The feeding and fat dynamics of resident juvenile Atlantic salmon (*Salmo salar* L.) during winter.

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Summary

This thesis investigated the relationships between the feeding behaviour and use of stored fat in overwintering Atlantic salmon parr remaining destined to remain resident in freshwater the following year. Experiments investigated the responses to periods of food shortage at different times of the year to assess the influence of season. Investigations were carried out to examine how feeding motivation and fat storage were influenced by changes in those environmental cues that indicate the change of season. The effect of the normal winter behavioural pattern upon feeding and fat was also investigated.

Chapter 2

Throughout the course of the thesis, the experimental designs required a non-intrusive technique to estimate the lipid content of a fish. Previous studies had identified that specific combinations of morphometric measurements could provide reliable estimates of lipid level in salmonids, although no existing estimator proved suitable for the small fish used in this study. Therefore, a biometric technique was tailored towards the appropriate range of fish sizes. The actual lipid content of salmon parr from hatchery and wild origin were established, and the body measurements taken used in multiple regression analyse, forming predictive equations. In every case, the technique allowed lipid content to be estimated and used a similar combination of measurements: fork length, wet weight, dorsal width, pelvic width and anal width.

Chapter 3

Juvenile salmon have been shown to respond to a period of food deprivation by increasing food consumption when food is made available.This chapter comprised four experiments in which this response was examined more fully.

The first experiment investigated changes in appetite and fat content following deprivations in early winter. Fish that incurred greater fat losses did not compensate by initially feeding more intensely than those incurring less severe losses when food was once again provided, but instead maintained this elevated intake for a longer time. Such a strategy would allow fish to forage at night and reduce predation risk.

In the second experiment, the responses of fish in the previous experiment were compared with those that experienced a similar period of deprivation in the summer. The results indicated that the allocation of energy to body components change seasonally as the need to maximise size in the summer gave way to the regulation of fat stores in winter.

The third experiment examined intra-seasonal differences in response to deprivation. Fish were subjected deprivation in early, middle and late winter. The appetite response and the rate of fat restoration were highest in early winter, and declined as the season progressed. The results indicated that the fish were responding not only to their current nutritional state, but to a projection of their lipid levels at the end of the winter.

The fourth experiment investigated the role of daylength in timing the seasonal responses shown in the previous experiment. Groups of fish were maintained under controlled photoperiods that either advanced or delayed their perceived calendar date and their responses to deprivations were recorded. Fish that perceived themselves to be at the beginning of winter behaved differently to those at the end of the winter, indicating that photoperiod change was used to time responses to deprivation.

Chapter 4

Studies on birds have highlighted that fat can be stored as insurance against starvation. This chapter investigated whether fish faced with an unpredictable supply of food during winter would compensate by increasing their levels of body fat. The results indicated that the fish did not elevate fat levels, but sacrificed somatic growth in order to maintain fat at a level appropriate for their size.

Chapter 5

Previous work has indicated that salmon exhibit a gradual reduction in appetite in late summer independent from the seasonal reduction in water temperature. This chapter examined the influence of temperature reduction on fishes feeding and fat levels throughout the autumn and early winter. One group of fish was maintained at near optimal water temperatures throughout whilst control fish experienced the normal seasonal temperature reduction. Those maintained at near optimal water temperature throughout differed little in their rate of growth from the controls, but did store more fat. All fish generally fed at a level below that physiologically possible. The results were consistent with the requirements for fish to regulate fat at the expense of increased body size, regardless of the environmental opportunity for growth during winter.

Chapter 6

Juvenile salmonids switch from mainly diurnal activity to daytime torpor in shelters as water temperatures fall. They emerge from these shelters under the cover of darkness, presumably to feed. This chapter comprised of four experiments that investigated the influence of this nocturnal behaviour pattern on the feeding and fat dynamics.

The first experiment investigated the influence of daytime sheltering behaviour on the conservation of body fat. By providing one group of fish with a shelter, and depriving access to the controls, the effect of sheltering on the normal seasonal decline in body fat levels was tested. The results indicated that the use of a shelter had no measureable effect on the rate of fat utilisation over the winter.

The second experiment examined the influence of sheltering on the timing and intensity of feeding. Feeding trials were conducted during the day and night on fish with or without access to a shelter. Although the results of statistical analyses proved inconclusive, the trend indicated that those fish denied access to a shelter were feeding more frequently during the day whereas those with access to a shelter were feeding more frequently at night. Both groups of fish consumed similar quantities of food, corroborating the results of the previous experiment in that there was little energetic advantage in adopting sheltering behaviour.

Animals have the ability to trade-off the risks of predation against those associated with the threat of starvation. The third experiment investigated whether juvenile salmon adopting a strategy of daytime sheltering and nocturnal emergence, would alter their pattern of emergence and leave the safety of their shelters during the day when faced with the threat of starvation. Fish were starved prior to being filmed moving between a food-tight shelter and the tank floor. The previously starved fish were more frequently found out of the shelter during the day than the well-fed controls during the first week in which food was provided. The results indicated the presence of a trade-off situation between avoiding predation and the need to restore fat losses.

The fourth experiment examined two points, namely the feasibility of night-time electrofishing as a means to sample juvenile salmon in the field, and whether salmon sampled out of their normal streambed shelters during day had different levels of body fat than those sampled at night. The results indicated that night-time electrofishing was more efficient at sampling populations than daytime electrofishing during winter. Day and night-sampled fish did not differ in their levels of body fat, indicating that fish were not leaving their shelters during the day in response to a fat deficit.

Chapter 7

The length of the intestine in birds and fishes is sensitive to diet and nutritional state. Starved animals may shorten the intestine as a means to mobilise energy. This chapter investigated whether the winter reduction in food intake results in intestinal shortening in salmon. The results indicated that there was no gross morphological change in the intestine between the start and end of winter. The reduction in energy intake may be offset by the mobilisation of stored fat.

Chapter 8

Salmon that are preparing to migrate to sea in spring behave differently to those opting to stay resident in freshwater during winter. This preliminary study investigated whether this difference in developmental strategy was reflected in the preference for environmental water temperature. The results indicated that the fish that are preparing to migrate prefered a higher water temperature than those that were to remain resident, reflecting the differences in developmental strategy during winter.

Chapter 9

The work in the previous chapters has illustrated that resident parr have evolved a range of complimentary behavioural adaptations to enable them to anticipate and survive the winter season. The results of the experiments described in the previous chapters are discussed in the context of optimal survival strategies.

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Chapter 1 - General introduction

1.1 Introduction

Many animals occupy an environment where the climatic conditions exert the ultimate control over the availability of food. As latitude increases, the climatic conditions become less constant and are replaced by a regular seasonal pattern. The severity of the seasons is influenced by a number of factors but in general the winter period, typified by low temperatures and short days, shows an increase in duration and severity with distance from the equator. Animals that occupy the mid-latitude temperate regions therefore regularly face periods when temperatures and food availability are low and have had to develop means by which to cope. The winter season is typically associated with high levels of mortality due to food shortages and freezing. The ways in which animals have adapted to face the hardships of winter depend upon their life history strategy, their locomotory capacity and their ability to utilise alternative energy sources.

With the predictable approach of unfavourable conditions in autumn, many animals adopt the general biological response to adversity - a migration to where conditions are more appropriate (Taylor & Taylor, 1977). Examples of migrations prompted by food shortages abound in the animal kingdom (see Aidley, 1981). When migration is not an option as a result of restrictions placed upon the animal by its own physiological capabilities or some natural barrier, animals have adapted to cope in other ways. Hibernation is an adaptation that can act to ensure survival through a period of the year which is unfavourable for an active mode of life (Nikolsky, 1963). Many endothermic animals opt to spend the winter in a state of torpor or hibernation where body temperature and metabolic rate are both reduced in order to conserve energy (see Lyman *et al.*, 1982). Amongst the ectotherms, a strategy of energy conservation is observed in wintering reptiles (Jenssen *et al.*, 1996), amphibians (Brenner, 1969; Bradford, 1983) and some freshwater fishes (Nikolsky, 1963). Some ectotherms have developed blood antifreezes that allow them to survive extremely low winter temperatures (see Storey & Storey, 1992 for review).

During animal migrations and hibernation, animals often reduce their food intake and enter a natural state of anorexia that can persist for long periods (see Mrosovsky & Sherry, 1980). This reduction in feeding motivation is an adaptation to a situation where feeding is either impossible or incompatible with the main activity (e.g. migrations across inhospitable terrain). Gray whales (see Appendix II for latin names) fast for approximately 6 months and lose up to 29% of their body weight during a migration between feeding and calving grounds (Rice & Wolman, 1971), Brent geese fly non-stop for 3500 km across the Pacific Ocean where no feeding grounds exist (Ogilvie, 1978) and many songbirds fly non-stop from Kazakhstan across two vast expanses of desert to Ethiopia, a distance of almost 4000km (Moreau, 1972). Egg incubation and offspring provision may lead to anorexia in parents as maintaining egg temperature or preventing predation both compete directly with feeding: male emperor penguins fast for 4 months and lose 40% of their body weight during egg incubation (Le Maho, 1977), female Burmese red junglefowl reduce food intake during incubation by 80% even when it is provided within reach of the nest (Sherry *et al.*, 1980) and mouth- brooding African cichlids are literally forced into appetite suppression during the 3 to 5 weeks of egg incubation (Goldstein, 1973). As an adaptation to long-term seasonal reduction in food, hibernating Golden-mantled ground squirrels that are periodically aroused eat sparingly or not at all even when food is freely available (Pengelley & Fisher, 1961; Mrosovsky, 1971).

Despite having evolved in response to different events, the anorexic periods exhibit broad similarities amongst taxa, including a gradual reduction in body weight that is closely controlled to allow weights to remain close to a pre-determined level or set-point (Mrosovsky & Sherry, 1980). Much of the weight loss reflects the gradual mobilisation of body fat stores, that have often peaked prior to the anorexic period, in preparation for use (Barnes & Mrosovsky, 1974; Alerstam, 1981; Sheridan, 1994). When the normal seasonal trajectory of fat use is disrupted, so that stores fall below the normal level, the motivation to feed is re-instated briefly, allowing them to be restored to the appropriate state (Mrosovsky & Sherry, 1980; Metcalfe & Thorpe, 1992).

The physiological regulation of fat levels has been proposed as being under the control of a negative feedback system with excess fat somehow signalling to the brain that the body is overweight. The control system has been increasingly studied in recent years with support for the theory coming from various experiments that have isolated specific neurotransmitters and receptors (see Scott, 1996). The action of a protein called leptin in the neuroendocrine system being highlighted as a potential regulator in mammals (Ahima *et al.*, 1996) as its total deficiency results in severe obesity, and falling concentrations in response to food deprivation initiate endocrinological responses to starvation. The role of a glucagon-like-peptide-1 (GLP-1) as a physiological mediator of satiety has also recently been proposed (Turton *et al.*, 1996). The control over appetite and mobilisation of fat stores in fishes is under multifactorial control, with both the nutritional status of the individual (as monitored by the peripheral nervous system) and specific neural areas of the CNS reported as integral to the response (see Fletcher, 1984 for review).

The use of fat as a source of metabolic fuel in fishes when demands exceed intake is widespread (Love, 1970; Sheridan, 1994), it is therefore no surprise that many species exhibit seasonal changes in the patterns of fat storage and utilisation (e.g. Newsome & Leduc, 1975; Gardiner & Geddes, 1980; Adams *et al.*, 1982; Flath & Diana, 1985; Higgins & Talbot, 1985; Booth & Keast, 1986; Simpson, 1993). The fat is stored in discrete depots, in both dark and light muscle, the liver and in the visceral cavity. The relative importance of each depot depends on whether it is ultimately intended for short or long-term storage, and upon the individual's life-history (Sheridan, 1994). In salmonid fishes, the most important and labile fat store is in the visceral cavity (Weatherley & Gill, 1981; Sheridan, 1994), where it is stored intracellularly in intestinal mucosa cells and also outwith the viscera where it surrounds the digestive tract (Love, 1970). The mobilisation of intracellular fat stores to fuel metabolism results in its replacement with water (Love, 1970; Gardiner & Gedddes, 1980).

This thesis deals with the patterns of feeding and fat utilisation during the winter in juvenile Atlantic salmon. This species has previously been shown to exhibit seasonal variation in appetite and rely upon stored fat reserves during the completion of its life-cycle; the background will now be discussed in more detail.

1.2 Salmonid life history strategies

The Atlantic salmon is an example of a species with a highly plastic life-history strategy (Thorpe, 1994). Like all salmon, this species spawns in freshwater, and juveniles hatch into the relatively cool, fast-flowing temperate streams in the spring, residing there for between 1 and 7 years (Metcalfe & Thorpe, 1990) before undertaking a seaward migration (termed smolting). During the smolting process, the juveniles (parr) lose their characteristic markings and adopt silvery flanks (Hoar, 1976) in preparation for a pelagic mode of life. Whilst in the more productive marine environment they grow and mature, and re-enter their natal rivers after at least one year, to spawn and complete the reproduction cycle. Alongside this anadromous strategy, male parr also have the ability to mature in freshwater, and occurence of sexually mature precocious male parr has been widely reported (see Simpson, 1993 for review).

In hatchery-reared sibling populations, an initially unimodal size distribution in sibling populations becomes increasingly bimodal during the first autumn until by late winter two distinct modes are distinguishable (Thorpe, 1977; Thorpe & Morgan, 1978; Bailey *et al.*, 1980; Thorpe *et al.*, 1980, 1982; Saunders *et al.*, 1982). This phenomena has also been reported as occurring in the natural situation (Bagliniere & Maisse, 1985; Heggenes & Metcalfe, 1991; Nicieza *et al.*, 1991) and results from a brief growth spurt in those individuals destined to make up the upper mode of the distribution (the upper modal group, hereafter referred to as the UMG), during September, whilst those destined to form the lower mode of the distribution (lower modal group, hereafter referred to as the LMG) exhibit a reduction in growth (Kristinsson *et al.*, 1985; Metcalfe *et al.*, 1988). Although the fishes' parentage will affect the developmental pathway taken (Thorpe & Morgan, 1980; Bailey *et al.*, 1980), all individuals initially appear capable of entering the UMG but whether they do so appears determined by a decision taken around midsummer (Wright *et al.*,

1990) based on the size achieved by this time and the prevailing environmental conditions (Thorpe, 1989). The proportions in each mode can be altered by changing the opportunity for growth, as represented by increases in temperature and the number of light hours in mid-late summer (Kristinsson *et al.*, 1985; Adams & Thorpe, 1989a,b; Thorpe *et al.*, 1989).

The proportions in each mode remain relatively constant throughout the course of their first winter (Bailey *et al.*, 1980; Thorpe *et al.*, 1980) during which time the UMG fish undergo the physiological changes necessary to allow a downstream smolt migration the following spring, whereas the LMG fish delay the process for at least one more year, staying as residents in freshwater during this time. During winter the UMG exhibit higher rates of metabolism, growth and food intake than the LMG (Higgins, 1985; Higgins & Talbot, 1985; Metcalfe *et al.*, 1988), increasing body size in preparation for smolting, as small smolts have been shown to suffer higher mortality rates (Hager & Noble, 1976; Bilton *et al.*, 1982; Hansen & Lea, 1982; Mahnken *et al.*, 1982). The LMG fish reduce their food intake in late summer (Metcalfe *et al.*, 1986) and enter a state of natural anorexia during the autumn (Metcalfe & Thorpe, 1992). The reduction in appetite occurs more rapidly than the autumnal decline in water temperature and its timing is, to some extent, under the influence of photoperiod change (Thorpe, 1986). During the winter, the growth of the LMG fish is arrested and internal stores of fat are depleted as they are required to fuel metabolism (Egglishaw & Shackley, 1977; Gardiner & Geddes, 1980; Higgins & Talbot, 1985). Food intake is subsequently regulated with regard to the depletion of internal fat stores: an acceleration of fat loss leads to a brief increase in appetite until losses have been restored (Metcalfe & Thorpe, 1992). Appetite is then restored the following spring (Simpson *et al.*, 1996).

In late autumn, juvenile salmon also exhibit a change in micro-habitat, moving from holding station in the current during the day, to hiding in stream-bed refuges, from which they emerge under the cover of darkness (Fraser *et al.*, 1993, 1995) to feed (Heggenes *et al.*, 1993; Fraser & Metcalfe, *submitted*). Fish stay concealed for most of the day whilst water temperatures remain below 10^{o}C. It has been proposed that such a behavioural switch at the onset of winter may may have developed in response to a reduction in the fishes' ability to hold station in a current at low temperatures (Rimmer & Paim, 1990; Graham, 1996). It may also offer potential advantages in terms of energy conservation (Pickering & Pottinger, 1988; Rimmer & Paim, 1990) and predator avoidance (Fraser *et al.*, 1993, 1995) at low water temperatures.

This thesis concentrates upon the behavioural ecology of LMG salmon during their first winter. This is of importance to the management of wild stocks in this country, as the majority of wild smolts in the U.K. have spent at least two years in freshwater prior to migrating to sea (Metcalfe & Thorpe, 1990). As stress brought about through temperature change and nutritional deficiency has been highlighted as contributing to overwintering mortality (Gardiner & Geddes, 1980; Cunjak & Power, 1987), a greater understanding of the winter biology of resident parr may therefore benefit the management of populations in nursery streams. In the U.K. aquaculture industry, most slow-growing LMG fish are graded out from stocks, but in Scandinavia (another region where salmon are reared, both for aquaculture and re-stocking) the majority of smolts are 2 years old. Information on the feeding and ecology of LMG fish during the first winter may therefore contribute to increased efficiency during this stage of production.

1.3 Aims and objectives

The overall aim of this thesis was to investigate the behavioural ecology of LMG Atlantic salmon parr during their first winter. The experiments were designed to examine the following questions;

1. How is the appetite response to a period of food deprivation sensitive to the severity of the deprivation? (chapter 3.2)
2. Does the appetite response and the requirement to restore a fat deficit differ both within and between seasons? (chapter 3.3, 3.4).
3. Does photoperiod act to time the appropriate response to a period of food shortage? (chapter 3.5).

4. Do salmon store more fat to act as an insurance when feeding opportunity is unpredictable? (chapter 4).

5. Does the autumnal decline in water temperature affect food intake and fat storage? (chapter 5).

6. Does the provision of a refuge affect the seasonal decline in fat reserves? (chapter 6.2).

7. Does the provision of a refuge affect the timing and intensity of feeding? (chapter 6.3).

8. Do fish trade-off predation risk against the threat of starvation during winter? (chapter 6.4).

9. Is this trade-off reflected by the low nutritional state of wild fish sampled out of refuges during the day in winter? (chapter 6.5).

10. Does the seasonal decline in appetite lead to intestinal shortening? (chapter 7).

11. Is the developmental strategy during winter reflected in the temperature preferenda of UMG and LMG fish? (chapter 8).

Chapter 2 - Biometric estimation of lipid levels in underyearling salmon parr

2.1 Introduction

A number of studies investigating the dynamics of lipid deposition and remobilization in salmonid fishes have relied upon sacrificing the animals involved and extracting the lipid from their various depots (Gardiner & Geddes, 1980; Weatherley & Gill, 1981; Higgins & Talbot, 1985; Cunjak & Power, 1986a; Cunjak, 1988b; Miglavs & Jobling, 1989b; Quinton & Blake, 1990; Rowe *et al.*, 1991). As an alternative to killing the subjects, changes in lipid content have been inferred from measurement of the length to weight ratio, or condition factor (Wootton, 1990; Bolger & Connolly, 1989), with fish exhibiting greater mass for a given size being deemed to have greater lipid stores (e.g. Cunjak & Power, 1987; Rowe & Thorpe, 1990). Both intrusive techniques and fat estimation via an index of condition have drawbacks for the purpose of the present study. Obviously, the need to sacrifice individuals precludes repeated sampling, requires large sample sizes and places limits on the design of experiments. Problems with the use of indices of condition as a measure of energy status have been pointed out by Wootton (1990) and any inferences made regarding lipid should be treated with caution. Both Simpson *et al.* (1992) and Adams *et al.* (1995) found condition factor to be a poor predictor of body fat content.

As an alternative to sacrificing animals, a number of non-intrusive techniques have been developed in order to estimate the lipid content of a variety of animals (see Simpson, 1993 for review). It appears that the use of morphometric body measurements can provide a good estimation of body lipid in Atlantic salmon (Simpson *et al.*, 1992; Graham, 1994; Kadri *et al.*, 1995) and Arctic charr (Adams *et al.*, 1995). However, differences in body shape between stocks of the same species (i.e. Winans, 1984; Taylor & Foote, 1991), and the necessity to adhere to the size range of fish within the original calibration sample are both limitations that require consideration prior to applying the predictive equations to any sample of fish.

The 'winter' predictive equation presented by Simpson *et al.* (1992) incorporates the size range of fish to be used in the subsequent chapters, but also included UMG fish in the original sample. An equation to predict fat levels specific to LMG salmon has not previously been developed.

The initial aim of this chapter was therefore to develop such an equation to estimate the total body lipid content of hatchery-reared LMG salmon specifically during their first winter, based on the technique described by Simpson *et al.* (1992). It was also necessary to predict lipid earlier in the season (see chapter 3.3) and in wild fish (see chapter 6.5) so resulting in a set of predictive equations, appropriate to the stage of development, source and nutritional history of the fish.

2.2 Materials and methods

Hatchery-reared fish: winter sample

On 6 October 1993, 40 small fish (<73mm forklength) from a hatchery reared population (the offspring of a pair of sea-run adults from the River Almond, in Perthshire) were selected and split into two size-matched groups of 20. Small individuals were selected in order to maximise the number of LMG fish in the sample (Metcalfe *et al.*, 1988). To increase the range of body fat levels for a given body size, and subsequently increase the robustness of a predictive equation based on the relationship between body shape and fat levels, one group was established in a 1m tangential flow tank where they were prevented from feeding on pelleted food for a period of three weeks. This length of food deprivation has been previously shown to significantly reduce the fat levels in juvenile salmonids (Weatherly & Gill, 1981, Metcalfe & Thorpe, 1992). During this time, the remaining 20 fish were placed in a separate tank where they were provided with *ad lib.* rations of pelleted food by way of an automated feeder providing a trickle of food every 20 minutes throughout the 24 hour period. On 27 November, all fish were killed by an overdose of benzocaine and weighed (to nearest 0.01g). Six measurements (table 2.1) were taken at positions along the body (to nearest 0.05mm using Vernier callipers; figure 2.1). These measurements, taken immediately after death, included those found to be important in predicting fat levels in juvenile salmon by Simpson *et al.*, (1992) along with one new body position (i.e. the leading edge of the pelvic fins) in the trunk region.

In order to control for differences in body size and changes in body shape with size, the height, width and adipose fin measurements were standardised for length by using a variation of Ricker's formula for estimation of condition factor (Bolger & Connolly, 1989; Wootton, 1990):

$$x' = x / f^{b} \qquad \textbf{(eqn. 2.1)}$$

where x' is the standardised measurement, x is the body measurement in question, f is the forklength and b is the slope parameter of the regression of $\log_{10}(x)$ on $\log_{10}(f)$ (table 1). The actual fat content of the fish was then measured as follows. The dead fish were scored along each flank with a scalpel, packaged individually in a single sheet of filter paper and secured with a staple. The packages were marked and dried in an oven at 50-55^{0}C for three days. At the end of this time, they were removed, weighed and placed back in the drying oven. They were weighed daily for the next three days until a stable dry weight was established. The packages were then placed into a Soxhlet fat extraction apparatus (Schifferli, 1976; Perdeck, 1985) through which hot chloroform was passed at least four times. Once the liquid had run clear, indicating that fat had been removed, the individual packages were returned to the drying oven for another two days. Weighing took place as before in order to establish a stable weight. Actual fat content was defined as the change in dry weight following Soxhlet extraction and expressed as either a weight (g) or a percentage of the wet weight of the individual fish (% fat).

The standardised measurements were used in conjunction with the actual fat measurement in a series of multiple regression analysis in order to establish the minimum number of measurements that could accurately predict the fat content of an individual fish. Multiple regression analysis proceeded using all possible combination of measurements (including log transformed data) in order to achieve the most accurate predictive equation.

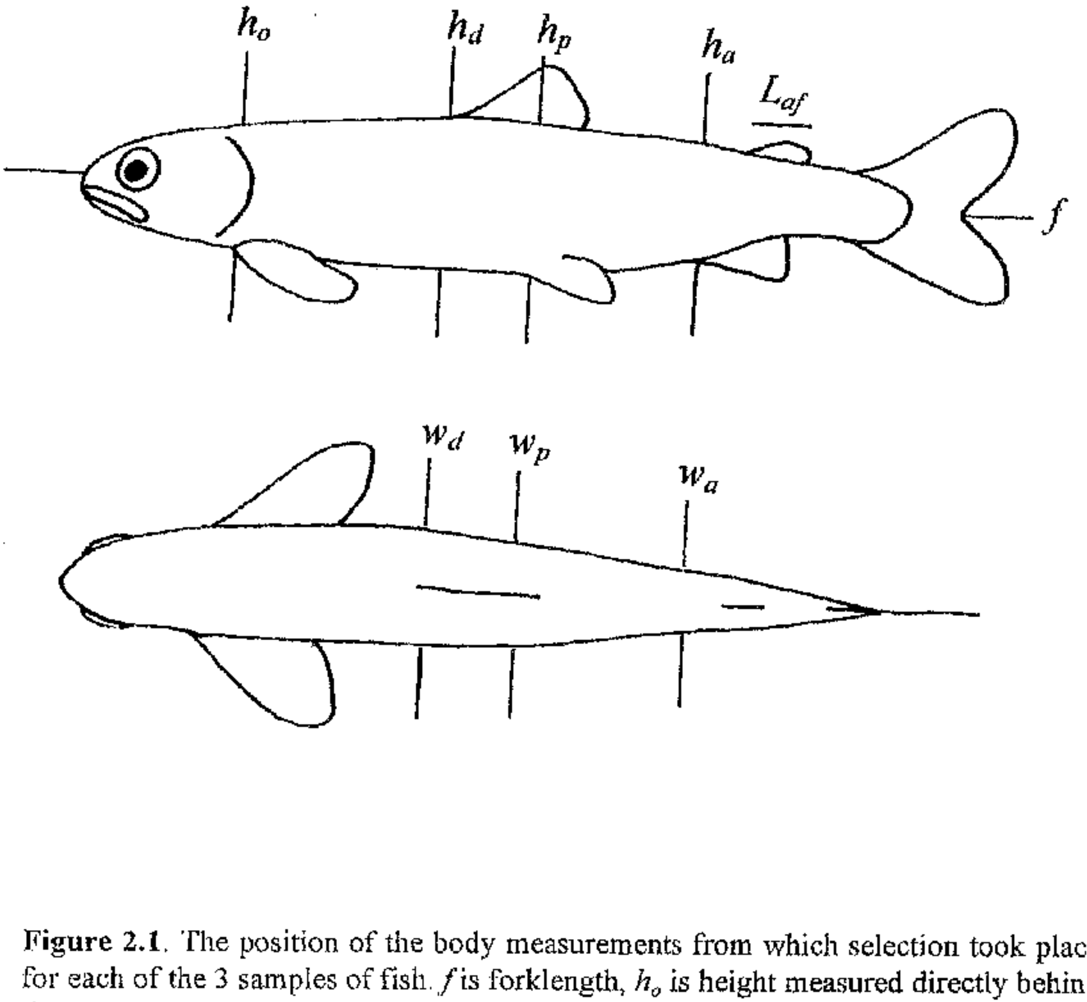

Figure 2.1. The position of the body measurements from which selection took place for each of the 3 samples of fish. f is forklength, h_o is height measured directly behind the operculum, h_d is the height at the dorsal fin, h_p is height at the pelvic fin, h_a is height at the anal fin, w_d is width at the dorsal fin, w_p is width at the pelvic fin and w_a is width at the anal fin (all measurements taken at the leading edge of the appropriate fins). L_{af} is adipose fin length.

On 17 January 1994, the original sample was supplemented by the accidental death of 20 LMG fish as a result of a pump failure (see chapter 6.2). These fish were from the same stock as used previously and had likewise been maintained on *ad lib.* rations of pelleted food. The 20 dead fish were weighed, measured and their fat extracted in a similar fashion to those sacrificed in October. As a result of the suppression of skeletal growth and the gradual depletion of fat stores in LMG fish during the course of winter (Gardiner & Geddes, 1980; chapters 3.3, 3.4, 6.2) these fish would be expected to have lower fat levels than those of a similar size in October. The combined sample using fish from both October and January (N=55, size range = 49-74mm forklength) was expected to cover the range of sizes and fat levels of overwintering LMG fish.

Hatchery-reared fish: summer sample.

Thirty underyearling salmon (33-58mm forklength) were selected from a sibling population (the progeny of a pair of sea-run adults from the Loch Lomond catchment) on 4 July, and divided into two size-matched groups. Fifteen were established in a tank where they were prevented from obtaining any pelleted food for the next two weeks in order to reduce their fat levels. During this time the remaining 15 fish were provided with *ad lib.* rations of pelleted food from an automated feeder providing a trickle of food every 20 minutes throughout the 24 hour period. On 20 July all fish were killed by an overdose of benzocaine, weighed (to the nearest 0.01g) and eight body measurements illustrated in figure 2.1 were taken. These body measurements were standardised as described for the winter sample (table 2.1). The actual fat content of the fish was measured by Soxhlet fat extraction as described earlier.

Wild underyearling salmon.

Thirty underyearling salmon parr were obtained from the Spittal Hill Burn, a tributary of the River Endrick (Loch Lomond catchment) situated approximately 2km south east of the village of Fintry, Central Scotland, (O.S. second series, sheet 57 grid ref. 653 864.) by electrofishing on 14 August 1995. Fish were deemed as underyearlings on the basis of size (mean forklength = 51.4mm ± 0.86, n = 30, range = 43-60mm) as a result of a separate investigation in the burn (see chapter 6.5 for further details).

Table 2.1. The slope parameters of the regression of $\log_{10}(x)$ on $\log_{10}(f)$ for the three samples of underyearling salmon. n refers to the number of fish in each analysis and missing values indicate that the measurement was not included in the analysis.

		Sample		
Body measurement		winter (n = 55)	summer (n= 28)	wild (n = 26)
Wet weight	m	3.200	3.160	2.716
Heights:				
Opercular	h_o	0.977	1.500	0.896
Dorsal	h_d		1.200	1.031
Pelvic	h_p	1.250		
Anal	h_a		1.450	1.389
Widths:				
Dorsal	w_d	1.270	1.170	1.197
Pelvic	w_p	1.360	1.060	0.996
Anal	w_a	1.320	0.760	1.044
Adipose fin length	l_{af}	0.691	0.052	0.896

The fish were moved to the University Field Station and 15 were placed in a 75cm diameter tangential flow tank where they were fed daily on a mixture of live and dead bloodworms (i.e. a near-natural diet) provide to excess. The remaining 15 fish were established in an identical tank, but were not fed for a period of two weeks (although the fish could feed on a small number of drifting invertebrates that entered the tank with the inflowing water). This length of food deprivation was chosen in order to increase the range of fat levels within the sample as a whole. On 28 August, all fish were killed by an overdose of benzocaine and weight (to the nearest 0.1g) and eight body measurements were obtained from every fish. The fish were subjected to Soxhlet extraction and the measurements standardised (table 2.1) as previously descibed.

2.3 Results

Hatchery-reared fish: winter sample

Multiple regression analysis was used on all the data collected from the combined October and January 'winter' sample to find the minimum number of combined morphometric measurements that best described the fat content of the fish (expressed in grams or as a percentage of wet weight). Fat content within the sample ranged from 0.01g-0.33g per fish with a mean of 0.13g ± 0.01 S.E. When fat was expressed as a percentage of the wet weight of the individual (% fat), it comprised an average of 5.17% ± 0.16 (n = 55), and covered a wide range, as a result of both the food deprivation and seasonal losses (range = 1.61-7.18%). The most accurate prediction of fat was given by relating the size, weight and widths at three positions along the body to the actual grams of fat as shown in equation 2.2 (see also figure 2.2a). The predicted weight of fat was subsequently expressed as % fat and was correlated with the actual fat content determined by Soxhlet extraction (figure 2.2b).

$$\text{Fat (g)} = 0.0976m - 0.00413f + 6.11w_d + 10.9w_p - 7.93w_a - 0.125 \qquad \textbf{(eqn. 2.2)}$$

$$(r^2 = 0.637,\ n = 55,\ P<0.001)$$

where m is wet weight, f is forklength, w_d is standardised width at the leading edge of the dorsal fin, w_p is standardised width at the leading edges of the pelvic fins and w_a is the standardised width at the leading edge of the anal fin.

The value of deriving an equation specific to the size range of the fish being studied was illustrated by comparing the results to those obtained by using the equations published by Simpson *et al.* (1992). When the morphometric measurements were standardised using the slope parameters given by these authors for a winter sample of fish (both UMG and LMG, and including the size range of the individuals used in the current study) and applied to their predictive equation, a significant correlation

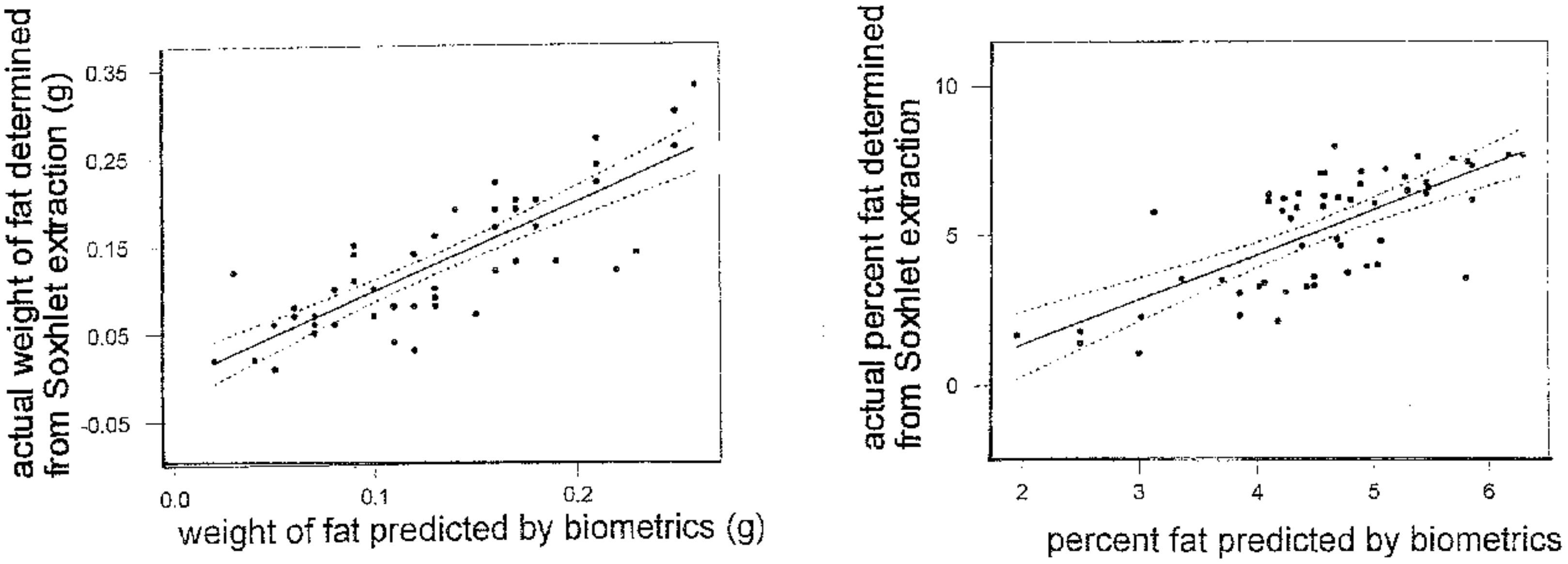

Figure 2.2. The correlation between actual and predicted body fat for the winter sample: a.) in terms of the weight of fat, $r^2 = 0.819$, $n = 55$, $P<0.001$ and b.) when expressed as % fat, $r^2 = 0.511$, $n = 55$, $P<0.001$.The dotted lines indicate the 95% confidence intervals.

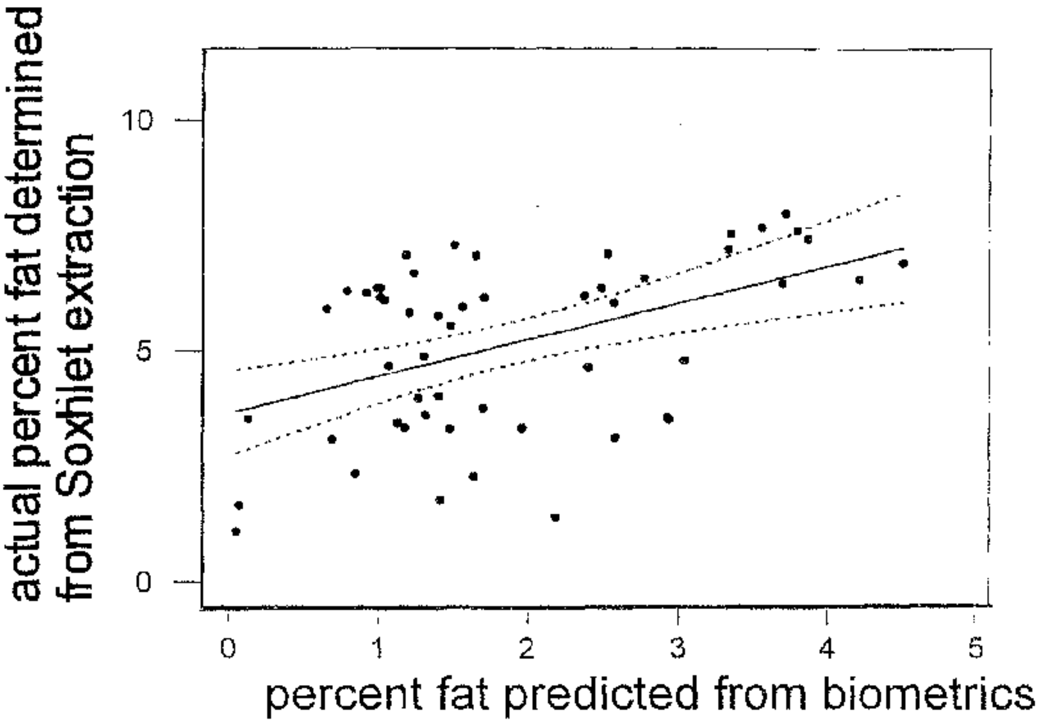

Figure 2.3. The correlation between the actual and predicted percentage body fat for the winter sample of hatchery-reared fish using the predictive equation of Simpson *et al.* (1992) ($r^2 = 0.153$, $n = 54$, $P<0.01$). The dotted lines indicate the 95% confidence intervals.

between predicted and actual %fat was apparent (figure 2.3). However, only 15.3% of the variation in %fat was explained by the predictive equation produced by these authors, lower than the 51.1% explained by the present equation (figure 2.2). In addition, the equation of Simpson *et al.* (1992) consistently underestimated an individual's % fat level, a trait not present with the current equation.

Hatchery-reared fish: summer sample

Two fish died during the course of the food deprivation period. The summer sample exhibited a wide range of fat levels as a result of the food deprivation period (mean weight of fat (g) = 0.03g ± 0.01 (n = 28), range = 0.003-0.103g; expressed as %fat, mean = 2.59% ± 0.31, range = 0.39-5.42%). Multiple regression analysis was carried out on the data obtained from the remaining summer fish to determine the combination of measurements that best predicted the fat content at this time. The most accurate prediction was obtained by using the same body measurements as the winter sample, but this time to predict the % fat content directly instead of the weight of fat (equation 2.3).

$$\%\text{fat} = -5.634m + 0.455f + 1.791w_d + 3.589w_p - 4.699w_a - 16.442 \qquad \textbf{(eqn. 2.3)}$$

$$(r^2 = 0.726,\ n = 28,\ P<0.001)$$

As with the winter sample, the predicted values were closely correlated with the actual values of % fat determined by Soxhlet extraction (figure 2.4).

Wild fish

Four fish died during the course of the experiment. The sample exhibited a wide range in fat levels (mean = 0.06g ± 0.02 (n = 26), range = 0.003 - 0.066g; expressed as %fat, mean = 2.42% ± 0.72, range = 0.44-3.77%) presumably as a result of the period of food deprivation. The most accurate prediction of fat level was obtained by using the same measurements as used in the hatchery-reared samples when relating them to the actual grams of fat, as shown in equation 2.4 (see also figure 2.5a).

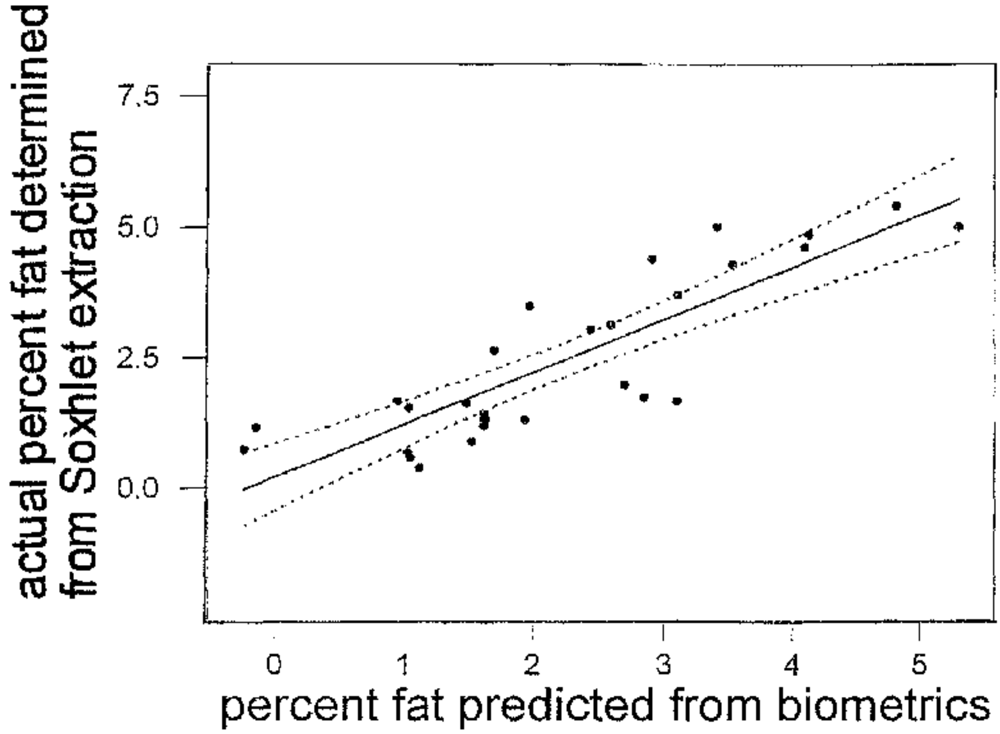

Figure 2.4. The correlation between the actual and predicted percentage body fat for the summer sample of hatchery-reared fish ($r^2 = 0.760$, $n = 28$, $P<0.001$). The dotted lines indicate the 95% confidence intervals.

$$\text{Fat (g)} = 0.0176m - 0.0013f + 2.955w_d + 0.778w_p - 0.888w_a - 0.08 \qquad \textbf{(eqn. 2.4)}$$

$$(r^2 = 0.61,\ n = 26,\ P<0.001)$$

When the resulting weights of fat were expressed as % fat, the predicted values were closely correlated with the actual % fat, determined from Soxhlet extraction (figure 2.5b).

When each of the predictive equations were used to calculate %fat for the other samples of fish, only one significant positive relationship out of six (using the equation for wild fish to predict the fat of fish from the winter sample) was found (table 2.2), indicating the limited application of each equation to its appropriate season and size range of fish.

Table 2.2 The correlation between %fat predicted from the biometric equation appropriate to a different sample, and the actual fat content of the fish determined from Soxhlet extraction.

equation	sample	correlation coefficient	n	P value
winter	summer	-0.344	28	N.S.
winter	wild	-0.448	26	<0.05
summer	winter	-0.096	55	N.S.
summer	wild	-0.0625	26	N.S.
wild	winter	0.339	55	<0.05
wild	summer	-0.369	28	N.S.

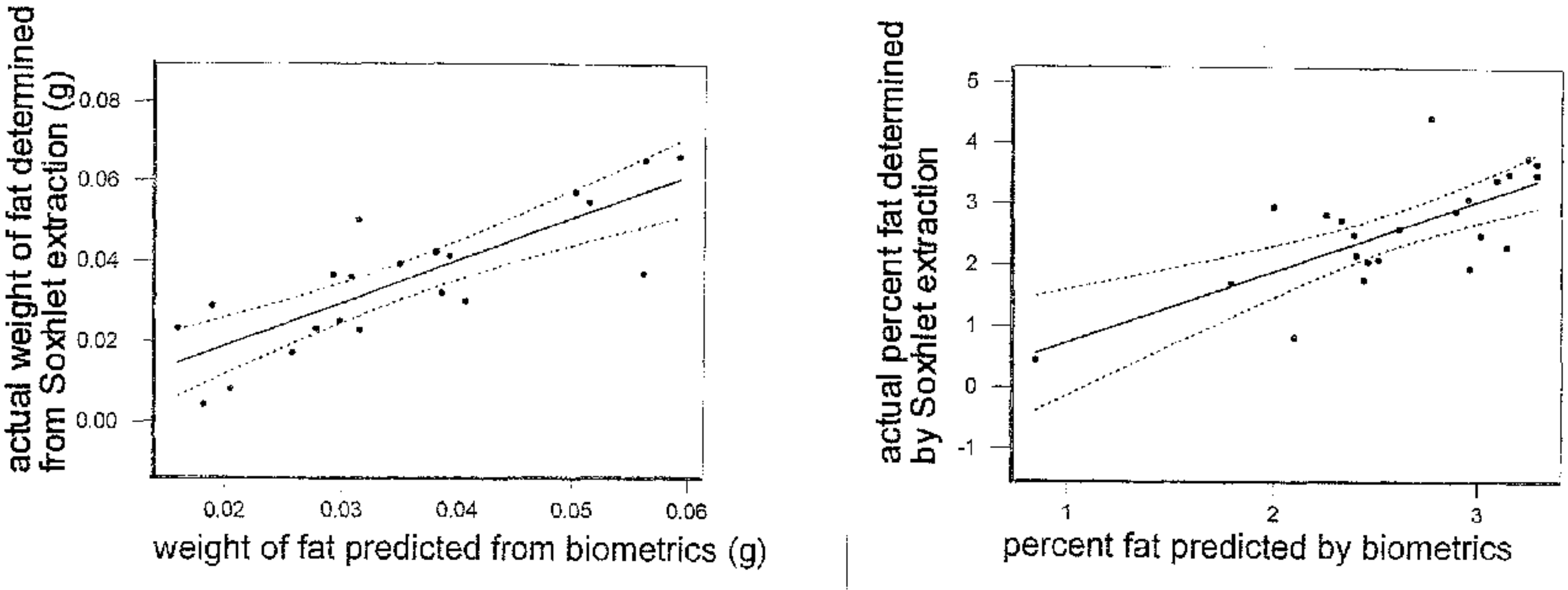

Figure 2.5. The correlation between actual and predicted body fat for the sample of wild fish from the Spittal Hill Burn: a.) in terms of the weight of fat, $r^2 = 0.829$, $n = 26$, $P<0.001$ and b.) when expressed as % fat, $r^2 = 0.557$, $n = 26$, $P<0.01$. The dotted lines indicate the 95% confidence intervals.

2.4 Discussion

The results indicate that the use of morphometrics provides an accurate, non-destructive technique for estimating the fat level in underyearling salmon of either hatchery reared or wild origin, that is sensitive to changes in nutritional status acting independently of body size. By using a period of food deprivation to decrease fat levels and increase the range of %body fat within each sample, it was possible to derive a set of appropriate predictive equations. The equation proposed for winter fish by Simpson *et al.* (1992) lacked accuracy with regard to underyearling LMG fish (figure 2.4), possibly due to the large range of sizes in their original sample. Although demonstrating that the use of simple non-destructive body measurements can be used to accurately predict the fat in overwintering juvenile salmon of either modal group, their equation had limited application with regard to underyearling fish. It consistently underestimated the fat level in the small size range of fish in the current study (figure 2.3). Accuracy may have been increased in the current study by using the actual weight of fat in the predictive equation and subsequently converting it to a percentage, rather than expressing it as a percentage of wet weight in the initial regression. A similar result was found by Adams *et al.* (1995) when developing a non-intrusive technique to predict the fat content of Arctic charr.

The incorporation of another trunk measurement (pelvic width) may have increased the sensitivity of the predictor to changes in visceral fat, a lipid depot utilised during periods of nutritional stress such as that typified by the winter season (Weatherley & Gill, 1981; Jezierska *et al.*, 1982; Currens *et al.*, 1989; Miglavs & Jobling, 1989a, see Cunjak & Power, 1987). Graham (1994) used a single measure of body height in this region to predict the visceral fat content in LMG fish, although the amount of variation in fat explained by the resulting equation was extremely low ($r^2 = 0.166$)

Although previously found to be important in predicting fat level in juvenile salmon (Simpson *et al.*, 1992) and to some extent in Arctic charr (Adams *et al.*, 1995), the adipose fin length was found to be of little importance in the current study. This may well have been due to the small size range of fish used and the difficulties in obtaining an accurate determination of the length of this small, flexible structure. By using the minimum number of measurements and omitting the adipose length measurement as a result of this difficulty, a more rapid collection of accurate data from an individual fish was possible. Such considerations must be considered advantageous not only from the point of view of the researcher, but also that of the animal.

The regression analysis selected the same three morphometric width measurements (w_d, w_p, w_a) out of a larger number of possibilities (see figure 2.1) in order to predict changes in body fat content in all three samples of fish. In addition, the partial regression coefficients associated with each measurement were allocated the same sign (w_d and w_p = positive, w_a = negative), in every predictive equation. This indicated that both w_d and w_p (in the area of visceral fat deposition) were larger where body fat content was greater, but that w_a (a measurement of the thickness of the posterior musculature) was reduced. In effect, the measure of w_a may well have only contributed to the equation by controlling for variation in the overall thickness of the fish, allowing differences in w_d and w_p to be attributed more convincingly to changes in body fat content. The results presented also indicated that the same set of body measurements, standardised for the appropriate size range of fish, are sensitive to body fat levels in fish reared under both hatchery-feeding and wild regimes. No previous attempt has been made to use morphometrics to predict fat in such small juvenile salmon during their first summer and autumn, with an estimation of energy status such as condition factor (Wootton, 1990) frequently being used in studies conducted either in the laboratory or in the field. However, due to the replacement of lipid in muscle depots by water (Parker & Vanstone, 1966; Love, 1980), and the fact that it cannot accurately predict tisue composition, condition factor must be used with caution when inferring changes in fat level. Simpson *et al.*, (1992), Graham (1994) and Adams *et al.*, (1995) all found morphometric techniques superior to condition factor in describing fat levels, although Herbinger & Friars (1991) indicate that it may be useful in some situations.

The resulting predictive equations have the advantage in that they can be repeatedly applied to the same individual, given that they originated from the same stock and size range. While there was some random error in estimating lipid levels by this technique, this might merely add 'noise' to any relationship between fat stores and behaviour, resulting in statistical tests becoming more conservative. The derivation of such predictive equations allowed an estimation of the fat dynamics of underyearling salmon in the subsequent experiments.

Chapter 3 - Temporal changes in responses to food deprivation

3.1 Introduction

Many animals respond to a period of food deprivation by displaying an increased consumption and growth rate once food is available again, typically referred to as compensatory or catch-up growth (Wilson & Osbourn, 1960, Bilton & Robins, 1973; Weatherley & Gill, 1981; Dobson & Holmes, 1984; Ashworth, 1986; Kindschi, 1988; Miglavs & Jobling, 1989a; 1989b; Metcalfe & Thorpe, 1992). During this time the pattern of energy allocation and the extent to which food consumption is elevated in a hyperphagic response varies between studies. For fish experiencing extended periods of food deprivation, internal energy stores (mainly in the form of lipid) are utilised as metabolic fuel, leading to their depletion (Love, 1980; Weatherley & Gill, 1981). Once food is available once more, the fish face a choice between allocating surplus energy into restoring this deficit in storage or into somatic growth.

Underyearling salmon parr have been shown to exhibit a compensatory response to food deprivation . They respond to deprivation in November by increasing their food intake and restoring fat losses to a level appropriate for the time of year (Metcalfe & Thorpe, 1992). However, the means by which they regulate their appetite with regard to the restoration of fat during the compensatory period remains unclear. Parr exhibit clear changes in their behaviour in response to the changing seasons (e.g. Higgins & Talbot, 1985; Thorpe, 1986; Metcalfe *et al.*, 1986; Fraser *et al.*, 1993; Simpson, 1993) and growth priorities (Metcalfe et al., 1988; Nicieza & Metcalfe, submitted), leading to the possibility of an array of responses to food deprivation at different times of the year.

In this chapter I monitor individual fishes' appetite and fat responses to food deprivation periods. By imposing deprivations of different severity and monitoring the compensatory period, the relationship between the rate of restoration of fat and the elevation in feeding was examined. By applying deprivation periods at different times of the year, the changes in feeding response and energy allocation was investigated.

Chapter 3.2 - Regulation of hyperphagia in response to varying energy deficits

3.2.1 Introduction

Many studies on endothermic animals have illustrated their ability to compensate for periods of reduced feeding opportunity by displaying a growth spurt on subsequent realimentation (Wilson & Osbourn, 1960; Ashworth, 1986; Mersman *et al.*, 1987; Summers *et al.*, 1990). The process of achieving normal body weight and composition following nutritional restriction (commonly termed catch-up or compensatory growth) can be achieved by increasing both food intake (i.e. hyperphagia) and food conversion efficiencies (Wilson & Osbourn, 1960; Bilton & Robins, 1973; Miglavs & Jobling, 1989*a*; Russell & Wootton, 1992).

Periods of starvation affect the feeding and digestive processes in fish (Fänge & Grove, 1979; Love, 1980). Due to environmental fluctuations, temperate teleost fish frequently face times when food supply is irregular, and many species have adapted to withstand long periods of starvation (e.g. Larsson & Lewander, 1973). Studies have shown that a wide variety of fish species experiencing food restriction often exhibit a compensatory growth spurt once food is made available (Bilton & Robins, 1973; Weatherley & Gill, 1981; Miglavs & Jobling, 1989*a,b*; Pederson *et al.*, 1990; Wieser *et al.*, 1991; Russell & Wootton, 1992; Nicieza & Metcalfe, submitted). However, the precise way in which growth is achieved and regulated is not understood.

In many studies examining the post-restriction period, the mechanisms responsible for this growth spurt are only implied, as individual food intake following starvation was not monitored (Weatherley & Gill, 1981; Kindschi, 1988; Quinton & Blake, 1990; Weiser *et al.*, 1991). Where feeding has been investigated, a hyperphagic response has been demonstrated for Arctic charr, Atlantic salmon and the European minnow, following experimentally imposed food restriction (Miglavs & Jobling, 1989*a*; Metcalfe & Thorpe, 1992; Russell & Wootton, 1992, 1993; Bull *et al.*, 1996; Nicieza

& Metcalfe submitted). However it is unclear how the hyperphagic response is regulated during the time in which losses are being regained.

Juvenile Atlantic salmon parr rely heavily on lipid reserves to survive their freshwater phase, drawing upon them during winter (Egglishaw & Shackley, 1977; Gardiner & Geddes, 1980) when food supply is inadequate and unpredictable due to environmental fluctuations. During this time fat stores and appetite are regulated with regard to nutritional state (Metcalfe & Thorpe, 1992; Bull *et al.*, 1996). Metcalfe & Thorpe (1992) showed that an accelerated depletion of fat reserves in early winter led to a hyperphagic response in order to replenish losses, but appetite soon fell once again to a low level. However, it is not clear from these previous studies of salmon how the intensity and duration of hyperphagia are related to the extent of the energy deficit.

Therefore, the aim of this study was to investigate more fully the changes in feeding responses of parr following artificial lowering of body energy reserves. Two variables were considered in regulating the extent of the hyperphagic response, namely the feeding intensity and the duration of appetite elevation. Compensatory growth responses could be achieved by varying either, or both simultaneously (figure 3.1). By imposing food deprivation periods of different lengths (and therefore, severity) and subsequently monitoring appetite and fat, I tested whether the energy deficit affected the duration and/or the intensity of the hyperphagic response.

3.2 2 Materials and methods

A sibling population of Atlantic salmon juveniles, the progeny of sea-run adults from the River Almond in Perthshire, Scotland, were reared at the SOEAFD hatchery at Almondbank and transferred to the University Field Station, Rowardennan during the summer of 1994. Sixty were selected from a stock holding tank on 19 October 1994 for use in the experiment. Fish were selected if they were <70 mm forklength in order to maximise the number of non-smolting fish (Metcalfe *et al.*, 1988). All fish received a combination of alcian blue marks on their ventral surface (Metcalfe *et al.*, 1988) to enable them to be identified, and a number of biometric measurements were

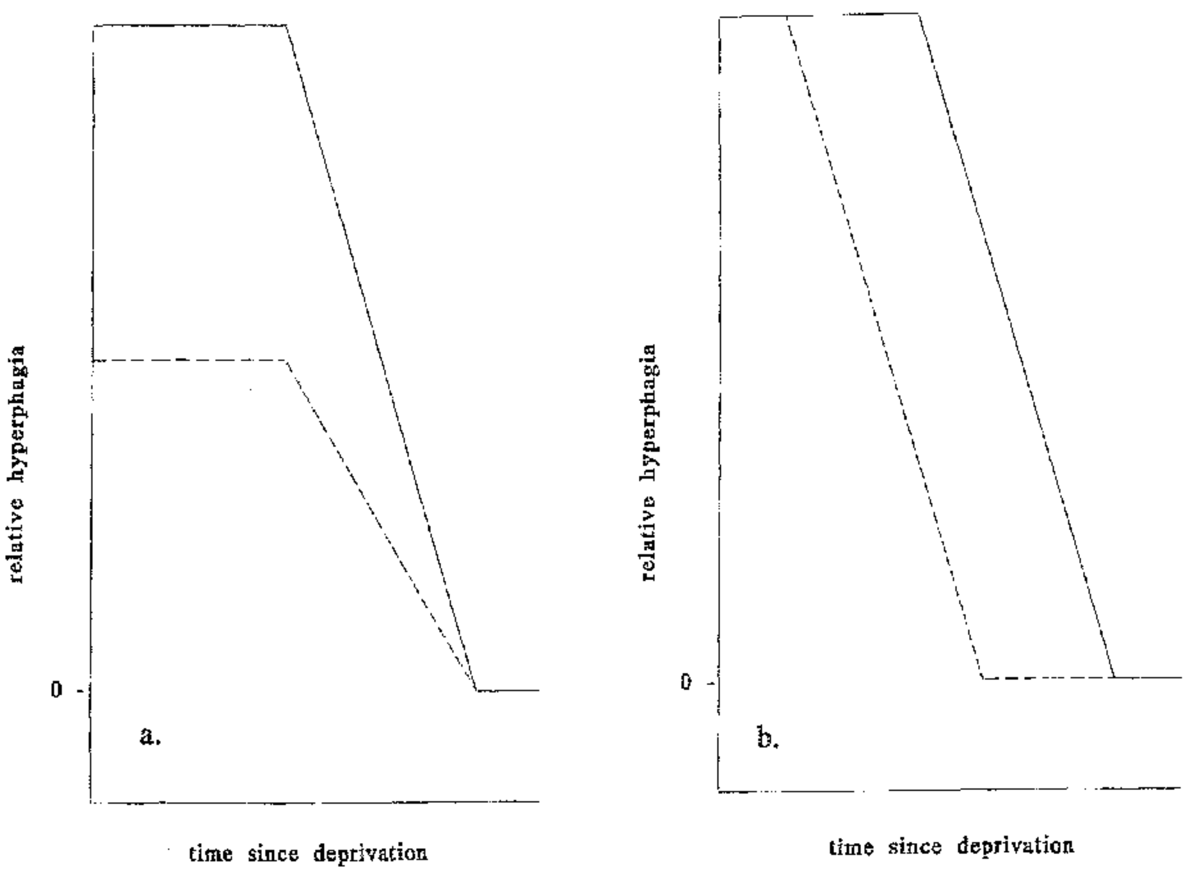

Figure 3.1. Hypothetical appetite dynamics following a period of food deprivation. The solid line denotes a fish experiencing a more severe reduction in body energy reserves than the dotted line. Values of relative hyperphagia greater than zero indicate that the fish is eating more than controls given ad lib. food throughout. In a.) the duration of hyperphagia is similar for the two fish, but the intensity varies in proportion to the energy deficit, whereas in b.) the initial appetite is similar but the duration of hyperphagia is variable.

taken whilst fish were under anaesthetic in order to estimate body fat levels from equation 2.2 (see chapter 2). Individual fat reserves were expressed as a percentage of wet weight to control for variation in body size.

Twenty fish were then assigned randomly to each of three groups; A, B and C. Those assigned to group A were transferred on 24 October to a separate 1m^2 holding tank where the normal supply of pelleted food was absent. Although the fish were not entirely starved of food (due to a small quantity of zooplankton entering through the source water) this technique has been shown to cause fish to utilise their body energy reserves (Weatherley & Gill, 1981; Metcalfe & Thorpe, 1992). Fish in groups B and C remained in the original 1m^2 tank where pelleted food was provided in quantities providing *ad lib.* rations by way of an electronically timed feeding system providing food every 20 min over the 24 h period. On 10 November (20 days since the beginning of group A's period of food deprivation) all fish were anaesthetised once again and body fat estimated.

Group B fish were then moved to join group A and consequently entered a period of food deprivation. Group C fish were designated as controls and remained with access to *ad lib.* food rations throughout this time. All groups experienced ambient water temperatures and simulated natural photoperiod during the course of the experiment. The deprivation period finished on 5 December, by which time groups A and B had experienced deprivations of 40 and 20 days respectively (Table 3.1).

Table 3.1. Lengths of feeding manipulation during the course of the experiment together with the appropriate ambient water temperature.

Group	length of food deprivation period	water temperature during deprivation (mean, max/min ^{0}C)		
A	40 days	9.8	max 10.9	min 8.2
B	20 days	8.8	max 9.7	min 8.2
C	none			

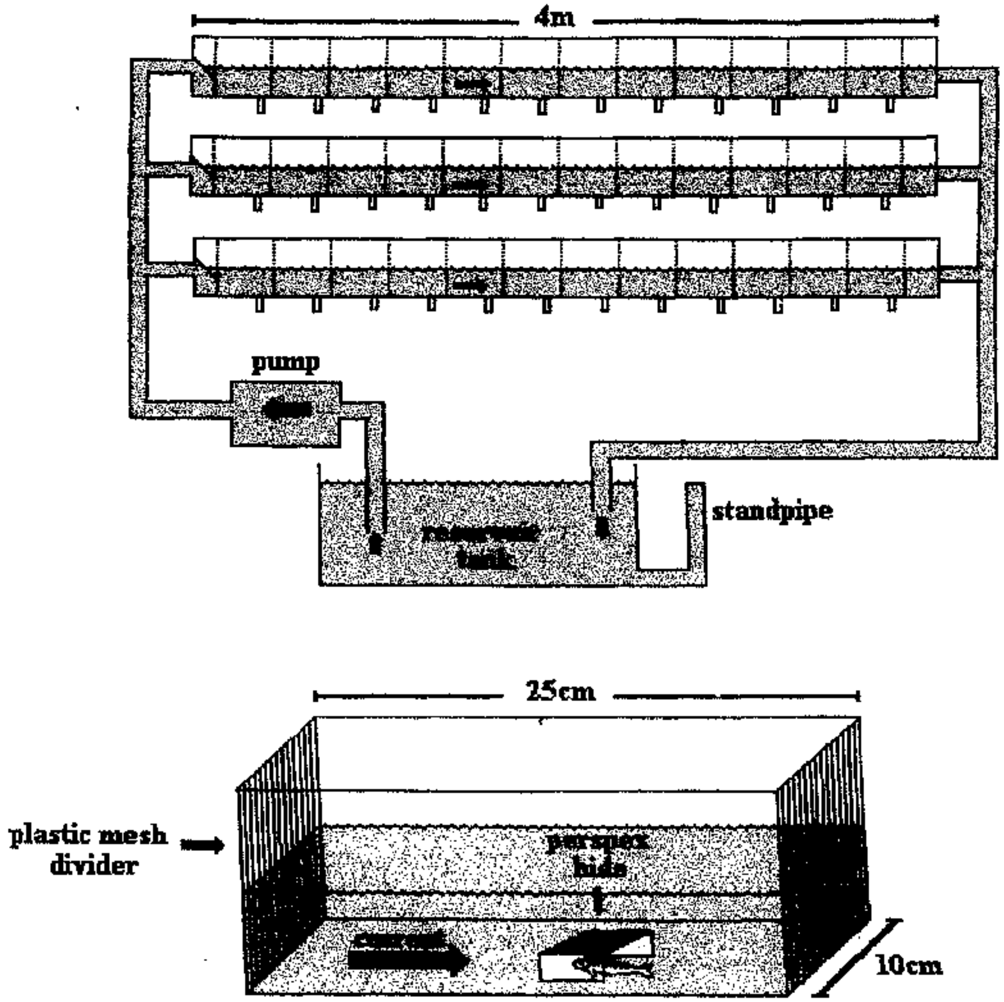

Figure 3.2 (Top) Diagramatic representation of apparatus used to house fish individually during appetite trials. Ambient temperature water entered the reservoir and was pumped through the raceways at a steady rate. (Bottom) Detail of a single compartment. Each compartment contained a centrally located darkened shelter under which the fish held station, facing into the current.

Fat content was then assessed and all fish were moved to a randomly assigned compartment within a specially designed tank system (figure 3.2) that allowed controlled feeding and observation of individual fish throughout a 15 day refeeding period. Water was pumped through a system of four raceways (each with one transparent side) each containing 15 fish. Each individual was separated from others by a plastic mesh screen so that it could maintain visual contact with others, but receive its own food supply. Each compartment contained a small black plastic shelter under which fish normally held station facing into the flow. Fish were allowed three days in which to settle, during which time food in the form of mixed live and frozen bloodworms was handfed to all fish twice daily. By introducing prey items at the upstream end of each compartment and allowing the slow water flow to distribute them, a sufficient number were retained to ensure excess rations for every fish. The majority of uneaten bloodworms were collected in a small plastic tube in the base of each compartment and removed daily. Providing excess feeding opportunities at the start of the appetite trials was necessary to prevent any subsequent recorded changes in appetite being attributable to differences in gut fullness.

Appetite was then measured by observations of feeding behaviour. Appetite trials were conducted daily between 1000 hours and midday for the next 15 days (9-24 December) except on the two days following estimations of fat content (12 and 18 December). Following fat estimation, fish were reassigned randomly to a new compartment prior to the next appetite trial to prevent any biases due to minor differences between compartments. An appetite score for the occupant of each numbered compartment was recorded from its responses to the presentation of five prey items (live bloodworms dropped into the water singly upstream of the fish, a minimum of 30 min apart) and scored as in Table 3.2 (*sensu* Metcalfe *et al.,* 1986).

Table 3.2. The scoring system employed to visually assess individual appetite responses

Score recorded	Response to prey item (live bloodworm) passing within 2cm of fishes head
0	no response to prey item
1	orientate towards item but no approach
2	turn back after moving towards prey
3	move towards item but miss
4	ingest but subsequently reject prey item
6	consume prey item

Any bloodworms not passing within 2cm of the fish's head were discarded from the results and replacement bloodworms were presented 30 minutes later. As the dye marks identifying each fish were not visible during these trials, appetite information was collected blind without knowing the treatment group of the fish. A minimum of four separate responses to the passage of a prey item were used to calculate an individual's mean appetite score each day. In the afternoon following each appetite trial and on days when trials were not performed (13, 14, 19 and 20 December), a minimum of 20 additional bloodworms were fed to each fish to maintain daily *ad lib.* rations throughout this time

Fat was assessed again following the final appetite trial on 24 December and all surviving fish were re-established in a holding tank. One final fat assessment was made on 10 January and all fish were assessed visually for external signs that they were preparing for smolting the following spring e.g. darkened fin edges and silvered flanks. Any fish showing these signs were removed from analysis as presmolts behave differently to non-smolting fish during winter (Thorpe *et al.*, 1980; Higgins & Talbot, 1985; Metcalfe *et al.*, 1986, 1988).

3.2.3 Results

Three fish continued to grow throughout the course of the experiment and exhibited external signs of smolting on 10 January. These were subsequently excluded from analysis. Five other fish died throughout the course of the experiment; mortality was independent of experimental group ($\chi^2 = 0.25$, d.f. = 2, N.S.).

Fat Dynamics

Given that there was no consistent positive relationship between fat content expressed as %body weight and body size in the control group throughout the course of the experiment (highest r^2 value = 0.17), fat levels for groups A and B were expressed as residual values from the mean of the controls at each sampling time. Prior to any experimental manipulations in October the three groups did not differ in fat level (ANOVA between treatments on residual fat level; $F_{(2,54)} = 0.51$, N.S.). The periods of food deprivation imposed on groups A and B (figure 3.3) acted to reduce the fat levels with regard to the controls (repeated measures ANOVA between treatment groups on residual fat levels over the course of deprivation, treatment effect, $F_{(2,50)} = 10.53$, $P<0.001$) with fat stores decreasing in groups A and B by an average of 2.1% ± 0.3 S.E. and 1.3% ± 0.3 respectively. Consequently by November (prior to appetite trials), the fat levels of groups A and B were lower than those of the controls (ANOVA between treatments on residual fat levels; $F_{(2,53)} = 34.04$, $P<0.001$; Tukey's HSD test, groups A and B differ from controls at $P<0.05$).

During the refeeding period, the fat dynamics of group A and B fish exhibited a similar pattern, (figure 3.3; repeated measures ANOVA on residual fat of the two deprivation groups during the course of refeeding, treatment effect, $F_{(1,31)} = 2.15$, N.S.) although group A fish increased their fat levels by an average of 2.0% ± 0.4 and group B by an average of 1.6% ± 0.8. As a result of their starting at a lower level than group B, group A fish had not fully regained their lost fat by the completion of the appetite trials (ANOVA between all three treatment groups on residual fat levels; $F_{(2,50)} = 3.89$, $P<0.05$, Tukey's HSD test, group A differs from controls at $P<0.05$). Group B fish had restored their losses by the end of the appetite trials. As a result of the restoration of fat losses displayed by both groups A and B, the fat levels of all

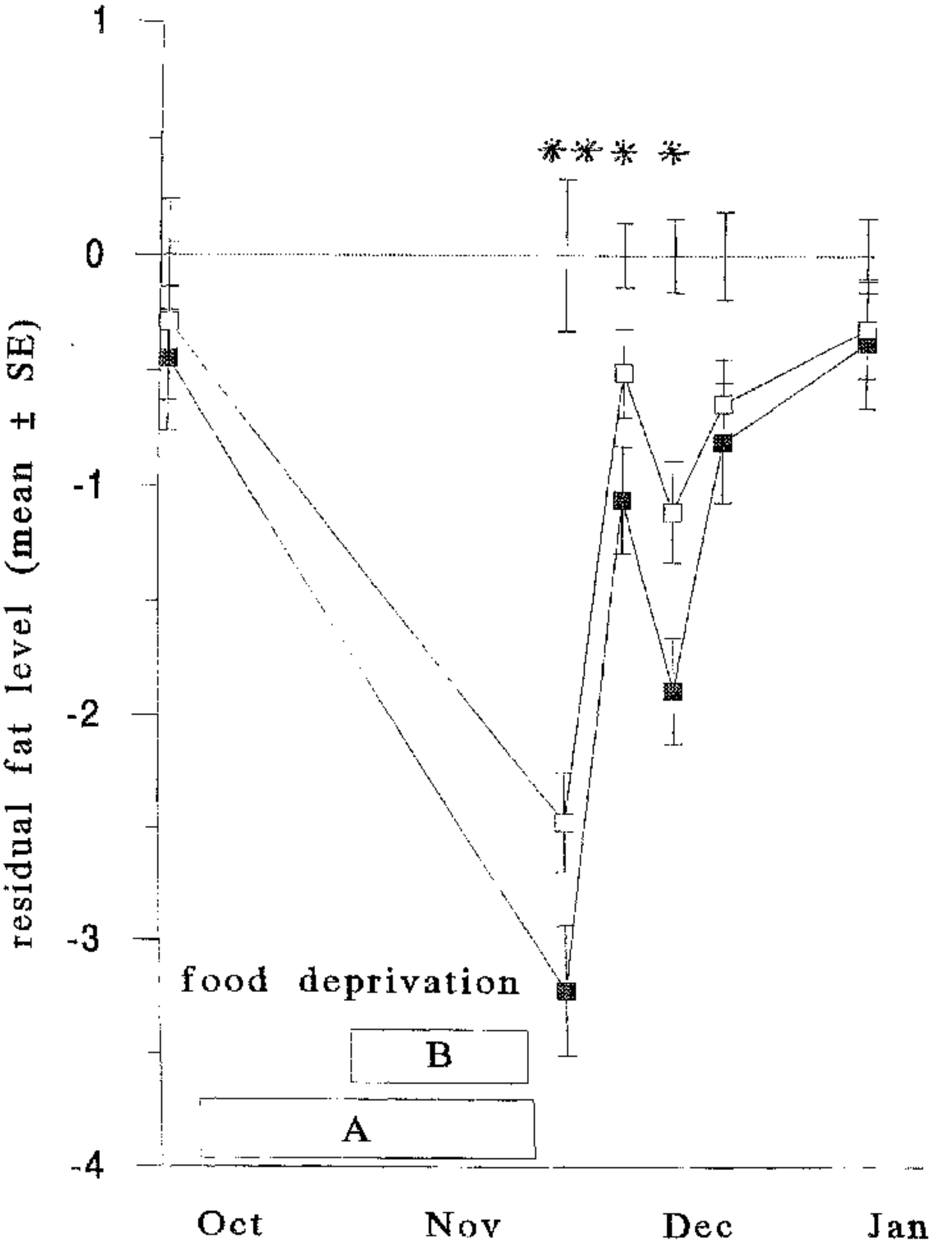

Figure 3.3. Change in lipid level during the course of the experiment. The bars indicate the duration of the deprivation period imposed on Group A and B fish. These treatments resulted in differences in the fat levels betweeen groups once food was again provided. Data plotted as residual values from the mean control fat level. Asterisks indicate the significance of ANOVA between treatments at different times, * = P<0.05, ** = P<0.01).

three groups were similar by the end of the experiment on 10 January (ANOVA between treatments on residual fat levels; $F_{(2,50)} = 0.82$, N.S.)

Appetite changes

During the course of the 15 daily appetite trials, a mean of 15% (range, 3.5 - 31.7%) of the fish tested consumed all the bloodworms presented. The appetite scores were pooled to produce a single mean value (based on a minimum of four daily scores) for each week for each fish to reduce the noise caused by large daily fluctuations. Appetite scores for groups A and B were expressed as residuals from the mean appetite score of control fish each day, to eliminate variation caused by parallel fluctuations in appetite between groups (as could be caused by slight daily fluctuations in temperature). The appetite of controls was low and exhibited the normal seasonal reduction throughout the course of the trial period (figure 3.4).

A measure of change in fat level for every fish during each of the weeks of refeeding, was calculated as follows:

$$\text{change in fat (per day)} = \frac{(f_2 - f_1) \times 100}{t_2 - t_1} \qquad \textbf{(eqn. 3.1)}$$

where $(f_2 - f_1)$ = change in estimated fat level during the period in question and $t_2 - t_1$ = duration of period in days. There was a weak relationship between residual appetite score and the change in fat during the first week of refeeding (figure 3.5). No such relationship was found during weeks 2 and 3 of refeeding (Pearson's $r = -0.24$, $n = 49$, N.S.; $r = 0.07$, $n = 49$, N.S. respectively).

Both groups of previously food-deprived fish displayed a hyperphagic response to food deprivation when compared to the control fish (figure 3.6; repeated measures ANOVA amongst treatments on residual appetite during refeeding period, treatment effect, $F_{(2,46)} = 8.53$, $P<0.01$). In accordance with the greater fat deficit at the beginning of the refeeding period (figure 3.3), group A fish maintained hyperphagy

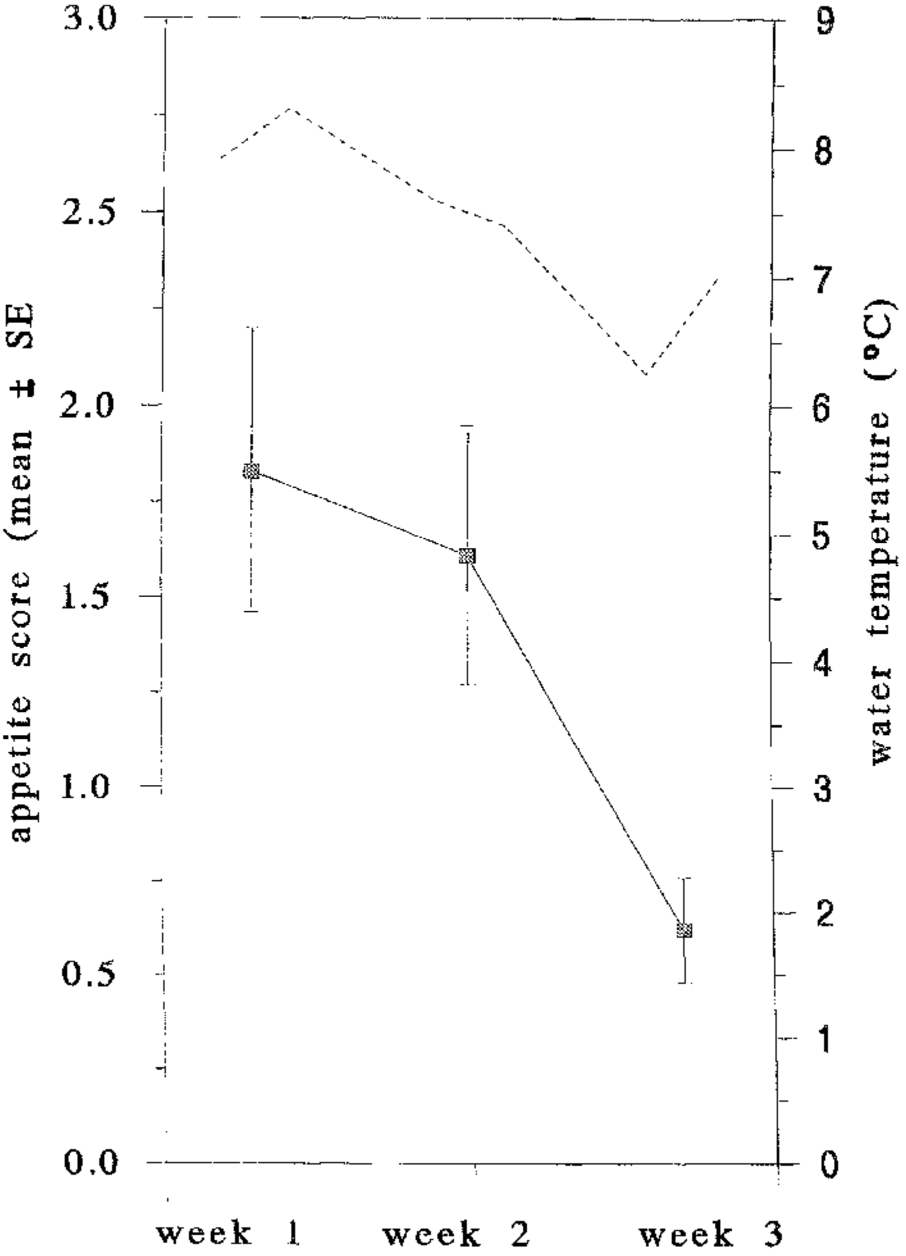

Figure 3.4. Decline in appetite of control fish throughout the December trial period. Data are pooled means for every week of refeeding. The dotted line shows the daily ambient water temperature in the raceways applicable to all three treatment groups during this period.

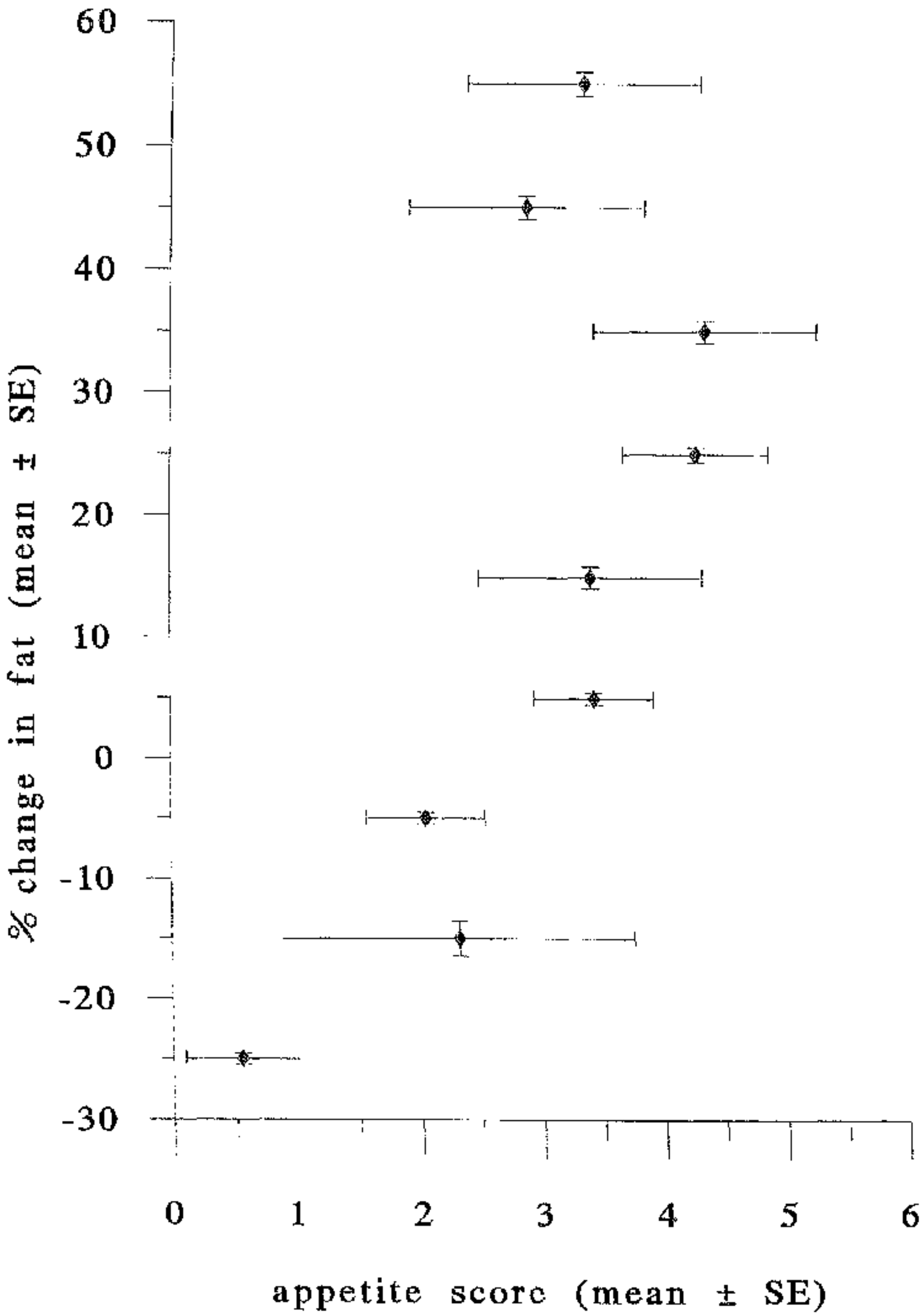

Figure 3.5. Relationship between the appetite score and the average daily change in fat (as a percentage of fat reserves) after the deprivation period (Pearson's correlation $r = 0.31$, $n = 50$, $P < 0.05$). Data are presented as mean appetite for each 10 % interval in change in fat for clarity.

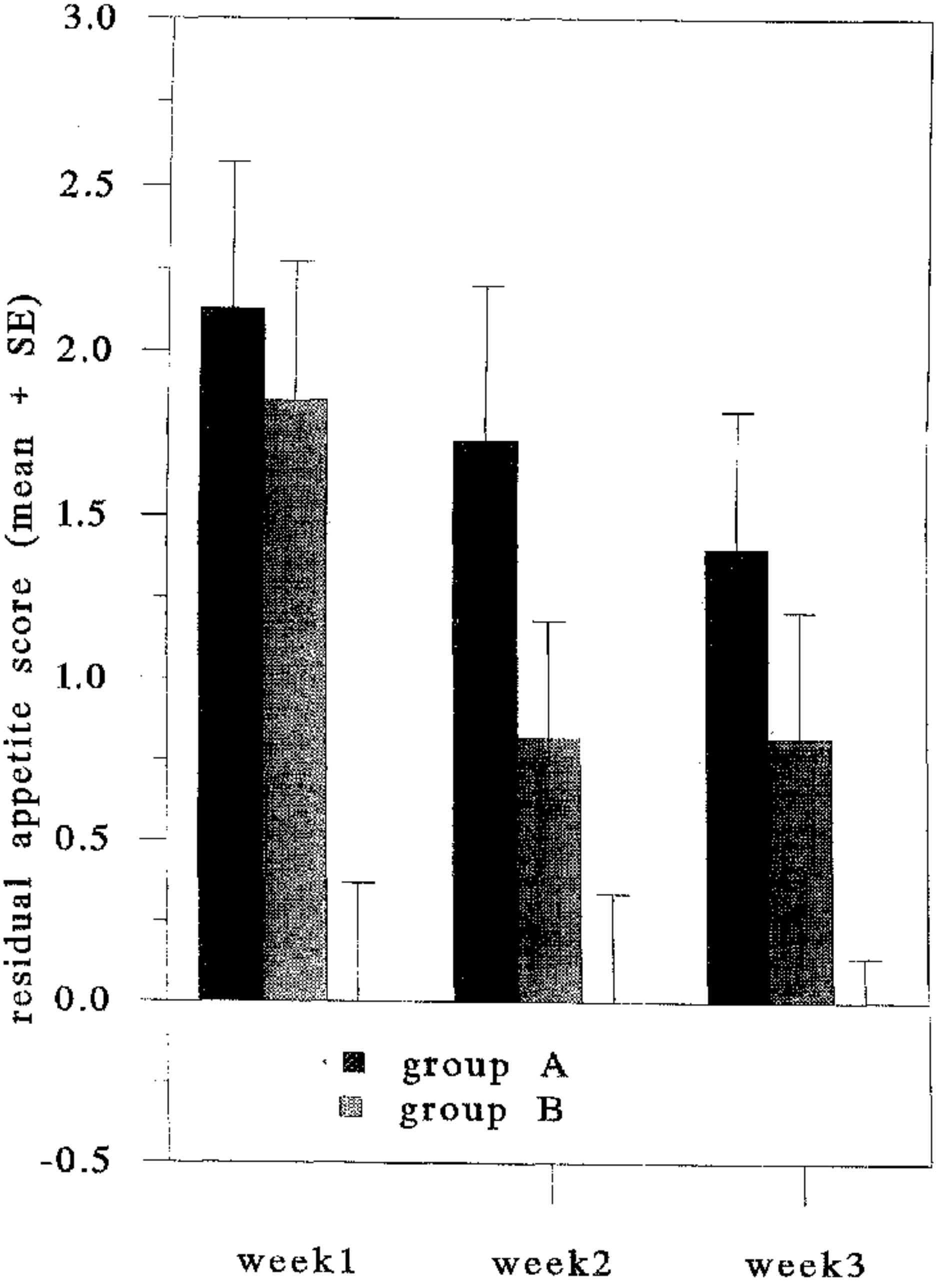

Figure 3.6. Appetite changes during the first three weeks of refeeding. Appetite is presented as a residual from the mean value of controls each week. The mean appetite of the controls (± S.E.) is depicted by the zero line for weeks 1, 2 and 3.

throughout the course of refeeding (separate ANOVAs between treatments on residual appetite during weeks 1, 2 and 3 all $P<0.05$; Tukey's HSD test: group A always differed from controls at $P<0.05$). Initially, the previously deprived groups showed no difference in their appetite responses, but the appetite of group B fish dropped rapidly to a level intermediate between groups A and controls during the second week. However, the pattern of change in appetite over time was not different between the treatment groups (repeated measures ANOVA on residual appetite changes, treatment x time interaction , $F_{(4,92)} = 1.58$, N.S.)

3.2.4 Discussion

Juvenile salmon parr responded to an artificial lowering of fat reserves in December by exhibiting a hyperphagic response, in agreement with other studies (Talbot *et al.*, 1984; Miglavs & Jobling, 1989a, Metcalfe & Thorpe, 1992; Russell & Wootton, 1992, 1993; Bull *et al.*, 1996; Nicieza & Metcalfe, submitted). This protective response was at least in part responsible for the restoration of body fat lost during food deprivation. Previous studies (Bilton & Robins, 1973; Miglavs & Jobling, 1989a; Russell & Wootton, 1992) have shown that food conversion efficiency can also be increased during compensatory feeding, and therefore this cannot be discounted as a contributing factor in allowing the rapid increases in body fat reserves following deprivation (figure 3.3).

The extent of the estimated energy deficit incurred during a period of food restriction appeared to affect primarily the duration of the hyperphagic response that occurred when food was once again available, rather than the intensity of feeding (figure 3.6). A similar result has been found following food restriction in the European minnow (Russell & Wootton, 1993). On first inspection this would appear maladaptive as increases in feeding intensity and therefore energy intake would allow more rapid fat restoration than would the extension of a lesser response. However, the functional significance of temporal control over hyperphagy during winter may be explained in terms of both proximate and ultimate constraints.

The seasonal reduction in water temperature exerts a proximate constraint on food intake in fish (Elliott, 1975; Love, 1980). Both the ability to capture (Webb, 1978), process (Brett, 1976; Elliott, 1976b; Priede, 1985; Wieser & Forstner, 1986; Nicieza *et al.*, 1994), and evacuate food (Elliott, 1972; Jobling, 1980) are all slowed as a result of reductions in metabolic rate. The rate of food intake and the motivation to feed are closely linked to both stomach fullness (Brett, 1971) and rate of gut emptying (Grove & Crawford, 1980; Godin, 1981), with appetite reduced by the presence of food in the stomach or its slow movement through the digestive system. Water temperatures during the course of the appetite trials (figure 3.4) were well below the optima for food intake rate; the initial hyperphagic response of the fish may therefore have been at (or close to) the upper physiological limit given the environmental constraints.

In addition to the constraints placed on feeding efficiency, declining water temperatures will also affect the ability of juvenile salmon to escape predators (Webb, 1978). As juvenile salmon are essentially sit and wait visual foragers (Stradmeyer & Thorpe, 1987), the actual process of feeding leads to a loss of crypsis and hence an increased likelihood of detection (Martel & Dill, 1995) Theoretical work on the trade-off between predation risk and feeding intensity (Lima, 1986; McNamara & Houston, 1987, 1990) suggests that the optimal level of body energy reserves will be that which minimizes mortality both from starvation and predation. A marked reduction in these reserves produces a disproportionately increased risk of starvation; the optimal strategy is therefore to increase foraging intensity in order to reduce the risk of starvation. However, in the presence of a perceived predation risk, the efficiency of foraging is markedly reduced (Metcalfe *et al.*, 1987; Huntingford *et al.*, 1988; Gotceitas & Godin, 1993). The similarity in initial feeding intensity exhibited by fish with markedly different energy deficits (figures 3.3, 3.6) may be explained therefore not only on the basis of proximal constraints, but from an ecological perspective as fish achieving their optimal feeding rate relative to the risk of predation.

Overwintering parr display other behaviours adapted towards minimizing predation at low water temperatures. In conjunction with a seasonal reduction in appetite

(Metcalfe *et al.*, 1986; Metcalfe & Thorpe, 1992; Bull *et al.*, 1996), resident parr switch to hiding in streambed refuges during the day, from which they emerge under the cover of darkness to feed (Fraser *et al.*, 1995). This latter behaviour is triggered by falling water temperatures, with the switch from daytime to night-time activity occuring at temperatures above that experienced during the refeeding period (Fraser *et al.*, 1993). The provision of overhead cover in the raceways in some respects accommodated this behaviour, but the procedure for scoring appetite did not lend itself easily to quantifying nocturnal feeding. Therefore a compromise was made, with appetite scores reflecting a slightly unnatural situation of sheltering fish being given free access to drifting food during the day. In the natural situation, fish feed little in daytime refuges (Cunjak, 1988) but emerge and feed on drifting invertebrates during darkness (Fraser *et al.*, 1995). However, despite this problem, the lack of variation in feeding intensity between treatment groups during hyperphagy may well be a reflection of the preference for nocturnal foraging during winter. If natural food restriction (e.g. as a result of a spate or drought) resulted in significant reductions in energy levels, a resulting hyperphagic response may be accommodated by the option of safer, but less efficient nocturnal foraging (Fraser & Metcalfe, submitted) whereas a higher intake rate may require more risky daytime foraging.

Chapter 3.3 - Seasonal change in the pattern of energy allocation

3.3.1 Introduction

Compensatory (or catch-up) growth following food restriction usually takes the form of a hyperphagic response and/or increased food conversion efficiency (Miglavs & Jobling, 1989a; Russell & Wootton, 1992) leading to restoration of body mass. Body mass has been viewed as consisting of two components: remobilizable tissues i.e energy stores of lipid (and to a lesser degree, muscle), and nonmobilizable tissues such as circulatory, neural and skeletal material (McCauley *et al.*, 1990; Broekhuizen *et al.*, 1994). The stores constitute those nutrients accumulated in anticipation of periods of adversity, whereas the structural tissues are unavailable as a source of nutrition during 'normal life' (van der Meer & Piersma, 1994). Channelling of resources to one component prohibits allocation to the other; animals exhibiting compensatory growth must therefore adopt a strategy of resource allocation that partitions resources between components appropriately.

For juvenile salmonids, the ratio of allocation of surplus nutritional reserves to stores and structural tissue growth is dependent upon both the nutritional state of the individual (e.g. Metcalfe & Thorpe, 1992; Nicieza & Metcalfe, submitted) and the developmental strategy adopted (Higgins & Talbot, 1985; Thorpe, 1989; Rowe & Thorpe, 1990; Thorpe *et al.*, 1990; Simpson, 1992). During their first year in freshwater, LMG fish exhibit changing short-term developmental goals in the form of size and energy reserves. Initially during the spring and summer, parr grow rapidly but with the approach of autumn, LMG fish exhibit a slowing and eventual cessation of growth (Thorpe, 1977; Higgins & Talbot, 1985; Metcalfe *et al.*, 1986, 1988) and enter a state of anorexia (Metcalfe & Thorpe, 1992). The period of reduced food intake is maintained throughout winter, during which time salmonids in general rely heavily on accumulated fat stores to survive (Gardiner & Geddes, 1980, Cunjak & Power, 1987; Cunjak, 1988b). Elliott (1976a) showed that larger brown trout had proportionally larger stores of body fat than smaller individuals. A similar relationship has been shown for salmon parr by Metcalfe & Thorpe (1992) and Simpson *et al.* (1992), with larger fish having available proportionally more fat stores. Larger parr will presumably therefore have a greater probability of avoiding overwinter starvation. Size related mortality during winter has been reported for both salmonid (Hutchings, 1994; Smith & Griffith, 1994) and non-salmonid species (Toneys & Cobble, 1979; Oliver *et al.,* 1979; Flath & Diana, 1985). Therefore, prior to winter it would appear adaptive for LMG fish to use surplus resources to increase structural tissue in order to maximise body size. However during winter, surplus resources might be best used to maintain mobilizable energy stores.

Here I aim to test the hypothesis that the preferential allocation of resources by LMG fish to tissue components will change seasonally, by comparing the compensatory responses of fish to a similar period of food deprivation in summer and winter.

3.3.2 Materials and Methods

Compensatory responses in summer.

Forty fish were selected from a stock holding tank and given an individual combination of alcian blue dye marks on 3 July, 1995. The fish (mean forklength 36mm; range 30-44mm) were also measured to enable fat level estimation using an equation derived for summer 0+ parr (equation 2.3). All fish were maintained in a 1m tangential flow tank where pelleted food was provided to excess by an automatic feeder dispensing food at twenty minute intervals throughout the day. Fish experienced ambient water temperatures and natural photoperiods throughout the course of the experiment.

Twenty fish were randomly allocated to the experimental group on 12 July and moved to a separate tank where they were prevented from feeding on pelleted food for a period of thirty days, sufficient to cause an appreciable reduction in body fat levels. Water temperatures during this deprivation period averaged 18.2^0C (range; 16.4 - 21.6, figure 3.7). Control fish were maintained on an *ad lib.* feeding regime throughout this time. On 11 August both groups of fish were once again re-measured for estimation of body fat and established in individual compartments in a raceway (see figure 3.2) where excess food (handfed live bloodworms) was provided twice a day for three days. From 15 - 21 August attempts were made to score individual

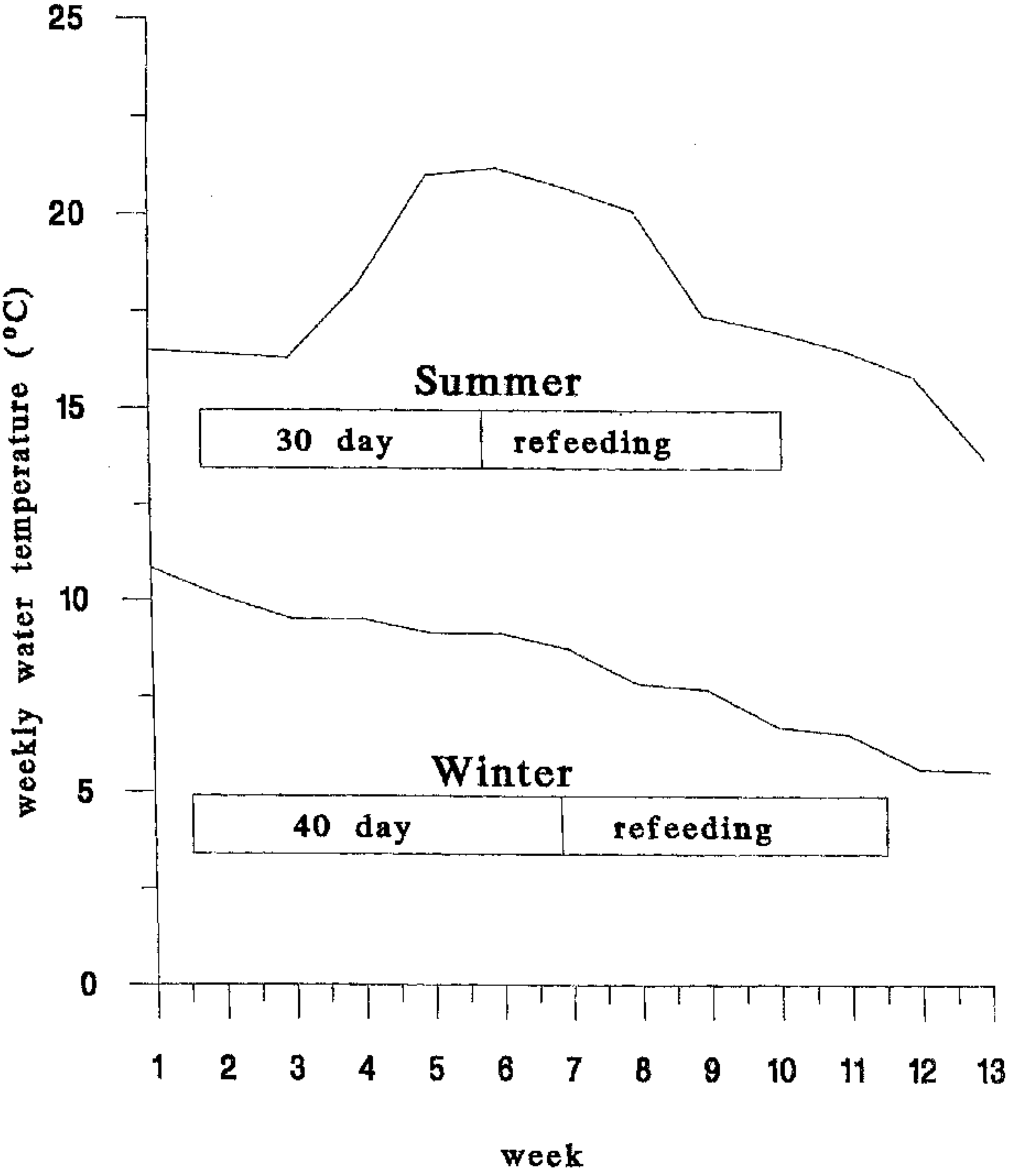

Figure 3.7. Water temperatures during the course of the experiment. The upper line denotes the temperature during the course of the summer deprivation and refeeding period (indicated by the open bars), while the bottom line applies to the winter manipulation.

appetite using the technique described in chapter 3.2, but proved ineffective due the higher intake rates associated with summer water temperatures (Elliott, 1975; Love, 1980). Fish were consuming every prey item presented to them, invalidating thre technique at this time. It therefore proved impossible to obtain appetite scores comparable with those obtained from fish deprived in winter using this technique, and so all fish were moved from the raceway to a holding tank on 21 August, following another fat estimation. Fish were maintained in this tank with excess pelleted food and two more fat estimates were made on 4 September and 13 September, following which all fish were remarked. On 5 November, all fish were measured for fork length and weighed and any fish exhibiting elevated growth rates and external signs of smoltification were removed from the analysis since they were deemed to be UMG fish, destined to smolt the following spring.

Compensatory responses in winter

Comparable information on the growth and fat dynamics of LMG fish after a period of food deprivation in winter came from a separate experiment (chapter 3.2) in which 20 fish (forming group A; chapter 3.2) were deprived of food for 40 days (from 19 October - 5 December, 1994). A longer deprivation period was needed in order to produce significant reductions in fat at the lower water temperatures of this winter trial (mean = 8.4^0C, range, 6.0 - 9.1^0C). Twenty control fish (group C) were allowed to feed *ad lib.* on pelleted food dispensed from a feeder every 20 minutes. On 5 December all fish were transfered to individual compartments of a raceway in which they remained for 30 days and were hand fed bloodworms. The fish were measured for estimation of body fat levels three times during this period (see chapter 3.2 for further details).

3.3.3 Results

Eight fish in the summer experiment were excluded from analysis as they exhibited external signs of smoltification, such as increased size, silvered flanks and darkened fin edges on 5 November.

Fat dynamics

Body fat levels of experimental (= deprived) fish, generated from either equation 2.3 (summer) or equation 2.2 (winter) were expressed as residual values from the mean fat levels of their respective control groups to allow direct comparisons between seasons. These residual values were calculated by firstly regressing fat on forklength for control fish on each sampling date since a significant relationship between fat and body size was found during summer (see appendix I). Residual values were then established by comparing the actual observed fat levels of experimental fish to that predicted for a fish of that forklength by the fitted regression line. No such positive relationship between fat and body size was apparent for either controls or experimental fish in winter (appendix I), presumably as a result of a smaller size range in body size within treatment groups. Fat residuals were therefore calculated as the residuals from the mean fat levels of control fish at each sampling time.

Despite the fact that experimental fish deprived of food during the summer lost less fat than those deprived in winter (mean ± S.E. residual fat of experimental fish after deprivation in summer = -1.52% ± 0.16, winter = -3.21% ± 0.29; *t*-test between treatments; $t = 5.20$, d.f. = 30, $P<0.01$), both groups exhibited a similar temporal pattern of restoration, with losses being regained within approximately five weeks of the fish being again given access to food (figure 3.8). However, the pattern of restoration differed seasonally, with fish in summer regaining lost fat at a slow but steady rate whereas winter fish exhibited a faster and more variable rate of restoration: the daily change in residual fat over the five week restoration period was significantly greater for winter fish (*t*-test between seasons; $t = -3.85$, d.f. = 26, $P<0.01$; figure 3.9) despite temperatures being lower (*t*-test on weekly water temperatures, $t = 14.19$, d.f. = 13, $P<0.01$).

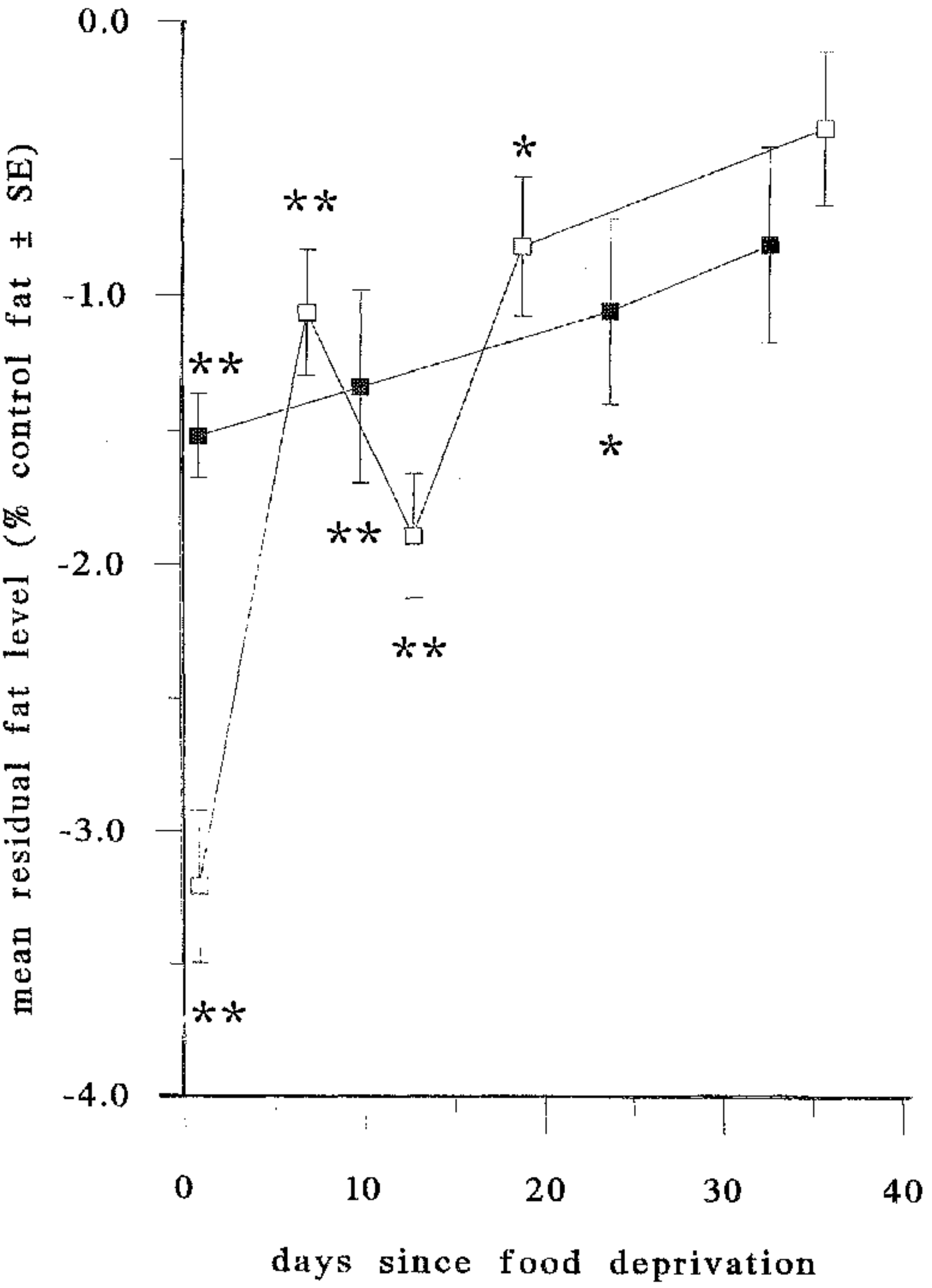

Figure 3.8. Change in body fat during the course of the experiment for summer (solid symbols) and winter deprived fish (open symbols). Data are presented as residual fat levels from the respective mean value for control body fat at that time as indicated by the zero point on the y-axis. Asterisks denote the significance of *t*-tests comparing fat level against the appropriate control value at each sampling time (* = $P<0.05$, ** = $P<0.01$).

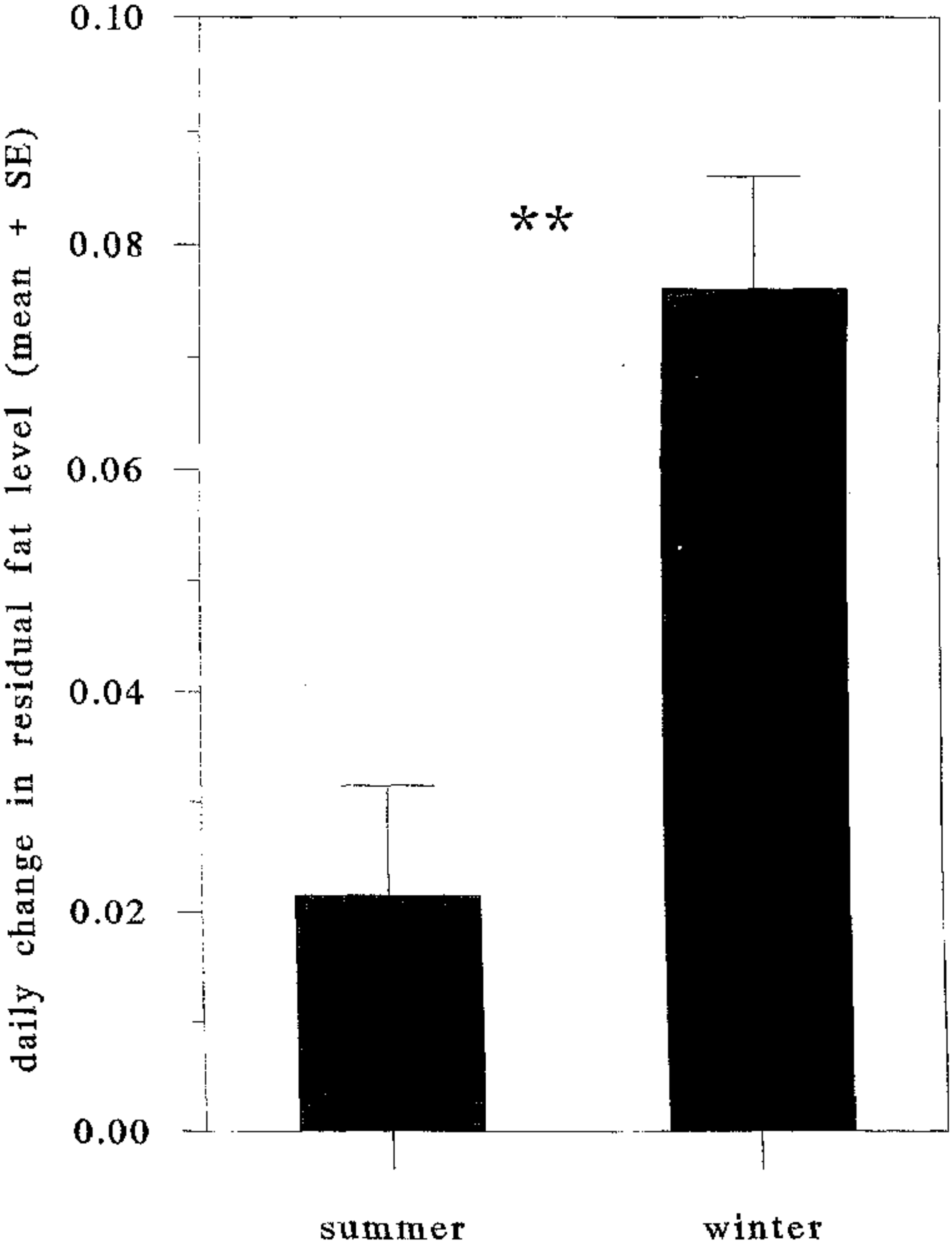

Figure 3.9 Daily change in body fat during the three week period following food deprivation in summer and winter. Fish in winter were regaining fat at a faster rate than those in the summer. Asterisks denote the significance of a *t*-test comparing the seasons in the rate of fat gain in the experimental fish (** = $P<0.01$).

Somatic growth dynamics.

Growth was assessed over the periods following food deprivation in summer (33 days) and winter (36 days) and expressed as specific growth rates, in terms of both body weight (SGR*w*) and forklength (SGR*l*) according to equation 3.2 (Ricker 1979; Wootton, 1990).

$$S.G.R.x \quad = \quad \frac{100\,(\log_e(x_2) - (\log_e(x_1))}{t} \qquad \textbf{(eqn. 3.2)}$$

where S.G.R.x is the specific growth rate (% change per day) of measurement x (either forklength (mm) or wet weight (g)) and t is the time in days between measuring x_1 and x_2.

Growth responses to a period of food deprivation differed seasonally. Summer experimental fish showed increased rates of growth in terms of both weight and body length following food deprivation when compared to controls (figure 3.10) (*t*-tests between experimental and control groups during five weeks of refeeding: SGR*w*, t = 4.81, d.f. = 26, P<0.01; SGR*l*, t = 4.72, d.f. = 26, P<0.01), indicating that these fish were not only restoring lost weight, but also allocating resources so as to allow enhanced structural growth at this time. Similarly, the restoration of fat following food deprivation in winter was reflected by an elevation in growth rate in terms of weight (*t*-tests between experimental and control groups during five weeks of refeeding: SGR*w*, t = 8.74, d.f. = 28, P< 0.01). However, no such increases in the allocation to structural/skeletal growth were found following winter food deprivation (SGR*l*, t = 0.73, d.f.= 28, N.S).

Not surprisingly, fish experiencing a period of food deprivation in summer exhibited higher growth rates in terms of weight and length than those in winter (*t*-tests between treatments during five weeks of refeeding : SGR*w*, t = 16.78, d.f. = 27, P<0.001; SGR*l*, t = 22.41, d.f. = 27, P<0.001).

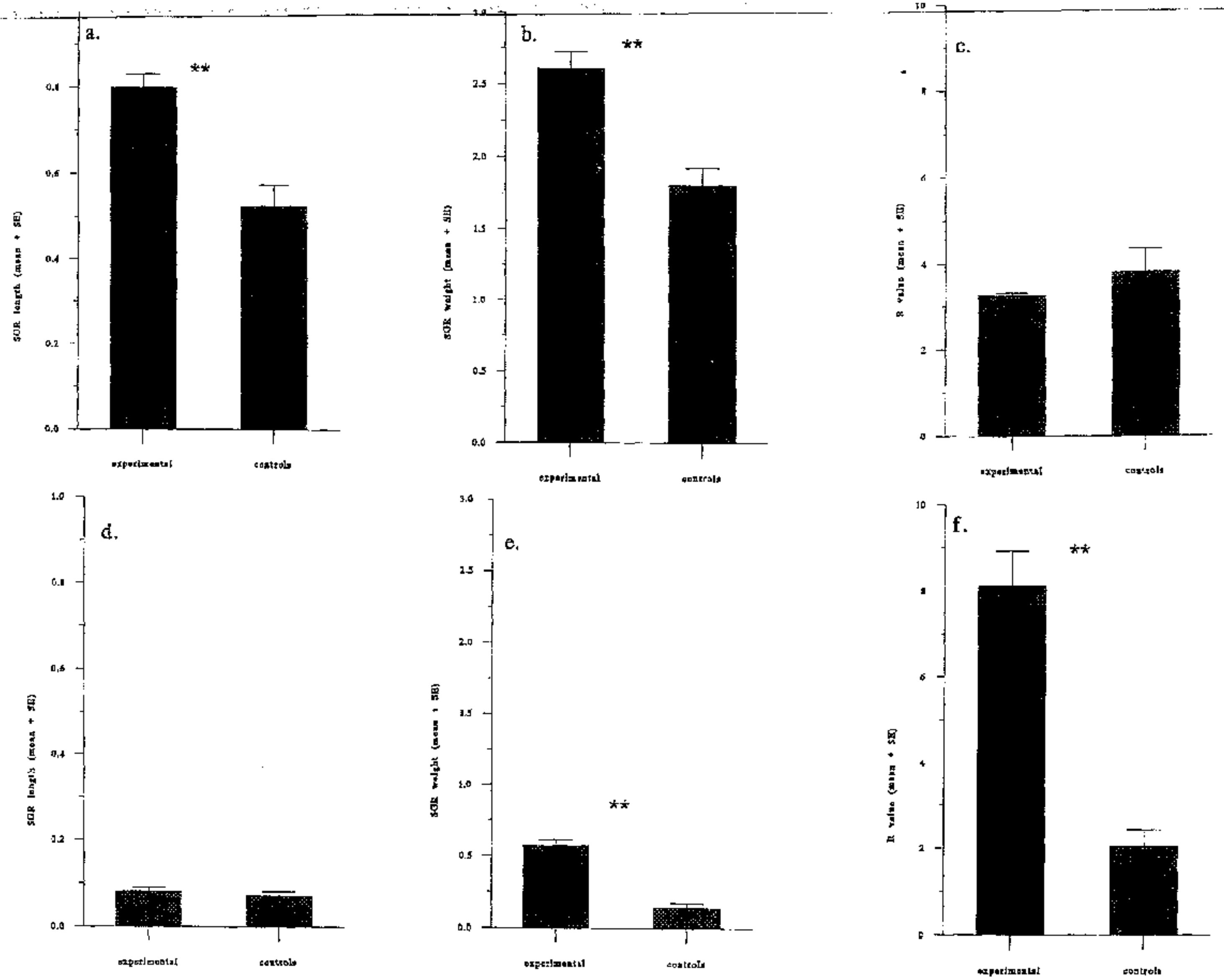

Figure 3.10. Patterns of growth and the value of *R* for the summer (a, b, c) and winter fish (d, e, f) during the three week period following a food deprivation period. Asterisks denote the significance of *t*-tests between treatment groups and the appropriate controls at each sampling time (*= $P<0.05$, ** = $P<0.01$).

By considering a ratio between growth rate in weight to that in length (equation 3.3) it was possible to examine the relative allocation of energy to both remobilizable and structural tissues following food deprivation in summer and winter:

$$\text{Ratio } (R) = \quad (\text{SGR}w) / (\text{SGR}l) \qquad \textbf{(eqn. 3.3)}$$

There was no relationship between body size and the value of R for the experimental fish in summer or winter, both prior to and following the deprivation period (summer: Pearson's $r = 0.12$, n = 14, N.S.; $r = -0.26$, n = 14, N.S.and winter: $r = -0.30$, n = 15, N.S.; $r = -0.28$, n = 15, N.S. respectively), indicating that the size of the fish had no effect upon the pattern of resources allocation.

Following food deprivation in summer, the experimental group did not differ from the controls in their pattern of allocation of resources: both were exhibiting the same increase in mass for a given length increment (t-tests comparing values of R for treatment and control groups: $t = 1.01$, d.f.=26, N.S., figure 3.10). However, during winter the pattern of allocation changed following food deprivation: the experimental group showed a higher increase in mass for a given length increment than the controls (t-tests comparing values of R for treatment and control groups: $t = 6.77$, d.f. = 27, $P<0.01$). Winter experimental fish were increasing in mass at a much higher rate for a given increase in length than those in the summer (t-tests comparing values of R for experimental groups in summer and winter: $t = 5.7$, d.f. = 27, $P<0.01$).

3.3.4 Discussion

The results indicate that the pattern of allocation to tissue components following a period of restricted growth potential differed seasonally, with fish in summer preferentially allocating more surplus energy to structural tissue and skeletal growth than in winter, when the rapid restoration of fat stores took precedent.

Many studies have reported that the relative sizes and importance of body tissue components in fish change seasonally (Gardiner & Geddes, 1980: Flath & Diana, 1985; Higgins & Talbot, 1985; Booth & Keast, 1986; Cunjak & Power, 1986; Rowe *et al.*, 1991; Shackley et al., 1994; Sheridan, 1994; Brown & Murphy, 1995; Luzzana *et al.*, 1995) depending upon the individual life-history strategy. Nicieza & Metcalfe (*submitted*) reported that during a period of experimentally reduced growth in September, juvenile salmon maintained their skeletal growth at the expense of their body fat. However, once a more favourable environment was provided, fish shifted allocation of surplus energy from skeletal growth towards the restoration of fat losses. In light of the marked differences in seasonal energy allocation illustrated by the results of the present study, the results presented by Nicieza & Metcalfe (*submitted*) may be viewed as a seasonal transition in a continuum of changes in the ratio of allocation between non-mobilizable and mobilizable body components, indicative of a change in the salmon's short-term developmental goal. Although care must be taken in interpretation of the results comparing ratios of growth in body components between seasons due to practical difficulties in measuring the small changes in axial size during the winter season, the results provide evidence for the statement proposed by Nicieza & Metcalfe (*submitted*) that "the ideal ratio between the remobilizable and nonmobilizable fractions of body mass should not be considered as fixed but a dynamic parameter that varies seasonally".

Both skeletal growth and body fat reserves of LMG salmon have been shown to peak in September (Higgins & Talbot, 1985; Kristinsson *et al.*, 1985) with little or no increase in the skeletal growth component during the following six months (Higgins & Talbot, 1985; Metcalfe *et al.*, 1988). Following the autumnal peak, body fat levels drop slowly throughout the course of the winter (Gardiner & Geddes, 1980, Higgins & Talbot, 1985; Metcalfe & Thorpe, 1992; Graham, 1994; see chapters 5 and 6.2) as they are utilised as an energy source (Cunjak & Power, 1987; see chapter 3.4).

The change in preferential energy allocation may be mediated by environmental constraints placed upon body functions involved with metabolism, such as enzyme activity (Sauer & Haider, 1977). Because fish with few exceptions are obligate ectotherms (Wootton, 1990), metabolic rates are reduced as water temperature declines (Elliot, 1976b: Brett & Groves, 1979). In the case of juvenile salmon, the developmental strategy adopted also appears to exert some control over metabolism, as during winter the LMG exhibit lower metabolic rates when compared to the UMG (Higgins, 1985). As well as reducing food intake at this time (Higgins & Talbot, 1985; Metcalfe *et al.*, 1986; Metcalfe & Thorpe, 1992) the LMG also achieve lower food conversion efficiencies when compared to the UMG (Higgins & Talbot, 1985) However, the results presented in this study show that even at low water temperatures, LMG fish have the capability to rapidly restore lost fat stores, suggesting that the synthesis of the normally more easily formed mobilizable tissue fractions (Love, 1980) are less constrained than sketetal tissue in LMG fish in winter. An investigation into the seasonal changes in activity of Glucose 6-phosphate dehydrogenase (G6PDH; a rate-limiting enzyme in the pentose phosphate pathway and an essential component in the process of lipid synthesis) in juvenile salmon revealed differences between the modal groups during winter; G6PDH activity was higher in LMG fish than UMG acclimated to winter water temperatures (Graham, 1994). The differences in the enzyme's substrate affinity were attributed to LMG fish having more forms of G6PDH than the UMG and reflected the differences in the immediate priorities of the two developmental pathways. The UMG fish maintain higher food intake during the winter (Higgins & Talbot, 1985; Metcalfe *et al.*, 1988) and subsequently can channel more excess resources to skeletal growth. The LMG eat less but are able to channel proportionally more resources into their fat stores as a result of having lower metabolic demands and possibly an increased efficiency of lipogenesis. Thus, when conditions improve following food deprivation at low temperatures it is easier and quicker for LMG fish to build up mobilizable tissues than to regain lost skeletal growth (Nicieza & Metcalfe, *submitted*).

As far as life history implications are concerned, these results illustrate the changes in the developmental goals throughout the first year of life. Although not exhibiting the fast-growth strategy of the Upper Modal Group (chapter 1) and not therefore under such intense selection pressure to grow fast enough to attain a critical size to maximise marine survival (Lundqvist *et al.*, 1994), those staying as residents preferentially still attempt to regain lost skeletal growth during the summer. It should be noted however, that the deprivation in July coincided with the timing of the decision regarding which developmental pathway is to be adopted (Wright *et al.*, 1990). It was therefore not possible to separate those fish that were ultimately destined to form the LMG prior to the deprivation, and which entered as a result of the reduced feeding opportunity at this time. However, the absence of any relationship between the ratio of resource allocation and body size in those fish deprived during this crucial time indicated that larger fish did not appear to maximise growth in order to attain a threshold size and subsequently enter the UMG. By allocating more surplus resources to structural tissue during summer the LMG fish may benefit from an increased capacity for storage of utilizable energy in the winter, since larger fish tend to carry more fat (Elliott, 1976a: Metcalfe & Thorpe, 1992; Simpson *et al.*, 1992). It therefore appears that the importance of maintaining an adequate store of mobilizable energy stores during the winter outweighs the requirement to increase body size as any lost growth potential can presumably be regained the following year. This seasonal response appears well adapted to maximise the survival chances of the LMG during the winter.

Chapter 3.4 - Seasonal matching of appetite to anticipated energy requirements

3.4.1 Introduction

Many animals use stored fat as an energy insurance, preferentially drawing on it (rather than protein or carbohydrate) when unable to match intake to demands. However, the means by which they regulate fat reserves through modulation of feeding is poorly understood. Recent theoretical work (e.g. Lima, 1986; reviewed by Witter & Cuthill, 1993) suggests that the size of an energy reserve will vary with the relative costs and benefits of maintaining it. Thus, in winter diurnal birds should increase their fat reserves due to long nights of fasting, less predictable food supplies and increased metabolic demands. This response to short-term needs is widespread (see Witter & Cuthill, 1993).

However in some situations animals may reduce appetite (and draw on stored reserves) because feeding is risky or difficult, or is in direct conflict with some other demand such as egg incubation (LeMaho, 1977; Sherry *et al.*, 1980), migration (Alerstam, 1990), hibernation (Mrosovsky & Barnes, 1974; Torke & Twente, 1977) or overwinter survival (Metcalfe & Thorpe, 1992). The question thus arises as to how appetite should be regulated and reserves used in these situations. There is some evidence that energy levels are actively defended by feedback controls on appetite (Mrosovsky & Sherry, 1980), but little is known about the ability to adjust appetite to match projected energy requirements.

Resident salmon parr show a suppressed appetite over their first winter (Metcalfe & Thorpe, 1992). This natural anorexia commences in early autumn independent of water temperature or food availability (Metcalfe *et al.*, 1986) and lasts until spring, when water temperature rises and food becomes more plentiful. Food intake during the anorexic interval is insufficient to maintain energy reserves (Gardiner & Geddes, 1980; Higgins & Talbot, 1985).

Previous work (Metcalfe & Thorpe, 1992) has shown that appetite at the commencement of overwinter anorexia is sensitive to energy levels. While glycogen and protein are used as supplementary energy stores, the most important and labile store is body lipid (Weatherley & Gill, 1987), which is depleted whenever food intake is suppressed (Miglavs & Jobling, 1989). Here I provide empirical data that tests the prediction that responses to deviations from the programmed path of reserve loss should vary across the season, demonstrating for the first time a connection between short-term feeding behaviour and long-term optimisation of survival through projection of energy requirements; the model providing these predictions is presented in Bull *et al.*, (1996; see Appendix III.).

3.4.2 Materials and Methods

Offspring of sea-run adults from the River Almond, Perthshire were reared at the SOAFD Almondbank hatchery prior to experiments at the University Field Station, Rowardennan, Loch Lomond. Forty fish of fork length < 70mm (to maximise the proportion that would delay smolting for another year; Metcalfe *et al.*, 1988), were selected on 28 September, 1994 and given individual combinations of alcian blue dye marks (Metcalfe *et al.*, 1988) on their undersides. They were divided into two size-matched groups of 20. The control group was maintained in a 1m^2 holding tank and experienced ambient water temperatures (figure 3.11), simulated natural photoperiod and excess food (dispensed every 10 min by automatic feeder) except during feeding trials.

All fish in the experimental group experienced three separate 3-week periods of food deprivation (termed Early, Mid- and Late Winter) commencing on 3 October, 5 December and 6 February respectively. During deprivation fish were held without food in a 1m^2 tank inside a controlled temperature cabinet (with simulated natural photoperiod) kept at 6.0 ± 1.0°C, ensuring a constant temperature during each period. Measurements allowing estimation of body fat level (using equation 2.2) were taken immediately before, and 0 and 30 days after each deprivation period.

At the end of each deprivation period, all experimental and control fish were established in randomly selected compartments of long raceways, (figure 3.2) and were left to settle for 4 days during which bloodworms were hand fed to excess twice a day. This was necessary to ensure that any subsequent observed differences in appetite between groups were not due to differences in gut fullness prior to trials.

Appetite trials were then conducted between 1000hr and 1200hr every second day for the next 3 weeks, following the procedure documented in chapter 3.2. Each fish was presented with a minimum of 20 bloodworms following each trial, and on days between trials, to ensure they received *ad lib.* rations.

At 10 and 20 days since establishment in the raceway all fish were moved to a new randomly-allocated compartment to control for variations in water flow between compartments. Following the final appetite trial all fish were transferred to a $1m^2$ holding tank where they were fed excess pelleted food. Two weeks later, fish in the experimental group experienced the next period of food deprivation and the cycle was repeated. Any dead fish were replaced at this point.

3.4.3 Results

Two fish grew throughout the winter (increasing > 20mm in length) and underwent smolt metamorphosis and were subsequently omitted from analyses. Five experimental group fish died throughout the course of the experiment.

Control fish entered anorexia in early winter (figure 3.11), and their body lipid levels consequently dropped from 5.32 ± 0.42% of body weight ($n = 18$) in September to 2.63 ± 0.40% ($n = 18$) in April (figure 3.13), despite food always being in excess.

The three deprivation periods caused significantly accelerated lipid depletion: the mean reductions in fat during the Early, Mid, and Late Winter periods were 1.12 ± 0.22% of body weight (paired *t*-tests, $t = 2.83$, d.f. = 19, $P<0.01$), 1.00 ± 0.33% ($t = 3.03$, d.f. = 18, $P<0.01$) and 1.94 ± 0.31% ($t = 6.18$, d.f. = 19, $P<0.001$) respectively. These reductions were significantly greater than the concurrent changes in control

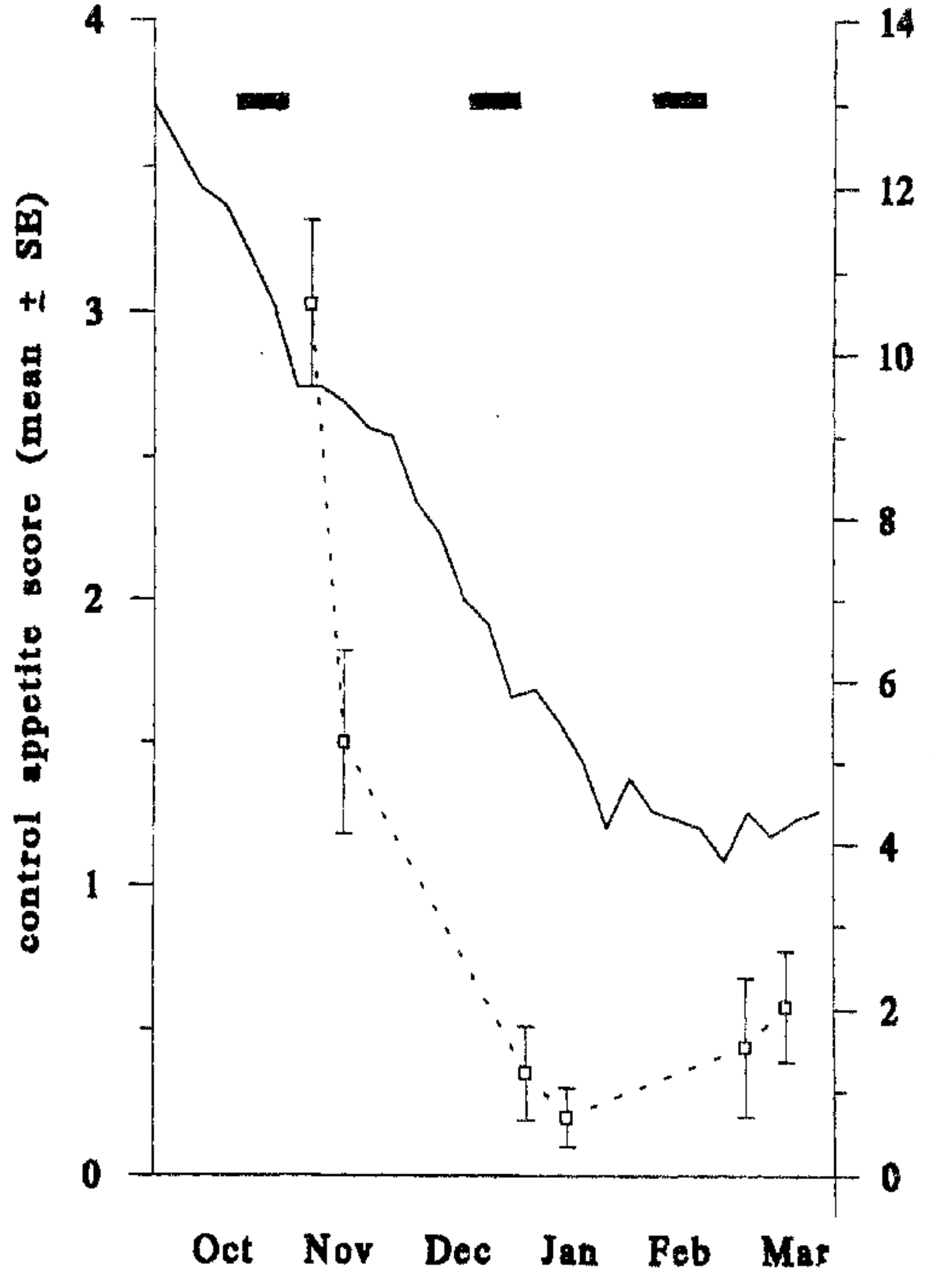

Figure 3.11. Seasonal decline in appetite of control fish for days 1-14 and 15-28 of trials in Early, Mid- and Late Winter; the solid line shows ambient water temperature and the solid bars indicate periods of food deprivation for experimental fish.

fish during the Mid- and Late Winter deprivations (*t*-tests on fat change between treatments, $t = 2.60$, d.f. = 33, $P<0.05$; $t = 4.56$, d.f. = 33, $P<0.001$ respectively) but not during the Early Winter period, owing to a reduction in control fat levels ($t = 1.07$, d.f. 34, N.S.).

Individual daily appetite scores for experimental fish during refeeding were calculated as residuals from the mean value for control fish on that day; elevated or reduced appetites were indicated as positive or negative values respectively. This eliminated variation due to parallel fluctuations in appetite in the two treatment groups of fish between trials. Following deprivation in Early Winter, experimental fish exhibited a marked elevation in appetite relative to controls over the first 14 days of refeeding. However no such effect was apparent after periods of deprivation in Mid- or Late Winter (figure 3.12), despite their body lipids being lower by this time. The Early Winter elevation in appetite was short-lived: appetite scores over the period 16 to 28 days of refeeding were no different from controls, regardless of season (figure 3.12).

These appetite responses resulted in fat levels of experimental fish increasing during the refeeding period in Early Winter (mean increase of 0.49 ± 0.23% of body weight; paired *t*-test between sampling dates, $t = 2.11$, d.f. = 18, $P<0.05$), but not following Mid- and Late Winter deprivations (mean reduction 0.05 ± 0.39%, $t = 0.13$, d.f. = 18, N.S.; 0.37 ± 0.34%, $t = 1.08$, d.f. = 18, N.S. respectively; figure 3.14).

Seasonal variation in appetite responses were compared by considering a ratio expressing the elevation in appetite over the first 14 days of refeeding per unit loss of fat:

$$R = \frac{\text{Mean residual appetite}}{\text{\% fat lost during deprivation period}} \qquad \textbf{(eqn. 3.4)}$$

As predicted, fish showed a relatively far greater elevation of appetite in response to an accelerated loss of lipid in Early Winter than later in the year (figure 3.15), despite

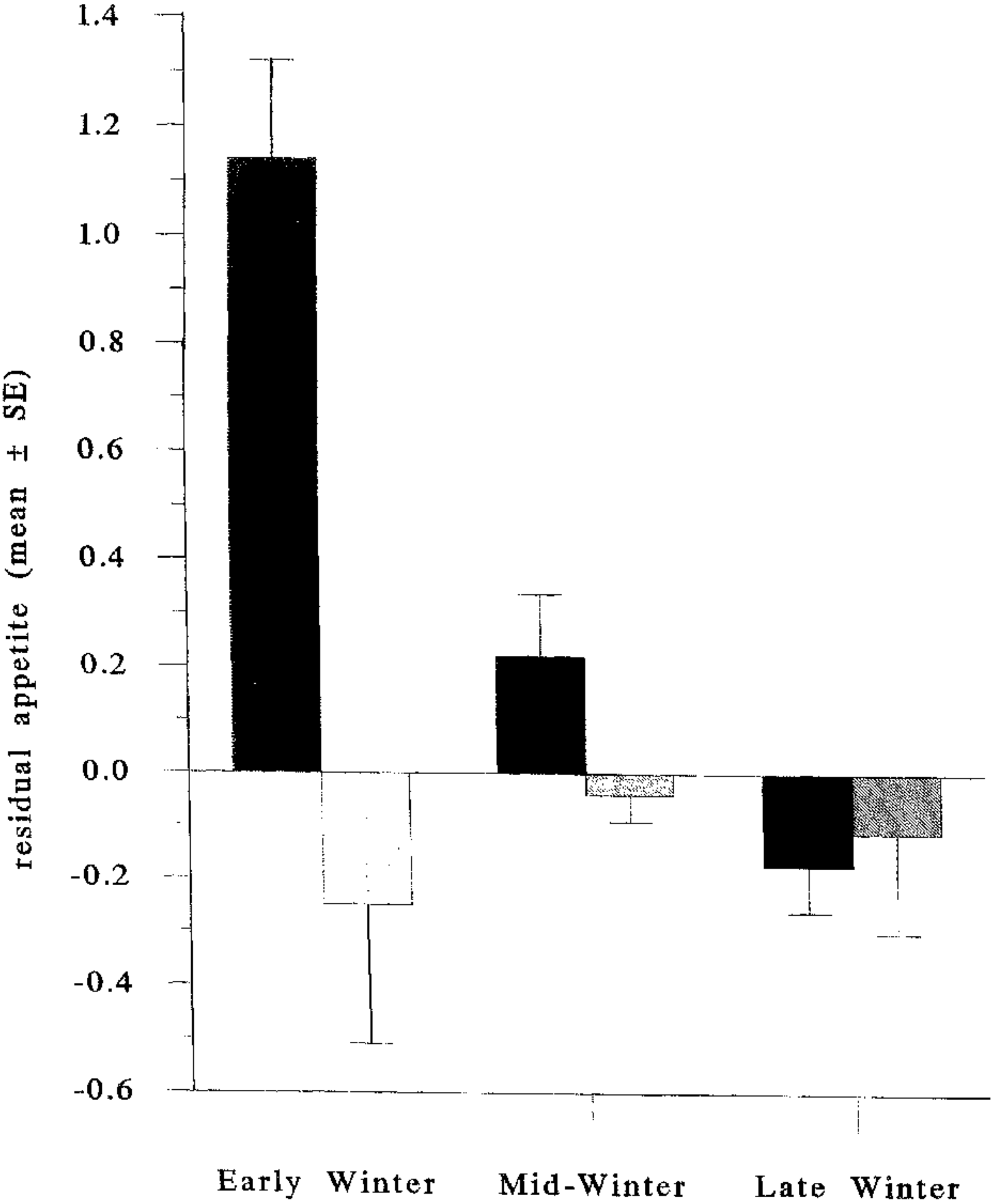

Figure 3.12. Changes in appetite of experimental fish following deprivations (means ± SE for days 1-14 (dark bars) and 15-28 (light bars) during refeeding). Experimental fish appetite was significantly higher than controls following Early Winter deprivation ($t_{33} = 3.11$, $p<0.01$), and was greater following Early Winter deprivation than either Mid- or Late (Kruskal-Wallis ANOVA, $\chi^2 = 14.61$, df = 2, $p<0.001$).

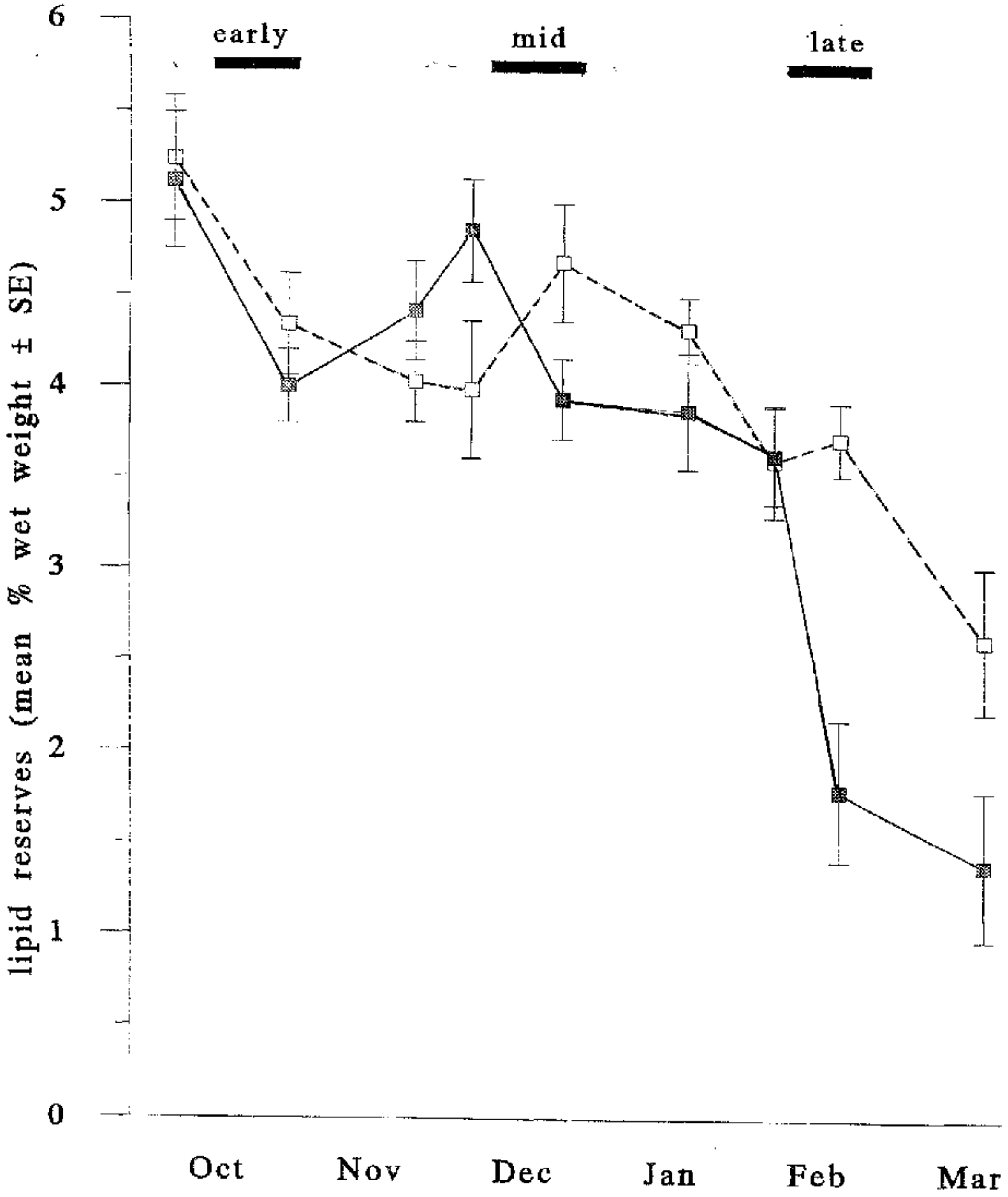

Figure 3.13. Changes in lipid level during the course of the experiment (solid squares represent experimental fish and open squares represent controls; periods of food deprivation indicated by black bars).

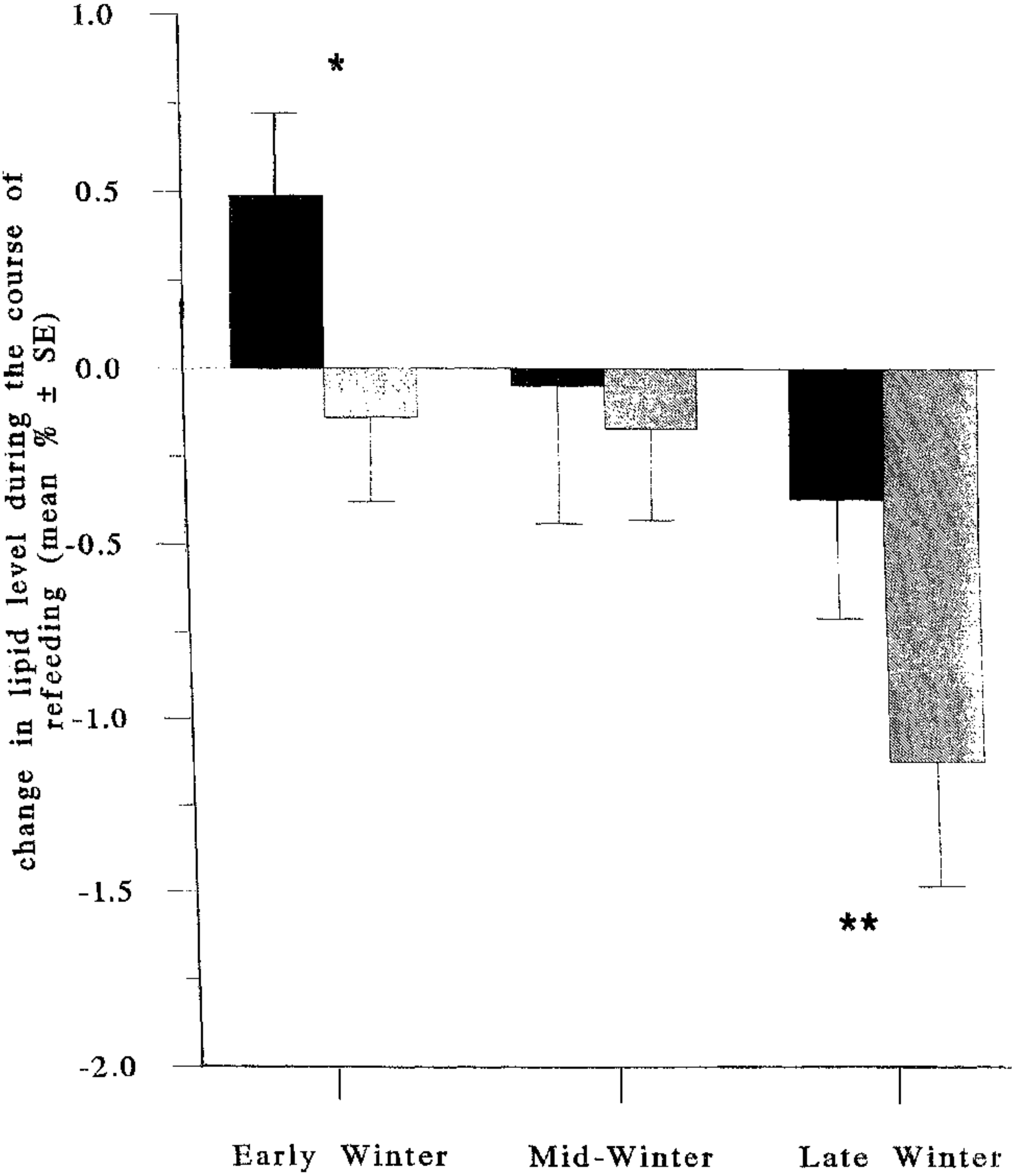

Figure 3.14. Changes in lipid during refeeding following deprivation in Early, Mid- and Late Winter for experimental (dark bars) and control fish (light bars). Asterisks indicate *t*-tests comparing treatments; *p<0.05, **p<0.01.

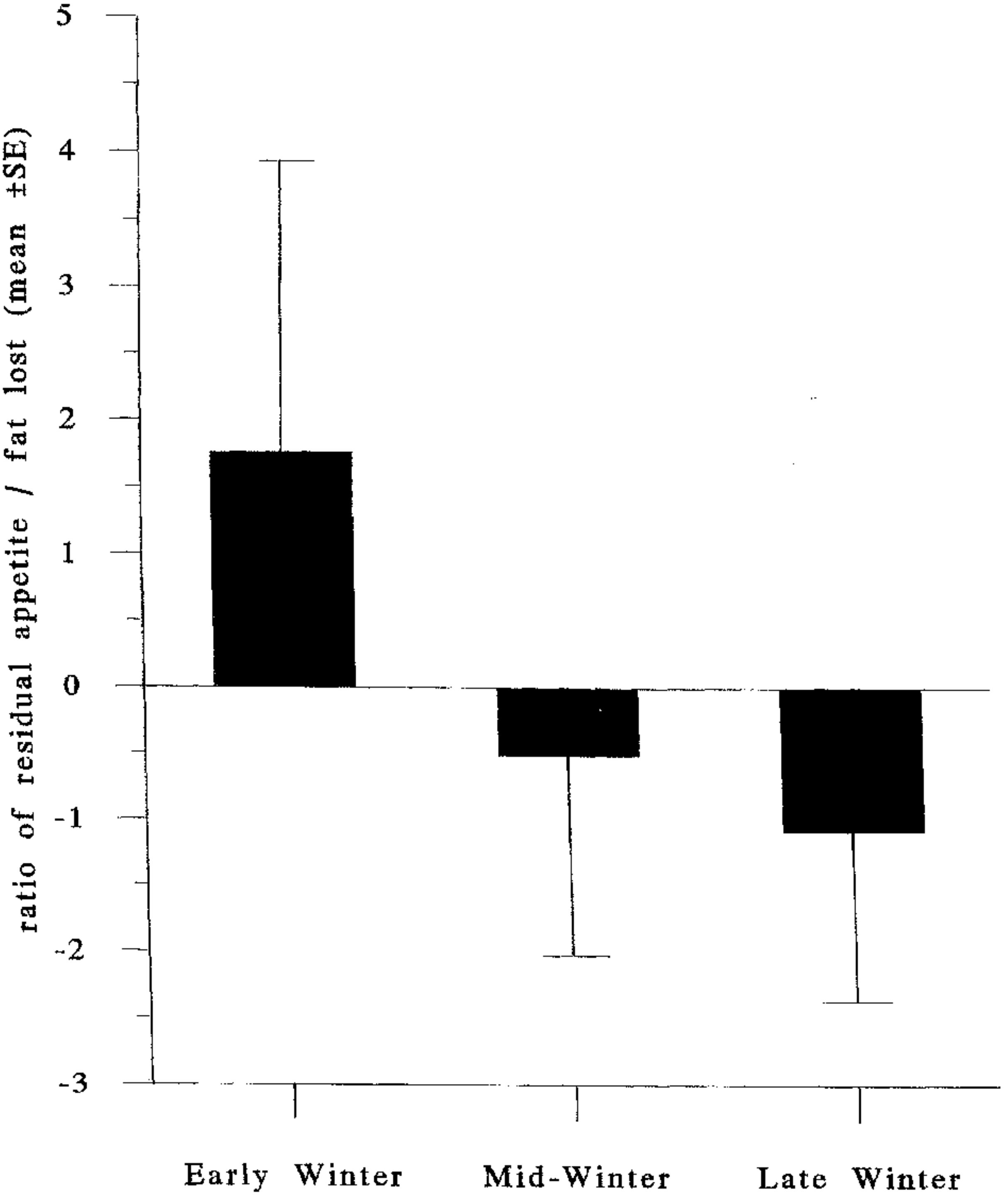

Figure 3.15. Values of R (elevation of appetite per unit loss of fat; see text) following deprivation periods in Early, Mid- and Late Winter. The appetite response to accelerated lipid loss declined over the course of the winter (Kruskal-Wallis ANOVA between times $\chi^2 = 6.53$, df = 2, $p<0.05$).

their body lipid levels being higher; their appetite therefore reflected future needs rather than current state.

3.4.4 Discussion

Despite excess food, all fish showed a depletion of energy reserves over the winter (figure 3.13), in agreement with other studies (Egglishaw & Shackley, 1977; Gardiner & Geddes, 1980; Higgins & Talbot, 1985). It therefore appeared that the fish were following seasonal trajectories towards a low target level of lipid early in the spring, when the expected improvement in conditions would allow rapid replenishment (Cunjak & Power, 1986). The seasonal variation in the appetite response to deviations from this lipid trajectory (figure 3.12) suggests that the fish facultatively responded not to their current reserve level, but to their projection of whether they would be above or below the target level at the end of the winter: thus they foraged harder to restore lost lipids earlier in the winter despite having greater reserves at the time. It might be argued that the colder temperatures later in the winter would prevent fish from expressing any increase in appetite after a period of deprivation. However, the fish would be physiologically capable of feeding at a higher rate since the intake by control fish appears much lower than that predicted from existing published data relating food intake by salmonids to temperature: Elliott (1976b), using brown trout (*Salmo trutta* L.) as subjects and *Asellus* spp. as food items. The magnitude of the change in appetite response between early and late winter (see figure 3.15) is therefore much greater than any metabolic constraints imposed by desreasing temperatures. The experimental data exactly match those predicted by a mathematical model that calculates the level of foraging effort that maximises overwinter survival for juvenile salmon (Bull *et al.*, 1996; see Appendix III); the fish therefore appear to regulate their appetite so as to optimise long term survival rather than short term gains.

Studies have shown that many juvenile salmonids exhibit a rapid decline of body lipid early in the winter (Egglishaw & Shackley, 1977; Gardiner & Geddes, 1980; Cunjak, 1988). However these results show a relatively constant level of body lipid between mid-October and January (figure 3.13). A possible explanation is that the stable low water temperatures reduce the costs of metabolism and temperature acclimatisation (Cunjak & Power, 1987; Cunjak, 1988), thus leading to a balance between assimilable energy and maintenance requirements.

An alternative explanation is that the fish are responding to the unpredictable nature of their environment during this time. While salmon respond to photoperiod as a cue for change of season (Villareal *et al.*, 1988), the arrival of better feeding conditions in spring is still unpredictable due to substantial interannual variation in spring temperatures. Studies with birds show that individuals faced with unpredictable feeding opportunities carry higher levels of fat as insurance (Rogers, 1987; Ekman & Hake, 1990; Ekman & Lilliendahl, 1993). Thus, if the salmon continued to lose fat at the early winter rate, the chances of survival when faced with a late spring might be minimal. A more cautious strategy (i.e. the maintenance of greater fat reserves in mid-winter) requires a higher foraging effort, and thus the acceptance of a greater predation risk, since escape responses are slowed by low water temperatures (Webb, 1978). As water temperatures drop below 10°C, salmon switch from diurnal to nocturnal foraging, hiding during the day in streambed refuges (Fraser *et al.*, 1995). This behavioural switch is controlled solely by temperature and is consistent with the idea of reducing foraging risks: nocturnal foraging may allow the maintenance of a higher lipid insurance without increased predation costs.

These empirical results would not have been predicted from previous studies on the dynamics and function of fat reserves. Indeed it would appear counter-intuitive that the animals exerted greater foraging effort when their lipid levels were higher. The results can be explained if the fish are anticipating future energy requirements, and responding appropriately to maximise their survival chances.

Chapter 3.5 - The use of photoperiod to time seasonal appetite responses

3.5.1 Introduction.

Under hatchery conditions, the length-frequency distribution of sibling populations of juvenile salmon becomes bimodal (see chapter 1). The decision regarding which developmental strategy is adopted has been shown to have both genetic and environmental components. The maintenance of appetite and its associated growth during the late summer exhibited by those destined to smolt the following year (Higgins & Talbot, 1985; Metcalfe *et al.*, 1988) has been found to be under the control of an endogenous rhythm synchronised by photoperiod in midsummer (Thorpe, 1986; Villarreal *et al.*, 1988; Adams & Thorpe, 1989*a,b*). By controlling the growth opportunity in terms of daylength and temperature prior to the crucial midsummer period, these authors were able to manipulate the proportions of fish in either the upper or lower modes of the bimodal distribution. With increased growth opportunity in late summer, more fish maintained appetite and growth and subsequently entered the upper mode. In addition to exerting control over the developmental strategy adopted, photoperiod also exerts control over the timing of the cessation of growth exhibited by the lower mode fish (Thorpe, 1986; Villarreal *et al.*, 1988) whose developmental pathway has been fixed. This reduction in growth rate is mediated by a reduction in feeding motivation during this time (Metcalfe *et al.*, 1986; Metcalfe & Thorpe, 1992).

Although the reduction in food intake displayed by the LMG fish during winter (Higgins & Talbot, 1985) will be affected by low water temperatures (Elliott, 1975; Brett, 1976; Love, 1980), their ability to match feeding effort to the appropriate phase of the winter (chapter 3.4) would appear to act independently of seasonal temperature changes, implying that some other environmental cue is used in order to judge the time of year. Although temperature acts to govern the rate of physiological response, it is not in itself an accurate cue to the changing season (Clarke *et al.*, 1978) as it exhibits significant interannual variation, and fish require a more consistent means of gauging the time of year. As photoperiod conveys more reliable seasonal information than temperature (Villarreal *et al.*, 1988) the fishes' sensitivity to this cue in the timing of appetite suppression may well extend into winter and act to synchronise the appropriate feeding effort.

Here I test this prediction by manipulating the 'perceived' seasonal photoperiod trajectory of the fish throughout the time when appetite and energy reserves are under internal control. By controlling for temperature and imposing a period of food deprivation on fish experiencing 'perceived' early and late winter photoperiod cues, I was able to investigate their use of the latter zeitgeber in synchronising the appropriate feeding behaviour.

3.5.2 Materials and Methods.

Eggs from a pair of wild sea-run adult Atlantic salmon from the Loch Lomond catchment were hatched at the University Field Station in the spring of 1995, and the juveniles reared in a 1m tangential flow tank on pelleted food. One hundred and fifty of the smallest fish were selected for the experiment on 30 August in order to maximise the proportion of potential LMG fish (Metcalfe *et al.*, 1988). Fish were measured (fork length to the nearest mm), weighed (to the nearest 0.01g) and assigned to one of three size-matched groups: accelerated, delayed and control (n = 50 each). Each group was established in a separate tank where food was provided every twenty minutes from identical automated feeders, providing *ad lib.* rations 24 hours a day to every group. Each tank received ambient temperature water from a common reservoir tank constantly renewed by water pumped from Loch Lomond. The three groups were housed inside a light-proof screen and were separated from each other by lightproof partitions ensuring that in the absence of an artificial light source, all groups were kept in complete darkness. A single fluorescent tube was suspended 40 cm above the water surface of each tank and connected to a separate electronic timer switch, programmed to provide a controlled period of light every 24 hours. The three groups of fish therefore experienced the same seasonally changing water temperatures (figure 3.16) and constant feeding regime, but differed only in perceived daylength. The timers were changed at regular intervals to provide photoperiod cues to

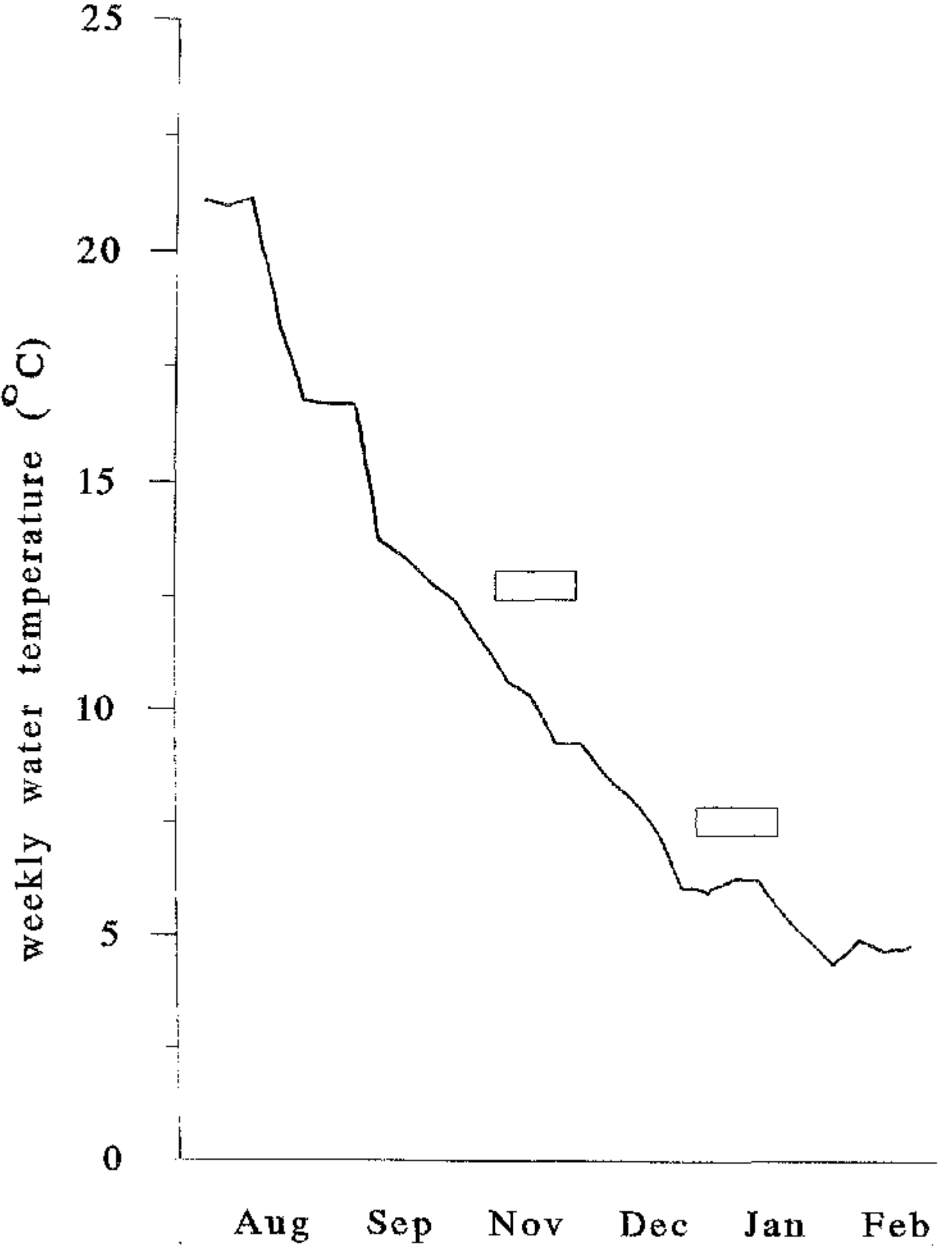

Figure 3.16. Water temperature throughout the course of the experiment. Open bars denote the timing of periods of food deprivation, applicable to all three groups.

accelerate, slow or maintain the seasonal daylength trajectory throughout the next six months (table 3.3).

Table 3.3. Manipulated daylengths experienced by the three groups of fish throughout the course of the experiment.

	Accelerated group		*Delayed group*		*Control group*	
true date	daylength (hours)	equivalent date	daylength (hours)	equivalent date	daylength (hours)	equivalent date
13 Sept	11.75	4 Oct	14.25	30 Aug	13.25	13 Sept
27 Sept	10.25	27 Oct	13.75	6 Sept	12.25	27 Sept
11 Oct	8.75	21 Nov	13.25	13 Sept	11.25	11 Oct
27 Oct	7.75	13 Dec	12.45	20 Sept	10.25	27 Oct
12 Nov	8.75	4 Jan	12.25	27 Sept	9.25	12 Nov
1 Dec	10.25	29 Jan	11.75	4 Oct	8.25	1 Dec
25 Dec	11.75	28 Feb	11.25	11 Oct	8.75	25 Dec
13 Jan	14.25	28 Mar	10.75	19 Oct	9.25	13 Jan
29 Jan	16.25	27 Apr	10.25	27 Oct	10.25	29 Jan
14 Feb	16.75	27 May	9.75	4 Nov	11.25	14 Feb

Fat estimation (see equation 2.2) commenced on 9 October and was carried out on all fish at approximately two week intervals throughout the experiment prior to any further manipulations. Following fat assessment on 30 October, the feeders on all tanks were switched off until 20 November; all three groups therefore experienced a food deprivation period of three weeks. During this time the accelerated group were experiencing a photoperiod regime equivalent to December while the delayed group were subject to a regime equivalent to September. Control fish received the photoperiod regime appropriate for the time of year. Fat assessment was made upon completion of the deprivation period.

On 20 November all feeders were switched on and food was made available to all fish for four days during which time they remained undisturbed. In order to assess feeding intensity following the deprivation period, food intake was then measured at five day intervals during the next two weeks (24 November, 29 November and 4 December). The large overall sample size ($N = 150$) prohibited individual appetite assessment by the techniques used in chapters 3.2-3.4 and a radiographic method developed by Talbot & Higgins (1983) was therefore employed. Labelled food was made incorporating X-ray dense glass beads (Ballotini size 9; Jencons Ltd., Leighton Buzzard, U.K.) at a concentration of 9% by weight. This concentration, although higher than that used in other studies (e.g. Simpson, 1993; Nicieza & Metcalfe, submitted), was chosen as a result of pilot trials whereby the inclusion of a lesser quantity of beads misrepresented the low rates of food intake experienced at winter water temperatures. Known weights of labelled food were X-rayed (using a Todd Research 80/20 X-ray unit and Kodak Industrex CX film) and the number of Ballotini counted. By regressing weight of food on the number of beads it was possible to estimate food intake (g) from the original count:

$$\text{weight of food (g)} = 0.0019\,(\text{number of Ballotini}) + 0.0007 \qquad \textbf{(eqn. 3.5)}$$

$n = 19$ samples of food, $r^2 = 0.979$, $P<0.0001$.

On the day of the trial, the normal unlabelled food was removed from all three feeders at 1000 h and replaced with the labelled food. After 4 hours (1400 h), the labelled food was removed and the original unlabelled food restored. The fish from each group were removed, identified and X-rayed (exposure time 1.5s) under anaesthetic (benzocaine). It took less than one hour to X-ray all the fish, after which time they were re-established in their original tank. A maximum of 5 hours therefore elapsed between the fish first being able to feed on the labelled food and their being X-rayed, considerably less than the gut evacuation time at winter temperatures (Higgins & Talbot, 1985). X-ray plates were developed and the number of fish feeding was established. The food ingested by these fish during the trial period was expressed as a percentage of their individual wet body weight (g) consumed per hour (% b.w.hr^{-1}). Wet weight was estimated on the day of the trial by interpolation of wet weight measurement taken on the closest sampling dates. Fish were reassessed for fat on 5 January after which time a second three week food deprivation period was started (8 - 29 January). During this second deprivation, the accelerated group were experiencing a photoperiod regime appropriate to April, while the delayed group were receiving the regime of October (table 3.5). Upon completion of the second deprivation, fat and food intake were estimated as before and a final fat estimation took place on 19 February, at which time all fish were assessed for external signs of smoltification (i.e. silvered flanks and darkened fin edges).

3.5.3 Results

Ten fish continued to grow throughout the course of the experiment (number in accelerated group = 4, delayed group = 5, controls = 1) and showed external signs of smoltification on 19 February; these were subsequently excluded from the analysis. An additional 21 fish died throughout the course of the experiment (accelerated group = 5, delayed group = 8, controls = 8), so that by the end of the experiment, the initial sample size of 150 had been reduced to 119 non-smolting survivors (number in accelerated group = 41, delayed group = 36, controls = 41). Both smolting and mortality were independent of group (χ^2 between treatments comparing proportion smolting, $\chi^2 = 3.36$, d.f. = 2, N.S; mortality, $\chi^2 = 0.98$, d.f. = 2, N.S) There were no differences in the overall size or weight of LMG survivors at the start, or completion of the experiment (ANOVA amongst treatments on length and weight, all N.S.; table 3.6).

Table 3.6. Body size (fork length) and weight of each treatment group at the start and completion of the experiment.

	Fork length						Wet weight					
	30 August			19 February			30 August			19 February		
	mean	S.E.	n	mean	S.E	n	mean	S.E.	n	mean	S.E.	n
Acc	49.3	0.55	46	70.6	0.72	41	1.39	0.05	46	3.32	0.10	41
Del.	48.7	0.51	45	70.0	0.99	36	1.27	0.04	45	3.42	0.15	36
Con	49.5	0.52	49	70.7	0.95	41	1.34	0.05	49	3.44	0.14	41

Fat dynamics

First deprivation period

There was a strong, positive relationship between body size (fork length) and fat levels in the control group during the course of the experiment (appendix I). To control for this effect, comparisons between groups were made using ANCOVA with fork length as covariate. Prior to the first deprivation period in November, all three groups had the same level of body fat (ANCOVA between treatments on fat level controlling for body size; $F_{(2,133)} = 0.38$, N.S.). The first deprivation period had a significant effect on the body fat levels of all three groups (figure 3.17) with mean reductions in body fat of 1.74% ± 0.11 S.E., 1.71% ± 0.13, and 2.10% ± 0.12 for the accelerated, delayed and control groups respectively (paired *t*-tests on fat level in individual fish before and immediately after the deprivation period: accelerated, $t = -16.18$, d.f. = 44, $P<0.01$; delayed, $t = 13.35$, d.f. = 42, $P<0.01$; controls, $t = 17.03$, d.f. = 44, $P<0.01$). Unexpectedly, the deprivation caused significant variation in fat loss between groups (ANOVA between treatments on fat loss during deprivation; $F_{(2,132)} = 3.32$, $P<0.05$) although the differences between individual groups were slight (Tukey's HSD test: no two groups differ at $P<0.05$). During the following three weeks of refeeding, all three groups responded to the deprivation by increasing their fat stores by an average of 1.02% ± 0.13, 1.27% ± 0.11, and 1.91% ± 0.13 for accelerated, delayed and control groups respectively. When the daily rate of change in fat was calculated (see chapter 3.2) during this first refeeding period, there was no difference between the gains exhibited by either the accelerated or delayed groups indicating that the photoperiod manipulation had no effect upon restoration of losses at this time (figure 3.18a). However, the control fish were gaining fat at a faster rate than either the accelerated or delayed groups, presumably as a result of their incurring a slightly greater fat loss (ANOVA between treatments comparing daily fat gain during refeeding; $F_{(2,127)} = 13.62$, $P<0.01$: Tukey's HSD test indicates that controls differ from both accelerated and delayed groups at $P<0.05$). However there were no differences between groups in the daily rate of fat gain when using the longer time period from the end of the first deprivation period on 20 November to the beginning

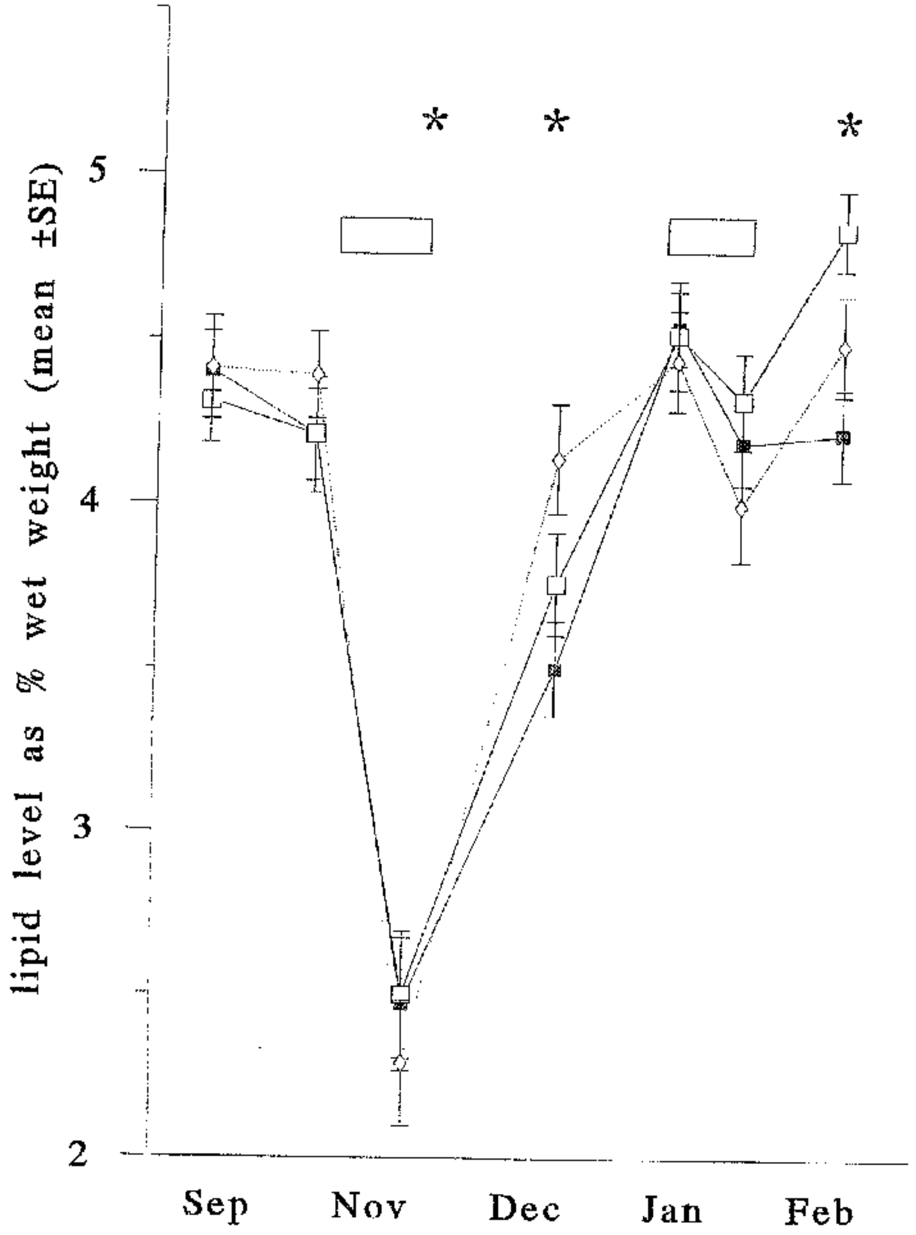

Figure 3.17. Changes in lipid level during the course of the experiment (solid squares represent accelerated fish, open squares represent delayed fish, and open triangles represent control fish). Open bars denote the timing of periods of food deprivation, applicable to all three groups. Asterisks indicate results of ANCOVA comparing lipid levels between groups, controlling for body size; * = P<0.05, ** = P<0.01.

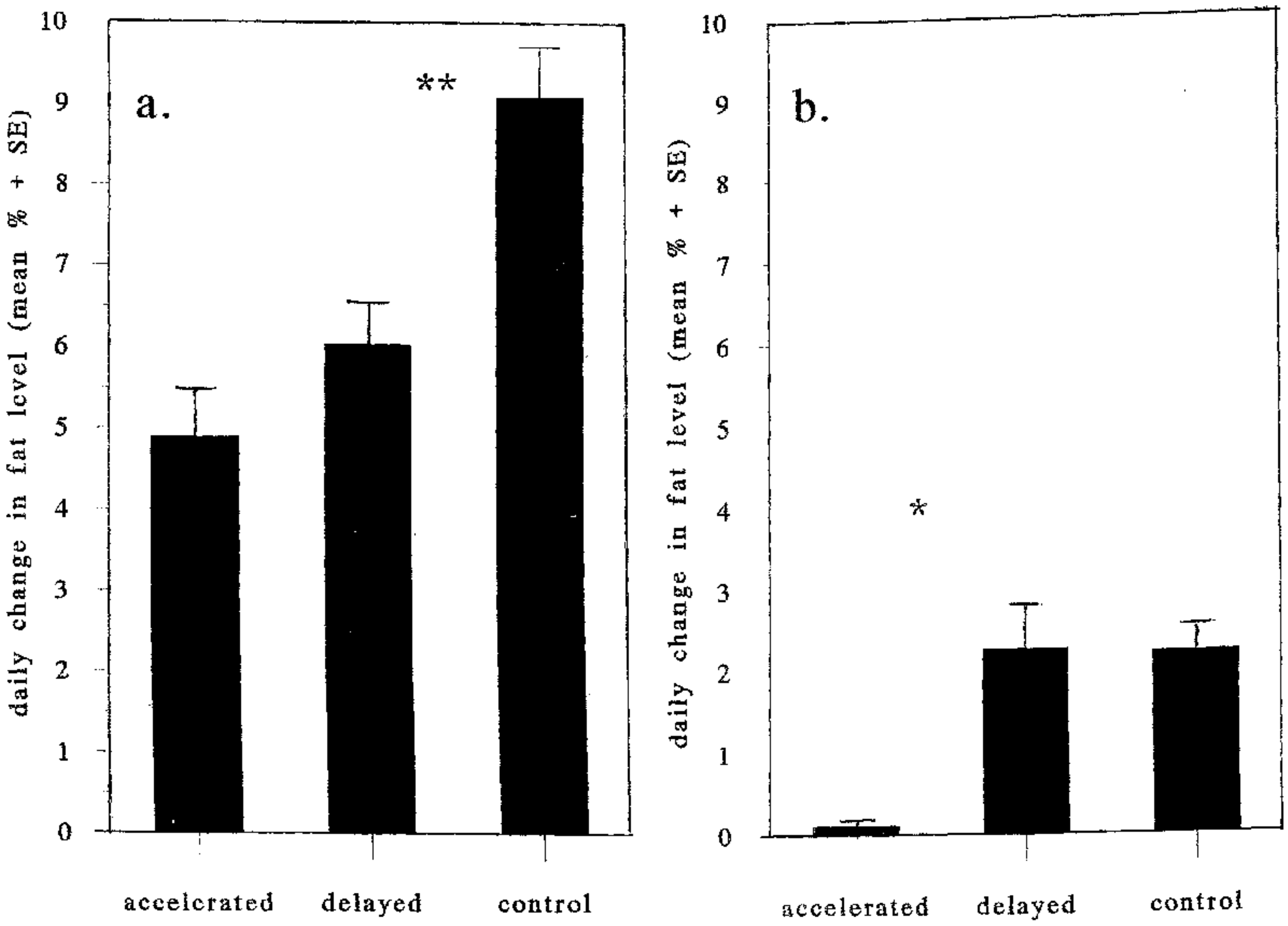

Figure 3.18. Daily changes in lipid level during the first three weeks following (a.) the first and (b.) the second deprivation periods for all three groups. Asterisks indicate results of ANOVA comparing between treatments, * = $P<0.05$, ** = $P<0.01$.

of the second on 5 January (ANOVA between treatments comparing daily fat gains; $F_{(2,121)} = 1.55$, N.S.).

Second deprivation period.

Fat losses incurred in November had been restored in all groups prior to the second deprivation period in January (figure 3.17), so that there were no differences between the groups in body fat levels on 5 January (ANCOVA between treatment fat levels controlling for body size; $F_{(2,121)} = 2.87$, N.S.). The second period of deprivation once again reduced the amount of body fat in all fish, with a mean reduction of 0.37% ± 0.11, 0.24 % ± 0.14 and 0.42% ± 0.07 for accelerated, delayed and control fish respectively (paired *t*-tests between the start and completion of the second deprivation: accelerated, $t = -3.43$, d.f. = 39, $p<0.01$; delayed, $t = -1.94$, d.f. = 36, $P<0.05$; controls, $t = -5.60$, d.f. = 38, $P<0.01$). However, the fat reductions were consistently smaller than those during the first deprivation (paired *t*-tests between the change in fat level experienced by the same fish during the first and second deprivations: accelerated, $t = 7.74$, d.f. = 39, $P<0.01$; delayed, $t = 6.86$, d.f. = 36, $P<0.01$; controls, $t = 13.84$, d.f. = 38, $P<0.01$), presumably as a result of the reduction in water temperature (figure 3.16). During the three week refeeding period following the second deprivation, both the delayed and control groups responded by increasing body fat (paired *t*-test between fat level on day one and day 21 of the refeeding period: delayed, $t = 4.21$, d.f. = 35, $P<0.01$; controls, $t = 6.70$, d.f. = 38, $P<0.01$) whereas the accelerated group did not restore losses and maintained body fat at their post-deprivation level ($t = 0.31$, d.f. = 39, N.S.). Accordingly, both the delayed and control groups were exhibiting a higher daily rate of fat gain during this time when compared to the accelerated group (figure 3.18b; ANOVA between treatments comparing daily fat gain during refeeding; $F_{(2,114)} = 8.83$, $P<0.01$; Tukey's HSD test indicates that both delayed and control groups differ from accelerated at $P<0.05$). As a result of these varied responses to fat loss, differences in body fat levels between the groups were still apparent by the end of the experiment in February (figure 3.17; ANCOVA between treatments on fat level controlling for body size, $F_{(2,117)} = 15.23$, $p<0.01$).

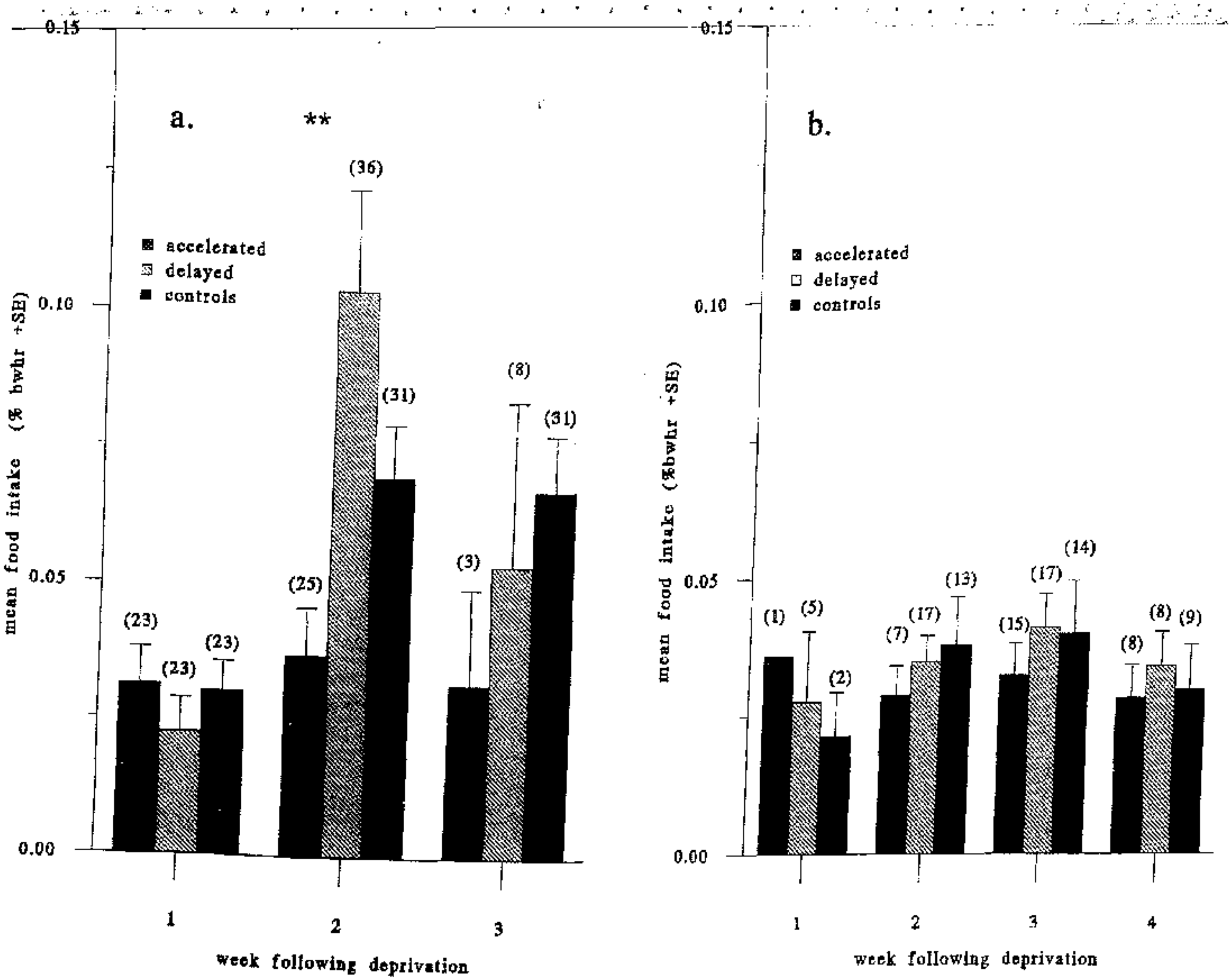

Figure 3.19. Food intake during X-ray trials in the weeks following (a.) the first and (b.) the second food deprivation periods for all three groups. The numbers in parentheses are the sample size of feeding fishes upon which the mean intake is calculated. Asterisks indicate ANOVA comparing between treatments, * = P<0.05, ** = P<0.01

Feeding intensity following food deprivation.

There were no consistent trends displayed in the feeding behaviour of any group following either of the deprivation periods.

Although there were always a number of fish feeding during every feeding trial following the deprivation period in November (figure 3.19a), analysis of the numbers of fish that consumed any of the labelled food during a given trial revealed that a greater proportion of the delayed fish were feeding during the second week of refeeding than of either the accelerated or control fish ($\chi^2 = 8.22$, d.f. = 2, $P<0.05$). However, during the third week, a higher proportion of control fish were feeding than in either the delayed or accelerated groups ($\chi^2 = 49.95$, d.f. = 2, $P<0.01$). In addition, the amount consumed by the delayed fish that did feed during the four hour trial was higher than that by the accelerated group (ANOVA between all three treatments on the quantity of food consumed; $F_{(2,92)} = 5.21$, $p<0.05$; Tukey's HSD indicates that delayed group differs from accelerated at $P<0.05$).

Following the deprivation period in January fewer fish were responding to food (figure 3.19b). The proportion of delayed fish feeding during the trial in the second week of refeeding was again higher than either the accelerated or control groups ($\chi^2 = 7.94$, d.f. = 2, $P<0.05$) but there were no differences in the numbers feeding during weeks 1, 3 or 4 (χ^2 all N.S.). There was no difference between groups in the individual quantities of food consumed by feeding fish during trials during the course of refeeding (ANOVA between all three treatments on the quantity of food consumed, all N.S.).

3.5.4 Discussion

The results indicate that photoperiod can act to synchronise the timing of the appropriate restoration of energy deficits, but proved inconclusive in terms of the feeding intensity during the restoration periods. The fat dynamics shown by all three groups following the second deprivation (figure 3.18b), when a discrepancy in photoperiod regime of five months between the delayed and accelerated groups had been established, are comparable with the responses to deprivations in 'early' and 'late' winter (see chapter 3.4). Fish that were receiving visual cues that the second deprivation to have taken place in early winter responded by restoring losses, whereas those that received cues to it having occurred in late winter maintained a low post-deprivation level of body fat. The response in terms of fat levels shown by the control fish following the second deprivation was higher than would be predicted from the results of chapter 3.4 but may in some respects have been elevated as a result of this group exhibiting the largest loss of fat during deprivation. As temperature was equal across all three groups, the differences in restoration of losses can only be attributed to seasonal information derived from photoperiod cues.

Previous studies have shown that growth, smolting and maturation in salmonids are influenced by photoperiod manipulations (Thorpe, 1986; Adams & Thorpe, 1988b; Villarreal *et al.*, 1988; Thorpe *et al.*, 1989). Clarke *et al.* (1994) and Duston & Saunders (1995) both reported a considerable advancement in smolting as a result of advancing photoperiod by two months, although the latter authors were using fish that had been maintained with both heated water and long days prior to the manipulation and would doubtless be at a more advanced stage as a result. Steffansson *et al.* (1991) found that both growth and the timing of smolting was advanced as a result of increasing the number of daylight hours experienced.

In the present study, the timing of the first deprivation period (two months since the start of photoperiod manipulations) may not have allowed sufficient phase-shifting to result in measurable differences in response between the groups. Villarreal *et al.* (1988) reported that a three month phase shift starting from first feeding, and advances of two, three and four months commencing after midsummer had no effect on the overall growth performance of juvenile salmon during the winter. Duston & Saunders (1992) were able to manipulate the timing of smoltification and maturation by using compressed cycles of the annual photoperiod (6, 12 and 18 months) but commenced these treatments from the eyed-egg stage. Thrush *et al.* (1994) used a similar compressed cycle on LMG fish commencing in December and reported successful advancement of smolting by up to five months. However, these authors found that the smolting characteristics appeared later in the manipulated photoperiod regime than would be expected under an ambient cycle i.e. on a decreasing photoperiod. This highlights an interesting feature in common with other studies (e.g. Bromage & Duston, 1986; Duston & Saunders, 1992), in that there is often a transitional acclimation period (termed a phase delay) in response to forcing an entraining zeitgeber such as photoperiod. This feature, typical of the behaviour of endogenous rhythms, cannot be discounted as influencing the results of the present study, with the phase shift caused by controlling the photoperiod leading to a response of lesser magnitude than anticipated from the results presented in chapter 3.4. If feeding and the control over body energy stores are controlled by an endogenous rhythm, entrained by photoperiod, further controlled investigations would be required for confirmation.

The lack of any conclusive evidence from the feeding trials (figure 3.19) may well have been influenced by the lack of sensitivity of the technique towards the motivation to feed. Although used successfully in other studies (Talbot & Higgins, 1983; Higgins & Talbot, 1985; Nicieza & Metcalfe *submitted*) the technique does not take account of any of the processes associated with the capture of prey items, and only records the number of captures that lead to ingestion. The appetite scoring procedure adopted in chapters 3.2 and 3.4 is a technique more suited for assessing the motivation to feed, and in all instances, the majority of scores include a number of precursor movements, with successful attacks being in the minority. As a result of the necessity to handle all fish during X-raying, the feeding intensity could only be estimated once per week of refeeding, whereas in previous investigations (chapters 3.2, 3.4), weekly measurements were based on the mean score of multiple trials giving a more robust estimate.

For any given temperature, the gut evacuation rate of juvenile salmon is faster on the ascending, as opposed to the descending arm of the seasonal photoperiod trajectory (Higgins & Talbot, 1985). As food intake is closely linked to the rate at which food is moved through the gut (Grove & Crawford, 1980; Godin, 1981), this may have led to higher recorded food intake in the accelerated group following both periods of food deprivation, and in the controls following the second as they were experiencing lengthening photoperiods.

Due to the small numbers of fish responding to food during the trials (figure 3.19), any individual differences due to such confounding factors as listed above may have contributed disproportionately to the appetite results. However, the results regarding the necessity to restore fat losses following deprivation do indicate that photoperiod is used to some degree by overwintering salmon to gauge the time of season, allowing optimal use of their energy stores.

Chapter 3.6 - Conclusions

The results presented in this chapter show that juvenile salmon exhibit varied responses in terms of elevating appetite and restoring energy reserves following periods of food deprivation at different times of the year. Following a deprivation period in early winter, food intake was regulated with regard to the nutritional status of the fish and the duration of any hyperphagic response was governed by the extent to which the energy stores had been depleted. This mechanism may allow the fish to maintain less efficient nocturnal foraging as a means of restoring fat losses, reducing the risk of predation at winter water temperatures.

The changing developmental goals of the LMG fish during their first year from maximising body size during the summer, to maintenance of internal fat stores at the expense of increased size in winter were reflected in their responses to periods of food deprivation. The fish exhibited preferential allocation of surplus energy into skeletal growth during the summer, and into fat restoration during the winter. Thus, the dynamics of feeding and fat during a period of compensatory growth were affected not only by the extent of energy loss, but by the season in which it occurred.

Within the winter season, the compensatory growth period changed with regard to the long-term projected energy state of the individual. These varied responses appeared to be cued by the change in daylength marking the advancement of the winter season. Both feeding and fat restoration following a deprivation period in early winter were greater than those exhibited following similar periods as winter progressed, despite them having greater reserves at this time. Fish seek to acquire large fat stores early in the winter to act as an insurance against long-term starvation, but the costs associated with restoring losses later on in the season outweigh the benefits of maintaining such a reserve. Thus, such a cautious strategy involving changing responses to food deprivation allows the fish to regulate their fat stores at levels that optimise overwinter survival chances.

Chapter 4 - Responses to an unpredictable feeding regime

4.1 Introduction

Many fish populations are subjected to natural periods of reduced food availability throughout the year. These may occur not only as a result of scarcity of prey, but also as a result of seasonal fluctuations in temperature, limiting the fishes' ability to acquire and process food (Elliott, 1972, 1976b; Brett, 1976; Webb, 1978; Jobling, 1980; Priede, 1985; Weiser & Forstner, 1986; Nicieza *et al,* 1994; Graham *et al.*, 1996). As a result, many species have developed the ability to withstand lengthy periods of food scarcity and rely upon internal energy stores to survive (Larsson & Lewander, 1973; Gardiner & Geddes, 1980; Flath & Diana, 1985; Booth & Keast, 1986; also see chapter 3). Seasonal changes in lipid content (the primary energy store for the majority of fish) in many species indicate that it is of great importance as a source of metabolic fuel during times when food is limited (Love, 1980; Young Cho & Bureau, 1995).

In temperate regions, the onset of winter and the associated prolonged period of food limitation may be anticipated by juvenile salmonids by using environmental cues such as seasonal reductions in water temperature and shortening of daylength (Thorpe, 1986; Villarreal *et al.*, 1988, see chapter 3.5). Autumnal increases in lipid stores in juvenile salmonids (Gardiner & Geddes, 1980; Cunjak & Power, 1986a; Simpson, 1992) may therefore be an adaptation to the approach of winter, with fish relying on internal energy sources throughout an extended period (see chapter 3.4). However, the nature of the physical environment that they occupy, namely high altitude steep gradient streams, in combination with an unpredictable climate, must superimpose short-term fluctuations upon normal seasonal changes in food availability. For example, spate conditions of high water flow and turbidity that may affect foraging success (Stradmeyer & Thorpe, 1987) cannot be anticipated much in advance by resident fish.

Recent work on overwintering birds has highlighted the importance of fat stores in preventing starvation (Lima, 1986, McNamara & Houston, 1990; Clark & Ekman, 1995, see chapter 3.4). Provided that starvation risk is an inverse function of fat reserves and a trade-off between starvation and predation risk exists, the optimal level of fat carried at any time will minimise both (Ekman & Hake, 1990; see Witter & Cuthill, 1993 for review). The value of fat stores increases when feeding opportunity becomes more uncertain (Lima, 1986; Gosler, 1987; Ekman & Hake, 1990; Ekman & Lilliendahl, 1993; Clark & Ekman, 1995) since they can act as a buffer or 'insurance' against starvation. Failure to hedge against short-term uncertainty in feeding opportunities by increasing stores of fat may result in death through starvation (Clark & Ekman, 1995).

The aim of this chapter was to investigate whether fish match energy reserves to the degree of stochasticity in food availability in a manner similar to that observed in birds. I therefore monitored the response of hatchery-reared juvenile salmon (previously reared on a predictable, abundant supply of food) to a prolonged period of unpredictable feeding opportunity during winter. Fat levels of fish exposed to an unpredictable sequence of feeding and fasting periods were compared to those of fish given continuous food throughout the winter.

4.2 Materials and methods

One hundred fish (mean forklength = 58.6mm, range = 52-65mm) were selected from a sibling population (the offspring of a pair of wild adults from the Loch Lomond catchment) on 13 September 1995. The fish were measured for length (to the nearest mm), weighed (to the nearest 0.01g) and measured for fat estimation (see chapter 2) prior to being separated into two size-matched groups (experimental and control groups, both n = 50) housed in identical 1m^2 tangential flow tanks. Both tanks were supplied with ambient temperature water (see figure 3.11) and were maintained under a simulated natural photoperiod regime. The experimental fish then experienced an alternating pattern of periods of food deprivation and refeeding over the next 6.5 months. The feeding regime was designed to provide an equal number of days of deprivation and feeding over the experimental period in total (200 days), but avoided the possibility of fish anticipating when food would be available by varying the lengths of both the deprivation and refeeding periods in a semi-random fashion (table

4.1). The control fish were provided with excess rations of food daily throughout the experiment.

The fish in each group were fed on pelleted food by way of an electronically timed feeder suspended above each tank, providing a trickle of food every 20 minutes throughout the 24 hour period. The amount of food available to each group was controlled by adjusting the aperture through which food pellets could leave the feeder. The control group received an amount of food approximating to 2% of their average wet body weight per day during the light period, in excess of the recommended maximum intake under good growing conditions. This level of food provision was maintained throughout the course of the experiment. During the periods of refeeding, the experimental fish were provided with approximately 4% of their average wet body weight per day during the light period. A greater quantity of food was made available to the experimental fish during their refeeding periods to ensure that both groups had received a similar total quantity of food by day 50, 100, 150 and 200 of the experiment, and any differences could not therefore be attributed to overall differences in food supply. The feeder was switched off during deprivation periods.

Both groups of fish were re-measured for fat estimation on the first day of each deprivation period experienced by the experimental fish. By measuring for body fat at the end of their refeeding periods, comparisons could be made with the control fishes, as both had been given access to food prior to estimation and had had their maximum opportunity to accumulate stores.

In addition to the routine measurement of fat levels, 5 additional measurements were taken for both groups of fish following food deprivation periods experienced by the experimental fish. These were taken to allow examination of fat dynamics during refeeding and seasonal changes in response to food deprivation.

Table 4.1. The design of the feeding regime imposed on the experimental fish during the course of the experiment. The initial food deprivation period commenced on 13 September. Asterisks indicate that fat was estimated at the end of the period.

Feeding regime	Duration (days)	Fat measurement
deprivation	15	*
ad lib.	10	*
deprivation	10	
ad lib.	15	*
deprivation	20	*
ad lib.	5	*
deprivation	5	
ad lib.	15	*
deprivation	10	*
ad lib.	10	*
deprivation	15	*
ad lib.	20	*
deprivation	5	*
ad lib.	5	*
total days of deprivation	**100**	
total days of *ad lib.* food	**100**	

All fish were re-weighed and measured on 1 April, 3 weeks (of *ad lib.* food) after the end of the controlled feeding regime. Any fish exhibiting darkened fin edges and silvering flanks at this time was assigned to the UMG and subsequently removed from the analysis.

4.3 Results

Three control and nine experimental fish died throughout the course of the experiment, although the treatment group had no significant effect on mortality rates ($\chi^2 = 2.19$, d.f. = 1, N.S.). Twelve control and two experimental fish exhibited external signs of smolting at the completion of the experiment and were removed from the analysis. There was a significant effect of treatment feeding regime on the proportion of surviving fish that entered the UMG (χ^2 test between groups on number of fish entering UMG; $\chi^2 = 9.09$, d.f. = 1, P<0.01.). A logistic regression correctly predicted in 91.67% of the cases whether a fish would join the UMG on a basis of initial size and the treatment group (P< 0.001). This left 35 experimental and 39 control LMG fish at the end of the experiment: all subsequent analyses are based on these fish.

Somatic growth

At the start of the experiment in September there was no difference in the size, weight or fat levels of the two groups of fish (*t*-tests comparing between treatments on fork length and wet weight, *t* = 1.19, d.f. = 81, N.S. and *t* = 1.02, d.f. = 81, N.S. respectively). The control fish showed a brief period of body growth during October before reducing the growth rate in both size and weight as winter progressed (figure 4.1 a, b). As a result of this growth, by the end of the experiment in April the control fish were both larger and heavier than at the start of the experiment (mean increase in length = 20.25mm ± 0.64 S.E., weight = 2.62g ± 0.13: paired *t*-tests comparing fork length and wet weight between sampling dates, *t* = 31.96, d.f. = 32, P<0.001 and *t* = 21.21, d.f. = 32, P<0.001 respectively). The experimental fish displayed a similar pattern of growth throughout the experimental period with increases in both body size and weight (paired *t*-tests comparing fork length and wet weight between sampling dates, *t* = 11.28, d.f. = 42, P<0.001 and *t* = 13.85, d.f. = 42, P<0.001 respectively)., However, the unpredictable feeding regime retarded the growth of the experimental fish in terms of both body size and weight (mean increase in fork length = 11.28mm ± 0.53 and wet weight = 1.21g ± 0.10; *t*-tests comparing between treatments on the gain in length and weight from 13 September to 1 April = 10.90, d.f. = 72, P<0.001 and *t* = 8.58, d.f. = 74, P<0.001 respectively), so that by the end of the experiment, they were both smaller and lighter than the control fish (*t*-tests between treatments on fork length and wet weight on 1 April, *t* = 7.42, d.f. = 72, P<0.01 and *t* = 6.64, d.f. = 72, P<0.01 respectively). The unpredictable feeding regime had a significant effect on the normal seasonal trajectories of both fork length and wet weight of the experimental fish throughout the course of the experiment (repeated measures ANOVA on the fork

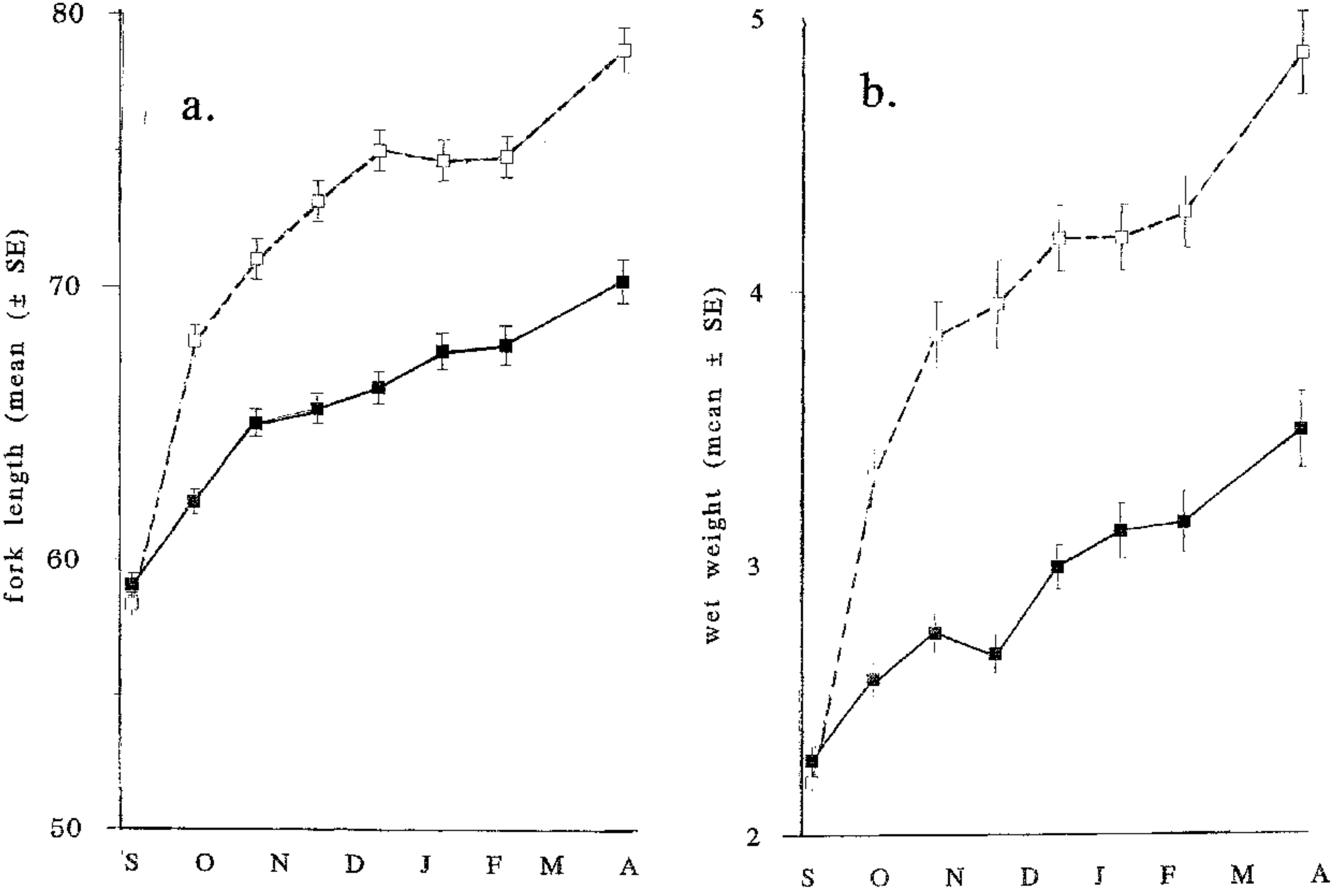

Figure 4.1. Changes in a) body size and b) body weight, throughout the course of the experiment. Values are shown as means for the experimental (solid symbols) and control fish (open symbols, dotted line).

length and wet weight at each sampling time during the course of the experiment; treatment effect, $F_{(7,63)} = 11.15$, $P<0.01$ and $F_{(7,63)} = 3.64$, $P<0.01$ respectively).

Fat dynamics

There was a consistent positive relationship between body size and the fat levels generated from equation 2.1 in the control fish at every sampling point throughout the course of the experiment, larger fish having more fat, but the relationship varied over the course of the winter (see Appendix I for regression equations). Therefore the fat levels of both the control and experimental fish were expressed as residual values from those predicted for control fish of the same forklength at that time (see chapter 3.3).

Prior to any manipulations in September, the two groups did not differ in fat level (*t*-test between treatments on residual fat levels on 19 September, $t = 1.43$, d.f. = 81, N.S.). Despite significant fluctuation in fat levels between consecutive sampling dates in both groups (changes in fat levels being significant on 6 and 4 of the 7 occasions for control and experimental fish respectively, paired *t*-tests, $P < 0.05$), the two groups of fish displayed some similarities in fat dynamics (figure 4.2a). Minimum fat levels occurred for both groups in November and there was no difference between the body fat levels measured at the start and completion of the experiment in either group (paired *t*-tests on the fat levels of the controls and experimental fish, $t = -1.50$, d.f. = 32, N.S. and $t = 1.47$, d.f. = 42, N.S. respectively). However, between these times, the two treatment groups diverged (figure 4.2b), so that overall the control fish maintained higher fat levels than did experimental fish (repeated measures ANOVA, treatment effect, $F_{(7,58)} = 2.97$, $P<0.01$). Thus, the initial increases in fat level displayed by the control fish during the first two months (figure 4.2a) were not fully mirrored by the experimental fish, and the difference between the groups was further accentuated by the 20 day deprivation period at the end of November. Thereafter the experimental fish restored lost fat so that for the rest of the experiment the differences between the groups, when controlling for fish body size, were minimal (figure 4.2b).

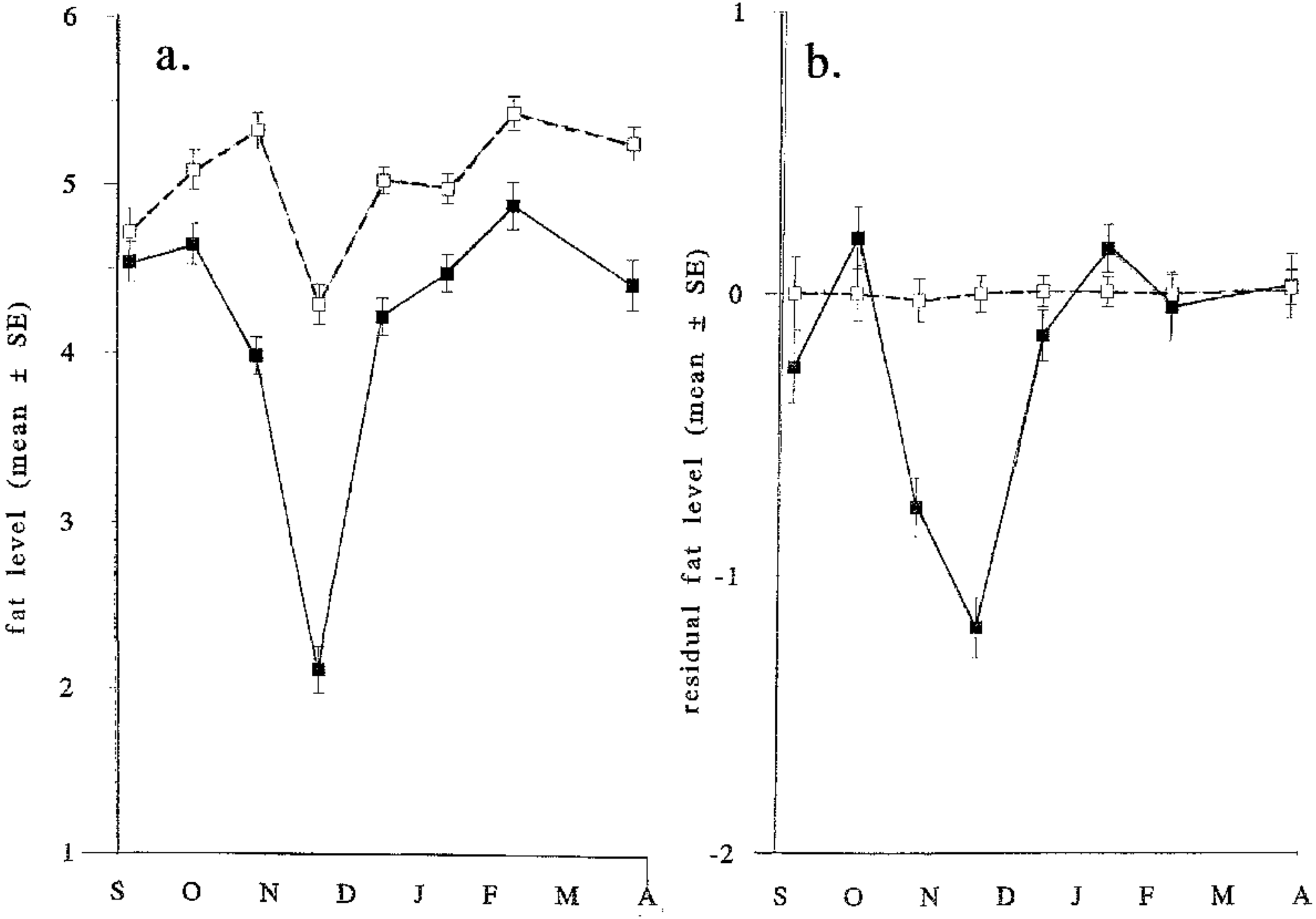

Figure 4.2. Changes in body fat content throughout the course of the experiment shown as a % of wet weight (a) and as residual values (b). Values are shown as means for the experimental (solid symbols) and control fish (open symbols, dotted line).

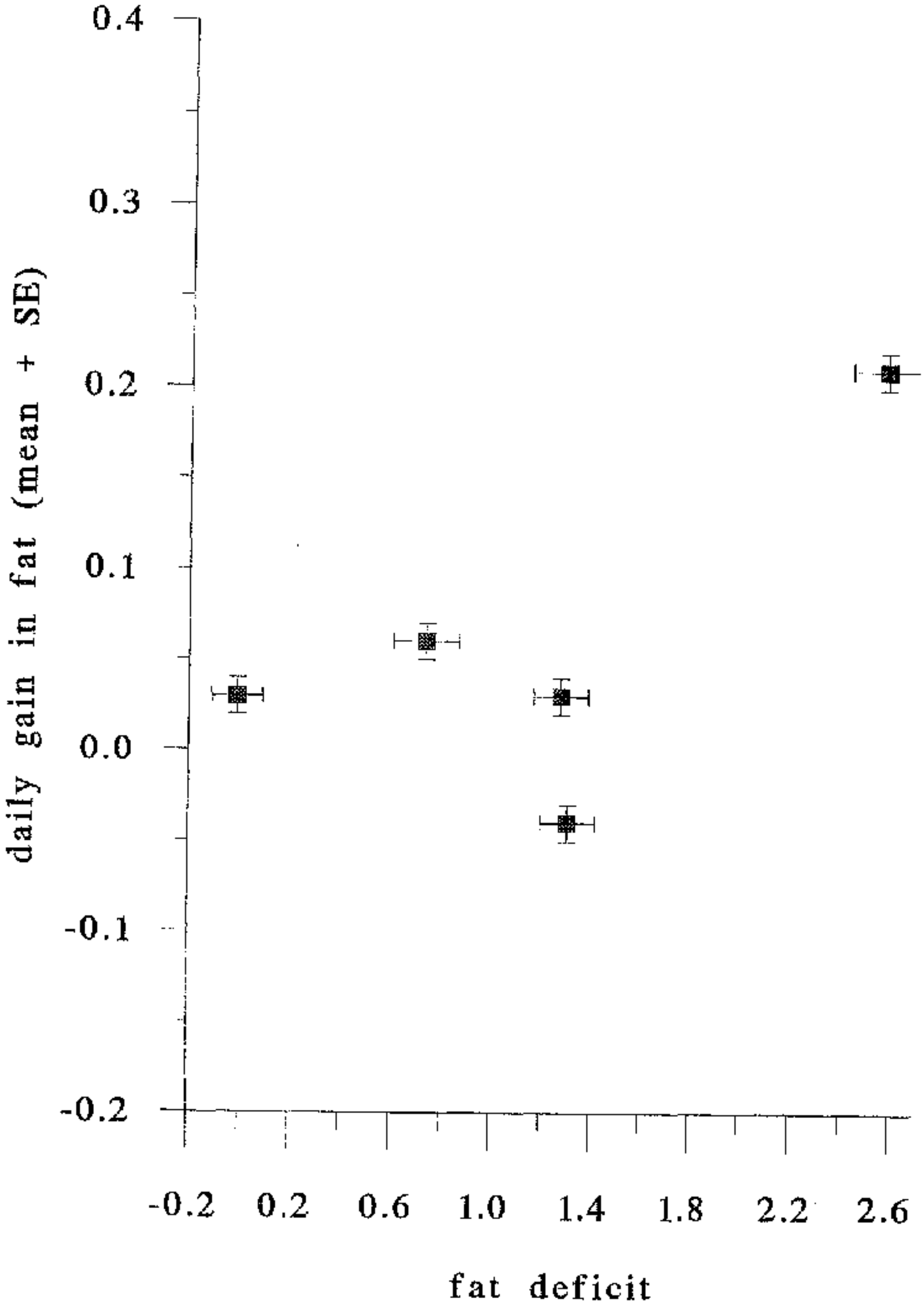

Figure 4.3. The relationship between the fat deficit incurred as a result of a period of food deprivation, and the daily rate of fat gain during the subsequent refeeding period. Zero relates to the appropriate mean fat level of the control fish. Values shown are means for the experimental fish.

In order to test whether fish showed an increasing response to an increasing loss of energy reserves, the observed fat levels of the experimental fish following the five food deprivation periods (table 4.1) were expressed as residual values from that expected from a similarly sized and constantly fed control fish at each time. These values were plotted against the subsequent rate of change of fat during the following refeeding period (figure 4.3). There was no significant correlation between the residual fat level and the subsequent rate of gain of fat during the following refeeding period (Spearmans rank correlation $r = -0.20$, n = 5, N.S.).

4.4 Discussion

Those fish subjected to an unpredictable feeding regime exhibited retarded body growth in both length and weight when compared to the continuously fed controls. This is contrary to the findings of Dobson & Holmes (1984) and Smith (1987) whereby rainbow trout fed intermittently exhibited full compensation during refeeding periods and were of a similar size to continuously fed controls at the end of 6 and 30 weeks respectively. However, the results of the current study are in broad agreement with those obtained by Miglavs & Jobling (1989b) and Jobling *et al.* (1993) on Arctic charr and Kindischi (1986) on rainbow trout, who found that cycling periods of feed and fast resulted in poorer growth. However, growth depression in the fish used by these authors can be attributable to restrictions in the total amount of food available throughout the entire experimental period. In the current study, an attempt was made to maintain an equal level of food provision to both the previously deprived and control fish over the entire period. However, the limitations placed on both food acquisition and processing as water temperatures fall to winter levels (Elliott, 1972,1976b; Brett, 1976; Webb, 1978; Jobling, 1980; Priede, 1985; Weiser & Forstner, 1986; Nicieza *et al*, 1994; Graham *et al.*, 1996) may have prevented previously deprived fish from taking full advantage of the additional food provided. The switch in developmental goal in the LMG in autumn from growth to regulation of fat stores (Nicieza & Metcalfe, *submitted*, see chapter 3.3) may also have resulting in reduced allocation of any surplus energy to somatic growth. It may well be that the physiological limitations rather than overall food availability produced the growth depression in the experimental fish.

Fish exposed to a series of short-term fast and feeding bouts in winter (akin to an unpredictable food supply) did not respond by increasing their levels of fat in order to hedge against the possibility of starvation. The severity of the regime imposed did have some effect on the fat levels over the entire timescale of the experiment: the experimental fish differed in trajectory from the controls (repeated measures ANOVA on fat level over time) but the instantaneous measurements of fat rarely differed between groups. As fish exhibited depressed growth they ended up smaller and so had a lower fat content (as a result of the positive relationship between % fat level and body size: see Elliott, 1976; Metcalfe & Thorpe, 1992; Simpson *et al.*, 1992), but rarely had less fat than would be predicted for a normally-fed fish of the same size. Therefore the fish appeared to sacrifice growth in order to maintain / restore lipid levels (see also chapter 3.3).

This runs contrary to both the theoretical (Lima, 1986; McNamara & Houston, 1990; Clark & Ekman, 1995; Lima, 1986) and empirical work (Ekman & Hake, 1990; Ekman & Lilliendahl, 1993; Bednekoff & Krebs, 1995) on wintering passerine birds and emphasises the differences between the overwintering energetic requirements of these taxa. Being endothermic, birds require energy to maintain body temperature, and have a high resting rate of metabolism, so that when energy intake is restricted, as in the case of visual foragers at night, they rapidly deplete fat stores (see Witter & Cuthill 1993 for review). This produces a pronounced cycle of an overnight loss followed by a daytime replenishment. The rate of energy utilisation is such that a single day without food could result in starvation; bet-hedging (Lima, 1986; McNamara & Houston, 1990; Clark & Ekman, 1995) would therefore be advantageous when the predictability of foraging is reduced. Cold-water fish on the other hand have a much lower metabolic cost and have adapted to the unpredictable nature of their environment so that some species can withstand extremely long periods of complete starvation. Larsson & Lewander (1973) reported that the European eel can withstand 5 months of starvation and Weatherley & Gill (1981) showed that juvenile rainbow trout could recover from 13 weeks of complete food starvation and a corresponding loss of 32.5% of their initial body weight. This ability, in combination with the fact that the rate of energy reserve depletion is reduced at low winter temperatures (Love, 1980; see Beck & Gropp, 1995 for review) may have negated the need for the fish in the current study to hedge against short-term starvation. Indeed, the results presented in chapter 3.4 indicate that the projected energy requirements over the winter as a whole (nominally taken as a 6 month period) are of greater importance in regulating foraging behaviour than short-term needs. The lack of any relationship between the fat deficit incurred during a period of food deprivation and the subsequent rate of restoration in the current study (figure 4.3) reflected this seasonal change in priority: a small deficit incurred early in the winter might be predicted to have a greater impact than a greater deficit occurring later (see chapter 3.4).

Juvenile LMG salmon appear to have adapted to the unpredictable nature of their environment, and do not require additional energy stores to insure against short-term starvation. Here I have shown that they have the ability to withstand repeated periods of food deprivation during the winter season. If they had been committed to smolting in the spring, the reduced growth of the experimental fish would have had a severe mortality cost upon entry to sea water (Lundqvist *et al.*, 1994). However, resident parr which have opted to stay in fresh water for another year can presumably make up the growth deficit during the following summer. By maintaining fat stores at levels appropriate for the time of year at the expense of body growth, the fish can maximise their long-term survival chances, previously shown to be of great importance in determining overwintering success.

Chapter 5 - The influence of temperature on seasonal appetite loss and fat use

5.1 Introduction

As suggested by Wootton (1990), temperature is perhaps the most pervasive of any abiotic factors influencing the overall energy budgets of ectotherms. Indeed, Brett (1971) labelled it the "ecological master factor" amongst abiotic environmental factors. As the majority of fish are strict thermal conformers (Fry, 1968) the regulation of the energetics of metabolism by temperature (Elliott, 1976b) ultimately determines food intake by influencing both the ability to acquire (Graham *et al.*, 1996; Johnson *et al.*, 1996), and process food (e.g. Edwards, 1971).

Although in some situations the thermal environment inhabited may be reasonably constant, as in the case with many marine species, the majority of freshwater fishes will experience some degree of temporal fluctuation in water temperature. Both short-term (Brett, 1971; Hokanson *et al.*, 1977; Cox & Coutant, 1981; Spigarelli *et al.*, 1982) and long-term seasonal changes in temperature (Flath & Diana, 1985; Cunjak & Power, 1986a, 1987) have been shown to affect the feeding and energetics of fishes. The deposition and mobilisation of fat stores have, in a number of studies, been shown to be influenced by environmental temperature (Flath & Diana, 1985; Spigarelli *et al.*, 1982; Brown & Murphy, 1995), although changes in daylength associated with the seasons are more likely to exert the ultimate control over the timing of life-history traits (Villareal *et al.*, 1988; see chapter 3.5). In general, whilst temperature acts to govern the rates of physiological response, it is not in itself a cue to the changing seasons (Clarke *et al.*, 1978).

For stream-dwelling salmonids, a range of preferred or optimal temperatures for feeding and growth has been proposed, varying in relation to species and location. Brett (1971) provided a comprehensive review of work on juvenile sockeye salmon, reporting that in 79% of the physiological and behavioural parameters measured, an optimum of 15^0C was apparent. Deviations from this optimum were reported for voluntary food intake (17^{0}C) and for growth rate in the presence of a restricted ration (between 15 and 5^{0}C). Similarly, in a series of studies on brown trout, Elliott (1975; 1976b) found differences between the temperature optima for growth (13^{0}C) and feeding (18^{0}C). However, more recent work with Norwegian populations of brown trout found that temperature optima for growth and maximum feeding rates were similar at 15-16^{0}C (L'Abée-Lund *et al.*, 1989; Jensen, 1990; Forseth & Jonsson, 1994). Dwyer & Piper (1987) found that growth efficiency, as measured by weight increase, was maximised at 16^{0}C in juvenile Atlantic salmon, but that a reduction to 13^{0}C did not significantly reduce growth. It thus appears that as a general guide, 15^{0}C could be taken as providing near optimal conditions for salmonid feeding and growth, providing that food is not limited.

The proportion of fish entering either the UMG or LMG of a bimodal length frequency distribution of hatchery-reared salmon parr during late summer of their first year (Thorpe, 1977) is to some extent, dependent on water temperature (Kristinsson *et al.*, 1985 Adams & Thorpe, 1989a, b) and its effect on the potential for growth. More fish were found to enter the UMG when temperatures during late September were maintained above 10^{0}C (Kristinsson *et al.*, 1985). The segregation appears due to a period of rapid growth in those fish destined to become the UMG (Kristinsson, 1985; Metcalfe *et al.*, 1988) at a time when both the appetite and growth of the LMG are in decline (Metcalfe *et al.*, 1986; Metcalfe & Thorpe, 1992). The onset of the LMG fishes' loss of appetite in August has been shown to occur before any seasonal temperature decline (Metcalfe *et al.*, 1986) and to be under the influence of photoperiod change (Thorpe, 1986). Food intake is subsequently regulated with regard to internal stores of energy (Metcalfe & Thorpe, 1992) and maintained at a low level throughout the winter (Gardiner & Geddes, 1980; Higgins & Talbot, 1985; Cunjak, 1988a). Body lipid levels are steadily utilised as an alternative source of energy throughout this time (see chapter 3.4). However, the influence exerted by the normal seasonal reduction in water temperature upon the maintenance of appetite suppression, its rate of decline, and the dynamics of fat regulation are little understood.

The aim of this chapter was to address this question by comparing the voluntary food intake and the dynamics of body fat levels in two groups of fish experiencing different thermal regimes throughout the autumn and winter. By maintaining one group of LMG fish at the estimated optimum temperature for growth and the other in water at ambient temperature (therefore showing the normal seasonal decline), I was able to examine the effects of temperature independently from those of seasonal photoperiod change.

5.2 Materials and methods

On 28 August 1994, 160 fish were selected from a sibling stock population (the progeny of a pair of sea-run adults from the River Dee catchment, Scotland) for use in the experiment. The smallest individuals in the population were chosen to maximise the likelihood that thay would be LMG fish (Metcalfe *et al.*, 1988; see chapter 3.2). All fish were measured for fork length (to the nearest mm; mean = 53.7mm ± 0.65 S.E., range = 44-60mm) and weighed (to the nearest 0.01g) and assigned to one of two size-matched groups (n = 80 fish per group). No estimation of body fat was possible at this time as a number of the fish in both groups were below the size range covered by the predictive equation (equation 2.2) developed to investigate fat changes in autumn and winter. Each group was established in a separate tank where food was provided throughout 24 hrs by way of an automated feeder that dispensed a trickle of food every 20 min. in quantities ensuring *ad lib.* rations. One tank of fish (the experimental group) was housed inside a temperature-controlled cabinet where water temperature was maintained close to the optimal temperature for growth throughout the next 5 months (figure 5.1) in an attempt to remove any possible entraining effect of seasonal temperature decline on feeding behaviour and fat dynamics. The other tank of fish (the controls) were supplied with ambient temperature water and so experienced the normal seasonal temperature decline (figure 5.1). Both tanks were maintained under a simulated natural photoperiod for the duration of the experiment.

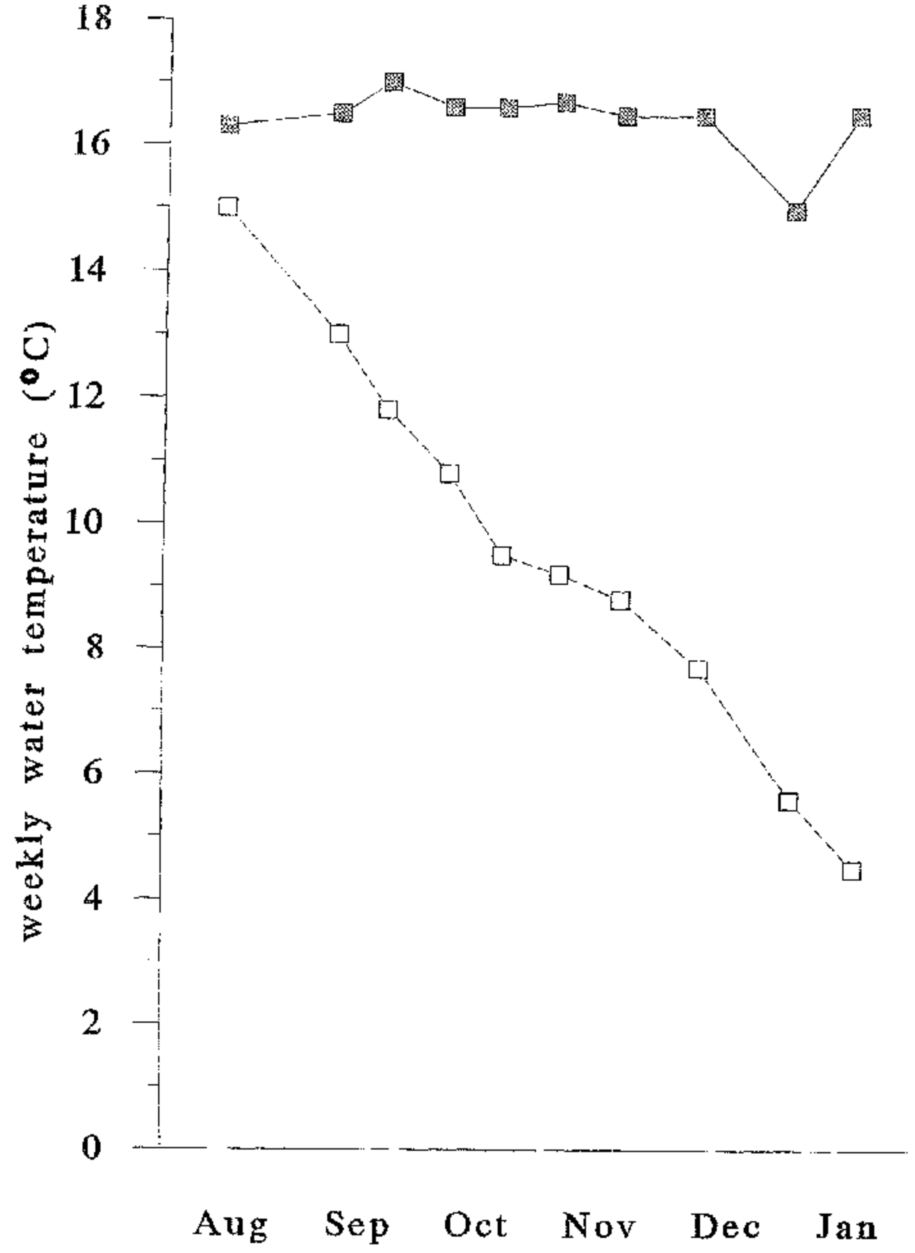

Figure 5.1. The water temperature experienced by the experimental fish (solid line) and the controls (dotted line) throughout the course of the experiment. Displayed values are weekly means centred around each sampling date.

At approximately two week intervals commencing on 6 September, the food intake of all fish was measured using the X-ray radiography technique described in chapter 3.5. Labelled food was provided to both groups of fish for 4 hours (1000- 1400hrs) and all fish were subsequently X-rayed. The quantity of food ingested during this period was then estimated from the number of marker beads in the stomach and intestine. An hourly feeding rate for each fish was calculated and expressed as a percentage of the fishes body weight at that time (%bw.hr^{-1}). Following the feeding trial on 22 September, all fish were measured for body fat content using equation 2.2 (chapter 2) as they were now of a suitable size to allow an accurate estimation (mean forklength = 57.8mm ± 0.67, range = 49-65mm).

Due to unknown causes, the mortality rate of fish in the experimental group was extremely high during September and October (mean loss of experimental fish = 5 per week during the 66 days between sampling on 28 August and 3 November: mean losses of control fish = 2 per week) resulting in a markedly reduced sample size in this group. On 26 January 1995, all fish were assessed for external signs of smoltification and any showing darkened fin edges and silvering flanks were deemed to be UMG fish and discarded from the analysis. The following results are based on only those LMG fish surviving until the termination of the experiment on 26 January ($n = 9$ experimental, $n = 34$ controls).

5.3 Results

Somatic growth

At the start of the experiment in August, there was no difference between the two groups of fish in either the size or weight (*t*-tests comparing between treatments on fork length and wet weight on 28 August: $t = 1.31$, d.f. = 41, N.S. and $t = 0.90$, d.f. = 41, N.S. respectively). Throughout the course of the experiment, both groups of fish continued to increase in length and weight (control fish mean increase in length = 11.6mm + 0.86 S.E. and weight = 1.36g + 0.123; experimental fish = 20.4mm + 2.25 and 2.76g + 0.44). Initially, both the experimental and control fish exhibited similar growth rates (SGR_W, see equation 3.2) prior to the control fish putting on a brief growth spurt in October, when the experimental fish were exhibiting a reduction in

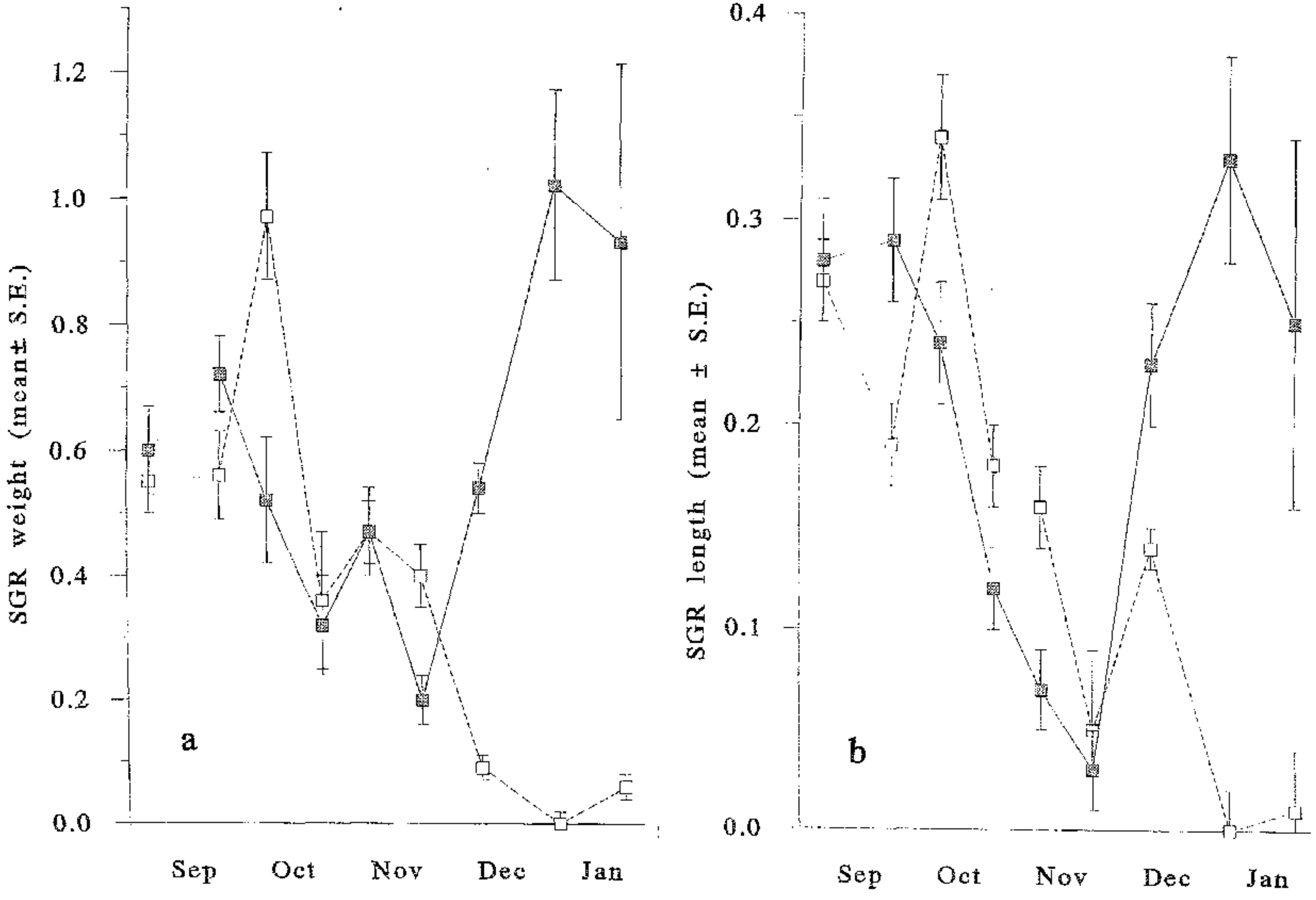

Figure 5.2. Changes in the specific growth rate (SGR) in terms of a.) gain in weight and b.) gain in length throughout the course of the experiment. Data are presented for both the experimental (solid symbols) and control fish (open symbols) and are plotted at the mid-point between sampling dates.

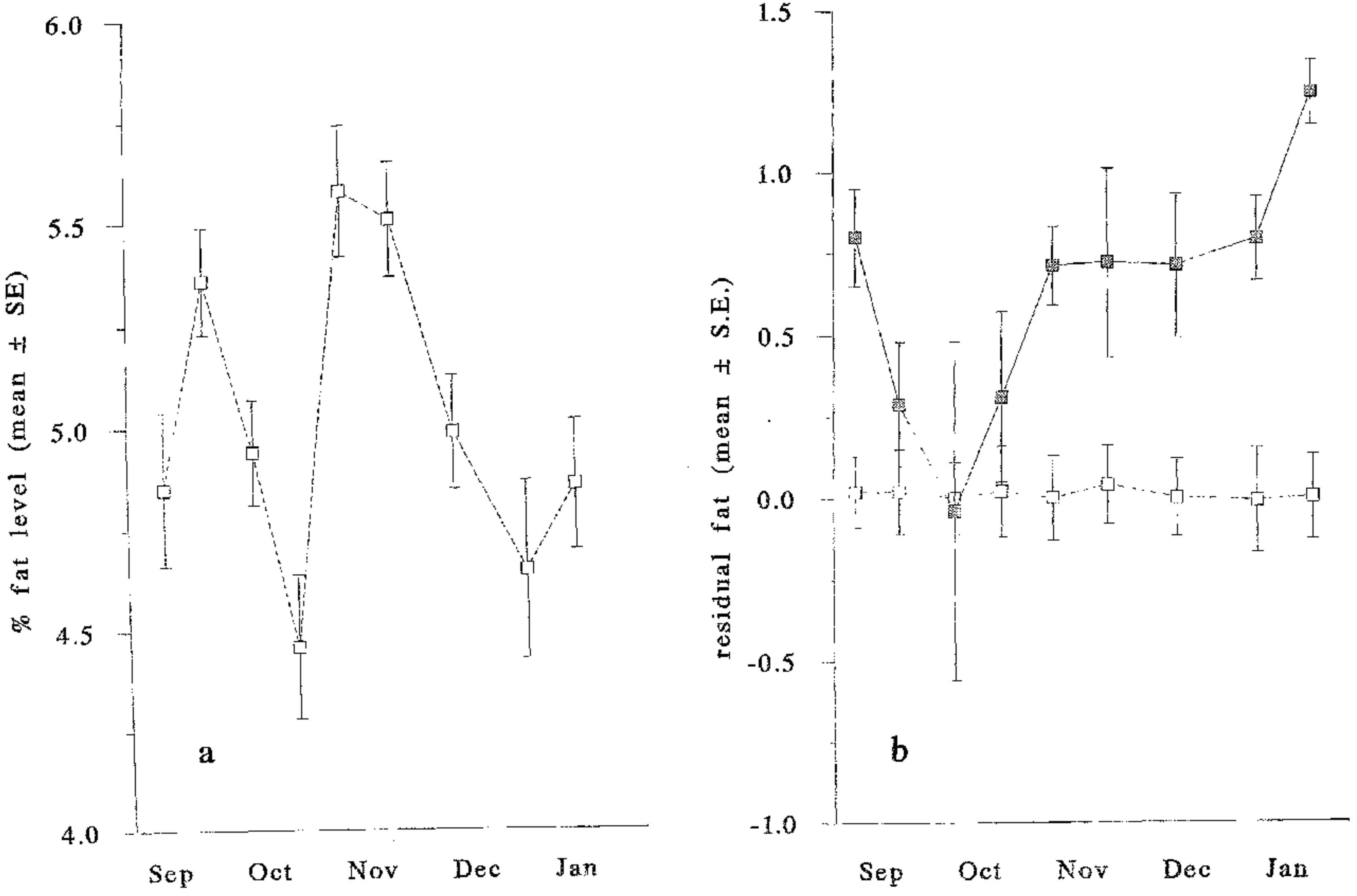

Figure 5.3. Fat dynamics throughout the course of the experiment a.) for the control fish and b.) plotted as residual fat values for both treatment groups (solid symbols = experimental, open symbols = controls). The residuals arre calculated as deviations from the regression of % fat on body size for control fish at each sampling time; values for control fish are therefore close to zero by definition.

their growth rate (figure 5.2a,b). However, following this brief peak, the control fish steadily slowed their growth until by December, they were hardly growing at all. The experimental fish started to increase their growth rate in late November, and were subsequently heavier than the controls at the termination of the experiment in January (*t*-test between treatments on wet weight on 26 January, $t = 2.90$, d.f. = 41, $P<0.01$). The differences in growth between the treatment groups were reflected in overall differences in the trajectories of weight change (repeated measures ANOVA on successive measures of SGR*w* during the course of the experiment; treatment effect, $F_{(8,29)} = 3.68$, $P<0.001$). The increase in weight was mirrored to some extent by skeletal growth as the body size trajectory of the experimental fish differed from the controls throughout the course of the experiment (repeated measures ANOVA on SGR*l* during the course of the experiment; treatment effect, $F_{(8,31)} = 1.69$, $P<0.001$). However the differences in the rate of skeletal growth were not large enough to lead to any difference in size between the groups at the end of the experiment (*t*-test between treatments on fork length on 26 January, $t = 1.77$, d.f. = 41, N.S.).

Fat dynamics.

Initially the body fat levels of the control fish showed small fluctuations, prior to peaking at 5.62% ± 0.18 in early November (figure 5.4a) following a growth spurt during October (figure 5.3). Fat levels then dropped steadily throughout the next three months resulting in their being significantly lower than the peak level at the start of the winter period (paired *t*-tests comparing fat levels on 3 November and 26 January; $t = 3.12$, d.f. = 28, $P<0.01$).

Body fat levels of the experimental fish, generated from equation 2.2 were expressed as residual values from the mean fat levels of the controls to allow direct comparison. Due to the presence of a significant positive relationship between body size and fat level in the control fishes on 6 of the 9 sampling dates (see appendix I), these residual values were calculated by comparing the actual observed fat levels to that predicted for a similarly-sized control fish from the fitted regression line, appropriate to each sampling date (figure 5.3b).

By one month after the start of the treatment the experimental fish had higher levels of body fat than the controls (*t*-test between treatment groups, comparing residual fat level on 24 September: $t = 3.35$, d.f. = 37, $P<0.01$). Although it is not possible to ascertain whether this initial difference between groups was due to chance (since fat could not be measured at the start of the experiment), the fact that during the first month the water temperature experienced by control fish had dropped by 3^0C (figure 5.1) may well have influenced their fat dynamics during this period. The fat levels of the experimental fish dropped markedly through October (possibly related to the high rates of mortality occuring at this time) but were higher than those of the controls during the latter three months of the experiment. The overall effect of the temperature manipulation was to maintain the fat levels of the experimental group at a higher level with regard to the normal lipid trajectory exhibited by the controls (repeated measures ANOVA on residual fat level at each sampling time during the course of the experiment; treatment effect, $F_{(1, 27)} = 10.76$, $P<0.01$).

Food intake

The initial food intake of the experimental fish on 6 September was higher than that of the controls (mean food consumed for experimental group = 0.12 %bw.hr^{-1} $\pm$ 0.06 S.E. and for control fish: 0.04 % bw.hr^{-1} $\pm$ 0.09, *t*-test comparing food intake between treatments: $t = 3.71$, d.f. = 38, $P<0.01$) presumably as a result of differences in water temperature between the treatments at this time (figure 5.1). Whilst the control fish exhibited a noticeable increase in food intake during late October and early November (figure 5.5) coinciding with increases in somatic growth and body fat (figure 5.3, figure 5.4), the experimental fish never rose above their initial feeding rate, and exhibited large fluctuations. The temperature regime influenced the normal seasonal trajectory of food intake, and the experimental fish maintained a higher feeding rate than the controls throughout (repeated measures ANOVA on the food intake at each sampling time throughout the course of the experiment, treatment effect, $F_{(9,16)} = 2.7$, $P<0.01$). Consequently, the intake rates of the experimental fish were still higher than those of the controls in January (*t*-test between treatments on the rate of food intake on 25 January, $t = 4.78$, d.f. = 37, $P<0.001$).

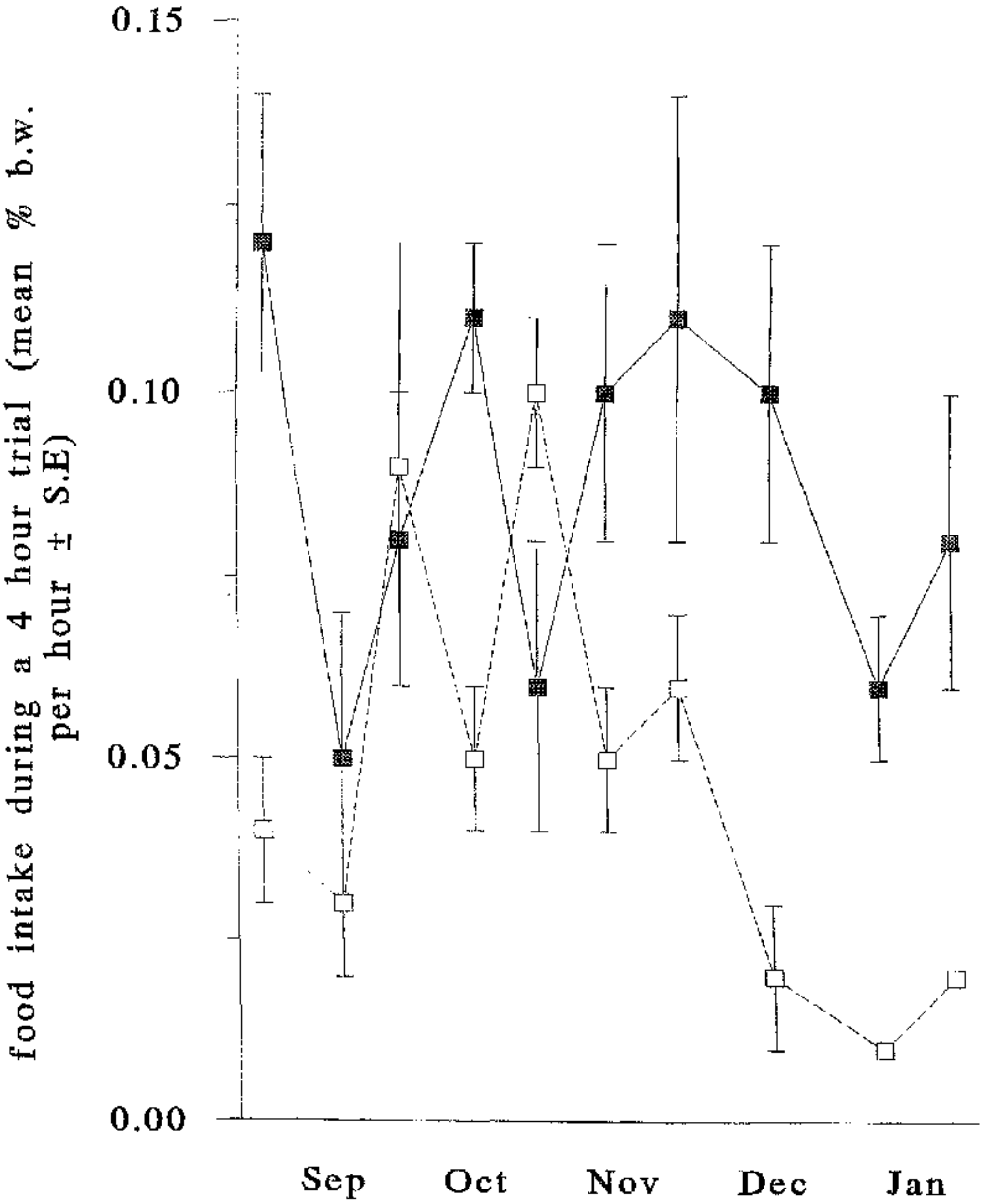

Figure 5.4. The food intake during the 4 hour daytime feeding trials throughout the course of the experiment. Data are presented as the mean intake rate per hour for the experimental (solid symbols) and control fish (open symbols) and expressed as a percentage of their wet weight.

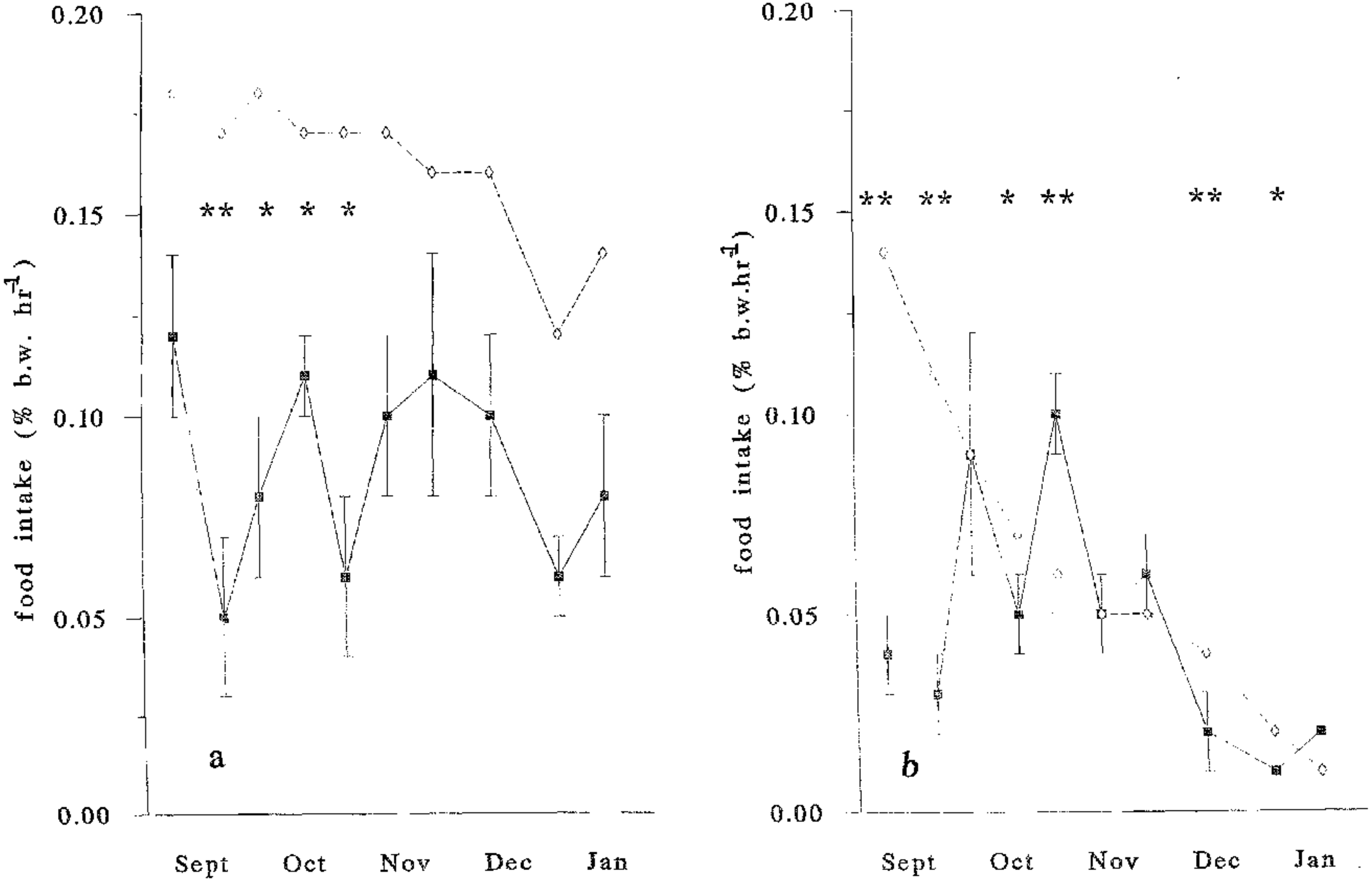

Figure 5.5. The relationship between the observed rate of food intake of the experimental and control fish (a. and b. respectively, both solid symbls) plotted alongside the predicted maximum rate for the appropriate temperature, derived from the equations of Elliott (1976) (open diamonds, dotted line). Asterisks indicate the significance of paired *t*-tests comparing between the observed and predicted rate of food intake ($* = P<0.05$, $** = P<0.01$).

The results of the feeding trials were compared to the physiological maximum food intake predicted from the equations of Elliott (1976b) for a similarly-sized brown trout maintained at the same temperature. The control fish were initially exhibiting appetite suppression (figure 5.5), but increased their food intake above that predicted in a noticeable peak during late October. Food intake then dropped again to below that predicted by the seasonal temperature decline. While the experimental fish maintained a higher rate of food intake than the controls, it was consistently below that which was physiologically possible, especially at the time when the controls were exhibiting a brief feeding spurt.

5.4 Discussion

The fish maintained at temperatures close to those optimal for body growth (Dwyer & Piper, 1987) differed little from the control fish in their rate of growth during the first 4 months of the study (figure 5.3) despite the latter experiencing a reduction in water temperature of 9^0C. When differences did appear later in the study, only a small increase in skeletal growth was apparent in the experimental fish. These results complement the findings of chapter 3.3 whereby during winter, the requirement to maximise somatic growth (as found during the summer) was reduced in comparison to the need to allocate surplus energy into storage. The similarities in growth rate during the initial period of the current study highlight that seasonal differences in somatic growth and energy storage are not governed entirely by limitations placed by temperature, but are subject to internal regulation regardless of environmental opportunity. Differences between the treatment groups did became apparent at the end of December, when the experimental fish rapidly increased their growth rate (mostly in terms of weight gain) whilst the controls were growing little, presumeably as a result of approaching their lower thermal limit for growth of approximately 4^0C (see Elliott, 1982 for review).

The fact that the increase in growth rate in the experimental group occurred when the photoperiod was increasing (from mid-winter onwards) may give some insight into its cause. Gross *et al.* (1965) reported that increasing daylength enhanced growth, while decreasing daylength inhibited growth in the green sunfish, and Higgins & Talbot

(1985) found that the gut evacuation time of LMG salmon during winter was faster on an increasing as opposed to a decreasing photoperiod regardless of water temperature. Although photoperiod is used by juvenile salmonids to time various life-history events (Villarreal *et al*, 1988; see chapter 3.5) including the cessation of feeding in later summer (Thorpe, 1986), temperature acts to govern the extent of the response. From the results of Higgins & Talbot (1985), a decrease in gut evacuation time with increasing photoperiod would have been expected in both the experimental and control fish in the current study. In the case of the experimental fish, the seasonal increase in evacuation rate (presumably initiated by either a neural or hormonal effect acting on peristalsis; Fänge & Grove, 1979) would have been complemented by higher water temperatures allowing more rapid allocation of excess energy into growth in terms of weight or storage of fat. The control fish, although possibly experiencing faster gut evacuation rates, would have been ultimately limited in terms of growth by the effects of low water temperature on overall food intake, (and consequently the energy available for allocation) and rate of processing (Edwards, 1971; Elliott, 1976).

Overall, the body fat levels of the control fish exhibited the normal seasonal pattern of accumulation in late autumn, and utilisation during the following winter months as found in wild and hatchery-reared stocks (Gardener & Geddes, 1980; Cunjak & Power, 1986a; Simpson, 1992). The peak in fat level displayed in early November (figure 4a) followed a brief increase in skeletal growth that may have an adaptive basis, since it would allow fish to accumulate more fat as a result of the positive relationship between body size and fat storage capacity (Elliott, 1976a; Metcalfe & Thorpe, 1992). The fat levels of the experimental fish were consistently higher than the ambient temperature controls, except during a brief period in October (figure 5.4b). This reduction in fat could possibly have been due to an unknown stressor contributing to high mortality rates in the group at this time. However, fat levels were quickly restored and maintained throughout the remainder of the experiment.

The reasons why these fish should maintain a higher level of body fat during the winter are difficult to ascertain. As the ability of fish to assimilate energy from food increases as water temperature rises (Brocksen & Bugge, 1974), the experimental fish may have been able to allocate more energy from their food into fat storage than the controls. By maintaining a higher level of fat, the experimental fish may have been hedging against the risk of suffering from more rapid fat depletion should they be required as metabolic fuel at the higher water temperature (Love, 1980). Laying down larger fat stores may have been less costly for the experimental fish than the controls in terms of predation risk, as they would have been less vulnerable at the higher water temperatures (Webb, 1978; Fraser *et al.*, 1993). This may have allowed them to forage more than the controls, but by allocating the excess energy into fat storage they were guarding against the possibilty that the optimal water temperature conditions would not persist. Thus an overwinter survival strategy dependent on fat storage took precedent over maximising skeletal growth even when the environmental conditions made it a possibility.

The food intake of the control fish during September was maintained at a level lower than the physiologically possible maximum for the closely-related brown trout (Elliott, 1976b), indicating that they were displaying voluntary appetite suppression (Metcalfe *et al.*, 1986l; Metcalfe & Thorpe, 1992). The results of the current study are in agreement with those of Metcalfe et al. (1986), who reported that the internal suppression of appetite at this time occurred independently from water temperature. However, following the initial period of suppression, food intake increased in a notable peak, coinciding with (and presumably contributing towards) a rapid growth spurt and deposition of fat (figure 5.5). A similarly-timed growth spurt has been found in UMG fish (Kristinsson *et al.*, 1985; Metcalfe *et al.*, 1988). Following this peak it would appear that the fish were regulating their food intake at a level below that dictated by water temperature.

The experimental fish, although maintaining a higher average food intake than the controls, were not feeding at a rate which was physiologically possible given the elevated water temperatures. Although a higher level of energy intake would be necessary to offset the increased demands of metabolism at this water temperature when compared to the control fish (Brett & Groves, 1979) it appeared that these fish were still not feeding maximally in order to realise their full somatic growth potential, instead of feeding at a rate which maintained their lipid stores but only resulted in a modest growth rate.

However, when discussing the maximum daily feeding level, it should be noted that the equations proposed by Elliott (1976b) from which the predicted values were calculated were based entirely upon daytime feeding and no mention was given to nocturnal feeding at low winter temperatures. This phenomenon has been observed both in the wild (Heggenes *et al.*, 1993; Riehle & Griffiths, 1993) and in the laboratory (Fraser et al., 1993, 1995, see chapter 6.3) and its exclusion in any estimation of total food intake at temperatures below 10^0C would potentially lead to underestimation of total intake rates throughout the 24hr period.

The occurrence of peak feeding rates and increases in both somatic growth and fat deposition displayed by the control fish in late autumn coincided with water temperatures reaching the threshold of 10^0C, at which point fish switch from a diurnal activity pattern to daytime sheltering and nocturnal emergence (Fraser *et al.*, 1993, 1995). Further work is needed to unravel the inter-relationships between feeding and allocation of energy at this crucial point, as the results of the current study point towards some interesting possibilities e.g. do fish feed maximally during the day when their efficiency is highest (Fraser & Metcalfe, *in press*) as temperatures drop toward 10^0C in anticipation of further temperature reduction, in order to maximise firstly body size and secondly, their fat levels?

In summary, the environmental temperatures to which LMG fish are exposed during winter have a limiting effect on both the feeding rate and utilisation of energy stores. However, temporal changes in both cannot be fully accounted for by concurrent temperature change, indicating that internal regulation of feeding and fat use remains an important facet of LMG wintering strategies. The increased opportunity for growth offered to experimental fish by increasing their water temperature during winter was not fully realised in terms of body growth or food intake, and fish opted for maintenance of enhanced body energy stores at the expense of skeletal growth.

Chapter 6 - The effect of refuge use on winter feeding and fat dynamics

6.1 Introduction

Throughout spring and summer, juvenile salmon spend the day maintaining and defending a feeding station in the current (Kalleberg, 1958) from which they dart out to intercept food items passing in the drift (Wankowski, 1981). However, during winter, when water temperatures fall to below 10^{0}C, juvenile salmonids switch to occupying stream-bed refuges by day, from which they emerge under the cover of darkness (Fraser *et al.*, 1993, 1995; Heggenes *et al.*, 1993).

Spending a proportion of the day in darkened shelters, where water velocity is reduced, may potentially offer advantages in terms of energy conservation (Pickering & Pottinger, 1988; Rimmer & Paim, 1990) and predator avoidance (Fraser *et al.*, 1993, 1995) at low water temperatures. However, this behaviour restricts feeding opportunity, as salmonids are essentially visual foragers (Hoar, 1942) and so feed little if at all whilst concealed during the day (Cunjak & Power, 1987). Feeding does occur under the cover of darkness (Fraser et al., 1993; Heggenes *et al.*, 1993) but success rate is markedly reduced (Fraser & Metcalfe, *in press*,). Juvenile salmon continue to feed throughout the winter (although at a reduced rate, see chapter 3 and 5) in order to supplement internal energy sources and fuel metabolism, and in the majority of circumstances, the level of energy intake required may be met by nocturnal foraging. However, when environmental conditions create poor foraging opportunities e.g. spate conditions with turbid water and high flows, fish may suffer accelerated depletion of internal energy stores. Following such events during winter there appears to be the potential for conflict between the need to restore a fat deficit to prevent starvation, and the adherence to strict nocturnal foraging that results in only a low energy intake. During these times the fish must trade-off the potential risk of emerging to feed during the daytime, where they will be highly vulnerable to predation (Webb, 1978; Fraser *et al.*, 1993) against the possibility of starvation, should energy stores become exhausted.

In this chapter I investigate the effect of daytime refuge-seeking behaviour on the normal winter utilisation of energy stores (estimated as body fat stores), and whether it offers some advantage in terms of energy conservation. I also attempt to examine the timing and intensity of feeding bouts when fish are provided with refuges inside which feeding cannot take place. In both cases the results are compared to fish denied access to daytime refuges. The presence of a trade-off involving abandoning strict nocturnal activity when faced with the risk of starvation is tested in the laboratory and compared to the results of fieldwork on wild fish.

Chapter 6.2 - The effect of refuge use on fat levels

6.2.1 Introduction.

Fish, in general, tend to show preferential deposition of lipids as water temperatures decline (Love, 1970; Spigarelli *et al.*, 1982). During the winter, juvenile salmonids reduce food intake and gradually deplete their energy stores (Egglishaw & Shackley, 1977; Gardiner & Geddes, 1980; Higgins & Talbot, 1985; Cunjak & Power, 1986, 1987; Metcalfe & Thorpe, 1992; see chapters 3 and 4). During this time, nutritional stress and a resulting metabolic deficit have been suggested as factors contributing to high mortality rates (Gardiner & Geddes, 1980; Cunjak & Power, 1987; Cunjak, 1988b; Shackley *et al.*, 1994; Smith & Griffith, 1994). Pickering & Pottinger (1988) found that the stress levels in hatchery reared salmon during their first winter (measured in terms of various haematological parameters) were higher in fish from the LMG as opposed to the UMG, contributing to mortality rates almost ten times higher in the LMG fish.

The reduction in feeding and decline in energy stores accompanies a behavioural switch to sheltering during the day, and emerging under the cover of darkness (Cunjak, 1988a; Heggenes *et al.*, 1993). The provision of overhead cover to hatchery tanks has been shown to enhance the growth rate of juvenile salmon during summer (Pickering & Pottinger, 1987) and has been suggested as a means of reducing mortality during winter when fish are naturally seeking cover (Pickering & Pottinger, 1988). One feature common to many daytime refuges is that water velocity is markedly reduced (Rimmer *et al.*, 1984), so almost removing the need for the fish to work so as to hold station against the current. As this ability to hold station is diminished at low temperatures (Graham *et al.*, 1996), spending a large proportion of the time in areas of low velocity would appear to be advantageous in terms of conservation of energy at a time when internal stores are at a premium (see chapter 3.3). It is possible that daytime sheltering may function to forestall the depletion of energy stores (Rimmer & Paim, 1990).

The aim of the current study was to examine whether the provision of suitable overwinter cover would influence the rate of depletion of body energy stores, allowing a larger store to be maintained throughout the winter period.

6.2.2 Materials and methods.

Forty fish were selected from a $1m^2$ stock holding tank and given an individual combination of alcian blue dye marks on 13 December, 1993. The fish were measured for body fat (equation 2.2) and assigned to one of two size-matched groups: the experimental or control group (both with n = 20 fish). Both groups were established in 60cm circular tangential flow tanks where pelleted commercial salmon food was provided to excess by an automatic feeder dispensing food at 20 minute intervals throughout 24 hours. The addition of a removable 'false bottom' into the tank containing the experimental fish provided a darkened refuge into which they could enter through four 3cm diameter holes in the upright section surrounding the central drain (figure 6.1). Food was prevented from entering the refuge space by the additon of flexible 1.5cm diameter plastic tubing that was split and added to the rim of the false bottom, blocking gaps caused by any irregularity in tank shape. The tubing was also necessary to reduce the water flow in the refuge space. Both tanks were positioned outside and all fish experienced both natural photoperiod and ambient water temperature (figure 6.2) throughout the course of the experiment.

During the following 5 months, all fish were re-measured at intervals of approximately 3 weeks to allow estimation of body fat. A pump failure on 17 January resulted in the death of the entire control group and a replacement group was measured for fat, marked and established on 19 January. Fish making up the replacement control group had been previously held in a similar tank and been given access to *ad lib.* rations of pelleted food. The replacement group was size-matched to those killed by pump failure (*t*-test between original and replacement control group on forklength and wet weight, t = 0.22, d.f. = 38, N.S.; *t* = 0.36, d.f. =38, N.S.). On 18 April all fish were measured for a final time and visually assessed for external signs of smoltification.

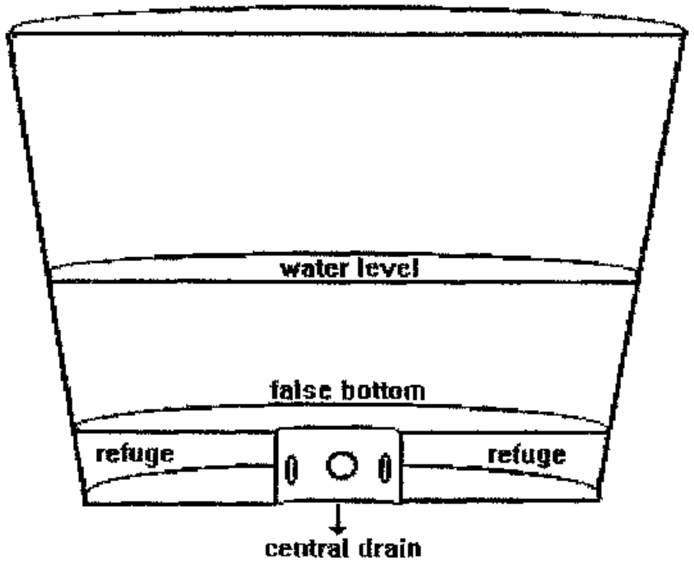

Figure 6.1. Cross section of the tank used to house the experimental group. The addition of a false bottom provided a darkened refuge with minimal water flow into which fish could move freely at any time.

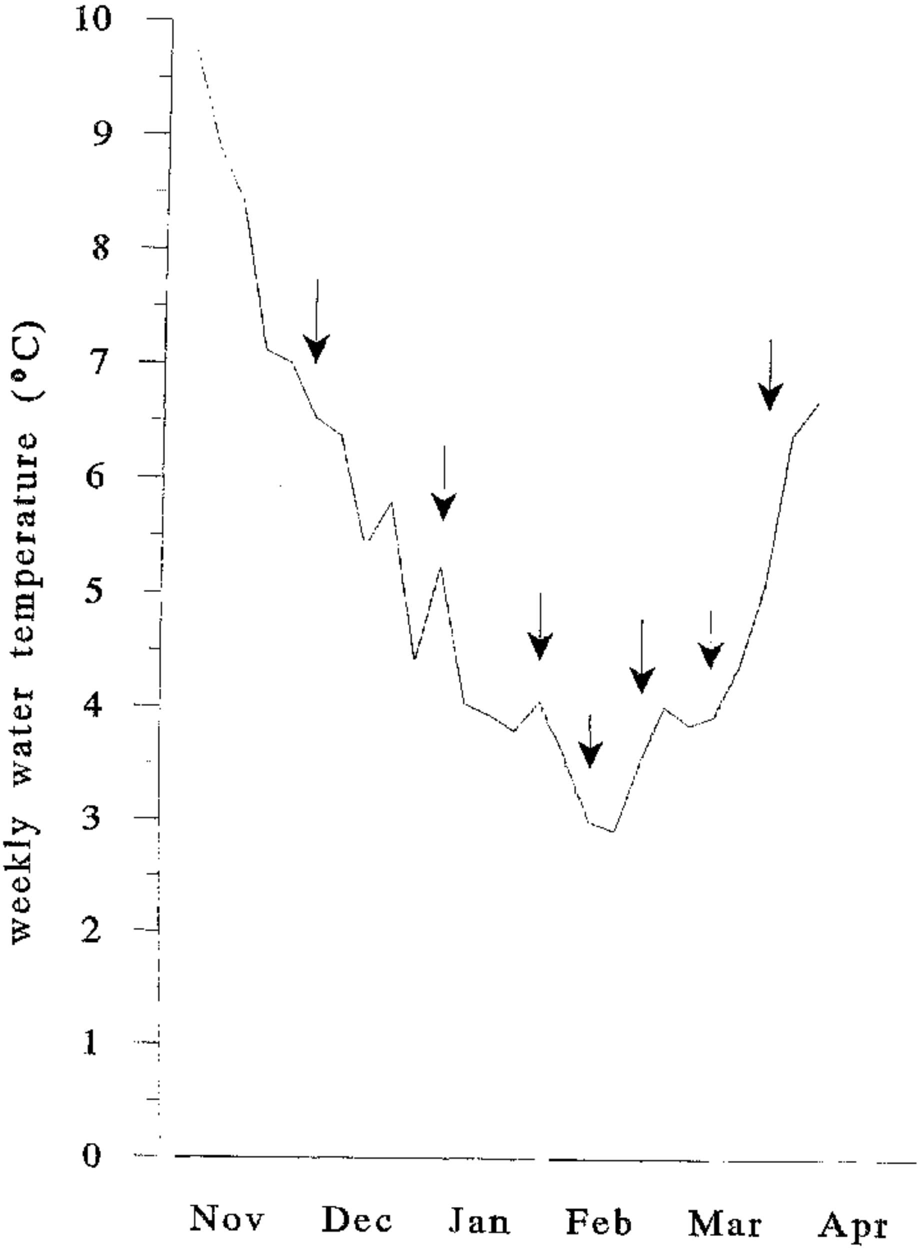

Figure 6.2. Weekly water temperature during the course of the experiment. Arrows indicate sampling dates when all fish were removed and measured for body fat content.

6.2.3 Results.

There were no fish displaying signs of smolting in April and so all were treated as LMG fish, that were delaying the smoltification process until the following year. In addition to those dying due to pump failure, three fish died during the experiment in both the experimental and the replacement control groups, reducing the final sample size to 17 in each group.

Fish in the experimental group were only rarely visible in the tank during the day, opting to spend the majority of the light period in the darkened shelter. Observations at night revealed that fish were leaving the refuge under the cover of darkness and holding station against the current on the tank floor. The addition of the false tank floors was therefore successful in causing the fish to adopt the typical winter pattern of predominantly nocturnal activity and diurnal hiding.

Fat dynamics.

There was a significant relationship between fat and body size in the control fish on four of the six sampling dates (see appendix I), and as a result, fat was compared between treatment groups using ANCOVA with forklength as the covariate.

The replacement control group did not differ in body fat from the original controls at the time of their establishment in the experiment (ANCOVA between control groups body fat, controlling for body size, $F_{(1,39)} = 0.32$, N.S.). Although both the experimental and control fish appeared to be reducing their fat levels throughout the course of the experiment (figure 6.3), only the decrease shown by the experimental group was significant (mean reduction in body fat from 3 Jan.-18 Apr. for replacement controls = 0.31% ± 0.38 S.E., paired t-test between sampling dates, $t = 0.83$, d.f. = 15, N.S.; for the experimental group: mean reduction of 0.78% ± 0.33, $t = 2.36$, d.f. = 16, $P<0.05$). However, the fat losses shown by those fish with access to a refuge (experimental group) were no different from those shown by fish without a refuge (replacement control group) over this period (t-test between treatments on fat

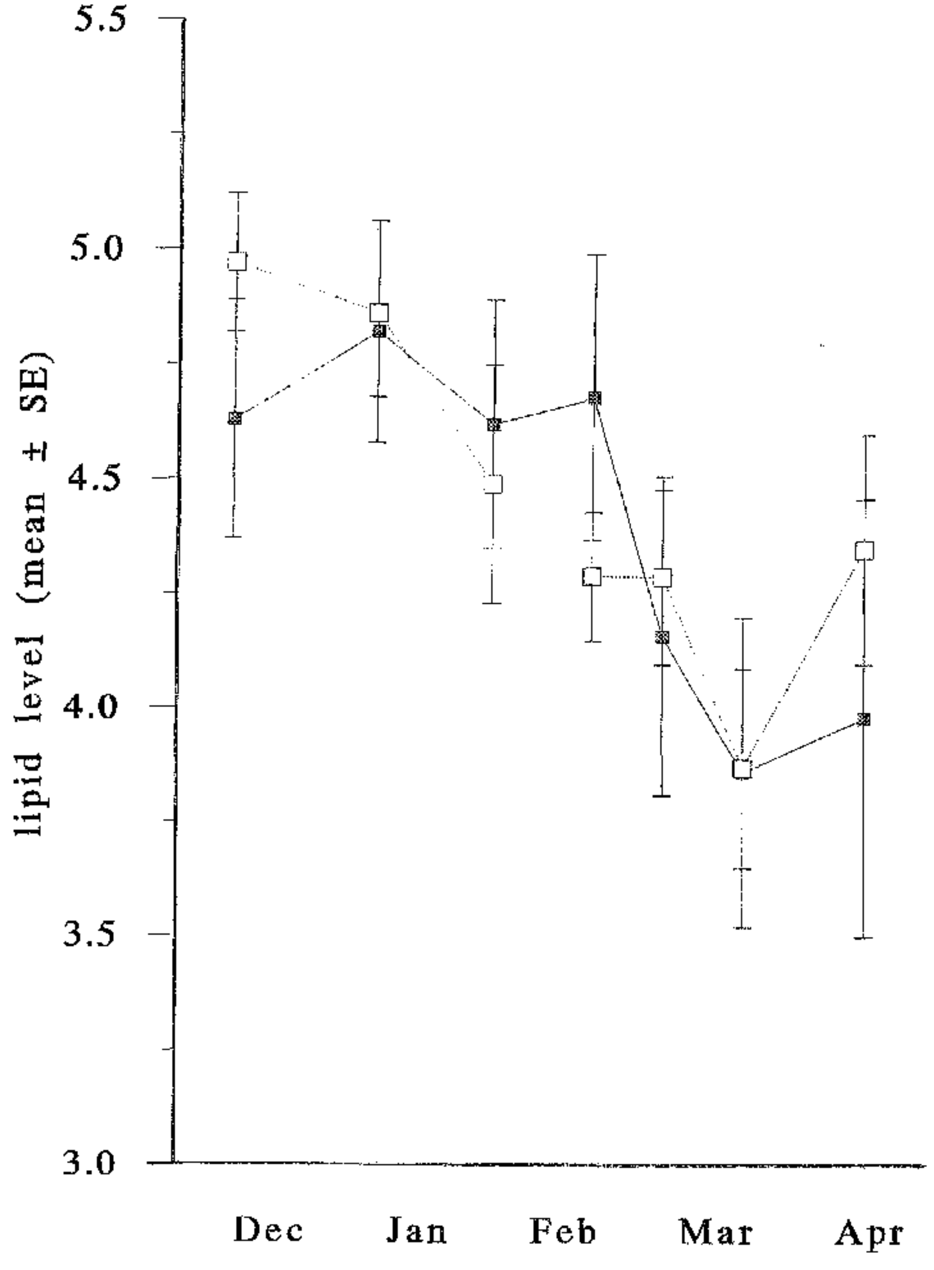

Figure 6.3. Changes in body fat throughout the course of the experiment. Data are presented as a percentage of wet body weight and are mean values for both the experimental (solid symbols) and original/replacement control groups (open symbols, dotted line). The discontinuity in the line representing the control group indicated the time of replacement of this group. Both groups displayed similar fat levels at all times and depleted fat at the same rate throughout.

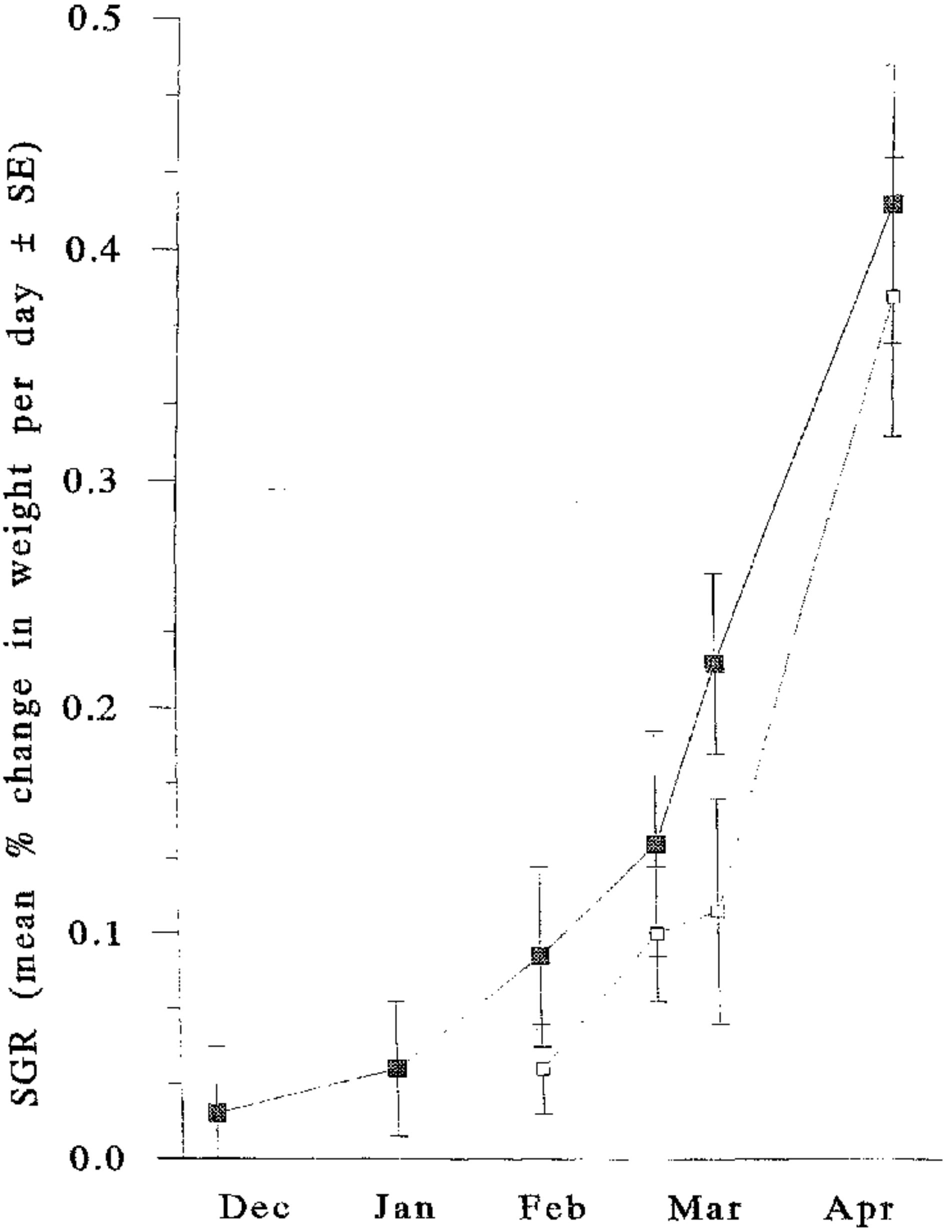

Figure 6.4. Changes in weight throughout the course of the experiment. Data are presented as mean values for both the experimental (solid symbols) and replacement control groups (open symbols, dotted line). Both groups displayed similar increases in body weight on every comparable sampling date.

loss between 3 Jan.-18 Apr., $t = 0.93$, d.f. = 31, N.S.). As a consequence, there was no difference in the body fat levels between groups at any time during the course of the experiment (ANCOVA between treatments controlling for body size, all N.S).

Somatic growth.

Both those fish with access to a refuge and those without continued to increase in weight throughout the course of the experiment, although the increases were extremely small for the majority of the time (figure 6.4). The provision of a refuge itself had no effect upon the rate at which fish were gaining weight (t-tests on SGRw (see chapter 3.3) between treatment groups, all N.S). The daily weight gain over the entire 86 day period between sampling points in January and April was the same for both groups (t-test on SGRw between treatment groups, $t = 0.86$, d.f. = 32, N.S.).

6.2.4 Discussion.

The daytime sheltering behaviour had no measureable effect upon the conservation of fat stores, as both the groups of fish were depleting their fat at a similar rate (figure 6.3). The loss of approximately 1% fat on average during the course of the experiment (a five month period) in both groups of fish, is in close agreement with the natural losses in the wild (Gardiner & Geddes, 1980).

Fausch (1984) provided evidence that juvenile salmonids will select a foraging position on a basis of water velocity characteristics and food supply so as to maximise their net energy gain. By using the refuge provided, the experimental fish would have benefitted by minimising the energy expended on holding station against a current; note that their ability to withstand currents reduces drastically over the range of water temperatures in the present study (Rimmer *et al.*, 1985; Graham *et al.*, 1996). However whilst in the refuge, fish would have been unable to obtain food. By emerging only under the cover of darkness, the experimental fish were losing out on the potential to feed efficiently, since feeding efficiency is greatly reduced at low light levels (Fraser & Metcalfe, *in press*) but this was not reflected in terms of an accelerated fat loss when compared to the control fish. Although experimental fish were not necessarily in the refuge for the whole of the light period, this result implies that the food intake necessary to maintain fish on their normal seasonal trajectory of fat depletion can be obtained by nocturnal foraging. The timing and intensity of feeding with or without the provision of a suitable refuge is investigated in detail in chapter 6.3.

Fish were depleting their fat stores, but still showed a slight increase in body weight (positive values for SGR*w*; figure 6.4) throughout the course of the experiment. The overwinter maintenance of body weight is in agreement with Simpson (1992) and was probably due to fat replacement by water (Love, 1980) and a slight increase in overall body size as water temperatures started to increase.

The suggestion that the use of cover by juvenile salmonids in winter offers some physiological advantage (Rimmer *et al.*, 1984; Pickering & Pottinger, 1988) was not reflected in the rate at which fat was depleted. Indeed, the results of the current study are in disagreement with a study conducted on juvenile cutthroat trout, where the provision of a cover above a raceway significantly increased the fat content of experimental fish during a period of 166 days (Wagner *et al.*, 1995). The advantage of adopting a strategy of daytime concealment and nocturnal emergence may therefore not be physiological, but ecological. Juvenile salmonids are more vulnerable to predation in winter as a result of a reduction in their overall performance at low temperatures (Webb, 1978; Rimmer *et al.*, 1985; Graham *et al.*, 1996). By seeking refuge in interstitial crevices during the day and emerging to feed at night, the risk of capture would be minimised (Fraser *et al.*, 1993; 1995) and sufficient food could be obtained under the cover of darkness to safeguard the normal rate of fat depletion.

Chapter 6.3 - The effect of refuge use on the timing and intensity of feeding

6.3.1 Introduction

Salmonids in general have long been considered as feeding predominantly during the hours of daylight (Hoar, 1942; Higgins & Talbot, 1985; Sagar & Glova, 1988; Thorpe *et al.*, 1988; Angradi & Griffith, 1989). In winter, whilst juvenile salmonids are concealed in stream-bed refuges during the day (chapter 6.1), some opportunistic feeding on benthic invertebrates has been found to occur (Cunjak & Power, 1987). However, as the majority of food items in the fishes' diet are drifting invertebrates, acquired by darting out into the current from a vantage point (Wankowski, 1981; Stradmeyer & Thorpe, 1987), the fish have to leave the refuges to occupy a suitable feeding station from which to intercept prey. The switch to seeking daytime refuge may offer advantages in terms of avoiding predation (Fraser *et al.*, 1993; 1995), but inhibits the daytime acquisition of food. Juvenile brown trout have been observed to emerge from their daytime shelters under the cover of darkness, to feed on drifting invertebrate prey (Heggenes *et al.*, 1993). Rainbow trout show a shift in feeding time from late afternoon in summer to night and early morning in winter, concurrent with adopting daytime sheltering behaviour (Riehle & Griffith, 1993). In a laboratory study investigating the factors controlling the shift to nocturnal activity in juvenile salmon, Fraser *et al.* (1993) noted that at low temperatures there was feeding activity during darkness in fish normally dormant in refuges by day.

As juvenile salmonids rely almost entirely on vision in order to detect and capture food (Stradmeyer & Thorpe, 1987), the efficiency of feeding by night is lower than that possible during the day (Fraser & Metcalfe, *in press*). However, this may be compensated for by the fact that in the wild situation, both the quantity and quality of drifting food items is increased at night (Elliott, 1965; 1970; Sagar & Glova 1988), increasing encounter rates. Fish may therefore be adopting a nocturnal feeding strategy in order to take advantage of the increase in drift plus the reduced predation rates associated with foraging under the cover of darkness (Fraser *et al.*, 1993; 1995).

In the normal hatchery situation, fish are denied access to daytime cover during winter, and Higgins & Talbot (1985) and Jørgensen & Jobling (1992) both reported that under these conditions the feeding rates of juvenile salmon were at a minimum during darkness. This occurred presumeably as a result of the fish being exposed to food items 24 hours per day and utilising the increased efficiency of daytime feeding. However, what is still unclear from the literature is whether the potential to sheltering during the day has any real effect on the choice of feeding times during winter by altering the time available in which to forage.

The aim of the current study was to investigate this effect by allowing one group of juvenile salmon unlimited access to daytime cover and comparing both the incidence and intensity of feeding to a control group denied the possibility of seeking cover.

6.3.2 Materials & methods

One hundred fish were selected from a stock holding tank on 13 October 1995 for use in the experiment. The hatchery-reared fish used were the progeny of a pair of wild adults from the Loch Lomond catchment. The fish were selected if <73 mm forklength to maximise the proportion of LMG fish in the treatment groups (see chapter 3.2). All fish were weighed (to the nearest 0.01g) and given a unique combination of alcian blue dye marks. They were assigned to one of two size-matched groups, each housed in a 1m tangential flow tank where pelleted food was provided to excess from an automated feeder dispensing food every 20 minutes throughout the 24 hours. The tanks were placed next to a window in the laboratory, allowing natural light from the moon and stars to illuminate the water on clear nights. Fish experienced ambient photoperiod and water temperature throughout the course of the experiment. One group of fish, hereafter termed the experimental group, was provided with a communal darkened refuge in which to shelter. The control group were denied any access to cover in their tank . The refuge was made from a standard 4 cm deep, 30 x 30 cm aluminium dissecting tray with a section (20 x 3cm) removed from one side. The tray was placed upturned on the tank floor and weighted down. To prevent food pellets from entering the refuge as they were carried around the tank in the water flow, the entrance was always located at the downstream side of the refuge. The refuge was removed, cleaned and replaced every day as part of the normal fish husbandry procedure.

The influence of daytime refuge use on the timing and intensity of feeding was investigated using the X-ray technique described in chapter 3.5. Labelled food replaced the normal pelleted food for a period of 4hr commencing at either 1000 hr (day) or 2200hr (night) at approximately monthly intervals throughout the next four months (table 6.1). All fish were then removed, identified and X-rayed under anaesthetic before being replaced back into their respective tanks, where normal pelleted food was once again provided.

Fish from both groups were weighed on three occasions during the course of the experiment (15 December, 6 January and 14 March). This allowed the quantity of food eaten during a given X-ray trial to be expressed as a percentage of body weight, by interpolation of an individual fish's weight to the date of the trial (see chapter 2.5). Following re-weighing on 14 March, all the fish were assessed for external signs of smoltification.

Table 6.1. Sampling dates for day and night X-ray feeding trials.

Day trials (1000 - 1400hr)		Night trials (10pm - 2am)	
Date	Water temperature	Date	Water temperature
6 November	10.5 °C	15-16 November	10.1 °C
8 December	8.0 °C	15 -16 December	8.0 °C
4 January	6.3 °C	11-12 January	6.0 °C
22 February	4.6 °C	10-11 March	5.0 °C

6.3.2 Results

A total of 16 fish showed signs of smolting in March (control group = 11, experimental group = 5) and were subsequently excluded form the analysis. Another 9 fish died throughout the course of the experiment (control group = 2, experimental group = 7) resulting in there being 38 experimental and 37 control fish by the end of the experiment.

The refuge was used extensively by the experimental group as a shelter, and once the water temperature was consistently below 8^0C, the experimental fish were rarely visible on the tank floor during the day. Occasional observations at night confirmed that fish were leaving the refuge.

Timing of feeding

The proportion of fish in both the control and the experimental groups that were feeding during trials at day and at night showed a general decline between November and January (figure 6.5). This decline continued during the day trials for the experimental group, with no fish feeding during the trial on 22 February. On every

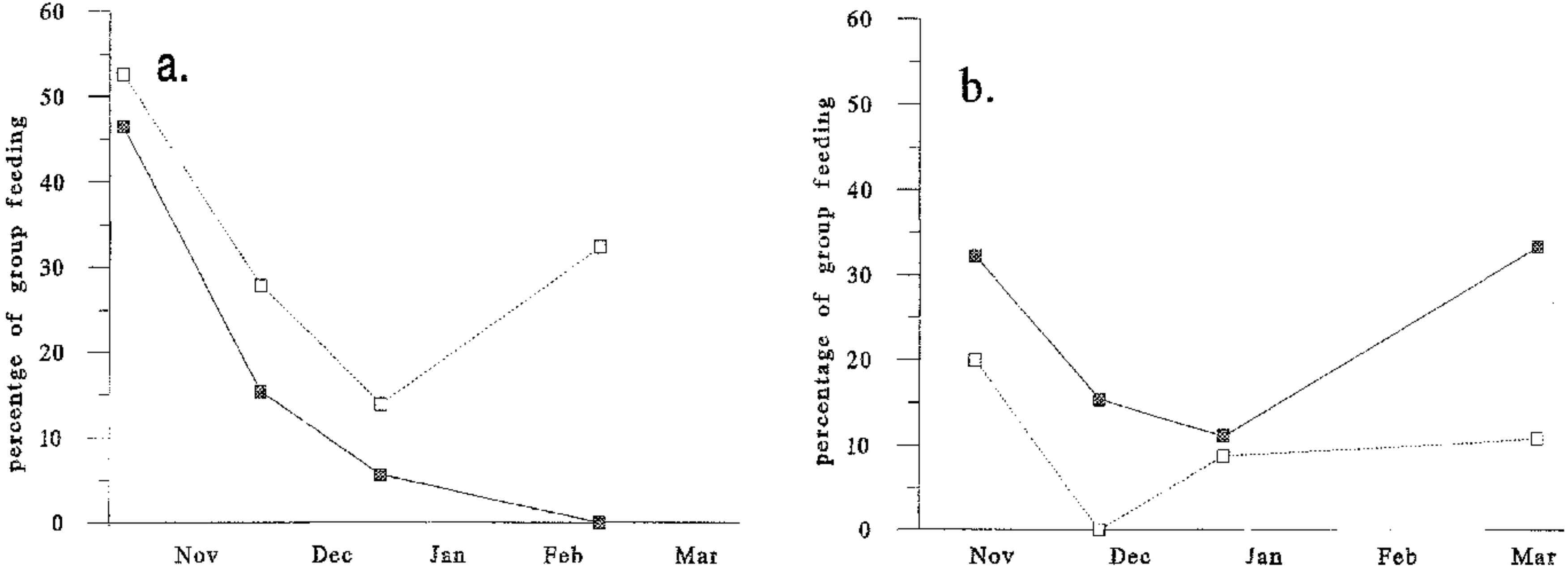

Figure 6.5. The proportions of each treatment group feeding during a 4 hour feeding trial during the day (a.) and night (b.) Data are presented for both the experimental fish (solid symbols) and controls (open symbols) throughout the course of the experiment.

daytime trial, more fish were feeding in the control group than in the experimental group with access to a suitable refuge. Conversely, on every X-ray trial conducted at night, more fish in the experimental group were found to have been feeding than in the control group. However, these trends were not statistically significant (χ^2 tests on proportion of the treatment groups feeding during both day and night trials, all N.S).

Intensity of feeding

The food consumption data were pooled across the four day and night feeding trials and included only those fish that survived to the end of the experiment in order to reduce noise (figure 6.6). Both groups of fish exhibited the normal seasonal reduction in the amount of food consumed as the winter progressed. The control fish consumed more food during the day than by night (paired *t*-test comparing day and night, t = 3.25, d.f. = 36, P<0.01), although there was no difference in the amount of food consumed by day and by night by the experimental fish (paired *t*-tests comparing day and night trials, t = 0.13, d.f. = 35, N.S.). When comparisons were made between groups, both were found to be consuming similar amounts of food by day (*t*-test comparing between treatments, t = 0.39, d.f. = 71, N.S.) but the experimental fish were eating more than the controls at night (t-test comparing between treatments, t = 2.69, d.f. = 71,P<0.01). When all data collected from day and night trials were pooled, there was no difference in the average food consumption during the duration of the experiment (*t*-test comparing between treatments, t = 0.75, d.f. = 71, N.S.).

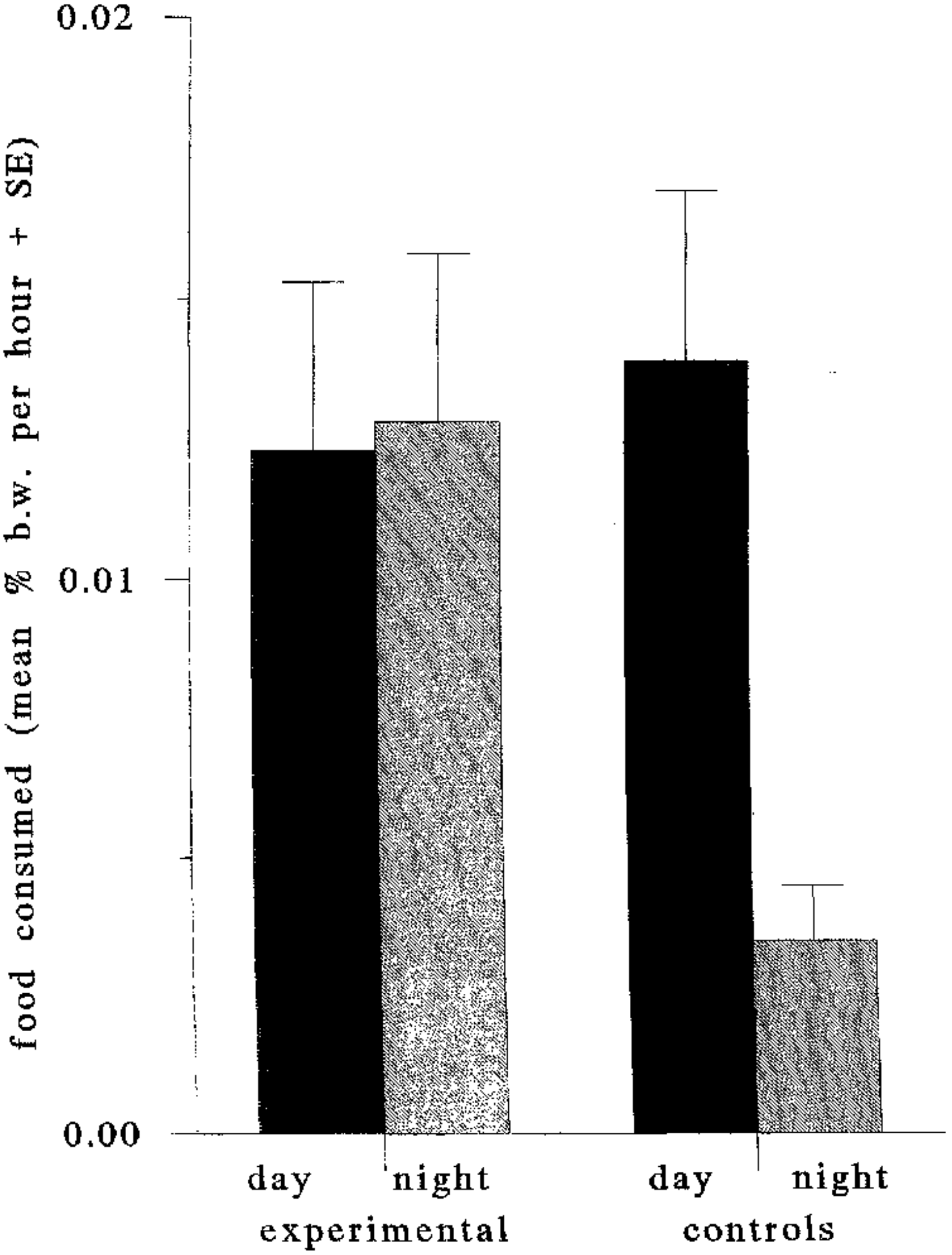

Figure 6.6. The quantity of pelleted food consumed by fish feeding during the 4 hour feeding trials during the day and night throughout the course of the experiment. Values are expressed as mean percentage of wet body weight for the total number feeding during the 4 day and night trials.

6.3.4 Discussion.

Juvenile salmon that opted to relinquish their feeding potential for the majority of the daylight hours by sheltering in a refuge did not differ statistically in either the timing of periods of feeding, or the intensity of feeding bouts. Higgins & Talbot (1985), using a similar technique to measure feeding in 1+ juvenile salmon during May, found that during darkness (2330-0230hr) fewer fish were feeding, but those that did feed were not consuming any less per hour than during the day. Starving a group of fish prior to testing food intake in darkness had little effect on the numbers of fish feeding, and the authors concluded that fish were predominantly feeding between dawn and dusk. The results of Higgins & Talbot (1985) show similarities to those of the control group presented here, in that culture conditions and experimental protocol were similar, with some feeding occurring during day and night. However, the fact that their trials were carried out in May when water temperatures were 8 - 11^{0}C (above the critical temperature when juveniles normally abandon nocturnal foraging, Fraser *et al.*, 1993) mean that their results cannot be extrapolated to the winter season, when lower water temperatures predominate and fish would normally be seeking refuge during the day.

Jørgensen & Jobling (1992) used a similar technique at 6^{0}C on a population of hatchery reared salmon under a simulated winter photoperiod and found that the feeding rate (the amount ingested per hour) was higher during the daylight hours, but that the total food intake of individual fish was higher during night. They concluded that feeding does occur during darkness and although the efficiency of feeding was markedly reduced (*sensu* Fraser & Metcalfe, *in press*) the total food intake was higher during the night than during the day, simply because the nights lasted longer. These results cannot be directly comparable to those presented here, as all fish used by Jørgensen & Jobling were from the UMG, so would have exhibited different feeding behaviours when compared to the LMG fish used in the present study (Metcalfe *et al.*, 1986; 1988).

Although not statistically significant as a result of few fish feeding due to low water temperatures at the time of many trials (Elliott, 1975; Brett, 1976; Love, 1980), more fish with access to a daytime refuge were emerging and feeding at night than the control fish (figure 6.5). Conversely the control fish, in constant visual contact with food pellets throughout 24 hours, were more inclined to feed during the day than those seeking refuge. The results of the investigation into the intensity of feeding indicated that during their brief excursions from the refuge during the day, those experimental fish emerging were feeding intensely and receiving a similar amount of food as the control fish. Such short 'sampling' trips have recently been found to be a common feature of juvenile salmon hiding in refuges, and are influenced by both light intensity and food availability (N.H.C. Fraser, unpublished data). Fish unable to achieve an adequate daily ration by merely foraging at night may therefore have been undertaking short forays from the refuge during the day to supplement their night-time intake. The similarity between groups in the total pooled individual food intake provided evidence for the requirement of a constant maintenance ration, regardless of the provision of a refuge.

In summary, the results although inconclusive due to environmental limitations placed on the technique employed, indicated that the daytime use of a refuge influenced the feeding times of overwintering salmon, such that fish compensated for the reduction in daytime feeding time by increasing their foraging rate at night. Those fish without access to a refuge and consequently in constant contact with food pellets, did not take advantage by consuming more during the daytime, indicating that daily intake rates during winter are under internal control (Metcalfe *et al.*, 1986; Metcalfe & Thorpe, 1992; see chapter 2) and are not limited by the behavioural switch to seek refuge during the day.

Chapter 6.4 - The effect of increased starvation risk on sheltering behaviour

6.4.1 Introduction.

The impact of predation risk on the feeding strategy adopted by animals has received much attention in the literature. Where there is a trade-off between feeding and predator avoidance, decisions regarding the optimal allocation to each behaviour often depends on both the risk of capture and the cost of lost feeding opportunities (Ydenberg & Dill, 1986). The balance between these (often conflicting) demands is affected by the animal's nutritional state (Lima, 1988; Mangel & Clarke, 1988).

Feeding involves a loss of crypsis, so making the fish more vulnerable to visual predators (Martel & Dill, 1995). Juvenile salmonids have been shown to take higher risks in terms of potential predation when hungry (Dill & Fraser, 1984; Magnhagen, 1988; Gotceitas & Godin, 1993) or when a high feeding rate and growth is desirable (Huntingford *et al.*, 1988). As the susceptibility of salmonids to predation varies with environmental water temperature as a result of its effect on their ability to accelerate quickly and so evade capture (Webb, 1978; Fraser, 1994; Johnson *et al.*, 1996), a strategy minimising this risk would be advantageous during the winter. The fishes' main avian predators (e.g sawbill ducks and the grey heron) rely heavily on vision and so are limited to hunting during the day (Cramp & Simmons, 1973), subsequently reducing the fishes' risk of capture during the night. Although the feeding efficiency of juvenile salmon is markedly reduced at the low light levels associated with even the brightest moonlit night (Fraser & Metcalfe, *in press*), the reduced physiological requirement for feeding (e.g. Elliott, 1975; Love, 1980) at low water temperatures in combination with an increase in the total quantity of drifting invertebrates available (Elliott, 1965; 1970; 1973; Sagar & Glova, 1988) may make a strategy of nocturnal foraging beneficial. The fish seek refuge during the daytime in stream-bed crevices (chapter 6.1) when the drifting food is naturally lower (Hynes, 1970) and emerge to feed at night when the risk of predation is reduced.

However, the unpredictable nature of food availability and rapidly changing environmental conditions associated with winter (e.g. ice formation and spate conditions) may result in fish experiencing periods of metabolic deficit that result in rapid depletion of energy stores. In this situation, nocturnal feeding alone may not be efficient enough to offset the risk of starvation. The aim of the current study was to examine the extent to which fish will maintain a nocturnal feeding regime when faced with the possibility of starvation at low water temperatures.

6.4.2 Materials & methods

Forty LMG fish were randomly selected from a stock population of siblings on 23 January, weighed and measured to allow fat estimation, and given a unique combination of dye marks. Fish were allocated to two size-matched groups: the experimental, and the control (both n = 20) and received a separate batch-coding mark on either the left or right pectoral fin to identify them as such. Both groups were maintained together for two weeks in a 1m holding tank where food was provided to excess by way of an automated feeder dispensing pelleted commercial salmon food every 10 minutes throughout the 24 hours. On 6 February, the experimental group was moved to a separate 1m tank without a feeder for 15 days. Both groups of fish were then re-weighed and measured for fat estimation on 21 February, and established in two separate white 60cm diameter tangential flow tanks fitted with white food-tight 'false bottoms' which provided a refuge (see figure 6.1). Food was provided in excess to both groups from automated feeders suspended above the tanks, releasing a trickle of food every 10 minutes throughout the 24 hours. No food was available in the refuge; the fish therefore had to choose between being in a safe refuge or a potentially risky feeding site.

Information regarding the numbers of fish out of the refuge during the day was collected by suspending a video camera from a gantry approximately 1.5m above the water surface of the tanks and fitting it with a wide angle lens that included both tanks in the field of view. The camera was connected to a video recorder that was programmed to film the tanks from dawn until dusk (simulated natural photoperiod). Filming commenced on 23 February, after allowing 48 hours for both groups of fish to settle in the new tanks, and continued until 1 March. Video tapes were analysed by counting the number of individuals in each tank that were fully emerged from their refuges every 5 minutes. The twelve counts made per hour were combined and expressed as a proportion of the number of fish remaining in the tank. A daily mean proportion was then calculated from these hourly values. The time of any disturbance to either tank as a result of cleaning or disruption of water flow was noted, and the data discarded until the pre-disturbance count of fish had once again stabilised. On 2 March both groups of fish were removed from their respective tanks and measured for a final estimation of body fat level.

6.4.3 Results

A total of four fish died during the course of the experiment (experimental = 3, controls = 1).

Fat dynamics

Presumably by chance, the experimental group had lower levels of body fat than controls prior to any manipulation (mean fat level of experimental group fish on 23 January = 2.22% body weight (b.w.) ± 0.48 S.E, n = 19; mean fat level of controls = 3.20% b.w. ± 0.26, n = 19: ANCOVA between treatment groups and controlling for body size (forklength), $F_{(1,37)}$ = 6.70, P<0.05). Although this initial discrepancy between groups was maintained throughout the experiment, the deprivation period imposed on the experimental group reduced their levels of body fat at a time when controls were putting on fat (mean change in body fat between 23 January and 21 February for the experimental group = -0.67% ± 0.47, and controls = +0.72% ± 0.31: *t*-test between treatments on the change in fat; *t* = 2.50, d.f. = 34, P<0.05). When food was once again provided to the experimental group following deprivation, they responded by slowing their rate of fat loss to a level no different from the controls (mean change in body fat between 21 February and 2 March for the experimental group = -0.13% ± 0.04, and controls = -0.04% ± 0.03: *t*-test between treatments on the change in fat: *t* = -1.71, d.f. = 33, N.S), and exhibited the typical fat response associted with a deprivation in late winter (see chapter 3.4).

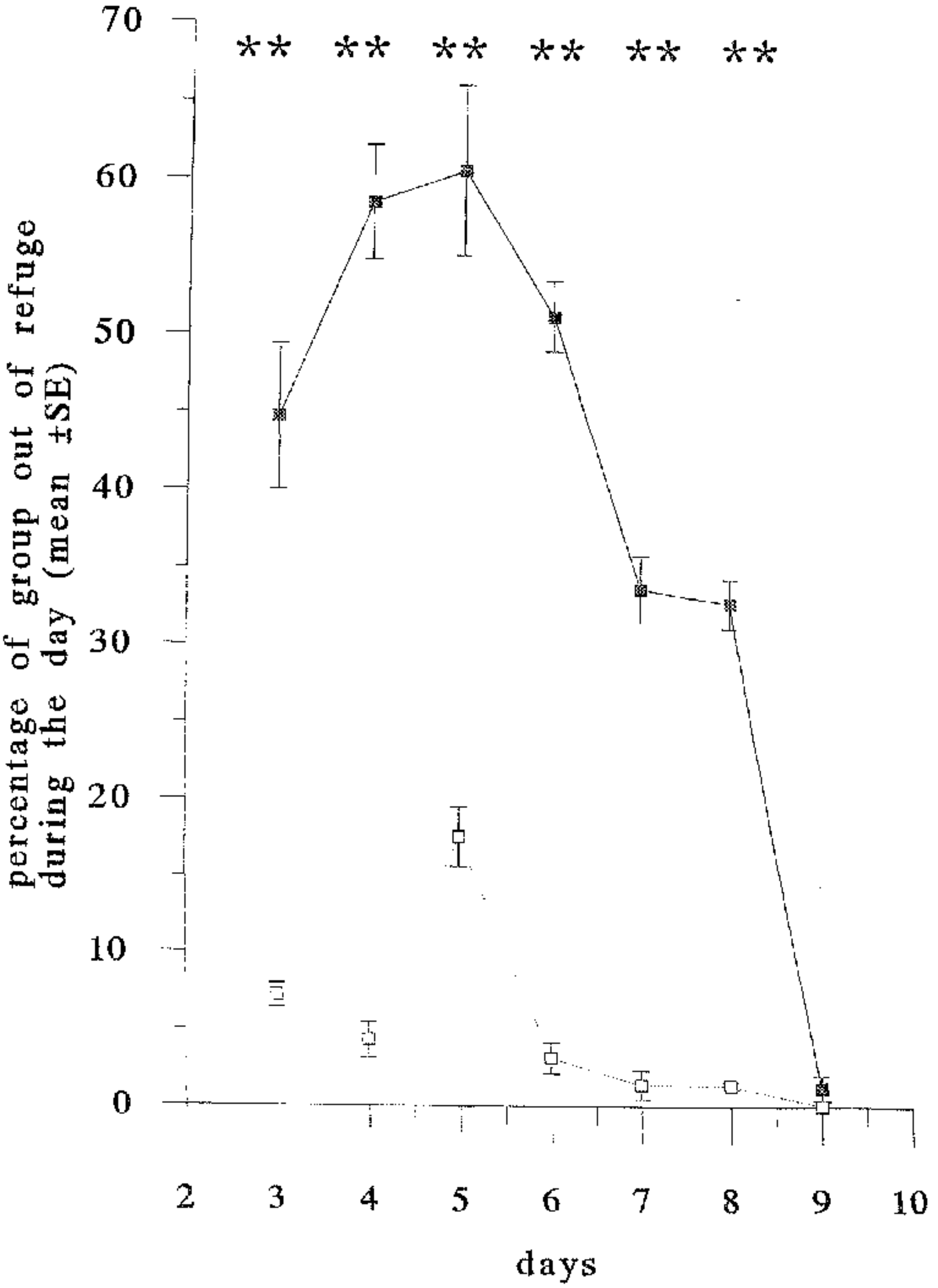

Figure 6.7. The percentage of the treatment groups observed out of the refuge during the hours of daylight.The x axis indicates the number of days since resumption of feeding, applicable to the experimental group (solid symbols). Control fish are represented by open symbols and a dotted line. Values are means calculated from repeated daily observations throughout the light period (+ SE). Asterisks indicate the significance of a Mann-Whitney *U* test comparing median numbers between treatment groups at each sampling date (** = P<0.01, * = P<0.05).

Sheltering behaviour

Water temperatures were low throughout the course of filming (see figure 6.2), so that many fish in both groups used the refuges during the day.

During the first 8 days of refeeding, the previously deprived experimental fish were more frequently observed out of the refuge during the day than the controls (figure 6.7) presumably as a result of their lower nutritional status. However, this response was short-lived and by day 9 the proportion leaving the refuge during the day had fallen rapidly and was no different from the controls.

6.4.4 Discussion

Fish that had previously experienced a rapid depletion in energy stores spent a greater proportion of the day out of the refuge once feeding conditions improved. However, this response was short-lived, and may be equated with a brief period of hyperphagy following food restriction (see chapter 3.2) as food acquisition could only occur when out of the refuge. The 'perceived' predation risk would have been much higher for fish exposed against the white tank floor (especially if they were foraging) as opposed to remaining in the refuge (Martel & Dill, 1995). Therefore these fish were increasing their risk of being predated in order to feed during the day. The increase in the potential mortality risk offered by exposure on the tank floor must have been overcome by the benefits of a sufficiently higher foraging profitability (Werner *et al.*, 1983; Abrahams & Dill, 1989).

When assessing predation risk and balancing the available information on the costs and benefits of refuge use, one of the proximate factors (Stephens & Krebs, 1986; Krebs & Kacelnik, 1992) involved in the decision must have been the effect of low water temperatures on the ability to escape from predators (Webb, 1978: Fraser, 1994; Johnson *et al.*, 1996). Thus, the normal refuge seeking behaviour during winter (as displayed by the control fish in the current study) is probably a direct result of the threat of predation (Fraser *et al.*, 1993; 1995). Predation risk has been shown to influence developmentally important decisions (Brännäs, 1995), habitat choice (Werner et al., 1983; Magnhagen, 1988; L'Abée-Lund *et al.*, 1993), reproductive behaviour (Magnahagen, 1991) and feeding behaviour (Huntingford *et al.*, 1988; Gotceitas & Godin, 1993; Martel & Dill, 1993) in fishes. However, by increasing the potential mortality risk due to the imminent exhaustion of energy stores, this experiment altered the balance of the trade-off between predator avoidance and feeding in experimental fish. The potential threat of starvation was accentuated in this group of fish as they had, by chance, lower fat levels than the controls prior to deprivation. Indeed this may well account for the discrepancy between the intense compensatory foraging effort displayed in the current study, and the weak compensatory feeding response predicted to follow a (more extensive) deprivation period at a similar time of year in chapter 3.4. Intense foraging would only be predicted if the fish in the current study were well below the threshold value for foraging at this stage in the winter (see Bull *et al.*, 1996).

In summary, the results firstly provide evidence for the presence of a trade-off between predation risk and the necessity to maintain a normal pattern of nocturnal emergence and secondly, that the balance of this trade-off is influenced by nutritional state. The optimal feeding strategy employed during the winter therefore takes account of both risks associated with capture and those associated with starvation in a manner which appears to maximise the probability of overwinter survival (Bull *et al.*, 1996).

Chapter 6.5 -Winter field study

6.5.1 Introduction

Much of the information relating to juvenile stock asssment in salmonid fisheries management comes from electrofishing surveys undertaken in the summer months. Standard serial removal techniques (Zippin, 1958) and more recently, a one run semi-quantitative technique (Strange *et al.*, 1989; Crozier & Kennedy, 1994) have been successfully employed to sample juvenile salmonids in their natural habitat. Winter water temperatures cause juvenile salmonids to undergo a behavioural switch from the normal pattern of diurnal activity, to lying dormant in streambed refuges during the day and emerging under the cover of darkness (Fraser *et al.*, 1993; 1995). During this time, nutritional stress has been highlighted as a factor resulting in the high overwinter mortality rates associated with the winter (Gardiner & Geddes, 1980; Cunjak, 1988b). Too often in the U.K, this season and its effect upon the salmonid's behaviour and physiology has been neglected by fisheries managers. Consequently there appears to be little information regarding survival rates and habitat preferences of juveniles in their nursery streams. It therefore seems important to obtain information regarding the whereabouts and health status of stocks during the winter.

This aspect of fisheries management has received some attention from researchers in North America and Scandinavia (Rimmer *et al.*, 1983; Cunjak & Power, 1986a; Cunjak, 1988a,b; Heggenes & Saltveit, 1990; Heggenes *et al*, 1993; Smith & Griffiths, 1994; Griffiths & Smith, 1995). However, studies concerned with the density and microhabitat preferences of stream-dwelling salmonids during the winter have been hindered by two things, namely that the efficiency of standard pulsed AC electrofishing equipment is markedly reduced at typical winter temperatures (Cowx, 1983), and that most of the fish present will be concealed in stream-bed refuges during the day (Fraser *et al.*, 1995). Consequently most investigations have relied upon direct observations using snorkeling techniques (Cunjak & Power, 1986; Cunjak, 1988a; Heggenes & Saltveit, 1990; Heggenes *et al.*, 1993). This technique has been successfully used by day to locate fish, and also at night in order to estimate the abundance of salmonid species. Night electrofishing has been successfully used to examine diurnal fish movements in wide, deep rivers and reservoir outflows in the United States (Sanders, 1992; Vanzee *et al.*, 1996) and in the River Morava, in the Czech Republic (Copp & Jurajda, 1993). Electrofishing has been used during the day in winter in Scotland, with limited success (R. Gardiner, SOAEFD: pers. comm.) but has never been attempted at night even though fish should be more susceptible to this sampling technique in darkness at this time of year, due to their nocturnal habits.

The purpose of the current field study was two-fold. The first aim was to check the feasibility of electrofishing at night as compared to the same technique carried out during the day, as a means to sample stream-dwelling juvenile salmon in mid-winter. The second aim was to compare the nutritional status of fish sampled out of the refuges during the day and at night. Since the fish appear to avoid diurnal activity at this time of year we can hypothesise that fish captured out of refuges during the day would be of lower nutritional status than those captured at night and were consequently taking a risk to supplement nocturnal feeding (see chapter 6.4). I tested this hypothesis by measuring the nutritional state of all captured fish.

Previous studies concerned with the nutritional status of stream-dwelling juvenile salmon in winter have either used destructive sampling followed by proximate analysis (Gardiner & Geddes, 1980; Cunjak & Power, 1986a; Cunjak, 1988a; Shackley *et al.*, 1994) or have estimated the well-being of the individuals by adopting an index of condition (Wootton, 1990; Cunkak & Power, 1987; Cunjak, 1988b). Both these approaches to estimating nutritional status have their own limitations (see chapter 2 for further discussion). Therefore I used the non-destructive technique of biometric measurements, here applied to wild fish for the first time.

6.5.2 Materials and methods

The study was carried out on the Spittal Hill Burn, a 2nd Order tributary of the River Endrick, Central Scotland (O.S. second series, sheet 57 grid ref. 653864.). The small burn (average width 2m) rises from an underground spring in the Campsie hills and flows for 4.2 km before reaching the main river 6 km east of the village of Fintry. The burn was chosen for its ease of access and its healthy population of juvenile salmon, assessed by fieldwork during the summer of 1995. Four replicate sampling reaches (each 18m in length) were identified, on the basis that they all contained a similar proportion of pool and riffle habitat. All four sites (A, B, C and D) were located in close proximity to a road to ease access (especially important at night). Site A was located furthest downstream, and was separated from section B by an easily distinguishable cascade. Section C commenced 150m upstream of B, and was separated from D by a similar cascade. Bankside vegetation was sparse, and consisted of coarse grass along the majority of the sections. The only daytime shade was provided by an ash tree overhanging a portion of section C, and undercutting of banks in all sections.

At midday on 23 January 1996 (water temperature = 4.4^{0}C), Section A was electrofished using backpack electrofishing equipment (24V, Pulsed D.C., Electracatch U.K. Ltd) The sampling technique required two operators, one working the electrofisher, the other maintaining station alongside and supporting two 32 x 22 cm hand-nets (Collins Nets, U.K.). Both operators entered the section from the downstream end, and timed one pass through it using the electrofishing equipment. The sampling technique was standardised as follows. The operators stood stationary while making three downstream sweeps of the anode (near the left bank, mid-stream and near the right bank). They then advanced one pace upstream and repeated the procedure. All fish were captured in the two nets held stationary downstream of the anode by the second operator, and no attempt was made to move the nets so as to capture any fish that either missed the nets, or recovered and swam out of them prior to removal. This approach was adopted so that the probability of catching a fish would not be influenced by the ability of the operators to see, so making day and night surveys of comparable efficiency. The whole procedure took approximately twenty minutes to complete. Section C was then sampled in an identical fashion. All captured juvenile salmon were anaesthetised, measured for length (to the nearest mm), weighed (to the nearest 0.1g by means of a portable balance) and measured for body widths at the leading edges of the dorsal, pelvic and anal fins, to allow fat estimation (see equation 2.4). Whilst anaesthetised, all salmon were marked on the right pectoral fin with an alcian blue dye spot to categorise them as individuals that had been sampled during the day, if subsequently recaptured. The fish were allowed 30 minutes to recover, and were returned to an area of reduced water flow at the upstream limit of their appropriate section only once all individuals were actively swimming. Fish were seen to move off into the current, and hold station comfortably in the flow.

At 2200hrs (water temperature = 3.8^{0}C), a similar sampling technique was undertaken on section B followed by section D; note that these sections were located upstream of those sampled during the day and so would have received minimal disturbance. Every effort was made to use the same protocol and sampling effort at night as during the day, although at night it was necessary to use red-filtered head-lamps to assist with the procedure. Preliminary trials at night whereby captured fish were illuminated under red-filtered lamps caused them only minimal disturbance. The electrofishing runs were timed and the procedure used identical to that adopted during the day. All fish were identified and the salmon measured as previously described. However, each night-caught fish received an alcian blue dye mark on the caudal fin in order to identify it as such if subsequently recaptured, and returned to the appropriate section.

One week later, at 1230 on 1 February 1996 (water temperature 3.0^{0}C), section B followed by section D were electrofished. Each run was timed to match the length of that done at night the previous week to maintain constant effort. All resulting fish were treated as before, and returned to the section. At 2200 hrs that night (water temperature = 2.6^{0}C), sections A followed by C were similarly electrofished. Therefore each of the four replicate sections had been sampled once during the day, and once at night, with each electrofishing pass on a given section separated by seven days to minimise disturbance.

The burn was revisited three weeks later, on 22 February (day water temperature = 6.7^{0}C, night = 6.0^{0}C), and again on 1 March (day = 7.6^{0}C, night = 7.0^{0}C), with electrofishing undertaken once more during the day and night at each site in order to estimate the retention of marked fish in the sections. Stream discharge was noticeably increased on both occasions although quantitative measures were not possible. No new measurements were collected from fish, although the presence or absence of any previous alcian blue marks was recorded.

6.5.3 Results

A total of 65 juvenile salmon were sampled from the four replicate sites during electrofishing runs on 23 January and 1 February (mean fork length = 65.8mm ± 1.8 S.E., range = 47-110mm). Only those belonging to the 0+ year class (mean fork length = 57.7mm ± 0.6, n = 48, range = 47-67mm: as determined from inspection of a length-frequency distribution, see figure 6.8) were used in the subsequent analysis.

Using the data from each of the four replicate sections sampled, the total number of salmon sampled by electrofishing at night exceeded that caught during the day (mean number of fish caught per site at night and day = 11.50 ± 1.85 and 4.25 ± 1.05 respectively; paired *t*-test comparing the number of fish caught at night and day, t = 4.26, d.f. = 3, $P<0.05$; figure 6.9). Three 0+ salmon were caught both by day and by night in a given section; these fish, although included in the previous results, were discarded from the analysis of fat level, as their diurnal pattern of refuge-use was undefined. This left a total of 45 0+ fish sampled exclusively either at night or by day in which fat level was estimated from body measurements using equation 2.4.

There was no difference in the size or weight between fish sampled by night or by day (mean fork length and wet weight = 57.8mm ± 0.81 S.E., 1.81g ± 0.08 and 57.4mm ± 1.02, 1.85g ± 1.85 respectively; *t*-tests between night and day-caught fish on fork-length and wet weight, t = 0.38, d.f. = 43, N.S. and t = 0.06, d.f. = 43, N.S. respectively). The body fat levels of fish caught by night or day (figure 6.10) were also not different (ANCOVA between night and day caught fish, controlling for the effect of body size (fork length); $F_{(1,44)} = 0.13$, N.S.).

Three weeks later, electrofishing resulted in the capture of 32 0+ salmon from all the sections. A total of 13 0+ salmon were caught during the daytime sampling, of which

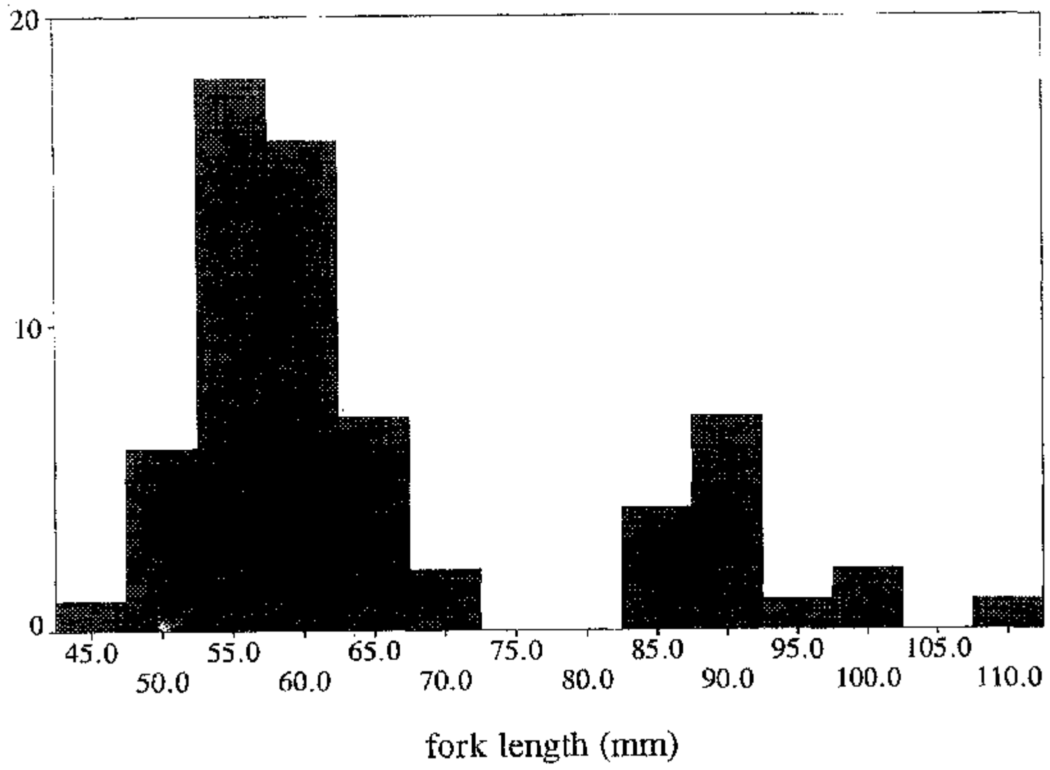

Figure 6.8. The length-frequency distribution of all juvenile salmon caught during the first day and night electrofishing runs (28 January and 1 February, 1996) at sites A,B,C, and D. Fish smaller than 75mm were confidently assigned as 0+ fish as a result of the clear separation into modes in the data.

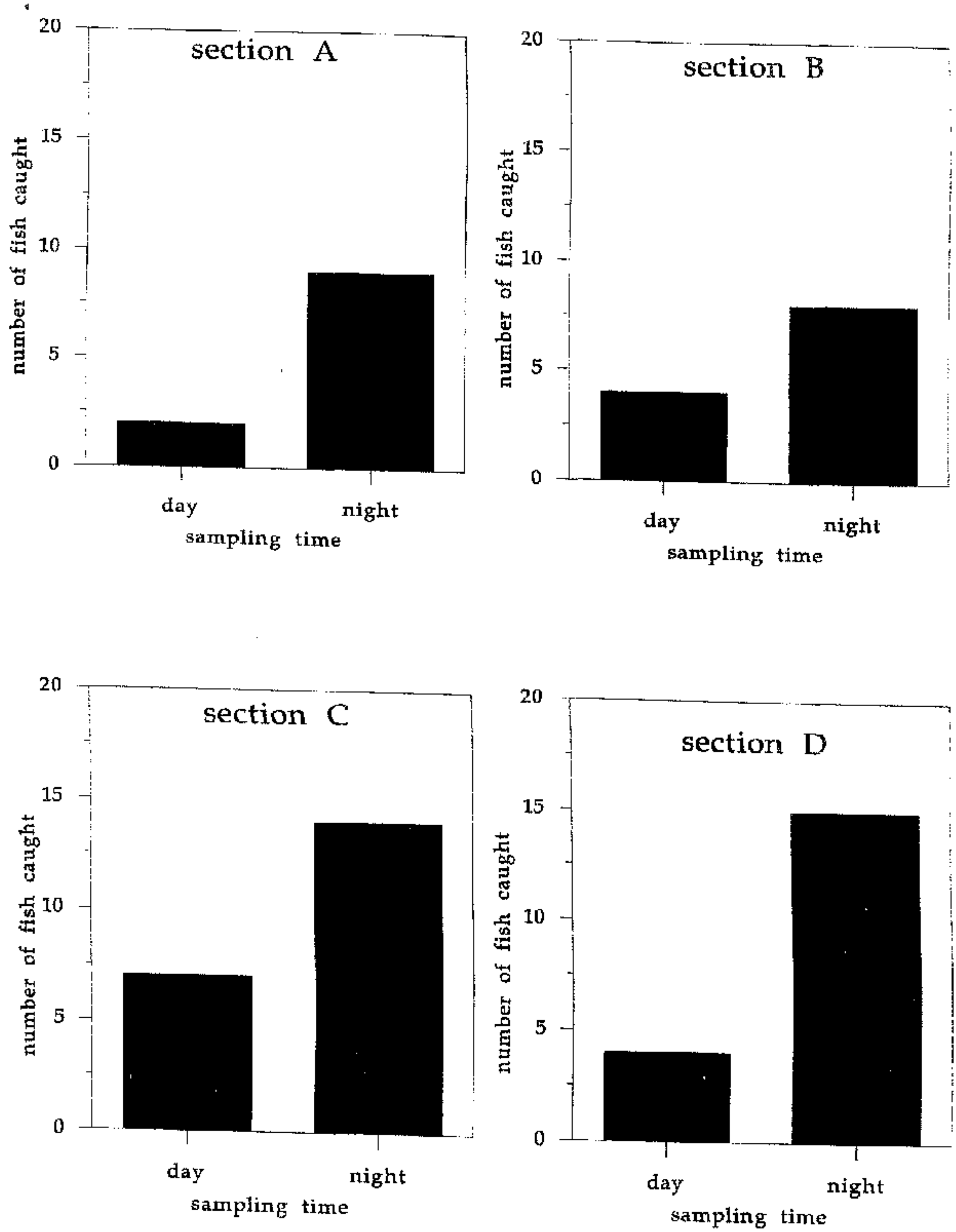

Figure 6.9. The number of 0+ salmon sampled by electrofishing by day and by night at the four sampling sites. Catch effort was the same by day and by night yet in every case, more fish were caught by night.

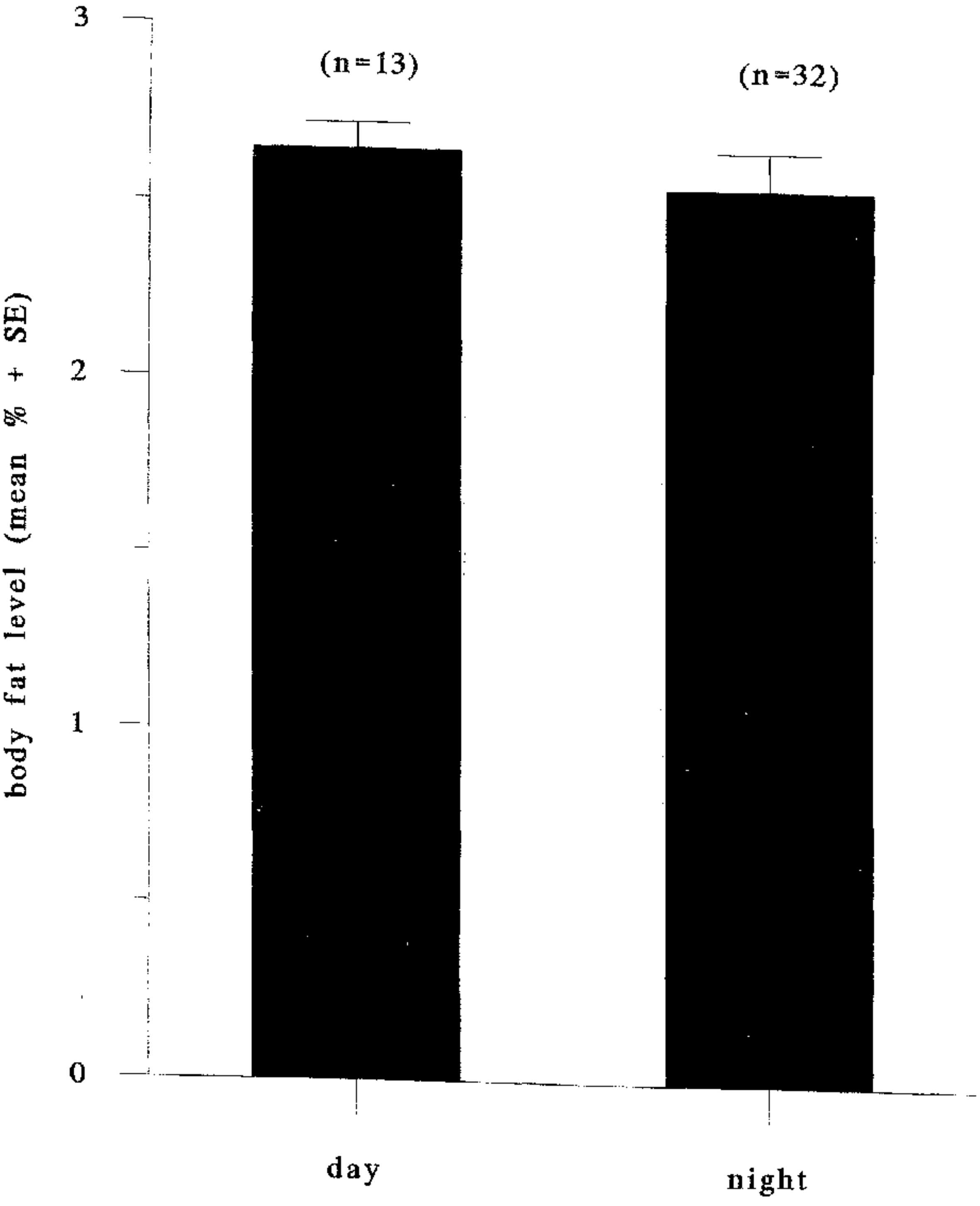

Figure 6.10. The percentage body fat of the 0+ salmon sampled exclusively by day and by night. Data are presented as a pooled average for all 4 replicate sites, and sample sizes are shown in parenthesis.

6 (50%) bore either a caudal or pectoral mark. Night electrofishing led to the capture of 19 additional fish, of which 5 (28%) were marked. Although night electrofishing resulted in more fish being captured than during the day, the difference proved non-significant (paired *t*-test comparing the number of fish caught by night and day, t = 1.24, d.f. = 3, N.S.). Overall retention in the four sampling sites was estimated as the percentage of the initial marked sample of fish (n = 48) that were recaptured; retention of marked fish was thus estimated as 22.9%.

6.5.4 Discussion

Electrofishing under the cover of darkness at low winter water temperatures proved a more effective technique of catching stream-dwelling juvenile salmon than the same procedure carried out during the day. The initial difficulties associated with working at night appeared to be worthwhile, since it more than doubled the number of fish caught, which would presumably lead to a more representative indication of population density. Nocturnal electrofishing in winter may therefore be a useful technique in small salmon nursery streams where juveniles can find daytime refuges in the stream-bed, as well being of use in larger rivers (e.g. Sanders, 1992; Copp & Jurajda, 1993)

The low incidence of recapture between night and day sampling may well have been due to fish adopting a strict regime of daytime sheltering in stream-bed refuges and subsequent nocturnal emergence (Heggenes *et al.*, 1993; Fraser *et al.*, 1993, 1995), leading to fish rarely being active (and so available to be caught) in both time periods. The range of water temperatures encountered during the course of the study were all below the threshold value (10^{0}C) at which fish adopt this nocturnal behaviour (Fraser et al, 1993). Alternatively, the disturbance caused by electrofishing and handling (Mesa & Schreck, 1989; Snyder, 1995) may have caused fish to leave the area, preventing their recapture the following week. The low estimate of retention in the sampling areas (22.9%) may have been indicative of the lattter explanation, although increased water temperature and velocity during this time will have influenced the comparability of the results (Cowx, 1983). The increased flows experienced during nocturnal sampling in March may have led to an under-estimation of the numbers of marked fish retained. In addition, the single-pass technique with no attempt to capture escaping fish would have been relatively poor at sampling all the fish in the sections.

The body fat levels of fish sampled by electrofishing by day and by night were not significantly different. On first inspection, this result must lead to the rejection of the initial hypothesis that assumes that fish exposed during the day are of lower nutritional status. However, a number of explanations for the observed result may be proposed. Hatchery-reared fish only left the relative safety of refuges to make prolonged daytime foraging sorties if they had experienced an extended period of deprivation, when energy stores were artificially lowered (an average reduction of 0.67% wet weight equivalent to 1/5 of the total body fat pre-deprivation, chapter 6.4). Moreover, in this artificial situation, the fish were faced with an "all or nothing" choice regarding location: they were either in a darkened refuge where they could not feed, or exposed on a white tank floor. The natural heterogeneity of a stream-bed will offer a wide range of microhabitats to a fish, varying in both the degree of exposure and the availability of suitable prey. As salmonids have been shown to select the most profitable in-stream location, governed by their particular requirements (Fausch,1984), it may be postulated that fish sampled during the day in the current study might have been occupying marginal habitats, intermediate between complete exposure and concealment, where some daytime feeding on drifting prey would be possible. These fish would have been more susceptible to the sampling technique than those occupying refuges deeper in the substratum, but they would not necessarily have been in as poor a nutritional state as would warrant full daytime exposure.

The large natural range of fat levels estimated in these 0+ wild fish (range = 1.59-3.83% of body weight) may have masked small (yet important) changes in fat. The possibility that wild fish were altering their behaviour at a different threshold level of energy reserve depletion than hatchery-reared fish (as a result of previous nutritional history) cannot be discounted. Therefore, another possible explanation lies in the fact that the day-caught fish were indeed responding to depleted energy reserves by opting for locations where daytime feeding would be possible, as predicted by the hatchery study, but the degree of fat depletion was too small to be detected by the biometric technique. Conversely, it could also be argued that these fish were occupying refuge areas that were as energetically profitable as those deeper areas, as the fish were not suffering from any appreciable energy deficit.

The results of chapter 6.3 indicate that although fish spend the majority of the daylight hours in refuges during winter, they will often emerge for short foraging trips. This may in part be due to their immediate nutritional demands exceeding that which can be achieved solely by nocturnal foraging (Fraser & Metcalfe, *in press*). The occurrence of short excursions from refuges during the day is an integral component of the normal wintering behaviour (N.H.C.Fraser, pers. comm), and the possibility remains that the day-caught fish in the current study were simply those undertaking such trips at the time of electrofishing, regardless of their energy stores.

In summary, the current study has verified the technique of nocturnal electrofishing as a valid means of sampling small salmon in shallow streams during winter. Fish sampled by day and by night did not differ in their estimated nutritional status, as would have been predicted from the results of a hatchery study (chapter 6.4). The results have highlighted some of the dangers of extrapolating the conclusions of finely-controlled hatchery-based experiments into the natural situation where a number of both biotic and abiotic factors combine to influence events.

Chapter 6.6 - Conclusions

The use of daytime refuges had little influence on the conservation of measureable body fat stores during winter. The seasonal decline in fat stores was found to be similar in fish either allowed or denied access to a suitable daytime refuge, indicating that winter refuge seeking offers no physiological advantage. However, in light of the results presented in chapter 3.4, the original hypothesis that refuge seeking may increase conservation of fat may not have been applicable, or at the very least, suffered from temporal changes as the season progressed. Indeed it appears maladaptive to slow the rate of fat utilisation towards the end of winter, as its value to the fish is diminishing as spring approaches. Thus, to achieve measureable differences between groups of fish on the basis of the provsion of a refuge may in retrospect have been unrealistic, considering the regulation of fat stores in response to other considerations.

The provision of a refuge did affect the timing and duration of feeding bouts, with those given acess to a daytime refuge feeding more under the cover of darkness, whereas those without a refuge fed more during the day. However, fish were found not to be exclusively nocturnally active, sometimes leaving the refuge during the day for short sorties during which time they were feeding. An interesting point arising from this study was that the total average food consumption throughout the winter was similar, regardless of whether a refuge was provided or not. This was indicative of there being a constant ration to which the fish were adhering, and fits with the previous results in that a steady rate of fat depletion would require the same level of energy intake, both being unaffected by daytime use of refuges.

Fish that were facing the possibility of starvation as a result of artificially reduced fat stores spent a greater proportion of the day out of the refuge, where they could feed to restore losses, than the constantly-fed fish. However, the response was short-lived, and after seven days the normal pattern of daytime sheltering and nocturnal emergence was re-established. This result provided evidence for the possibility of there being a trade-off between the risks associated with starvation and predation, with fish occupying daytime refuges as a result of increased vulnerability to diurnal predators. Once the risk of starvation overcame that of predation, the normal behavioural pattern was broken until fat was restored and the threat had passed.

The field-test of this laboratory-based hypothesis proved less conclusive. The use of electro-fishing during the night in winter proved highly successful at capturing more salmon than normal daytime sampling in shallow streams, and may be of benefit as a tool for fisheries management. The fat levels of salmon caught using the technique were no different by day or by night, initially indicating that the nutritional status of the fish had little bearing on its requirement to leave daytime refuges, contrary to the laboratory result. However, the heterogeneity of a natural stream-bed and the occurrence of short daytime feeding sorties from the refuges may have influenced this result. Both possibilities highlight the difficulties of identifying behavioural traits in populations of juvenile salmon in-stream and the necessity for controlled laboratory experiments, where confounding variables can be held constant.

Chapter 7 - Changes in gut morphology during winter

7.1 Introduction

The gastrointestinal tract is a dynamic and energetically expensive organ (Brugger, 1991) typically characterised by fast cell turnover rates. Kapoor *et al.* (1975) have described the length of the gut as "a variable entity which reacts sensitively to changes in feeding condition". The morphology of the intestine has been shown to be affected by food quality and quantity in birds (Drobney, 1984; Kehoe *et al.*, 1988; Brugger, 1991), small mammals (Sibly *et al.*, 1990) and fishes (Love, 1970; Gas & Noaillac-Depeyre, 1976; Hall & Bellwood, 1995). The generalized response to a reduction in food quality is to increase the length of the gut in order to maximise digestive efficiency, whereas animals experiencing a severe shortage in the quantity of food reduce their gut lengths. In the case of birds and mammals, it has been suggested that the need to maintain a constant rate of metabolism when food quantity is reduced leads to reduced energy allocation to cellular regeneration in the gut as a means of reducing costs (Sibly, 1981). In fishes, Love (1970) has suggested that cellular degeneration in the gut following starvation is due to mobilisation of epithelial cells for nourishment. In some cases the response to food shortage can be dramatic: the intestine of common carp has been found to both shorten by 18% and decrease in diameter by 67% during extended starvation (Noaillac-Depeyre, 1974).

Juvenile salmon that delay the smolting process enter into a natural state of anorexia during their first winter (Metcalfe *et al.*, 1986; Metcalfe &Thorpe, 1992) during which time food intake is suppressed below that which is physically possible given the seasonal reduction in water tempertature (Elliott, 1976; see chapter 5). During this time growth is arrested (Higgins & Talbot, 1985; Metcalfe *et al.*, 1988) and fat stores are utilised (see chapters 3 and 4). In adult sea-run salmon a similar type of anorexia occurs as the individual matures (Kadri *et al.*, 1995) and the stomach, intestine and pyloric caecae all degenerate after cessation of feeding in preparation for migration to freshwater (Love, 1970).

The purpose of this preliminary study was to establish whether the morphological response to (voluntary) suppression of food intake later in life is mirrored during the juvenile anorexic period, by comparing the intestine lengths of LMG salmon at the start and end of the winter.

7.2 Materials and methods

Several samples of similarly-sized subordinate salmon seemingly soundly situated in the suppressed-smolting section of the size range were collected from the early and late winter. The early winter sample was comprised of 34 fish that died in two separate incidents (on the 9 October (n = 20) and 3 November, 1995 (n = 14)) when the water supply to their tank was interrupted. The late winter sample of 21 fish was similarly obtained from incidents on 30 March and 2 April, 1996. On all occasions the fish that died were apparently a random sample of the population. All fish had been maintained in a $1m^2$ tangential flow stock tank where both water temperature and photoperiod were ambient. Prior to death, all fish had previously been maintained on *ad lib.* rations of pelleted food. The samples of fish were collected on the day of their death and frozen at -20^0C.

The fish were then defrosted and measured for fork length (to the nearest mm) prior to dissection. The entire viscera were removed and the length of the straightened, but unstretched intestine measured (to the nearest 0.05mm) from the last pyloric caecum to the anus using vernier calipers.

7.7.3 Results

The samples of fish were comprised of individuals of approximately the same size (mean fork length of fish in early winter sample = 63.0mm ± 1.18 S.E., late winter sample = 62.1mm ± 1.70: $t = 0.42$, d.f. =53, N.S.). There was no difference between the gut lengths of the subsamples in either the early or late samples of fish (ANCOVA between subsamples in the early winter sample, with fish fork length as covariate, slope: $F_{(1,31)} = 2.61$, N.S, elevation: $F_{(1,30)} = 0.39$, N.S and for the late sample, slope: $F_{(1,18)} = 2.42$, N.S. and elevation: $F_{(1,17)} = 3.61$, N.S.) allowing them to be pooled. For both the early and late winter samples of fish there was a positive relationship between fish size and the length of the intestine (figure 7.1); larger fish therefore had longer intestine lengths. The relationship between fork length and intestine length for both samples were best described by the following equations:

Early winter: $\log_{10}$ intestine length (mm) = (1.459 log10 fork length) - 1.143
n = 34, $r^2 = 74.0\%$, P<0.001
Late winter: $\log_{10}$ intestine length (mm) = (1.204 log10 fork length) - 0.670
n = 21, $r^2 = 68.0\%$, P<0.001

There was however no difference between the intestine lengths of LMG fish in early and late winter (ANCOVA between samples, with fish fork length as covariate; slope, $F_{(1,52)} = 1.86$, N.S. and elevation, $F_{(1,52)} = 1.32$, N.S).

7.4 Discussion

The lack of any measureable difference between the intestine lengths of hatchery-reared LMG salmon in early and late winter indicated that the fish are not undergoing an extended period of starvation that requires a reduction in intestine length to act as metabolic fuel, as reported in a number of fish species (Gas & Noailliac-Depeyre, 1976; Montgomery & Pollak, 1988; Hall & Bellwood, 1995). However, prolonged periods of reduced food availablity might not necessarily result in changes in the gross morphology of the intestine, but instead affect the intracellular structure of the tissue in more subtle ways. Histological changes in the intestinal mucosa, such as a shortening of the mucosal folds (Gas & Noailliac-Depeyre, 1976) or reduction in

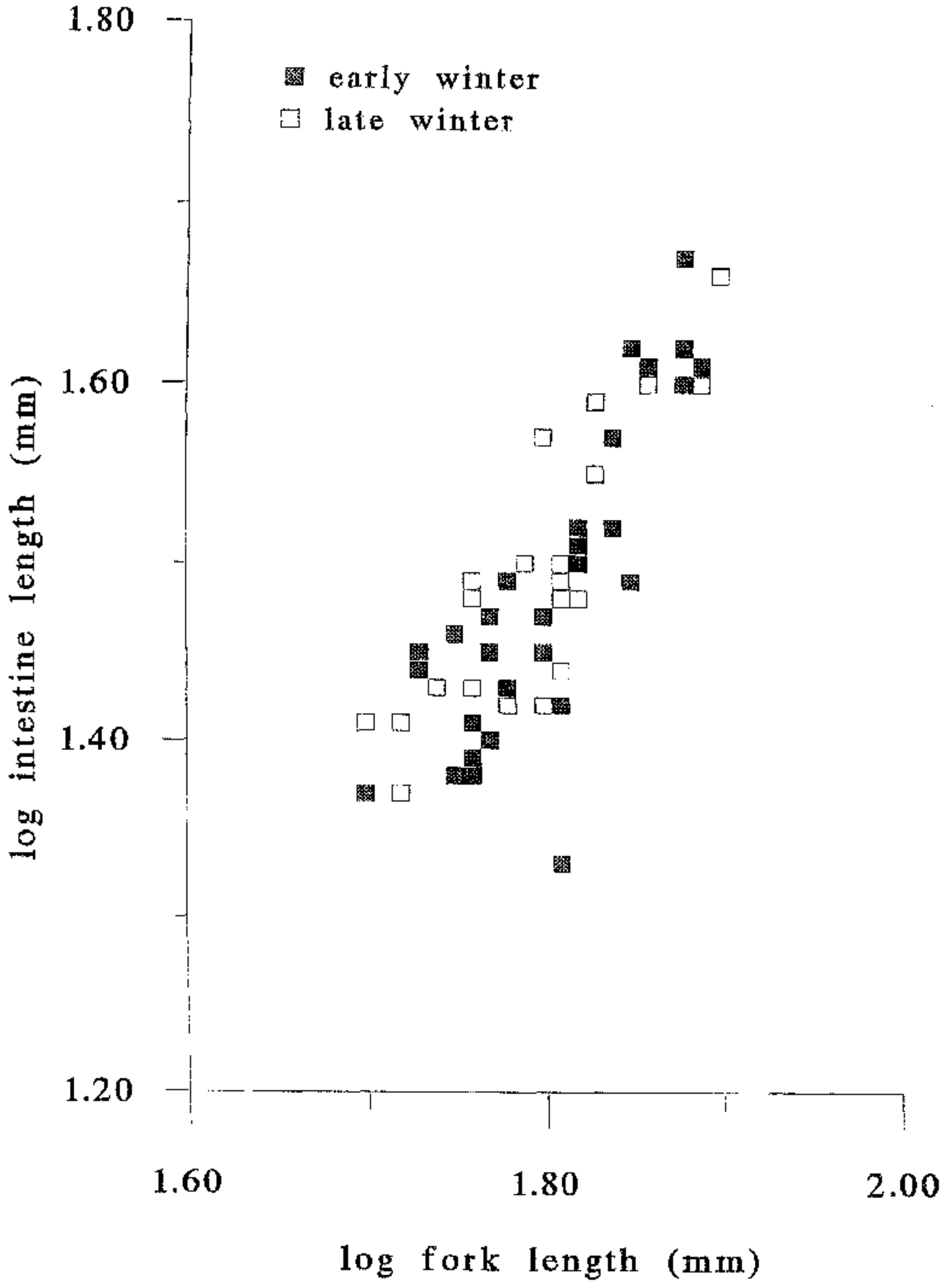

Figure 7.1. The relationship between $\log_{10}$ fish size and the $\log_{10}$ length of the intestine for samples at the beginning and end of the wimnter (solid and open squares). Each symbol represents one fish.

mucous cells (McLeod, 1978; McLeese & Moon, 1989) may well have been present, but could not have been detected in the current study. It is possible that the fish comprising the late winter sample were already growing and may have therefore been able to extend a previously shrunken gut. Simpson (1993) found that growth rates of LMG fish were rapidly increasing at this time. However, the negative results of this pilot study suggested that sacrificing additional fish would not have been justifiable.

Although juvenile LMG salmon do continue to feed at low levels throughout the winter (see chapter 3.4 and chapter 5) they have been reported as showing very low food conversion efficiences (Higgins & Talbot, 1985). This was partly due to a weight loss in the fish used by these authors, but the results of the current study indicate that low efficiencies are not mediated by any gross changes in intestinal morphology. Indeed, the LMG fish appear capable of avoiding the need to reduce intestinal length to maintain an energy balance. This may well be achieved by the careful regulation of food intake with regards to water temperature and the rate of utilisation of fat stores (see chapter 3.4).

In the hatchery environment the mechanism that regulates the pattern of energy allocation during winter may be permitted to achieve an equilibrium state whereby no drastic morphological energy reserves are required, as food availability is never limiting and fish therefore can choose when and how much food to ingest. In the natural situation this may not be the case, as the availability of suitable food in winter is reduced (Maitland, 1964; Elliott, 1967, 1968; Elliott & Minshall, 1968) and starvation may well be an important factor determining survival (Gardiner & Geddes, 1980; Elliott, 1986; Titus & Mosegaard, 1991; Shackley *et al.*, 1994). A study conducted using wild fish as subjects might therefore produce different findings to those presented here.

Chapter 8 - The temperature preference of UMG and LMG fish during winter

8.1 Introduction

Temperature is one of the most important environmental factors controlling not only the energy budgets of fish (Wootton, 1990), but their distribution and behaviour (Magnusson *et al.*, 1979). As fish are mobile organisms living in a thermally heterogeneous environment they have the potential to exercise substantial behavioural control over the temperatures they experience. Bardach & Bjorklund (1957) found that several species of freshwater fish could detect changes in temperature as slight as 0.05^0C and studies have shown that fish do select a preferred temperature (Fry, 1947) when faced with a range of environmental temperatures in the laboratory (Fry, 1947; Ogilvie & Anderson, 1965; Javaid & Anderson, 1967; Neill *et al.*, 1972; Richards *et al.*, 1977; Medvick *et al.*, 1981; Clark & Green, 1991; Deacon & Hecht, 1995; Konecki *et al.*, 1995; Kita *et al.*, 1996) and in the field (Kaya *et al.*, 1977; Bermann & Quinn, 1991; Nielson *et al.*, 1994; Snucins & Gunn, 1995). The temperature preference varies with fish age and season (McCauley & Huggins, 1979) and may well be balanced by other ecological constraints such as social hierachy and competition (Brett, 1971; Coutant & Carroll, 1980).

The thermal heterogeneity of the streams inhabited by juvenile salmonids results from groundwater seepage, tributaries, emerging streambed flow, deep water impoundment and shading (Bilby, 1984). The preferred daytime habitat of juvenile salmonids during winter, namely interstitial spaces between the stream-bed substratum (see chapter 6), has been shown to differ in temperature from the overlying water column (Shepherd *et al.*, 1986; Smith & Griffiths, 1994) throughout the year, being warmer on average during the winter and cooler in summer. Smith & Griffiths (1994) found that the overwinter survival rate of juvenile rainbow trout was higher when allowed access to streambed refuges and attributed the result partly to the increased water temperature in these areas offsetting the effects of a metabolic deficit brought about by the need for acclimation as temperatures decline (Cunjak *et al.*, 1987; Cunjak &

Power, 1987). Hunt (1969) also found a positive relationship between the number of hours in January in which temperature exceeded 4.5^0C and the survival of brook trout.

As winter approaches, juvenile salmon have different short-term developmental growth strategies that determine their behaviour throughout this season. Those destined to smolt the following spring (the UMG) continue to grow, feed at a higher rate and have higher metabolic rates than those that delay smolting for at least one more year (the LMG) (Higgins, 1985; Higgins & Talbot, 1985; Metcalfe *et al.*, 1988). Although both modes utilise fat stores during the winter (Higgins & Talbot, 1985), and adopt broadly similar behavioural patterns of nocturnal emergence from daytime refuges (Valdimarsson *et al.*, in prep.), differences between modes can and do occur due to the growth requirements of the UMG and the need for energy conservation in the LMG. Increases in water temperature result in an increase in metabolic rate (see Elliott, 1982 for review) and in the rate of utilisation of internal energy stores, necessary for fish eating less than a maintenance ration (Love, 1980), as in the case of the LMG during winter. It would therefore appear adaptive for these LMG fish to seek cooler water than their larger UMG counterparts in order to conserve energy stores. Therefore the differences between the developmental strategies of the UMG and the LMG have the potential to be reflected in their respective choice of environmental water temperature.

8.2 Materials and methods

Thirty fish were selected from a stock population of siblings (the offspring of a pair of sea-run adults from the Loch Lomond catchment) on 4 December, 1995 and maintained in a 1m^2 tangential flow tank. The tank's normal mesh anti-predator lid was covered completely with black plastic to prevent light reaching the fish, and a flourescent light was suspended above the surface of the water from the lid. The light period experienced by the fish was manipulated by way of an electronic timer, providing the seasonally adjusted number of daylight hours to the fish, but 12 hours out of phase. Therefore, the fish were experiencing an inverted photoperiod, so that observations of the nocturnal behaviour could be carried out during the normal

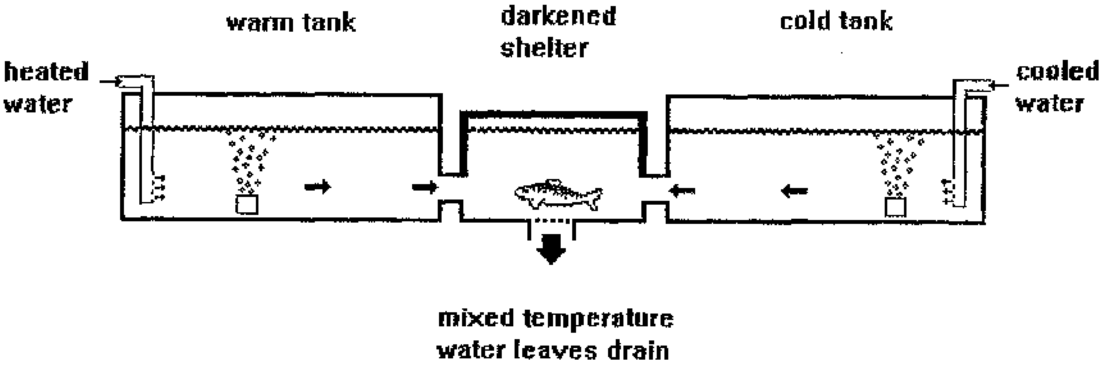

Figure 8.1. A diagramatic representation of the temperature choice chamber used in the experiment. Heated or cooled water flowed in from either end and into a central drain, located within a darkened shelter. This resulted in a gradual thermal gradient throughout the whole tank complex. Fish were therefore presented with a choice of water temperature when leaving the shelter: exiting into the flow of water coming from the right would lead to a cooler environment, and exiting to the left would result in a warmer environment.

working day. Whilst maintained in this tank, food was provided throughout the 24 hour period by way of an electronically timed feeder system, providing pelleted food every 20 minutes, in quantities ensuring *ad lib.* rations, and water temperature was ambient (see figure 3.16, chapter 3 for details).

At intervals of approximately 5 days during the next 10 weeks, a single fish was removed from the holding tank and placed into the central darkened shelter of a choice-chamber tank housed inside a temperature-controlled cabinet (figure 8.1). The tank was set up with the same inverted photoperiod regime as the stock tank and fish were moved during their perceived daytime to ensure that they would initially remain in the shelter. The water entering the tank at either end was either warmer or cooler than the normal ambient water temperature that the fish had previously been exposed to in the stock tank. On average, the temperature of the inflowing warm water was approximately 3^0C higher than ambient and the cooled water, approximately 2^0C lower. The discrepancy of approximately 1^0C between the extent to which water was heated or cooled arose due to technical limitations placed on the ability to cool water below ambient. The temperature of both inflows of water was monitored at the point of entry and were significantly different from the ambient (mean = 5.7^0C ± 0.14 S.E.) throughout the course of the experiment (mean temperature of warmed water = 9.7^0C ± 0.15: paired *t*-tests between warmed and ambient water, t = 35.88, d.f. = 61, $P<0.001$; cooled water = 3.6^0C ± 0.04: paired *t*-test between cooled and ambient water, t = 18.17, d.f. = 61, $P<0.001$). Both the warmed and cooled water flowed slowly into the refuge through circular openings of 35mm diameter, creating approximate ambient temperatures inside the shelter. Water then left through a drain in the floor of the refuge that was connected to a standpipe to maintain water levels.

Each tested fish was designated as belonging to the upper or lower modal group on the basis of body size and the degree of silvering and placed into the central shelter in the temperature-choice chamber. It was then left to settle for 48 hours during which time no food was provided. Fish would therefore have experienced two complete light and dark periods without disturbance prior to assessment of position. The procedure used to extablish the whereabouts of the fish in relation to the water temperature

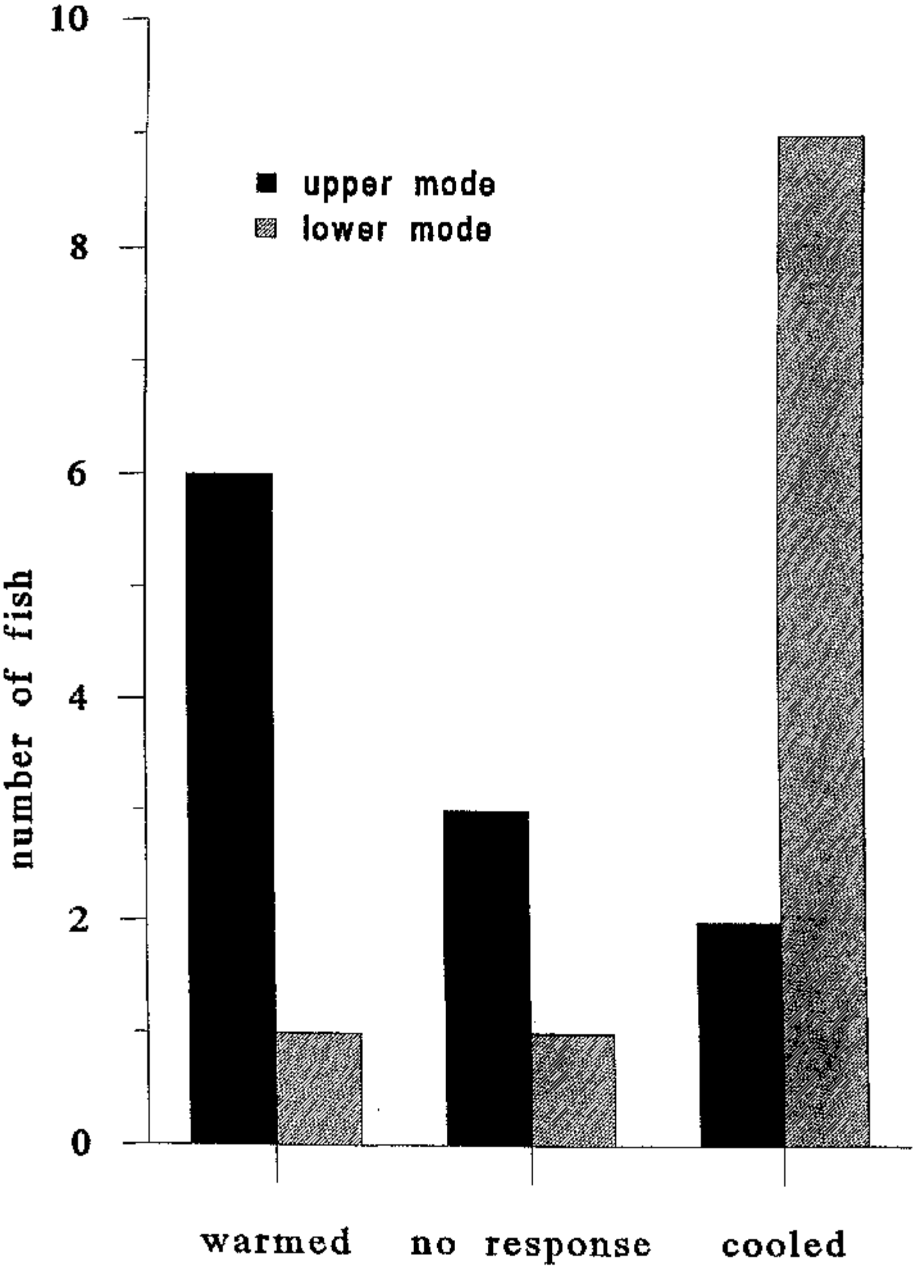

Figure 8.2. The temperature preferences recorded for 11 UMG and 11 LMG fish during the course of the experiment.

gradient utilised the nocturnal emergence behaviour displayed at low temperatures (see chapter 6). The fish's position was recorded approximately one hour following the beginning of the third dark period. The light above the tank was switched on, and the fish's location noted before any fright response was noted. The water temperatures in each of the tanks was also recorded at this time. Fish were recorded as seeking warmer or cooler water if they were fully out of the refuge, in either of the two tanks, or had a visible portion of their bodies out, facing into either of the water currents. No response was recorded if the fish was completely inside the refuge and not facing a water current. Once its position had been established, the fish were removed and returned to the original sibling population; thus each fish was only tested once.

8.3 Results

Four fish died throughout the course of the experiment, and four either jumped out of the choice-chamber tank prior to determining their position, or were exhibiting signs of stress such as erratic swimming and jumping at the water surface. These fish were subsequently removed from the analysis. A total of 11 LMG and 11 UMG were successfully tested (figure 8.2).

The LMG fish exhibited a tendancy to orientate towards, and settle in the cooler than ambient temperature water during darkness whereas the UMG tended to orientate towards the warmer water (χ^2 between treatment groups on the positions recorded during darkness, $\chi^2 = 7.91$, d.f. = 1, $P<0.01$).

8.4 Discussion

The results from this preliminary study indicated that differences in developmental sstrategy in overwintering salmon are reflected in the choice of preferrred temperature. The LMG fish preferred cooler than ambient, and the UMG preferred warmer water. The selection by the LMG for cooler water during winter is contrary to that predicted to increase overwinter survival by Smith & Griffiths (1994). Other laboratory studies on juvenile salmon have investigated temperature preference

(Ogilvie & Anderson, 1965; Javaid & Anderson, 1967; but have not taken into account the differences in developmental strategy.

Using the Q_{10} law and bioenergetic data collected for sockeye salmon (Brett, 1970), a salmon occupying water of 3.6^0C and then 9.7^0C (the mean temperatures of the two tanks in the choice chamber of the current study) would experience an increase in basal metabolic rate of approximately 60%. Selecting the higher temperature may allow growth rates to be elevated (Elliot, 1976b) and may well be an advantage to the UMG fish, maintaing growth during the winter (Metcalfe *et al.*, 1988). Selection of the cooler water might be adaptive for the LMG as they grow little and rely on a steady utilisation of internal energy reserves to survive (Metcalfe & Thorpe, 1992). An optimal overwintering strategy for these LMG fish would therefore presumeably utilise any available means of energy conservation to slow the rate of resource depletion.

The daytime refuges used by juvenile salmon during the day in winter (chapter 6) have been shown to have a daily average temperature that is 1^0C higher than the overlying water column (Shepherd *et al.*, 1986; Smith & Griffiths, 1994). The metabolic rates of LMG fish are lower than those of the UMG during the winter months (Higgins, 1985), presumeably reflecting the need for energy conservation, and this may in part offset the increased metabolic rate experienced by sheltering during the day, allowing both modes to occupy the same refuge areas within the streambed (Valdimarsson *et al.*, in prep). Although, on average the refuges are warmer than the water column during the winter, large diurnal fluctuations in temperature are common in streams as a result of solar radiation and are accentuated in the water column; the extent of the diurnal variation in temperature is lower in the streambed gravel and lags behind that of the water column (Shepherd *et al.*, 1986). Thus, the refuge may well provide both a warmer, or cooler environment with regards to the water column depending on the time of day and intensity of solar radiation. Fish may therefore be presented with the need to accommodate a changing thermal environment (and its effect on metabolism) along with the other ecological aspects of adopting refuge seeking behaviour (e.g. predation risk; Fraser *et al.*, 1993; 1995). Given that differences of 0.05^0C can be detected by freshwater fish (Bardach & Bjorklund, 1957), differences in the requirements for behavioural thermoregulation between the developmental pathways may help in the interpretation of complex fish movements in and out of refuges throughout the course of a 24 hour period (N.H.C. Fraser, unpublished data.).

On the wider scale, macrohabitat selection by juvenile salmon within streams during winter may well be influenced by the selection for either warmer or cooler water as required by the developmental strategy adopted. Neighbouring tributaries differing in water source and extent of riparian cover may well provide marked differences in thermal properties that offer optimal conditions for some, but not others. Habitat choices may then offer a greater opportunity for behavioural thermoregulation in overwintering salmon. However, further work in this area is needed before the results of such a preliminary study are extrapolated to the natural situation.

Chapter 9 - General discussion and conclusions

The overall aim of this thesis was to examine some of the behavioural and environmental factors that influence the overwintering strategy adopted by resident Atlantic salmon parr. Temporal change in the control of fat stores and appetite, the role of sheltering behaviour and the effects of temperature and photoperiod change have been examined.

This study has shown that the suppression of appetite, as found previously during late summer (Metcalfe *et al.*, 1986) and early winter (Metcalfe & Thorpe, 1992), is maintained throughout the course of the winter (chapter 3.4 and chapter 5). Food intake in fishes is invariably reduced at the low water temperature associated with winter conditions (Love, 1980; Wootton, 1990) but this study has found that LMG salmon parr feed at levels below their physiological capability as dictated by water temperature (chapter 5). This result implies that the fishes' motivation to feed in winter is being suppressed as suggested by Metcalfe & Thorpe (1992), a situation akin to the anorexias found in other species (see Mrosovsky & Sherry, 1980). It may be suggested that such an anorexic strategy has been adopted in response to the seasonal reduction in drifting food during in winter (Elliott, 1967; 1968; Elliott & Minshall, 1968). This seasonal reduction in the number of available prey items, and the fact that the actual process of prey capture is more energetically costly at low water temperatures (Webb, 1976; Fraser, 1994; Johnson *et al.*, 1996) may have provided the evolutionary pressures that have ultimately promoted such an anorexic strategy. The results presented in chapter 3.2 and 3.4 indicated that the food intake in LMG fish during winter is not exclusively regulated by proximate constraints on food intake such as seasonal temperature decline, but may be sensitive to ultimate considerations such as the vulnerability to predation during foraging (Martel & Dill, 1995). The results of chapter 5 provide further evidence that the overwinter suppression of appetite in the LMG salmon is in some way pre-programmed as it was maintained even when faced with optimal feeding conditions.

Reductions in food intake, be they naturally occurring or enforced through experimental starvation, can lead to an energy deficit that requires the utilisation of body tissue to maintain cell function (Love, 1980). One such reserve occurs in the digestive system, where starvation can result in reductions in the numbers of mucosal cells, and an overall shortening of the intestine (see chapter 7 for references). The length of the intestine in LMG salmon was not found to be reduced throughout the course of the winter (chapter 7) indicating that the reduction in food intake was being regulated to prevent the need for structural breakdown of digestive tissues. Fish would appear to have been mobilising an alternative source of energy during this time.

Although feeding does continue at a low level throughout the winter in the wild (Cunjak & Power, 1987) and in the hatchery situation (Higgins & Talbot, 1985; chapter 5 and 6.3), salmonids mobilise fat stores to provide energy to fuel metabolism (Gardiner & Geddes, 1980; Higgins & Talbot, 1985; Cunjak, 1988b; Metcalfe & Thorpe, 1992). They utilise fat stores not only as juveniles during the winter, but as adults during the spawning migration (Jonsson *et al.*, 1991) when appetite is suppressed. Precocious male parr also rely heavily upon fat during their movement to the redds and territory defence (Järvi & Petterson, 1991) as they exhibit reductions in food intake during this period (Simpson, 1993). The use of fat as an alternative energy source during some life-history stage is found in other fish species (see chapter 3.3 for references and Shulman, (1974) for review), and is a general strategy in the animal kingdom to be adopted whenever energy demands are greater than those possible through intake, as is often the case in winter (see Sheridan, 1994; Witter & Cuthill, 1993). The reliance upon fat is taken to the ultimate extremes during hibernation in mammals, and on long-distance migrations over terrain that prevents foraging (see Lyman *et al.*, 1981; Aidley, 1981).

Although long established that juvenile salmon deplete their internal stores of fat during winter, few studies have investigated how fat depletion and feeding rates are controlled with regard to each other. The careful regulation of fat stores is fundamental to the success of any strategy that relies upon them as an energy source, and this thesis has shown that juvenile salmon exhibit behavioural adaptations that are geared towards the careful control of their body stores during winter. The physiological mechanisms that allow animals to make an assessment of their fat status are still unknown (see Scott, 1996), but the results of this thesis indicate that fish must possess the ability to continually assess their fat stores in order to exhibit the range of responses to their accelerated depletion. Fish would require a negative-feedback control system between fat stores and appetite that could be adjusted with regard to season and the developmental pathway adopted.

The results of chapter 3.4 indicated that the value of fat stores decline as the winter progresses, with fish displaying a corresponding reduction in their foraging effort in response to an energy deficit. This occurred despite the actual levels of fat being highest during early winter. Previous theoretical work on the value of fat stores (Lima, 1986; see Witter & Cuthill, 1993) would not have predicted the observed results, and it appears that the fish are responding not only to their current nutritional state, but to a projection of their future energy needs (Bull *et al.*, 1996). Thus, fat at the start of winter is of the highest value as it may be used to offset the metabolic demands of appetite reduction or falling water temperatures (Cunjak & Power, 1987; Cunjak, 1988b). A brief increase in food consumption and subsequent growth rate at the beginning of the winter season (Higgins & Talbot, 1985; Kristinsson *et al.*, 1985; see chapter 5), may act as a means to maximise the storage capacity for fat in preparation for winter as larger fish can store more fat (Elliott, 1976; Metcalfe & Thorpe, 1992; Simpson *et al.*, 1992). A positive relationship between the amount of stored fat and subsequent survival during winter has been reported for smallmouth bass (Oliver *et al.*, 1979).

Although carried out under hatchery conditions, such a result has implications for the management of wild stocks occupying a less predictable environment, in that the timing of any event that reduces a fishes' foraging efficiency (such as a prolonged spate that increases flows and water turbidity; Stradmeyer & Thorpe, (1987)) may be crucial to overwinter survival. If such a spate occurs in autumn when the value of stored fat is maximal and fish are forced into depleting stores, the ability to carry out the compensatory feeding response (see chapter 3.1 for references) might be restricted by lower prey numbers. Salmon that therefore start the winter with reduced fat stores might then suffer from a greater threat of mortality through premature depletion of their stores of fat. Such nutritional stress has been indicated as a possible factor contributing to overwinter mortality (Gardiner & Geddes, 1980; Cunjak, 1988b; Pickering & Pottinger, 1988; Shackley *et al.*, 1994; Smith & Griffiths, 1994). However, the same event occurring later in the winter may have less impact on the resulting survival rates.

In order for seasonal changes in foraging effort and energy allocation to occur, fish require a means by which to assess the time of year. Previous work has highlighted that photoperiod can act to synchronise certain life-history events in salmonids (see chapter 3.5 for references). The results presented in chapter 3.5 indicated that the LMG are responsive to the changing pattern of daylength during winter, and exhibit the feeding and fat responses to an energy deficit that is appropriate to whether they perceived themselves to be either commencing, or approaching the end of a winter season. This means of assessing the calendar month is more reliable than using seasonal changes in water temperature, allowing fish to synchronise their foraging effort.

This thesis has indicated that the appropriate regulation of fat stores and the importance placed upon their restoration appear to be of the utmost importance during winter when body growth in the LMG fish is normally arrested (Gardiner & Geddes, 1980; Higgins & Talbot, 1985; Metcalfe *et al.*, 1988). The results of chapter 3.3 showed that the compensatory responses to food restriction differed between seasons, illustrating a change in the short-term developmental goals between attainment of body size in the summer and maintenance of energy reserves in the winter of the first year (e.g. Nicieza & Metcalfe, *submitted*). Thus it appears that the 'desired' growth rate (Calow, 1973) of LMG fish during summer is high, but reduced to a minimum during winter (Metcalfe & Thorpe, 1992). This switch during winter would allow the allocation of any available energy from a (necessarily reduced) food intake to be channelled into the appropriate regulation of fat stores. The losses in terms of growth opportunity by adopting such a strategy are offset by increased survival chances and the fact that LMG fish spend a further year in freshwater during which time losses can be made up. A rapid increase in the food intake of LMG fish during spring (Simpson et al., 1996) has been proposed to act as a means of compensating for the fat deficit incurred during the previous winter, presumably allowing subsequent energy allocation to increased body growth. Subsequently LMG fish that smolt after two years tend to do so at a larger size than their faster growing, earlier smolting UMG siblings. By having increased time to make up the deficit, they suffer less from the size-selective attentions of predators (Feltham, 1990) or physiological pressures placed on small migrants (Lundqvist et al., 1994).

Thus the principal physiological decision as to whether to smolt or not (Thorpe, 1986) undertaken sometime around midsummer (Wright *et al.*, 1990) leads to a switch in short-term developmental goal in the LMG fish during the subsequent months. This switch to energy conservation at the expense of body growth was highlighted further by their preference for colder environmental temperatures during winter than UMG fish (chapter 8) whose winter strategy may be likened to the LMG during summer as they maintain the need to increase body size (Metcalfe *et al.*, 1988). Temperature has an overwhelming effect on the energetics of fish (see chapter 6 and 8 for references) and although previous work has indicated that aspects of the biochemistry (Graham, 1994), physiology (Higgins, 1985; Higgins & Talbot, 1985), and behaviour (Huntingford *et al.*, 1988; Metcalfe *et al.*, 1988) of the two modes of salmon differ during winter, their respective preferences for temperature have not previously been examined. The result of chapter 8 clearly shows the developmental strategy influences the choice for environmental temperature, and might therefore influence habitat selection during winter in a thermally heterogeneous environment. Such thermal variation exist in nursery streams (Bilby, 1984), where salmon either preparing to smolt, or remaining resident may occupy spatially separate areas. Future research is needed to investigate this possibility, and the next step in this area might be to continue to examine the differences in microhabitat selection between the developmental pathways not only in the laboratory but in the field, to assist stock management practices during winter.

The suggestion that the switch to occupying streambed refuges during the day in winter has arisen in response to the requirement to conserve energy (see chapter 6.2 for references) was not borne out in the results of chapters 6.2 and 6.3. Fish using daytime shelters did not have increased stores of body fat, or require less food than those without shelters, both indicating no energetic advantage. However, the current holding performance of juveniles declines markedly at winter temperatures (Graham *et al.*, 1996), and although no measurable energetic advantages were recorded in this study, the switching to spending a large portion of time in areas of low water velocity should act increase energetic efficiency (Gibson, 1978) and lessen the chances of downstream displacement. The fact that fish predominantly undertake a rhythmic pattern of nocturnal emergence from shelters (during which time feeding occurs, Fraser & Metcalfe, in press; see chapter 6.3) might provide some indication as to the adaptive basis of this behaviour. As escape responses are reduced at low water temperatures (Webb, 1978; Fraser, 1994: Johnson *et al.*, 1996), and the majority of the juvenile salmon's avian predators rely on vision to feed (Cramp & Simmons, 1977), adopting such a pattern during winter might lessen the chances of being captured (Fraser *et al.*, 1993; 1995). Even though foraging efficiency at night is markedly reduced (Fraser & Metcalfe, *in press*), the low levels of food made necessary by adopting an anorexic strategy might be successfully acquired under the cover of darkness, without incurring the risks of predation. The results of this thesis have shown that the food intake of LMG salmon is lower than that physiologically possible, indicating a possible matching of energetic requirements from feeding to a less efficient, but safer strategy of nocturnal foraging. The result of chapter 6.4 demonstrated that the fishes' need to constantly assess the costs and benefits in a trade-off (see chapter 6.4 for references) between the threat of starvation and predation influenced the normal pattern of daytime sheltering and nocturnal emergence. Fish therefore appear to preferentially seek shelter during the day in order to avoid predation, but when fat stores are depleted to a level threatening starvation, fish abandon this normal behavioural pattern and briefly risk daytime foraging to restore a nutritional equilibrium.

This thesis has examined the behavioural regulations involved in the overwintering strategy of resident juvenile Atlantic salmon. The results have illustrated how the pattern of energy allocation changes with the onset of winter, and how the careful control of food intake is geared towards the maintenance of an optimal level of body fat as the season progresses. Maximising fat storage capacity in preparation for winter may explain a peak in appetite and growth exhibited in the autumn.This peak appears to signal a change in the seasonal developmental goal from increasing body size, to forgoing growth in order to regulate internal stores of fat. By reducing the energy requirement from foraging, and utilising internal fat stores, a strategy that reduces the risk of predation at low water temperatures can be maintained.

Appendix I

Table 1. The relationship between body size and fat level in the summer control fish used in chapter 3.3

Date	Regression equation
3 July	%Fat = 0.311forklength - 10.378 n = 19 r^2=73% $P < 0.001$
11 August	%Fat = 0.1021forklength - 1.040 n = 18 r^2 = 46% $P < 0.05$
21 August	%Fat = -0.045forklength + 5.108 n = 19 r^2 = 11% NS
4 September	%Fat = -0.076forklength + 7.192 n = 17 r^2 = 9% NS
13 September	%Fat = - 0.177forklength + 13.192 n = 14 r^2 = 66% $P < 0.001$

Table 2. The relationship between body size and fat level in the winter control fish used in chapter 3.3

Date	Regression equation
19 October	%Fat = 0.121forklength - 3.410 n = 18 r^2 =3% NS
5 December	%Fat = 0.177forklength - 8.564 n = 17 r^2 = 19% NS
12 December	%Fat = forklength - 1.44 n = 16 r^2 = 0% NS
18 December	%Fat = 0.203forklength - 9.24 n = 15 r^2 = 42% $P < 0.01$
24 December	%Fat = -0.018forklength + 5.086 n = 16 r^2 = -7% NS

Table 3. The relationship between body size and fat level in the control fish used in chapter 3.5

Date	Regression equation
18 September	%Fat = 0.065forklength + 0.724 n = 43 $r^2 = 3\%$ NS
9 October	%Fat = 0.135forklength - 4.207 n = 42 $r^2 = 41\%$ $P < 0.001$
31 October	%Fat = 0.116forklength - 3.384 n = 44 $r^2 = 44\%$ $P < 0.001$
20 November	%Fat = 0.212forklength - 12.0.31 n = 44 $r^2 = 70\%$ $P < 0.001$
11 December	%Fat = 0.125forklength - 4.379 n = 41 $r^2 = 60\%$ $P < 0.001$
5 January	%Fat = 0.150forklength - 6.289 n = 39 $r^2 = 78\%$ $P < 0.001$
29 January	%Fat = 0.156forklength - 70020 n = 38 $r^2 = 76\%$ $P < 0.001$
19 Febuary	%Fat = 0.132forklength - 5.084 n = 39 $r^2 = 75\%$ $P < 0.001$

Table 4. The relationship between body size and fat level in the control fish used in chapter 5

Date	Regression equation
24 September	%Fat = 0.023forklength + 3.63 n = 30 $r^2 = 3\%$ NS
6 October	%Fat = -0.032forklength + 7.261 n = 30 $r^2 = 5\%$ NS
21 October	%Fat = 0.063forklength + 0.975 n = 31 $r^2 = 20\%$ $P < 0.01$
3 November	%Fat = 0.101forklength + 0.975 n = 28 $r^2 = 40\%$ $P < 0.001$
17 November	%Fat = 0.021forklength + 4.088 n = 30 $r^2 = 2\%$ NS
1 December	%Fat = 0.052forklength + 2.038 n = 31 $r^2 = 19\%$ $P < 0.01$
20 December	%Fat = 0.0656forklength + 0.541 n = 30 $r^2 = 33\%$ $P < 0.001$
11 January	%Fat = 0.066forklength + 0.323 n = 29 $r^2 = 21\%$ $P < 0.01$
26 January	%Fat = 0.0676forklength + 0.275 n = 31 $r^2 = 26\%$ $P < 0.01$

Table 5. The relationship between body size and fat level in the control fish used in chapter 6.2

Date	Regression equation
13 December	%Fat = 0.051forklength + 1.640 n = 19 r^2 = 13% P = 0.063
11 January	%Fat = 0.014forklength +3.950 n =19 r^2 = -5% NS
23 Febuary	%Fat = 0.071forklength - 3.257 n = 18 r^2 = 23% $P < 0.05$
9 March	%Fat = 0.114forklength - 3.257 n = 17 r^2 = 34% $P < 0.01$
25 March	%Fat = 0.135forklength - 5.104 n = 17 r^2 = 35% $P < 0.01$
18 April	%Fat = 0.135forklength - 4.782 n = 16 r^2 = 32% $P < 0.01$

Appendix II

Common name	Latin name
Arctic charr	*Salvelinus alpinus*
Bloodworm	*Chironomidae spp.*
Brent goose	*Branta bernicula*
Brook trout	*Salvelinus fontinalus*
Brown trout	*Salmo trutta*
Common carp	*Cyprinus carpio*
Cutthroat trout	*Oncorhynchus clarki*
Emperor penguin	*Aptenodytes forsteri*
European eel	*Anguilla anguilla*
European minnow	*Phoxinus phoxinus*
Golden-mantled ground squirrel	*Citellus lateralis tescorum*
Gray whale	*Eschrichtius robustus*
Green sunfish	*Lepomis cyanellus*
Rainbow trout	*Oncorhynchus mykiss*
Red junglefowl	*Gallus gallus spadiceus*

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