eye.

HBJ - height behind the jaw.

HBO - height at the back of the operculum.

UMW - upper mandibular width.

Two ratios were calculated to give indices of relative head length to head width or depth:

SO/WBE - ratio of SO to WBE, head length to width ratio from the top.

SJ/HBE - ratio of SBJ to HBE, head length to depth ratio from the side.

All measurements except the two ratios were standardised for fork length by using Equation 1.

$$X' = Log_{10}X - b(Log_{10}FL - Log_{10}ML) \quad (1)$$

where X' is the standardised measurement, X is the original measurement, b is the slope of the regression of $Log_{10}X$ on $Log_{10}FL$, FL is fork length and ML is the mean fork length for the sample (Table 7.1).

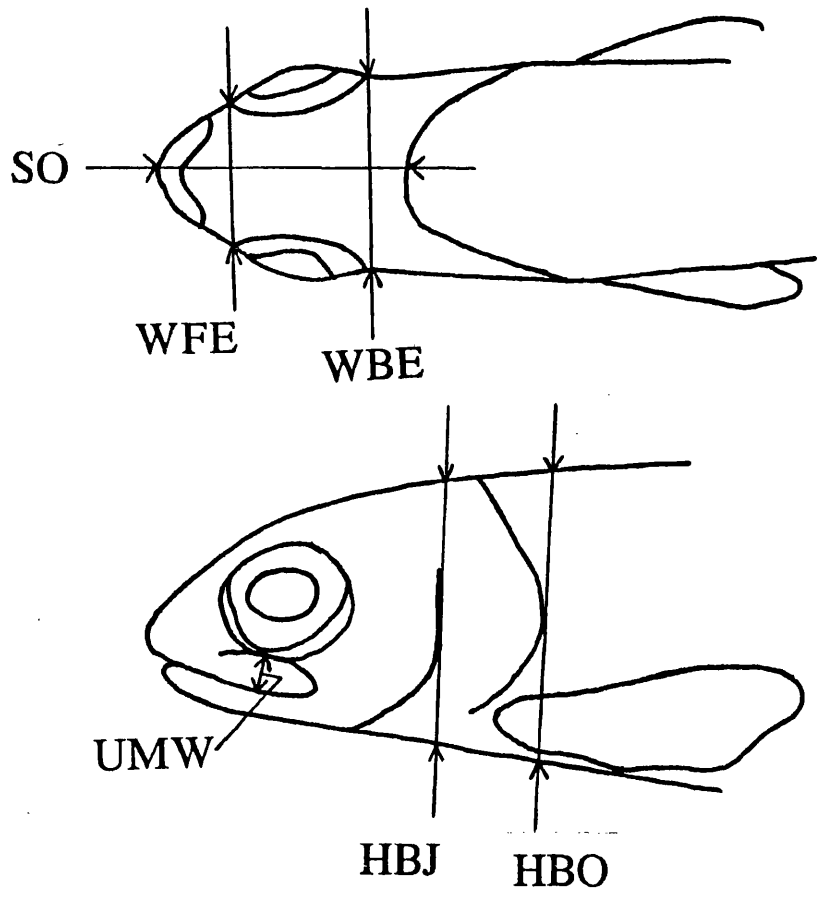


Fig. 7.2. The measurement positions for distinguishing between males and females, irrespective of maturity. SO, length from snout to top of operculum; WFE, width in front of eyes; WBE, width behind eyes; HBJ, height behind jaw; HBO, height at back of operculum; UMW, upper mandibular width;

Table 7.1. The slope of the regressions of $Log_{10}X$ on Log_{10}(fork length), where X is a head morphometric.

Measurement	Slope
Top view:	
SO, length from snout to top of operculum.	0.6057
WFE, width at front of eyes.	1.0592
WBE, width behind eyes.	0.9327
WO, width at operculum.	0.9709
Side view:	
SBO, length from snout to back of operculum.	0.7686
SBJ, length from snout to back of jaw.	0.7205
HBE, height behind eye.	0.8538
HBJ, height behind jaw.	0.9912
HBO, height at back of operculum.	1.1174
UMW, upper mandibular width.	0.9483

Discriminant analysis was used to see if a combination of these measurements could distinguish firstly between males and females irrespective of maturity, secondly between maturing and non-maturing fish, and thirdly between immature males and females.

7.3 Results

Head measurements from 60 fish were used in the analysis, 20 from each of the following groups; mature males, immature males and immature females.

72.9% of the fish were classified into the correct sex group using the discriminant function given in Equation 2 (HBO, $F_{1,1} = 19.17$, $P = 0.0001$; HBJ, $F_{2,2} = 11.73$, $P = 0.0001$; UMW, $F_{3,3} = 9.21$, $P < 0.0001$; WFE, $F_{4,4} = 7.33$, $P = 0.0001$; SO/WBE, $F_{5,5} = 6.30$, $P = 0.0001$).

$$D = -15.17 - 30.95(WFE) - 43.41(HBJ) + 104.59(HBO) + 9.91(UMW) - 21.70(SO/WBE) \quad (2)$$

where D is the discriminant score (Fig. 7.2, see Table 7.2 for the classification table). Mean scores for

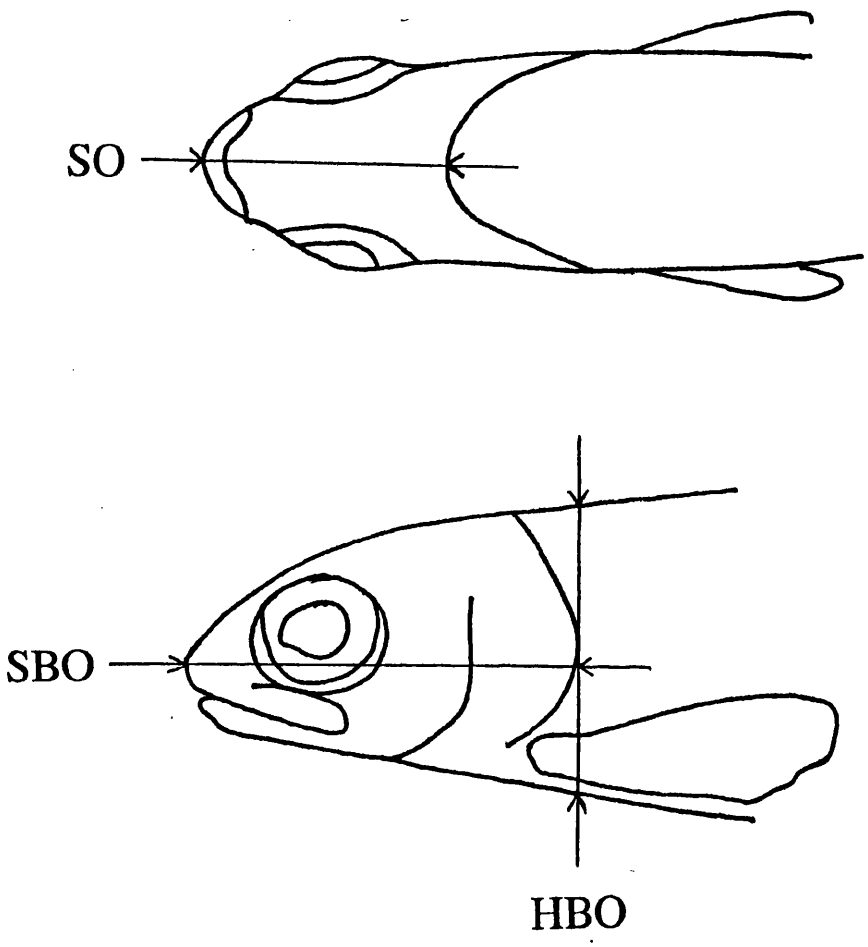


Fig. 7.3. The measurement positions for distinguishing between maturing and non-maturing fish. SO, length from snout to top of operculum; SBO, length from snout to back of operculum; HBO, height at back of operculum.

males and females were D = 0.54 and D = -1.06, respectively. The males having shorter, wider and deeper heads than the females. Females were classified 47% more accurately than by chance. Many of the males were classified as females and accuracy was only increased by 3% from that gained by chance.

Table 7.2. The classification table from discriminant analysis on head morphometrics of male versus female 1+ salmon, irrespective of maturity, in September.

		Predicted Group	
Actual Group	N	Males	Females
Males	39	27 (69.2%)	12 (30.8%)
Females	20	4 (20.0%)	16 (80.0%)

percent cases correctly classified = 72.88%

Mature males and immature parr could be correctly distinguished with 93% accuracy using the discriminant function given in Equation 3 (HBO, $F_{1,1} = 50.24$, $P < 0.0001$; SBO, $F_{2,2} = 55.30$, $P < 0.0001$; SO, $F_{3,3} = 38.49$, $P < 0.0001$).

$$D = -12.56 + 15.12(SO) - 51.59(SBO) + 96.98(HBO) \quad (3)$$

(Fig. 7.3, see Table 7.3 for the classification table). Mean scores for mature and immature fish were D = 2.07 and D = -0.98, respectively. Mature males having shorter, deeper heads than the immature fish.

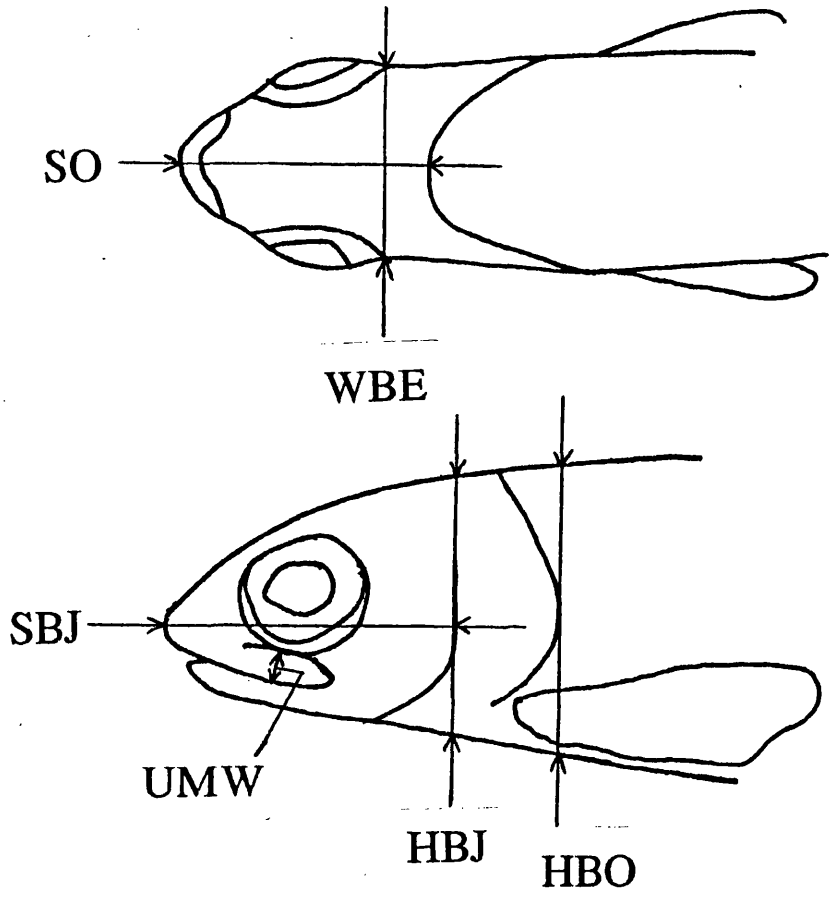


Fig. 7.4. The measurement positions for distinguishing between male and female immature salmon. SO, length from snout to top of operculum; WBE, width behind eyes; SBJ, length from snout to back of jaw; HBJ, height at back of jaw; HBO, height at back of operculum; UMW, upper mandibular width.

Table 7.3. The classification table from discriminant analysis on head morphometrics of maturing 1+ males versus non-maturing salmon (of both sexes) in September.

		Predicted Group	
Actual Group	N	Mature	Non-mature
Mature	20	19 (95.0%)	1 (5.0%)
Non-mature	40	3 (7.5%)	37 (92.5%)

percent cases correctly classified = 93.33%

Within immature fish, males and females could only be distinguished with 77.5% accuracy using the discriminant function shown in Equation 4 (HBO, $F_{1,1} = 5.32$, P = 0.0266; HBJ, $F_{2,2} = 5.64$, P = 0.0073; SBJ, $F_{3,3} = 4.74$, P = 0.0069; UMW, $F_{4,4} = 4.17$, P = 0.0072; SO/WBE, $F_{5,5} = 3.65$, P = 0.0094).

$$D = -17.46 + 65.54(SBJ) - 166.71(HBJ) + 159.15(HBO) + 9.45(UMW) - 22.11(SO/WBE) \quad (4)$$

(Fig. 7.4, see Table 7.4 for the classification table). Mean scores for males and females were D = 0.714 and D = -0.714, respectively. The males having shorter, wider and deeper heads than females.

Table 7.4. The classification table from discriminant analysis on head morphometrics of immature 1+ salmon males versus immature females, in September.

		Predicted Group	
Actual Group	N	Males	Females
Males	20	14 (70.0%)	6 (30.0%)
Females	20	3 (15.0%)	17 (85.0%)

percent cases correctly classified = 77.5%

7.4 Discussion

This experiment has shown that, although head measurements could be used to sex Atlantic salmon parr, irrespective of maturity, with only 73% accuracy (many of the males being classified as females), great accuracy (93%) was obtained in identifying maturing male parr. However, this experiment was carried out in September when maturing fish could already be distinguished from non-maturing fish with reasonable accuracy using other visual features (e.g. plump soft belly; yellow colouring). It was not possible at this time to distinguish with certainty between immature males and females. Using the above measurements, the probability of putting an immature fish in the correct sex group only increases by 27.5% from that gained by chance. Thus this morphometric technique obviously could not be used instead of dissection to sex immature fish. However, it could be used to bias the sex ratio in a particular tank. If it could be used earlier in the year, before the obvious visual changes in maturing fish occur, then it would be a very valuable and useful technique with wide potential. Maturing males could then be separated from immature fish thereby allowing them to be treated differently and so perhaps reduce the eventual maturation rate. All male or all female tanks could also be created if desired.

It is interesting to note which measurements were most important in distinguishing the categories of fish. For distinguishing sex, irrespective of maturity, males had shorter, wider and deeper heads with a wider upper mandibular width than females. Maturity was distinguished by the general head length and depth measurements, maturing males having shorter, deeper heads for their body size then immature individuals. Early maturing male chinook salmon also show this characteristic of having deeper heads (Taylor, 1989). For immature fish, it was depth and length/width ratios that became important in distinguishing males from females. Immature males had deeper, shorter and wider heads for their body size than did females. Both these results contrast with those found for the adults of several other salmonid species where the males have longer heads than the females (*Salmon trutta*, Yevsin, 1978; Pink salmon, *Oncorhynchus gorbuscha*, Berg, 1979; chum salmon, *O. keta*, Beacham, 1984). Although there were only minor differences in jaw length in this study, males had larger upper mandibular widths than females. Both in adult Atlantic salmon (Bodington, 1987) and pink salmon, *O.g.*, (Berg, 1979), males had longer lower jaws than females. Maisse *et al.* (1988) found that the upper mandibular length of male sea-run salmon was greater than females. Beacham and Murray (1986) included juvenile fish in their study and found that sexual dimorphism in adipose fin and upper jaw length of Pacific salmon became more marked at larger body lengths and in individuals closer to sexual maturation. The results of the present study show that sexual differences in head morphology exist in Atlantic salmon parr but that these cannot be used with great accuracy to predict sex.

Chapter 8 - General Discussion

8.1 Aims and objectives

The overall aim of this thesis was to investigate the factors influencing maturation in male Atlantic salmon parr. The relative effects of size and lipid levels as determinants of maturation within sibling groups and the possibility of using these determinants to predict which fish would mature and of using them to control maturation rates have been examined.

8.2 Lipid levels and size as determinants of maturation

Since lipid levels had been implicated in the control of maturation it was first necessary to develop a non-destructive method for estimating the fat content of live salmon. The biometric method described in Chapter 2 allowed the fat dynamics of individuals to be followed in the year preceding maturation. This technique is quick and easy to use and has wide application for future studies on other fish species as well as sea-run Atlantic salmon.

Chapters 3 and 4 dealt with the identification of factors important for maturation, their manipulation and effect on eventual maturation rates. Length, and to a certain extent fat content, were both important for maturation. However, growth rates and changes in total fat content were not significantly different between maturity groups until relatively close to the breeding season.

Those males that were the largest and fattest in their first November were most likely to mature a year later. November was also the time at which the gonads of these fish began growing (Chapter 5). Differences in length between maturing and immature trout have been detected at least one year prior to maturation (Naevdal *et al.*, 1981). Similarly, mature Arctic charr aged 2+ were those that had been heaviest at age 1+ (Nilsson, 1990). It therefore appears that maturation is switched on at least one year before spawning takes place. Previous experiments (e.g. Rowe, 1989), showing that maturation could be influenced by manipulation in early spring, are therefore not preventing the onset of maturation but the

continuation of a process that was initiated several months previously. Future studies would need to look at the fish's first summer to get a clearer picture of the factors that initiate maturation and the time at which they occur.

From all this evidence it is still not possible to pinpoint the beginning of the maturation process. Chapter 5 showed an increase in gonad growth from October to November. Villarreal and Thorpe (1985) showed that gonadal increases occurred in late September in all 0+ parr and, given increased growth opportunity through elevated water temperatures, 0+ fry showed spermatogenic activity in June (Saunders *et al.*, 1982). This leads to the same conclusion as suggested by Thorpe (1994b), that the maturation process has no starting point as such, in that it is present from hatching. All male parr invested proportionally more in gonadal than in somatic tissue as they grew, since G.S.I. was positively correlated with fork length over the experimental period (Chapter 5). If certain criteria, probably associated with accumulation of lipid reserves, are met at several points during the year when hormonal systems are sensitive to photoperiodic and perhaps temperature stimulation, then gonadal investment will continue or even increase. Conversely, if such criteria are not met, investment will be suppressed. From the evidence given above it seems that there are two major periods of sensitivity; the spring increase in temperature and photoperiod leading to increases in gonadal investment from early summer (May-June), and the autumnal decrease in temperature and photoperiod leading to increases from October-November. The summer period is also the time at which smolting decisions are made (Thorpe, 1977). Therefore there is a direct conflict between maturation and smolting (Thorpe, 1986; 1987). If, as suggested, maturation is present from hatching, smolting would then be a subsidiary process that may be initiated when maturation cannot be fulfilled in a particular year, due to criteria not being met at the appropriate times (Thorpe, 1994a). However, the smolting process itself will require that certain criteria are met at the appropriate time before it is initiated. Therefore some fish are able to remain in the river for several years without maturing before they smolt. The differences in size and growth rates that we see in Chapter 3 therefore, are indicators that certain fish have delayed maturation. Each individual fish will have its own threshold criteria relating to its genotype. Therefore within a population there will always be a size

range of maturing males that overlaps to a certain extent with that of immature males.

8.3 Predictors of maturation and sex

Logistic regressions could be used to predict the probability that males would subsequently mature based on their length and fat content earlier in the year. However the application of this prediction relied upon accurate discrimination between immature males and females. A morphometric technique was developed that allows discrimination between males and females with 79% accuracy based on several head measurements (Chapter 7). This is not precise enough to use experimentally, but shows that morphometric differences do occur in parr as have been found in adult salmon (see Chapter 7 for references).

8.4 Effects of dietary manipulation

Neither the use of a low fat diet from January to April nor a starvation regime over the winter caused a reduction in male maturation rates (Chapter 3) (even though those fish that had been on the starvation regime were smaller than their siblings in the control tanks at the end of the experiment). This could be due to the replenishment of fat reserves, once food ration was increased, in preference to achieving a larger size by growth in the starved group. This preferential replenishment of lipid reserves at the expense of growth has also been reported for bluegill sunfish, *Lepomis macrochirus* (Booth and Keast, 1986). Experiments designed to reduce fat levels in fish will need to take this preferential replenishment into account.

8.5 Appetite, lipid levels and maturation

Appetite did not differ between maturity groups even though maturing males had lower growth rates than non-maturing fish in July, the time of peak gonad growth. Therefore, energy was being diverted from somatic growth to gonad production at this time. This did not affect fat reserves, which continued to increase until the start of the breeding season in October, indicating that lipid is not important for the

production of the gonad tissue itself, which is mostly protein. Therefore, fat reserves must be needed for some other function associated with maturation. The breeding season is an active time for wild mature parr as they move, sometimes quite large distances to suitable spawning areas to arrive before the adult females (Garcia de Leaniz, 1990). At spawning time they compete amongst themselves to gain the best position in a dominance hierarchy established immediately downstream of the courting adult pair (Jones, 1959; Myers and Hutchings, 1987). Food intake is very low at this time (Chapter 4), so fat reserves are probably needed to sustain the fish through this active, but non-feeding, period. In this study (Chapter 3, Fig. 3.13) the largest mature males showed the greatest reduction in fat levels at the onset of the breeding season. There is evidence that these mature males are better competitors than their immature siblings (Järvi and Pettersen, 1991) and if they are dominant, they may use up more energy in aggressive interactions. In sea-run Atlantic salmon males use up much larger amounts of lipid reserves while spawning than females (Jonsson *et al.*, 1991). Fat levels therefore, seem to be important as an energy reserve during the breeding season when food is scarce and activity is high rather than providing the energy for gonad production, or allowing the fish to grow larger or faster. In addition, lipid may be needed for overwinter survival since maturing male parr tend to have a higher winter mortality than their immature siblings (Saunders, 1972). It has already been shown that maturing males develop a preference for gravel as the breeding season approaches (Garcia de Leaniz, 1990). Furthermore, mature male parr remained upstream while spawning females were present and only moved downstream later in the autumn (Buck and Youngson, 1982). It would be interesting to look at the activity of maturing and non-maturing parr to see whether there is a general increase in locomotor activity in maturing fish as the breeding season approaches, even in a laboratory situation with no suitable spawning substrate. This has been partly investigated by Thorpe *et al.* (1988), who found that maturing males were not displaced in a smooth flume tank during October while immature parr were. They concluded that this difference was due to maintained activity by maturing males when immature parr had reduced their activity.

Other species also exhibit an association between fat reserves and maturation (Shul'man, 1974). The onset of maturation in whitefish (*Coregonus lavaretus*) is connected with the attainment of fat reserves,

the threshold for maturation being specific to each population (Reshetnikov *et al.*, 1970). Some mature anadromous Arctic charr (*Salvelinus alpinus*) deplete their energy reserves to such an extent over the breeding season that they require more than one summer season at sea to replenish these reserves before returning to fresh water to spawn again (Dutil, 1986).

8.6 Appetite, lipid levels and season

Appetite peaked sharply for all fish in May, the first month that temperatures rose above the critical level for activity in juvenile parr (7-10°C) (Chapter 4). From June onwards there was a marked decline in appetite whilst the temperature remained suitable for feeding. Both total numbers and numbers of optimally sized drifting invertebrates decreased from June to August (Chapter 6), which would increase feeding costs and reduce returns making a reduction in appetite adaptive. However, the values for May were not higher than those for June, so why the peak in appetite in May ? Since May is the first month in the year that temperatures exceed the activity threshold (Chapter 4; Allen, 1940), and fat reserves are still relatively low (Chapter 3), I suggest that this appetite peak in May is a compensatory response to the fat deficit that has arisen over the winter (Metcalfe and Thorpe, 1992). As in other compensatory responses (Russell, 1991), this sharp increase in appetite leads to a peak in growth rate and rate of change in lipid levels (Chapter 3). The decline in appetite after May would then be a combination of the controlling effect of negative feedback on appetite and the adaptive appetite response to the reduction in natural prey at this time.

The hypothesis that salmon should be adapted to seasonal variation in food availability has a sound evolutionary base. Seasonal cycling of lipid reserves occurs in other fish species in Northern latitudes (large-mouth bass, *Micropterus salmoides*, Adams *et al.*, 1982; smelt, *Osmerus mordax*, Foltz and Norden, 1977; yellow perch, *Perca flavescens*, Newsome and Leduc, 1975; anadromous Arctic charr, Dutil, 1986). Similarly, bluegill sunfish build up fat reserves over the summer to sustain them through the winter (Booth and Keast, 1986), a period when there is little feeding activity (Moffet and Hunt,

1945). Since the amount of stored lipid is correlated with survival of starved fish, especially at low temperatures (Oliver *et al.*, 1979), overwinter mortality is higher for fish with low fat levels (Gardiner and Geddes, 1980).

8.7 Life history implications

There has been considerable speculation as to how the alternative reproductive strategy of maturing as male parr evolved and is maintained. By avoiding the seaward migration the parr are able to escape the higher mortality which occurs during migration and at sea (Gross *et al.*, 1988; Feltham, 1990) and reproduce sooner than their sea-run siblings. Several models have been put forward to explain the early maturation of parr in terms of an evolutionary stable strategy (Maynard-Smith, 1982; Gross, 1984, 1985; Bohlin *et al.*, 1985). All these models suggest that if the majority of males migrate it is better to stay behind and mature early and conversely it is better to be a large sea-run male if most males have matured early. This implies that age at first maturity is not based purely on physiological criteria, but must also involve an assessment of the abundance and behaviour of conspecifics. However, these models do not explain all the variations that exist in wild salmonid populations e.g. populations where all the males mature in freshwater early while all the females migrate and return as large adults (Nikolskii *et al.*, 1947). In this case it seems that salmon have fully adapted to make the best use of the freshwater environment, the high productivity allowing them to gain sufficient energy reserves to do so. The genetic variability in the population results in there always being some fish that migrate. However, recently there has been concern that fishing pressure on the large sea-run salmon may lead to a higher proportion of the population maturing as parr, a scenario that is unappealing to fishermen, salmon ranchers and anglers alike (Nikulin, 1970; Krogius, 1979; Caswell *et al.*, 1984).

8.8 Conclusions

Condition-dependent developmental switches -

This thesis has examined condition-dependent reproductive decisions in juvenile Atlantic salmon.

Similar condition-dependency can be seen throughout the animal kingdom associated not only with reproduction but a range of developmental switches. Good growing conditions in May induce nymphs of the red-legged grasshopper, (*Melanoplus ferurrubrum*) to undergo a further metamorphic phase, increasing the number of instars before pupation (Belinger and Pienkowski, 1987). Although not proven this could lead to a greater adult size and therefore greater reproductive success relating to increased fecundity (Roff, 1992). Reproductive success in vertebrates is also positively related to fat stores during early maturation stages (Shul'man, 1974; Frisch, 1988). Maturation in newts is suppressed when their fat bodies are removed (Adams and Rae, 1929). In snakes of temperate regions the probability of reproducing in one year is dependent on the amount of lipid stored before winter (Roff, 1992). Biennial reproduction occurs in some lizards as a result of reproduction in one year preventing sufficient lipid storage for the next year (Derickson, 1976). Various evidence from avian work shows that both reproduction and migration are lipid dependent. For example, the body condition of female Eastern Kingbirds (*Tyrannus tyrannus*) influences egg composition, especially lipid content (Murphy, 1986) and in Lesser Snow geese (*Chen caerulescens caerulescens*), clutch size is limited by nutrient reserves (Ankney, 1977). Lipid has also been shown to be the major substrate fuelling long flights in several bird species (Dawson *et al.*, 1983). Mammals also show this condition-dependency e.g. male squirrel monkeys increase in weight by up to 40% during the breeding season which is attributed to an increase in upper body fat (Siiteri, 1987). These developmental switches are therefore related to seasonal changes in body condition that presumably result from appetite changes that are adaptively timed to the cycling of productivity in the local environment. These have evolved to enable the animal to survive predictable annual periods of high energetic need, e.g. migration, hibernation, competition, maturation and reproduction.

Implications for aquaculture -

The results from this thesis have several implications for the commercial salmonid industry. The biometric technique (Chapter 2) could be adapted and used to assess the lipid reserves of all fish stages. Maturation rates are presently controlled by reduction of feeding in the early spring prior to maturation.

Results from this thesis now suggest that to reduce maturation rates most effectively restrictions should occur towards the end of the summer in the year prior to maturation. Feeding regimes would have to take into account the effect of preferential fat deposition on growth after a period of food restriction. As far as preventing maturation is concerned (as opposed to producing lean fish), this would not be important if, once the maturation process was switched off, it could not be restarted. This would enable optimum growth conditions to be applied after the switching-off point creating larger fish earlier in the year without the problem of maturation.

The next step therefore is to discover the best time (July, August, September) to apply restrictions, what these restrictions should be and establish whether maturation can be restarted later in the same reproductive year that it has been suppressed.

Appendix I - Experiment 1, 1990; Evaluating factors affecting maturation.

Table I.1. The mean length (mm) for mature males (Mm), immature males (Im) and immature females (If) from January to October. The F-value, D.F. and P-value are from oneway ANOVA.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Mm	63.4	64.6	69.0	72.9	82.6	94.1	103.0	110.0	119.3	123.7
Im	56.1	57.8	60.9	65.3	74.2	84.7	92.8	98.8	108.2	112.5
If	57.0	58.5	62.4	66.4	76.4	86.9	94.0	100.6	111.7	116.1
F-value	5.28	4.57	4.62	3.74	3.97	4.61	6.06	5.38	6.24	4.65
D.F.	2,92	2,92	2,92	2,92	2,92	2,92	2,92	2,85	2,70	2,66
P-value	0.007	0.013	0.012	0.028	0.022	0.012	0.003	0.006	0.003	0.013

Table I.2. The mean %fat for mature males (Mm), immature males (Im) and immature females (If) from January to October. The F-value, D.F. and P-value are from oneway ANOVA.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Mm	2.65	2.92	3.23	3.12	3.75	4.58	5.97	6.78	6.97	5.75
Im	1.78	1.70	2.32	2.07	2.87	3.61	4.67	5.16	5.97	5.81
If	1.98	2.13	2.51	2.34	3.32	3.83	4.73	5.38	6.14	5.79
F-value	2.36	4.83	3.65	5.34	7.42	6.42	6.40	9.48	9.38	0.04
D.F.	2,92	2,91	2,92	2,92	2,92	2,92	2,92	2,85	2,70	2,66
P-value	n.s.	0.010	0.030	0.006	0.001	0.002	0.003	0.000	0.000	n.s.

Table I.3. The mean specific growth rate (based on weight, SGR_W) for mature males (Mm), immature males (Im) and immature females (If) from January to October. The F-value, D.F. and P-value are from oneway ANOVA.

	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Aug	Aug-Sep	Sep-Oct
Mm	0.219	0.483	0.704	1.570	1.712	1.311	0.907	0.848	0.297
Im	0.157	0.434	0.850	1.584	1.628	1.205	0.673	0.761	0.350
If	0.246	0.467	0.852	1.661	1.568	1.075	0.852	0.896	0.366
F-value	2.54	0.75	3.12	1.02	0.86	1.37	2.18	1.22	0.21
D.F.	2,91	2,91	2,92	2,92	2,92	2,92	2,85	2,70	2,66
P-value	n.s.	n.s.	0.049	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table I.4. Mean specific growth rate (based on length, SGR_L) for mature males (Mm), immature males (Im) and immature females (If) from January to October. The F-value, D.F. and P-value are from oneway ANOVA.

	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Aug	Aug-Sep	Sep-Oct
Mm	0.0680	0.1844	0.2218	0.4746	0.4842	0.3613	0.2194	0.1997	0.1592
Im	0.1018	0.1601	0.2553	0.4692	0.4862	0.3490	0.2047	0.2106	0.1262
If	0.0810	0.2067	0.2402	0.4944	0.4656	0.3263	0.2357	0.2441	0.1374
F-value	0.54	2.22	0.95	0.92	0.50	0.80	0.53	1.06	0.77
D.F.	2,92	2,92	2,92	2,92	2,92	2,92	2,85	2,70	2,66
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table I.5. The mean rate of change in %fat for mature males (Mm), immature males (Im) and immature females (If) from January to October. The F-value, D.F. and P-value are from oneway ANOVA.

	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Aug	Aug-Sep	Sep-Oct
Mm	0.0125	0.0108	-0.0066	0.0227	0.0320	0.0590	0.0334	0.0121	-0.0515
Im	-0.0022	0.0210	-0.0089	0.0313	0.0287	0.0405	0.0221	0.0192	-0.0056
If	0.0048	0.0098	-0.0052	0.0343	0.0180	0.0382	0.0245	0.0170	-0.0116
F-value	1.10	1.98	0.19	0.87	2.13	1.22	0.57	0.44	30.48
D.F.	2,91	2,91	2,92	2,92	2,92	2,92	2,85	2,70	2,66
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.000

Table I.6. Differences in the fat/forklength relationship between mature and immature males from January to October 1990. F-values, D.F. and P-values are from covariance analysis.

	Slope			Intercept		
	F-value	D.F.	P-value	F-value	D.F.	P-value
January	0.09	1,52	n.s.	0.25	1,53	n.s.
February	0.14	1,51	n.s.	0.87	1,52	n.s.
March	0.06	1,52	n.s.	0.46	1,53	n.s.
April	1.81	1,52	n.s.	2.42	1,53	n.s.
May	0.14	1,52	n.s.	2.02	1,53	n.s.
June	0.99	1,52	n.s.	3.89	1,53	n.s.
July	0.05	1,52	n.s.	2.26	1,53	n.s.
August	11.93	1,48	0.001	*	*	*
September	34.97	1,40	0.000	*	*	*
October	28.02	1,37	0.000	*	*	*

Table I.7. F-values, D.F., and P-values from stepwise logistic regressions to predict maturation from fork length and percentage fat, based on data from males only in each month from January to April 1990.

Month	January		February		March		April	
Variable	FL	%Fat	FL	%Fat	FL	%Fat	FL	%Fat
F-value	10.05	0.08	1.98	8.73	10.15	0.76	1.14	10.33
D.F.	1,53	1,53	1,52	1,52	1,53	1,53	1,53	1,53
P-value	0.0025	n.s.	n.s.	0.0047	0.0024	n.s.	n.s.	0.0022

Table I.8. F-values, D.F., and P-values from stepwise logistic regressions to predict maturation from fork length and percentage fat, based on data from all fish in each month from January to April 1990.

Month	January		February		March		April	
Variable	FL	%Fat	FL	%Fat	FL	%Fat	FL	%Fat
F-value	11.26	0.06	10.19	0.23	9.64	1.06	1.32	9.44
D.F.	1,90	1,90	1,89	1,89	1,90	1,90	1,90	1,90
P-value	0.0012	n.s.	0.0019	n.s.	0.0025	n.s.	n.s.	0.0028

Table I.9. Median appetite scores for mature males (Mm), immature males (Im) and immature females (If) from January to October 1990. H-values, D.F. and P-values from the Kruskal Wallis non-parametric equivalent of oneway ANOVA.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct
Mm	0.50	0.24	0.14	0.15	4.76	0.72	0.00	0.25	0.00	0.00
Im	0.37	0.69	0.81	1.00	3.44	1.70	0.30	0.11	0.30	0.10
If	0.70	0.50	0.57	0.58	3.67	1.33	0.40	0.28	0.10	0.00
H-value	0.47	1.79	2.11	1.56	0.24	2.32	6.62	0.25	5.12	4.42
D.F.	2,74	2,55	2,40	2,78	2,90	2,81	2,90	2,72	2,64	2,64
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.037	n.s.	n.s.	n.s.

Table I.10. Spearman rank correlations between appetite and fat levels.

Month	r_s	n	P
January	0.135	74	n.s.
February	-0.007	55	n.s.
March	0.134	40	n.s.
April	-0.167	78	n.s.
May	-0.033	90	n.s.
June	-0.043	81	n.s.
July	-0.084	90	n.s.
August	-0.138	72	n.s.
September	-0.200	64	n.s.
October	0.231	64	n.s.

Table I.11. Spearman rank correlations between appetite and specific growth rate (SGR_W).

Appetite	SGR	r_s	n	P
Jan	Jan - Feb	-0.005	74	n.s.
Feb	Jan - Feb	0.150	55	n.s.
Feb	Feb - Mar	0.143	55	n.s.
Mar	Feb - Mar	0.333	40	0.05
Mar	Mar - Apr	0.221	40	n.s.
Apr	Mar - Apr	0.245	78	0.05
Apr	Apr - May	0.102	78	n.s.
May	Apr - May	0.082	90	n.s.
May	May - Jun	0.223	90	0.05
Jun	May - Jun	-0.058	81	n.s.
Jun	Jun - Jul	0.010	81	n.s.
Jul	Jun - Jul	0.023	90	n.s.
Jul	Jul - Aug	0.118	90	n.s.
Aug	Jul - Aug	-0.181	72	n.s.
Aug	Aug - Sep	0.392	72	<0.001
Sep	Aug - Sep	-0.038	64	n.s.
Sep	Sep - Oct	-0.092	64	n.s.

Table I.12. Spearman rank correlations between appetite and rate of change in percent fat.

Appetite	Rate of change in %fat	r_s	n	P
Jan	Jan - Feb	-0.074	74	n.s.
Feb	Jan - Feb	0.010	55	n.s.
Feb	Feb - Mar	0.033	55	n.s.
Mar	Feb - Mar	0.012	40	n.s.
Mar	Mar - Apr	-0.055	40	n.s.
Apr	Mar - Apr	0.020	78	n.s.
Apr	Apr - May	0.114	78	n.s.
May	Apr - May	0.079	90	n.s.
May	May - Jun	0.027	90	n.s.
Jun	May - Jun	-0.064	81	n.s.
Jun	Jun - Jul	0.135	81	n.s.
Jul	Jun - Jul	-0.061	90	n.s.
Jul	Jul - Aug	0.059	90	n.s.
Aug	Jul - Aug	0.102	72	n.s.
Aug	Aug - Sep	0.202	72	n.s.
Sep	Aug - Sep	0.061	64	n.s.
Sep	Sep - Oct	0.113	64	n.s.

Appendix II - Experiment 2, 1991; Dietary fat manipulation.

Table II.1. Diet differences in mean length (mm) for mature males (Mm), immature males (Im) and immature females (If) from January to September. The F-value, D.F. and P-value are from oneway ANOVA (no analysis for immature males due to small sample size).

		Diet 1	Diet 2	Diet 3	F-value	D.F.	P-value
	Mm	68.26	67.15	68.06	0.27	2,111	n.s.
Jan	If	67.68	65.54	66.71	1.31	2,140	n.s.
	Im	62.00	58.25	57.50			
	Mm	67.98	66.82	68.13	0.38	2,111	n.s.
Feb	If	67.40	65.46	66.62	1.09	2,139	n.s.
	Im	62.00	58.00	57.00			
	Mm	68.55	67.15	68.81	0.56	2,111	n.s.
Mar	If	67.85	65.84	67.16	1.18	2,140	n.s.
	Im	63.00	58.50	57.50			
	Mm	73.24	73.84	75.48	0.79	2,107	n.s.
Apr	If	72.30	71.61	72.86	0.36	2,135	n.s.
	Im	67.00	63.75	62.00			
	Mm	82.68	84.64	85.34	0.98	2,111	n.s.
May	If	82.05	81.82	82.82	0.20	2,140	n.s.
	Im	76.00	73.75	71.00			
	Mm	99.94	101.85	101.06	0.45	2,111	n.s.
Jun	If	99.10	97.76	97.31	0.51	2,140	n.s.
	Im	93.00	89.00	85.50			
	Mm	108.57	109.87	108.78	0.22	2,110	n.s.
Jul	If	106.40	104.30	103.57	1.27	2,140	n.s.
	Im	99.00	97.00	93.00			
	Mm	114.98	115.75	113.81	0.39	2,109	n.s.
Aug	If	111.35	109.20	107.02	2.99	2,140	n.s.
	Im	107.00	102.75	97.00			
	Mm	121.47	122.94	120.19	0.72	2,111	n.s.
Sep	If	119.70	118.92	116.20	2.46	2,140	n.s.
	Im	115.00	113.00	106.50			

Table II.2. Mean length (mm) and tests of differences between maturity groups (all diets combined). Mm, maturing males; If, immature females; Im immature males. F-values, D.F. and P-values are from oneway ANOVA.

Month	Mm	If	Im	F-value	D.F.	P-value
Jan	67.88	66.54	58.57	7.07	2,258	0.001
Feb	67.68	66.40	58.29	7.17	2,257	0.001
Mar	68.21	66.86	58.86	6.91	2,258	0.001
Apr	74.07	72.22	63.71	6.95	2,249	0.001
May	84.02	82.21	73.29	5.79	2,258	0.003
June	100.82	97.94	88.57	8.34	2,258	0.000
July	109.01	104.60	94.14	12.80	2,257	0.000
Aug	114.86	108.99	101.71	18.29	2,256	0.000
Sep	121.54	118.12	111.43	7.84	2,258	0.000

Table II.3. Diet differences in mean %fat for mature males (Mm), immature males (Im) and immature females (If) from January to May. The F-value, D.F. and P-value are from oneway ANOVA (no analysis of immature males due to small sample size).

		Diet 1	Diet 2	Diet 3	F-value	D.F.	P-value
	Mm	1.83	1.95	1.94	0.24	2,111	n.s.
Jan	Im	0.60	0.65	0.63			
	If	1.69	1.70	1.85	0.45	2,140	n.s.
	Mm	2.21	2.13	1.84	1.66	2,111	n.s.
Feb	Im	1.08	1.04	1.08			
	If	2.26	2.05	1.81	3.62	2,139	0.029
	Mm	2.52	2.65	2.53	0.26	2,111	n.s.
Mar	Im	1.13	1.47	1.83			
	If	2.58	2.49	2.58	0.15	2,140	n.s.
	Mm	3.77	4.13	4.16	4.01	2,107	0.021
Apr	Im	3.09	3.51	3.53			
	If	3.91	4.09	4.10	0.86	2,135	n.s.
	Mm	4.62	4.94	4.80	2.54	2,111	n.s.
May	Im	4.31	3.87	3.73			
	If	4.84	4.79	4.71	0.42	2,140	n.s.

Table II.4. The differences in %fat between mature males and immature females in each diet. The t-value, D.F. and P-value are from t-tests.

	Jan	Feb	Mar	Apr	May
Diet 1:					
t-value	0.69	-0.24	-0.34	-0.91	-1.71
D.F.	73	83	76	79	84
P-value	n.s.	n.s.	n.s.	n.s.	n.s.
Diet 2:					
t-value	1.35	0.49	0.81	0.27	0.99
D.F.	68	66	77	71	76
P-value	n.s.	n.s.	n.s.	n.s.	n.s.
Diet 3:					
t-value	0.46	0.19	-0.22	0.39	0.61
D.F.	65	71	70	74	73
P-value	n.s.	n.s.	n.s.	n.s.	n.s.

Table II.5. Diet differences in specific growth rate (based on weight, SGR_W) for mature males (Mm), immature males (Im) and immature females (If) from January to September. The F-value, D.F. and P-value are from oneway ANOVA (no analysis for immature males due to small sample size).

		Diet 1	Diet 2	Diet 3	F-value	D.F.	P-value
	Mm	0.0431	0.0781	0.0734	4.84	2,111	0.010
Jan-Feb	Im	0.0856	0.0852	0.0620			
	If	0.0247	0.0847	0.0227	2.01	2,139	n.s.
	Mm	0.1561	0.1622	0.1761	0.33	2,111	n.s.
Feb-Mar	Im	0.1590	0.1012	0.0625			
	If	0.1411	0.1335	0.1906	3.10	2,139	n.s.
	Mm	0.7472	0.9538	0.9584	21.47	2,111	0.000
Mar-Apr	Im	0.8652	0.9973	0.9088			
	If	0.7500	0.8790	0.9013	7.30	2,140	0.001
	Mm	1.6854	1.8298	1.7131	4.43	2,111	0.014
Apr-May	Im	1.5897	1.8164	1.5690			
	If	1.6873	1.7714	1.6276	5.69	2,140	0.004
	Mm	1.8299	1.8661	1.6942	3.33	2,111	0.040
May-Jun	Im	1.8443	1.8424	1.9079			
	If	1.7524	1.7118	1.6107	3.82	2,140	0.024
	Mm	1.1293	1.0897	1.0927	0.26	2,111	n.s.
Jun-Jul	Im	0.9662	1.1323	1.0851			
	If	0.9247	0.8406	0.8421	1.26	2,140	n.s.
	Mm	0.6847	0.6679	0.4843	10.04	2,109	0.000
Jul-Aug	Im	0.7069	0.6622	0.5188			
	If	0.4649	0.5536	0.2861	16.20	2,140	0.000
	Mm	0.3888	0.3965	0.3927	0.02	2,109	n.s.
Aug-Sep	Im	0.4471	0.5918	0.6453			
	If	0.5138	0.5490	0.6190	4.47	2,140	0.013

Table II.6. The differences in specific growth rate (based on weight, SGR_W) between mature males and immature females in each diet. The t-value, D.F. and P-value are from t-tests.

	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Aug	Aug-Sep
Diet 1:								
t-value	1.73	0.64	-0.06	-0.04	1.81	3.07	4.72	-4.17
D.F.	68	76	77	79	80	63	61	78
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	0.003	0.000	0.0001
Diet 2:								
t-value	-0.47	0.95	1.89	0.95	2.10	4.42	2.05	-5.49
D.F.	67	71	80	60	49	62	72	79
P-value	n.s.	n.s.	n.s.	n.s.	0.041	0.0000	0.044	0.0000
Diet 3:								
t-value	1.24	-0.63	1.76	2.15	1.24	3.54	3.88	-4.21
D.F.	56	73	72	69	67	50	55	54
P-value	n.s.	n.s.	n.s.	0.035	n.s.	0.0009	0.0003	0.0001

Table II.7. Diet differences in specific growth rate (based on length, SGR_L) for mature males (Mm), immature males (Im) and immature females (If) from January to September. The F-value, D.F. and P-value are from oneway ANOVA (no analysis for immature males due to small sample size).

		Diet 1	Diet 2	Diet 3	F-value	D.F.	P-value
	Mm	-0.0133	-0.0148	0.0038	0.92	2,111	n.s.
Jan-Feb	If	-0.0131	-0.0048	-0.0069	0.32	2,138	n.s.
	Im	0.0000	-0.0175	-0.0363			
	Mm	0.0340	0.0178	0.0373	2.74	2,111	n.s.
Feb-Mar	If	0.0283	0.0220	0.0340	1.31	2,138	n.s.
	Im	0.0000	0.0370	0.0285			
	Mm	0.1852	0.2730	0.2487	24.65	2,107	0.000
Mar-Apr	If	0.1915	0.2366	0.2398	6.14	2,134	0.003
	Im	0.1811	0.2458	0.2153			
	Mm	0.5145	0.5792	0.5325	6.42	2,107	0.002
Apr-May	If	0.5336	0.5596	0.5276	1.73	2,134	n.s.
	Im	0.5252	0.6048	0.5392			
	Mm	0.5023	0.4981	0.4632	1.97	2,111	n.s.
May-Jun	If	0.4999	0.4799	0.4382	6.42	2,139	0.002
	Im	0.5312	0.5178	0.5107			
	Mm	0.3335	0.3108	0.2987	1.45	2,110	n.s.
Jun-Jul	If	0.2852	0.2616	0.2526	1.77	2,139	n.s.
	Im	0.2501	0.3482	0.3570			
	Mm	0.1945	0.1823	0.1622	1.90	2,108	n.s.
Jul-Aug	If	0.1645	0.1653	0.1180	6.67	2,139	0.002
	Im	0.2775	0.2071	0.1481			
	Mm	0.1328	0.1354	0.1251	0.55	2,109	n.s.
Aug-Sep	If	0.1694	0.1986	0.1922	3.18	2,139	0.045
	Im	0.1677	0.2204	0.2237			

Table II.8. The differences in specific growth rate (based on length, SGR_L) between mature males and immature females in each diet. The t-value, D.F. and P-value are from t-tests.

	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Aug	Aug-Sep
Diet 1:								
t-value	-0.03	0.69	-0.48	-1.09	0.17	2.70	1.91	-4.05
D.F.	81	79	71	79	77	75	80	77
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	0.009	n.s.	0.0001
Diet 2:								
t-value	-0.54	-0.48	2.60	1.04	0.93	2.26	0.96	-5.84
D.F.	48	69	78	73	53	49	70	78
P-value	n.s.	n.s.	0.011	n.s.	n.s.	0.028	n.s.	0.0000
Diet 3:								
t-value	0.81	0.43	0.59	0.27	0.99	2.15	2.86	-5.56
D.F.	61	60	65	77	60	59	54	73
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	0.036	0.0059	0.0000

Table II.9. Diet differences in mean rate of change in %fat for mature males (Mm), immature females (If) and immature males (Im). F-values, D.F. and P-values are from oneway ANOVA (no analysis of immature males due to low sample size).

		Diet 1	Diet 2	Diet 3	F-value	D.F.	P-value
	Mm	0.01188	0.00580	-0.00312	3.84	2,111	0.024
Jan-Feb	If	0.01776	0.01121	-0.00129	11.32	2,139	0.000
	Im	0.01483	0.01308	0.01507			
	Mm	0.01270	0.02020	0.02615	1.94	2,111	n.s.
Feb-Mar	If	0.01300	0.01723	0.02888	5.64	2,139	0.004
	Im	0.00204	0.01652	0.02908			
	Mm	0.03608	0.04269	0.04576	3.33	2,107	0.039
Mar-Apr	If	0.03951	0.04638	0.04485	1.39	2,135	n.s.
	Im	0.05770	0.05870	0.04997			
	Mm	0.03507	0.03294	0.02645	0.89	2,107	n.s.
Apr-May	If	0.03656	0.02835	0.02452	2.38	2,135	n.s.
	Im	0.05078	0.01481	0.00806			

Table II.10. The differences in rate of change in %fat between mature males and immature females for each diet. t-values, D.F. and P-values from t-tests.

	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May
Diet 1:				
t-value	-1.05	-0.05	-0.95	-0.26
D.F.	84	74	77	79
P-value	n.s.	n.s.	n.s.	n.s.
Diet 2:				
t-value	-1.16	0.56	-1.04	0.70
D.F.	63	67	75	66
P-value	n.s.	n.s.	n.s.	n.s.
Diet 3:				
t-value	-0.48	-0.54	0.19	0.33
D.F.	58	75	72	57
P-value	n.s.	n.s.	n.s.	n.s.

Table II.11. Covariance analysis of the %fat/forklength relationship between maturing and non-maturing males.

	Slope			Intercept		
Month	F-value	D.F.	P-value	F-value	D.F.	P-value
January	4.49	1,115	0.036	*	*	*
February	3.56	1,115	n.s.	0.41	1,116	n.s.
March	10.21	1,115	0.002	*	*	*
April	1.18	1,111	n.s.	0.30	1,112	n.s.
May	4.75	1,115	0.031	*	*	*

Table II.12. F-values, D.F., and P-values from stepwise logistic regressions to predict maturation from fork length and percentage fat, based on data from males only in each month from January to April 1991.

Month	January		February		March		April	
Variable	FL	%Fat	FL	%Fat	FL	%Fat	FL	%Fat
F-value	0.65	6.72	11.11	0.03	10.81	0.06	9.69	0.70
D.F.	1,112	1,112	1,112	1,112	1,112	1,112	1,112	1,112
P-value	n.s.	0.0108	0.0012	n.s.	0.0013	n.s.	0.0023	n.s.

Table II.13. Diet differences in mean appetite (%body weight per hour) for mature males (Mm), immature males (Im) and immature females (If). F-values, D.F. and P-values are from oneway ANOVA (no analysis of immature males due to small sample size).

		Diet 1	Diet 2	Diet 3	F-value	D.F.	P-value
	Mm	0.01515	0.01483	0.01990	0.52	2,111	n.s.
Jan	Im	0.00241	0.02010	-0.00060			
	If	0.01337	0.01915	0.01718	0.74	2,137	n.s.
	Mm	0.00706	0.01494	0.02581	8.80	2,111	0.000
Feb	Im	-0.01400	0.00969	0.02920			
	If	0.00436	0.01673	0.02885	16.41	2,136	0.000
	Mm	0.02458	0.03704	0.03020	2.18	2,109	n.s.
Mar	Im	0.04351	0.06040	0.02992			
	If	0.02261	0.02620	0.03477	2.95	2,134	n.s.
	Mm	0.06474	0.04526	0.05180	5.78	2,109	0.004
Apr	Im	0.06118	0.04049	0.06152			
	If	0.06746	0.03866	0.04817	16.88	2,139	0.000
	Mm	0.14216	0.16689	0.13860	1.41	2,89	n.s.
May	Im	0.08025	0.11520	0.09870			
	If	0.14233	0.13377	0.12682	0.91	2,120	n.s.
	Mm	0.07298	0.05860	0.08276	3.95	2,111	0.022
Jun	Im	0.12776	0.07509	0.07102			
	If	0.06107	0.05926	0.07761	4.37	2,139	0.014
	Mm	0.04046	0.03406	0.05366	2.70	2,109	n.s.
Jul	Im	0.01841	0.03210	0.02042			
	If	0.03574	0.03014	0.05856	10.70	2,136	0.000
	Mm	0.03038	0.02652	0.03992	1.94	2,104	n.s.
Aug	Im	0.02972	0.06940	0.10500			
	If	0.03545	0.05348	0.06230	6.10	2,136	0.003

Table II.14. The differences in appetite between mature males and immature females in each diet. The t-value, D.F. and P-value are from t-tests.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Diet 1:								
t-value	0.38	0.65	0.37	-0.47	-0.01	1.92	0.61	-0.77
D.F.	83	77	81	81	76	81	79	80
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Diet 2:								
t-value	-1.13	-0.38	1.48	1.08	2.29	-0.10	0.66	-4.14
D.F.	61	59	57	59	30	61	73	75
P-value	n.s.	n.s.	n.s.	n.s.	0.029	n.s.	n.s.	0.0001
Diet 3:								
t-value	0.44	-0.68	-1.17	0.77	1.00	0.52	-0.64	-2.97
D.F.	71	70	77	58	68	61	73	74
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0041

Table II.15. Correlations between appetite and fat levels.

Month	r	n	P
January	0.026	258	n.s.
February	-0.018	257	n.s.
March	0.021	252	n.s.
April	-0.088	258	n.s.
May	0.192	217	0.05

Table II.16. Correlations between appetite and specific growth rates (SGR_W).

Appetite	SGR	r	n	P
January	Jan - Feb	0.063	257	n.s.
February	Jan - Feb	0.071	257	n.s.
February	Feb - Mar	0.000	257	n.s
March	Feb - Mar	0.071	252	n.s.
March	Mar - Apr	0.158	252	0.013
April	Mar - Apr	0.084	258	n.s.
April	Apr - May	0.114	258	n.s.
May	Apr - May	0.303	217	< 0.001
May	May - Jun	0.032	217	n.s.
June	May - Jun	0.100	260	n.s.
June	Jun - Jul	0.110	260	n.s.
July	Jun - Jul	0.089	260	n.s.
July	Jul - Aug	0.032	253	n.s.
August	Jul - Aug	0.000	249	n.s.
August	Aug - Sep	0.438	249	< 0.000

Table II.17. Correlations between appetite and rate of change in fat.

Appetite	Rate of change in Fat	r	n	P
January	Jan - Feb	0.100	257	n.s.
February	Jan - Feb	0.000	257	n.s.
February	Feb - Mar	0.000	257	n.s.
March	Feb - Mar	0.095	252	n.s.
March	Mar - Apr	0.100	243	n.s.
April	Mar - Apr	0.105	249	n.s.
April	Apr - May	0.161	249	0.010
May	Apr - May	0.032	209	n.s.

Appendix III - Experiment 3, 1991-1992; Winter starvation.

Table III.1. The mean length of maturing males (Mm), immature males (Im) and immature females (If) from November 1991 to September 1992. The F-value, D.F. and P-value are from oneway ANOVA.

	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Sep
Controls:										
Mm	61	61	61	62	63	67	75	88	102	121
Im	56	56	56	57	57	60	66	77	88	108
If	58	58	58	59	60	63	70	81	93	113
F-value	13.34	13.52	13.67	14.54	15.34	17.68	21.63	26.44	21.48	17.11
D.F.	2,189	2,189	2,189	2,189	2,189	2,188	2,187	2,183	2,151	2,148
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Starved:										
Mm	60	60	60	60	61	62	70	85	97	115
Im	56	55	55	55	55	57	62	75	85	102
If	60	59	59	59	60	62	69	84	96	113
F-value	22.22	22.33	22.30	21.62	23.19	24.68	29.32	33.04	38.18	29.77
D.F.	2,186	2,186	2,186	2,186	2,186	2,186	2,185	2,184	2,177	2,178
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table III.2. Results of t-tests for differences in length between control and starved fish in each maturity group.

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep
Maturing males:										
t =	0.67	1.52	1.73	1.86	2.71	4.71	4.07	2.37	2.05	2.64
D.F.	100	103	103	104	100	95	99	97	85	77
P =	n.s.	n.s.	n.s.	n.s.	0.0079	0.0000	0.0001	0.0200	0.0440	0.0100
Immature males:										
t =	0.61	1.53	1.85	1.71	2.28	3.55	3.29	1.30	1.72	2.80
D.F.	124	124	124	124	123	120	121	118	74	87
P =	n.s.	n.s.	n.s.	n.s.	0.0240	0.0006	0.0013	n.s.	n.s.	0.0062
Immature females:										
t =	-2.23	-1.26	-0.82	-0.71	-0.21	1.39	0.38	-1.87	-1.80	0.06
D.F.	131	131	130	130	131	132	129	125	113	114
P =	0.0270	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table III.3. The mean percentage fat for mature males (Mm), immature males (Im) and immature females (If) from November 1991 to July 1992. The F-value, D.F. and P-value are from oneway ANOVA.

	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July
Controls:									
Mm	2.76	2.60	1.76	2.26	2.47	3.10	4.16	5.24	5.14
Im	2.12	2.14	1.29	1.65	1.73	2.37	3.51	4.50	5.05
If	2.48	2.47	1.36	2.07	2.13	2.70	3.88	4.96	5.21
F-value	6.243	5.996	3.763	8.236	9.641	10.343	9.504	13.254	0.526
D.F.	2,189	2,189	2,189	2,189	2,189	2,188	2,187	2,183	2,151
P-value	0.0024	0.0030	0.0250	0.0004	0.0001	0.0001	0.0001	0.0000	n.s.
Starved:									
Mm	2.53	1.42	0.96	1.13	1.41	2.48	4.11	5.40	5.45
Im	2.08	0.95	0.34	0.53	0.61	1.39	3.38	5.02	5.33
If	2.59	1.49	0.94	1.12	1.47	2.41	3.87	5.24	5.36
F-value	5.747	7.840	8.946	9.502	17.127	26.099	15.790	4.707	0.626
D.F.	2,186	2,186	2,186	2,186	2,186	2,186	2,185	2,184	2,177
P-value	0.0038	0.0005	0.0002	0.0001	0.0000	0.0000	0.0000	0.0102	n.s.

Table III.4. Results of t-tests for differences in percentage fat between control and starved fish in each maturity group.

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Maturing males:									
t-value	1.45	7.99	5.54	6.97	6.87	4.00	0.39	-1.50	-1.90
D.F.	99	105	103	95	105	106	98	103	65
P-value	n.s.	0.0000	0.0000	0.0000	0.0000	0.0001	n.s.	n.s.	n.s.
Immature males:									
t-value	0.24	8.68	5.08	6.74	6.43	5.43	0.88	-3.70	-2.11
D.F.	126	112	126	123	122	114	125	125	101
P-value	n.s.	0.0000	0.0000	0.0000	0.0000	0.0000	n.s.	0.0003	0.0370
Immature females:									
t-value	-0.62	6.88	2.48	6.77	4.25	2.01	0.12	-1.92	-1.45
D.F.	131	133	127	123	133	131	125	126	104
P-value	n.s.	0.0000	0.0150	0.0000	0.0000	0.0470	n.s.	n.s.	n.s.

Table III.5. Mean specific growth rates (based on weight, SGR_W) for maturing males (Mm), immature males (Im) and immature females (If) from November to July 1991-1992. F-values, D.F. and P-values are from oneway ANOVA.

	Nov-Dec	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul
Controls:								
Mm	-0.0639	0.0352	0.0966	0.2787	0.7136	1.0617	2.0958	1.4173
Im	-0.1011	0.0566	0.0759	0.1819	0.5945	0.8661	1.8952	1.4134
If	-0.0838	0.0601	0.0906	0.2392	0.6538	0.9620	1.9171	1.2806
F-value	1.3011	0.7564	1.0993	17.2831	13.8677	19.6081	6.9078	2.1381
D.F.	2,189	2,189	2,189	2,189	2,189	2,188	2,184	2,151
P-value	n.s.	n.s.	n.s.	0.0000	0.0000	0.0000	0.0013	n.s.
Starved:								
Mm	-0.3685	-0.2032	0.1490	0.2338	0.5778	1.1384	2.5191	1.3820
Im	-0.3930	-0.2341	0.1388	0.1386	0.5301	1.0207	2.4072	1.3140
If	-0.3489	-0.2023	0.1831	0.2251	0.5798	1.1162	2.3702	1.3148
F-value	1.9405	3.9393	4.3014	12.7438	2.1767	10.6016	3.5588	1.1376
D.F.	2,186	2,186	2,186	2,186	2,186	2,186	2,184	2,177
P-value	n.s.	0.0211	0.0149	0.0000	n.s.	0.0000	0.0305	n.s.

Table III.6. Results of t-tests for differences in specific growth rates (SGR_W) between control and starved fish in each maturity group (Mm = maturing males, Im = immature males, If = immature females).

	Nov-Dec	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul
t-value	15.07	13.32	-3.16	2.42	5.51	-2.65	-6.09	0.53
Mm:D.F.	87	80	98	102	109	100	93	76
P-value	0.0000	0.0000	0.0021	0.0170	0.0000	0.0093	0.0000	n.s.
t-value	10.52	16.16	-3.97	2.03	2.45	-5.34	-9.94	1.57
Im:D.F.	100	119	109	97	105	126	122	82
P-value	0.0000	0.0000	0.0001	0.0450	0.0160	0.0000	0.0000	n.s.
t-value	14.53	17.11	-6.82	0.87	3.35	-5.62	-8.36	-0.51
If:D.F.	119	108	127	121	130	132	112	108
P-value	0.0000	0.0000	0.0000	n.s.	0.0011	0.0000	0.0000	n.s.

Table III.7. Mean specific growth rate (based on length, SGR_L) for maturing males, immature males and immature females from November 1991 to September 1992. F-values, D.F. and P-values are from oneway ANOVA.

	Nov-Dec	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Sep
Controls:									
Mm	0.0028	0.0084	0.0311	0.0621	0.2045	0.3249	0.6183	0.4462	0.2143
Im	0.0031	0.0055	0.0211	0.0417	0.1519	0.2601	0.5943	0.4381	0.2378
If	0.0014	0.0099	0.0275	0.0560	0.1825	0.2905	0.5949	0.4158	0.2345
F-value	0.0441	0.6403	0.8392	3.2491	14.1867	14.4622	1.1135	0.9003	1.9616
D.F.	2,189	2,189	2,189	2,189	2,188	2,187	2,183	2,150	2,148
P-value	n.s.	n.s.	n.s.	0.0410	0.0000	0.0000	n.s.	n.s.	n.s.
Starved:									
Mm	-0.0364	-0.0011	0.0257	0.0173	0.0935	0.3244	0.7335	0.4377	0.2021
Im	-0.0463	-0.0102	0.0284	0.0063	0.0724	0.2589	0.7312	0.4084	0.2265
If	-0.0455	-0.0092	0.0229	0.0297	0.1025	0.3240	0.7236	0.4117	0.2043
F-value	0.4604	2.2444	0.2725	4.3341	7.5396	19.0128	0.1719	1.8314	2.3302
D.F.	2,186	2,186	2,186	2,186	2,186	2,185	2,184	2,177	2,177
P-value	n.s.	n.s.	n.s.	0.0145	0.0007	0.0000	n.s.	n.s.	n.s.

Table III.8. Results of t-tests for differences in specific growth rate (SGR_L) between control and starved fish in each maturity group.

	Nov-Dec	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul	Jul-Sep
Maturing males:									
t =	8.00	2.12	0.84	5.55	11.45	0.04	-5.75	0.38	1.06
D.F.	106	108	109	103	95	109	103	68	86
P =	0.0000	0.0360	n.s.	0.0000	0.0000	n.s.	0.0000	n.s.	n.s.
Immature males:									
t =	5.46	3.52	-0.80	3.84	8.68	0.10	-7.63	1.41	0.72
D.F.	118	118	124	126	126	126	125	94	93
P =	0.0000	0.0006	n.s.	0.0002	0.0000	n.s.	0.0000	n.s.	n.s.
Immature females:									
t =	3.89	4.26	0.74	3.97	10.40	-2.95	-8.57	0.21	2.90
D.F.	78	127	116	129	129	130	117	110	101
P =	0.0002	0.0000	n.s.	0.0001	0.0000	0.0038	0.0000	n.s.	0.0046

Table III.9. Mean rate of change in percent fat for maturing males, immature males and immature females from November to July 1991-1992. F-values, D.F. and P-values are from oneway ANOVA.

	Nov-Dec	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul
Controls:								
Mm	-0.0053	-0.0263	0.0171	0.0080	0.0207	0.0305	0.0404	-0.0033
Im	0.0008	-0.0268	0.0124	0.0031	0.0212	0.0326	0.0382	0.0188
If	-0.0002	-0.0353	0.0247	0.0023	0.0189	0.0342	0.0415	0.0055
F-value	0.9652	1.8569	2.9696	0.5333	0.1225	0.5746	0.2475	7.7111
D.F.	2,189	2,189	2,189	2,189	2,188	2,187	2,183	2,150
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0007
Starved:								
Mm	-0.0398	-0.0132	0.0061	0.0101	0.0357	0.0467	0.0488	0.0014
Im	-0.0402	-0.0175	0.0066	0.0028	0.0261	0.0567	0.0618	0.0108
If	-0.0394	-0.0159	0.0061	0.0130	0.0312	0.0414	0.0522	0.0032
F-value	0.0159	0.7802	0.0074	2.5930	2.2586	7.9570	4.1844	2.8484
D.F.	2,186	2,186	2,186	2,186	2,186	2,185	2,184	2,177
P-value	n.s.	n.s.	n.s.	n.s.	n.s.	0.0005	0.0167	n.s.

Table III.10. Results of t-tests for differences in rate of change in %fat between control and starved fish in each maturity group.

	Nov-Dec	Dec-Jan	Jan-Feb	Feb-Mar	Mar-Apr	Apr-May	May-Jun	Jun-Jul
Maturing males:								
t =	7.47	-3.11	2.36	-0.45	-3.97	-4.34	-1.69	-0.87
D.F.	107	96	92	97	102	107	87	75
P =	0.0000	0.0025	0.0200	n.s.	0.0001	0.0000	n.s.	n.s.
Immature males:								
t =	10.27	-2.08	1.14	0.06	-0.95	-6.40	-5.03	1.65
D.F.	124	108	125	126	119	110	124	82
P =	0.0000	0.0400	n.s.	n.s.	n.s.	0.0000	0.0000	n.s.
Immature females:								
t =	8.65	-4.23	3.73	-2.02	-2.47	-2.16	-2.14	0.51
D.F.	132	119	133	132	127	127	126	113
P =	0.0000	0.0000	0.0003	0.046	0.015	0.033	0.035	n.s.

Table III.11. Tests of the differences between control and starved fish in the %fat/forklength relationship, using covariance analysis.

	Slope			Intercept		
	F-value	D.F.	P-value	F-value	D.F.	P-value
November	0.00	1,373	n.s.	1.24	1,374	n.s.
December	3.29	1,373	n.s.	260.03	1,374	< 0.001
January	6.45	1,373	0.012	69.26	1,374	< 0.001
February	0.73	1,373	n.s.	191.36	1,374	< 0.001
March	7.17	1,373	0.008	*	*	*
April	6.67	1,372	0.010	*	*	*
May	0.20	1,370	n.s.	6.34	1,371	0.012
June	0.37	1,365	n.s.	28.01	1,366	< 0.001
July	6.07	1,326	0.014	10.13	1,327	0.002

Table III.12. Tests of the differences between maturing and non-maturing males from the control tanks in the %fat/forklength relationship, using covariance analysis.

	Slope			Intercept		
	F-value	D.F.	P-value	F-value	D.F.	P-value
November	0.97	1,114	n.s.	0.44	1,115	n.s.
December	0.89	1,114	n.s.	0.18	1,115	n.s.
January	0.21	1,114	n.s.	0.15	1,115	n.s.
February	0.01	1,114	n.s.	0.04	1,115	n.s.
March	0.49	1,114	n.s.	1.13	1,115	n.s.
April	0.17	1,114	n.s.	0.02	1,115	n.s.
May	4.35	1,114	0.039	0.11	1,115	n.s.
June	15.37	1,113	< 0.001	*	*	*
July	42.49	1,92	< 0.001	*	*	*

Table III.13. Tests of the differences between maturing and non-maturing males from the starved tanks in the %fat/forklength relationship, using covariance analysis.

	Slope			Intercept		
	F-value	D.F.	P-value	F-value	D.F.	P-value
November	0.50	1,119	n.s.	0.81	1,120	n.s.
December	0.80	1,119	n.s.	0.32	1,120	n.s.
January	1.93	1,119	n.s.	1.38	1,120	n.s.
February	0.02	1,119	n.s.	0.00	1,120	n.s.
March	0.00	1,119	n.s.	0.18	1,120	n.s.
April	0.00	1,119	n.s.	3.79	1,120	n.s.
May	2.24	1,119	n.s.	2.74	1,120	n.s.
June	2.59	1,119	n.s.	0.01	1,120	n.s.
July	1.95	1,114	n.s.	1.99	1,120	n.s.

Table III.14. F-values, D.F., and P-values from stepwise logistic regressions to predict maturation from fork length and percentage fat, based on data from males only in each month from November to June 1991-1992.

	Fork length			Percent Fat		
	F-value	D.F.	P-value	F-value	D.F.	P-value
November	40.97	1,238	0.0000	0.00	1,238	n.s.
December	35.72	1,237	0.0000	2.82	1,237	n.s.
January	34.47	1,237	0.0000	3.40	1,237	n.s.
February	42.91	1,238	0.0000	1.61	1,238	n.s.
March	42.75	1,238	0.0000	0.21	1,238	n.s.
April	40.62	1,238	0.0000	0.92	1,238	n.s.
May	47.04	1,238	0.0000	2.57	1,238	n.s.
June	33.72	1,236	0.0000	3.13	1,236	n.s.

Table III.15. The mean food intakes (%body weight/hr) for maturing males (Mm), immature males (Im) and immature females (If) from 1991-1992. Means, F-values and P-values from oneway ANOVAs of the arcsine-transformed data.

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Controls:									
Mm	0.0645	0.0534	0.0524	0.0529	0.0317	0.0396	0.1926	0.0650	0.0466
Im	0.0718	0.0697	0.0686	0.0681	0.0433	0.0441	0.2478	0.1075	0.0852
If	0.0829	0.0707	0.0713	0.0681	0.0454	0.0488	0.2264	0.0864	0.0586
F-value	3.4706	3.2241	4.1073	2.8273	2.9370	0.3948	0.8382	2.0754	7.0136
D.F.	2,58	2,58	2,59	2,57	2,58	2,59	2,27	2,61	2,59
P-value	0.0379	0.0473	0.0216	n.s.	n.s.	n.s.	n.s.	n.s.	0.0019
Starved:									
Mm	-	-	-	-	-	-	0.2806	0.1253	0.0692
Im	-	-	-	-	-	-	0.2743	0.1149	0.0818
If	-	-	-	-	-	-	0.2508	0.1368	0.0586
F-value	-	-	-	-	-	-	0.5588	0.4576	1.4636
D.F.	-	-	-	-	-	-	2,56	2,58	2,57
P-value	-	-	-	-	-	-	n.s.	n.s.	n.s.

Table III.16. Results of t-tests for differences in food intake between control and starved fish in each maturity group.

	May	June	July
Maturing males:			
t-value	-2.10	-2.89	-1.96
D.F.	8	20	37
P-value	n.s.	0.009	n.s.
Immature males:			
t-value	-0.71	-0.38	0.24
D.F.	10	39	37
P-value	n.s.	n.s.	n.s.
Immature females:			
t-value	-0.70	-2.39	0.01
D.F.	26	39	26
P-value	n.s.	0.022	n.s.

Table III.17. Correlations between appetite and fat levels (control fish only).

	Control		
Month	r	n	P
November	0.663	44	< 0.001
December	0.525	43	< 0.001
January	0.643	43	< 0.001
February	0.552	43	< 0.001
March	0.475	42	0.001
April	0.352	43	0.021
May	0.071	28	n.s.
June	0.286	45	n.s.
July	0.089	45	n.s.

Table III.18. Correlations between appetite and specific growth rates (SGR_W) (control fish only).

Appetite	SGR	r	n	P
November	Nov - Dec	0.000	44	n.s.
December	Nov - Dec	0.184	43	n.s.
December	Dec - Jan	0.319	43	0.036
January	Dec - Jan	0.276	43	n.s.
January	Jan - Feb	0.045	43	n.s.
February	Jan - Feb	0.126	43	n.s.
February	Feb - Mar	0.367	43	0.015
March	Feb - Mar	0.302	42	n.s.
March	Mar - Apr	0.182	42	n.s.
April	Mar - Apr	0.045	43	n.s.
April	Apr - May	0.122	43	n.s.
May	Apr - May	0.161	28	n.s.
May	May - Jun	0.224	28	n.s.
June	May - Jun	0.077	45	n.s.
June	Jun - Jul	0.045	27	n.s.
July	Jun - Jul	0.084	45	n.s.

Table III.19. Correlations between appetite and rate of change in fat (control fish only).

Appetite	Rate of change in fat	r	n	P
November	Nov - Dec	0.431	44	0.004
December	Nov - Dec	0.327	43	0.032
December	Dec - Jan	0.077	43	n.s.
January	Dec - Jan	0.055	43	n.s.
January	Jan - Feb	0.089	43	n.s.
February	Jan - Feb	0.084	43	n.s.
February	Feb - Mar	0.089	43	n.s.
March	Feb - Mar	0.089	42	n.s.
March	Mar - Apr	0.355	42	0.021
April	Mar - Apr	0.161	43	n.s.
April	Apr - May	0.100	43	n.s.
May	Apr - May	0.367	28	n.s.
May	May - Jun	0.130	28	n.s.
June	May - Jun	0.032	45	n.s.
June	Jun - Jul	0.297	27	n.s.
July	Jun - Jul	0.184	45	n.s.

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