TEMPORAL FACTORS & MATURATION STATUS

AS DETERMINANTS OF APPETITE

IN ATLANTIC SALMON.

SUNIL KADRI

Presented in candidature for the degree of Doctor of Philosophy to the Faculty of
Science, University of Glasgow, September 1995.

© Sunil Kadri 1995

ProQuest Number: 13818420

ProQuest 13818420

FOR
ROMI
AND THE
SALMON

ACKNOWLEDGEMENTS

SUMMARY

This thesis examines the patterns of variation in appetite in Atlantic salmon, *Salmo salar*, their causes and consequences. The emphasis of this study is on both their biological bases and their significance for the aquaculture industry.

Chapter II: Experiments are described here that relate to daily feeding patterns and competitive interactions under both ambient and constant light and temperature regimes. Results were as follows:

For yearling salmon parr under ambient light and temperature conditions:

a) Consistent daily feeding rhythms with an afternoon peak were recorded in spring, while in autumn fish fed synchronously but without a consistent pattern between days.

b) There was also a diurnal rhythm in frequency of aggressive interactions that was similar in both seasons.

c) In spring aggression and feeding rates were negatively correlated.

For salmon smolts under constant light and temperature conditions (12L:12D photoperiod):

d) A clear diurnal rhythm was observed in feeding activity, with appetite peaks in early morning and late afternoon.

e) When the data described in d) were separated according to fish size categories, significant patterns of diurnal variation in appetite were found for the small and medium fish but not for the larger fish. Medium-sized fish fed in a daily pattern similar to that observed at the population level, while small fish showed the highest level of feeding activity at first light and at the end of the light phase, with a marked reduction in feeding in the afternoon. Large fish maintained a relatively consistent level of feeding activity throughout the day.

Chapter III: An experiment is described that was aimed at investigating the relationships between food intake, digestive efficiency and feeding regime, and to examine individual variation with respect to food intake and digestive efficiency. Results were as follows:

a) Food intake and digestive efficiency varied widely between individuals under both continuous and meal-feeding regimes.

b) However, while digestive efficiency was higher for continuous feeding, there was no difference between feeding regimes for food intake.

c) Continuous feeding produced a negative relationship between food intake rate and digestive efficiency but no such relationship was found for meal-feeding.

d) Under the meal-feeding regime there was a positive relationship between relative protein intake rate and body weight, with a weaker relationship between these two parameters under continuous feeding, suggesting a more pronounced feeding hierarchy under meal-feeding conditions.

Chapter IV: This chapter describes an experiment to test whether the amount of food obtained by individual one sea-winter Atlantic salmon was related to social status.

a) Food was distributed unevenly among fish; no significant relationship was found between food intake and either weight or gender.

b) More successful fish fed earlier in a feeding session than their companions, and smaller fish tended to avoid competitors when approaching pellets.

c) There was a positive relationship between frequency of being beaten to pellets and food intake, but no relationship between proportion of feeding attempts in which a fish was beaten and food intake. This suggests that fish scrambling for food were of a similar status.

d) Previous studies have shown a negative relationship between the coefficient of variation in daily food intake and total food intake, but did not control for fish size. Here I found the same relationship after controlling for fish size, showing that more successful fish had less variation in their day to day consumption (corrected for body weight) than did less successful conspecifics.

e) These data suggest that, in order to prevent food monopolisation by few individuals, the food in production cages should be presented so that it is unpredictable in time and space.

Chapter V: In this chapter patterns of feeding behaviour, growth and accumulation of lipid reserves were examined in maturing fish and immature fish of the same age from the onset of the maturation cycle (autumn) to the onset of anorexia (summer).

a) Maturing fish were larger and in better body condition than non-maturing fish from October until the end of the trial in July.

b) Maturing fish were over-represented in samples of feeding fish from April to May (end of trial) suggesting they had a greater motivation to feed than non-maturing fish over this period.

c) The period of greatest differentials in growth rate, body lipids and condition factor between the two groups of fish coincided with the period of differential feeding responses.

Chapter VI: An investigation of the most useful morphometric predictors of sexual maturation in one sea-winter fish is described here, with results as follows:

a) A combination of forklength and condition factor proved to be the strongest and most consistent predictor of maturation (over 88% accurate) from May until August (by which time maturing fish were losing condition and in any case could be easily distinguished by shape and colour).

b) Anal height and dorsal height also gave strong predictions of reproductive status, but these parameters were of secondary importance to forklength and condition factor.

c) A second trial was performed on a separate stock of fish for which forklength and condition factor were able to predict maturation to a similar level of accuracy. However, the discriminant function derived from one stock of fish did not produce the same discriminatory power when applied to the other stock.

Chapter VII: An experiment was performed to quantify in detail the phenomenon of anorexia in maturing sea-run salmon.

a) During May to August (a period of increasing water temperatures), maturing one-sea-winter Atlantic salmon showed decreasing appetite from early June onwards, consuming little or no food by early July.

b) By contrast, immature siblings increased their food intake steadily from early June until early August.

c) There was variation in the date on which individual fish became anorexic, suggesting that anorexia is dependent on an internal cue.

Chapter VIII: An experiment examining whether the onset of anorexia in female maturing fish is related to body condition and/or gonad development is described. Results showed the following:

a) The date on which individual fish stopped feeding varied greatly.

b) After ceasing to feed all lost weight at a consistent rate of 0.1% per day.

c) The onset of anorexia was significantly correlated with estimates of fat reserves, lean mass and gonad size; however multivariate analysis showed that lean mass was most important. Female salmon thus appear to continue feeding until their lean mass has reached threshold levels.

CHAPTER I

INTRODUCTION

This thesis is based upon a series of experiments conducted in order to answer fundamental questions about the biology of fishes, in the broad framework of behavioural ecology and life history theory that are of particular interest to aquaculturists. Specifically, it is concerned with potential biological solutions to the problems of wasted fish feed and 'early' maturers in cultured populations.

Life history theory

Life history theory has been developed to explain the action of natural selection upon traits related to reproduction by predicting the consequences for fitness of variation in life history patterns. The basis of this approach is to devise optimality models by which the costs and benefits of particular life history strategies are identified and trade-offs established. It has been applied successfully to life history variation in a number of species (Roff 1992, Stearns 1992), fish having proved particularly useful as models (Wootton 1990).

With respect to age at maturity, for example, life history theory predicts that selection for early maturation will bring the benefits of shorter generation time and higher survival to maturity, while delayed maturation will result in higher initial fecundity (due to greater body size), lower instantaneous juvenile mortality rates, and higher overall lifetime fecundity (Stearns 1992). Both within and between species, variation in traits such as age at maturity is characterised as a trade-off between the costs and benefits of different life history strategies with respect to the environment concerned. This has been demonstrated, for example, in salmonids, which generally inhabit an uncertain freshwater environment for at least part of their lives, and hence are characterised by flexible life history patterns (Schaffer & Elson 1975, Schaffer 1979, Thorpe 1988, 1989, 1994).

It is becoming increasingly clear in many branches of evolutionary theory that biologists need to investigate the mechanisms that generate adaptive outcomes, in conjunction with investigations of the outcomes themselves (Krebs & Davies 1991). Hence Thorpe (1989) proposed a developmental model whereby salmonids are subject to performance-dependent decision points at which future life history patterns are decided on the basis of past and current performance. In this respect,

accumulation of energy reserves has been identified as a key indicator of performance (Thurow 1966, Thorpe 1989, Rowe & Thorpe 1990, Rowe *et al.* 1991, Simpson 1992) and therefore a major influence on the development of individual life history strategies. The accumulation of energy reserves depends upon a complex of factors, e.g. water temperature, photoperiod, season, food availability and appetite. The last reflects a response to both environmental and endogenous variables and is the main subject matter of this thesis, which uses Atlantic salmon (*Salmo salar* L.) as a subject.

Determinants of variation in appetite among salmonids.

There is wide variation in patterns of appetite among salmonids, for many different reasons, relating to both endogenous and environmental factors. The nature and causes of this variation are of both fundamental and applied interest, hence these phenomena have been the subject of many studies.

Temporal factors: Over the last thirty years there has been an increasing interest in the study of daily feeding patterns in salmonids, in both field and laboratory environments. The results of these studies are not always in agreement, so the factors identified as major determinants of the patterns observed have also varied. These have included: temperature (Hoar 1942, Fraser *et al.* 1993), photoperiod (Higgins & Talbot 1985, Boujard & Leatherland 1992a, Smith *et al.* 1993) and changing light intensities (Gibson & Keenleyside 1966, Chaston 1968, Landless 1976, Rawlings 1994). Temperature however, is generally considered to have only a secondary influence in synchronising circadian rhythms in fish (Müller 1978, Rawlings 1989, Boujard & Leatherland 1992b, but see Fraser *et al.* 1993). The light/dark cycle is clearly of major import in entrainment of these rhythms (Spieler & Noeske 1984, Sundararaj *et al.* 1982), as it provides the most reliable natural source of information on time of day. The relative importance of dawn and dusk periods in entrainment however, remains unclear (Boujard & Leatherland 1992b, Eriksson & Alanärä 1992, Rawlings 1994). Similarly, inter-individual variability in digestive efficiency has received little attention. Digestive efficiency could well be under the influence of temporal feeding patterns, as these may affect the rate at which food moves through

the alimentary canal, and thus the time available for digestion of food (Jobling 1994, De Silva & Andersen 1995).

Social factors: In spite of the aggressive territoriality observed in salmonid parr (Keenleyside & Yamamoto 1962, Bachman 1984) and reports of the influence of social interactions upon the ability of salmonids to optimise food intake and minimise energy costs (Noakes & Leatherland 1977, Li & Brocksen 1977, Metcalfe 1986, Abbott & Dill 1989, McCarthy *et al.* 1992), there have been no studies of the effect of social interactions and dominance hierarchies upon temporal feeding patterns among individual salmonids or, for that matter, in any other fish species.

Reproductive factors: The life cycle of Atlantic salmon involves major shifts in ecological niche and consequently changes in food requirements and availability. Many studies have related differences in food intake to such life history events; for example, during the first autumn in the life cycle of Atlantic salmon, parr belonging to the lower modal group of the size distribution become anorexic as an over-wintering strategy (Kristinsson *et al.* 1985, Metcalfe *et al.* 1986, 1988, Metcalfe & Thorpe 1992). Another life history event known to influence appetite is maturation. Maturing Atlantic salmon in sea water commence investment in gonadal tissue in autumn, approximately one year prior to spawning (Thorpe 1994). As maturation progresses, they are known to grow faster than their immature siblings during the spring and early summer, with the weight difference being largest around June (Aksnes *et al.* 1986). However, little is known about the mechanism by which breeding reserves are accumulated rapidly at a time (late spring/early summer) when water temperatures are relatively low. In addition, there has been no published investigation of the patterns of accumulation of reserves in maturing salmon from the time of onset of gonadal investment. Selective forces may well act differently upon maturing salmon according to gender, so the male and female components of the maturing population do not necessarily follow the same pattern of accumulation of reserves. There have been several studies of sexual dimorphism in maturing Atlantic salmon (Jones 1959, Naevdal *et al.* 1981, Bodington 1987, Maisse *et al.* 1988, Naesje *et al.* 1988), but there has been scant attention paid to the morphological characters that distinguish maturing from immature fish (see Simpson 1993 for an exception). The latter study was performed on parr, and hence work on 'maturational dimorphism' in sea-run

salmon (in which morphological changes associated with maturation are much greater) is lacking. For salmon farmers, it is important to distinguish maturing from immature one sea-winter fish (regardless of gender) in the spring.

After the spring/early summer period of accelerated growth mentioned above maturing fish stop growing at some stage in the summer (Aksnes *et al.* 1986). It has long been known that maturing salmon do not feed during the period of several months that they spend in the river system when migrating to spawning grounds (Grey & Tosh 1894, Calderwood 1907). However, there is very little published information available on this subject, as wild salmon are so difficult to track and study at sea. The advent of salmon farming has meant it is now possible to collect such data from populations in sea cages and so provide information of interest to both fish biologists and aquaculturists.

Aims

Therefore there is still uncertainty in a number of key areas regarding feeding patterns in Atlantic salmon and how these relate to temporal and social factors as well as maturation in sea water. The present thesis aims to clarify some of them by addressing two basic sets of questions:

1) *Temporal factors and appetite.*

How does food intake vary with time of day in juvenile and post-smolt Atlantic salmon? (Chapter II, submitted to *Journal of Fish Biology* as two papers). How do food intake and digestive efficiency vary (both among individuals and between feeding regimes)? (Chapter III). Does competition affect individual feeding patterns and food distribution? (Chapter IV, *Aquaculture* in press).

2) *Maturation status and appetite.*

How do feeding patterns, growth and body lipid accumulation vary between maturing and immature adult Atlantic salmon? (Chapter V, *Aquaculture* in press). What are the most suitable external morphological predictors of maturity in adult Atlantic salmon? (Chapter VI, submitted to *Aquaculture International*). The phenomenon of anorexia in maturing adult salmon (already well known among salmon farmers) is quantified in the experiment reported in Chapter VII (*Proceedings of the 6th International*

Symposium on Feeding and Nutrition of Fish, in press). What determines the cessation of feeding in these fish? . (Chapter VIII, *Functional Ecology* 9, 790-797)

N.B. Individual chapters will be referred to by their respective roman numerals alone throughout the body of this thesis.

REFERENCES

Abbott, J.C. & Dill, M. (1989) The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. *Behaviour* **108,** 104-113.

Aksnes, A., Gjerde, B. & Roald, S.O. (1986) Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **53,** 7-20.

Bachman, R.A. (1984) Foraging behaviour of free-ranging wild and hatchery brown trout in a stream. *Trans. Am. Fish. Soc.* **113,** 1-32.

Bodington, P. (1987) *A Study on the Feasibility of Externally Sexing Farmed Atlantic Salmon (*Salmo salar*) prior to the Development of Secondary Sexual Characteristics*. Unpubl. MSc. thesis, University of Stirling, Scotland.

Boujard, T. & Leatherland, J.F. (1992a) Demand feeding behaviour and diel pattern of feeding activity in *Oncorhynchus mykiss* held under different photoperiod regimes. *J. Fish Biol.* **40,** 535-544.

Boujard, T. & Leatherland, J.F. (1992b) Circadian rhythms and feeding time in fishes. *Environ. Biol. Fish.* **35,** 109-131

Calderwood, W.L. (1907) *The Life of the Salmon*. Edward Arnold, London, cited in Mills (1989).

Chaston, I. (1968) Influence of light on activity of brown trout (*Salmo trutta*). *J. Fish. Res. Bd. Can.* **25,** 1285-1289.

De Silva, S.S. & Anderson, T.A. (1995) *Fish Nutrition in Aquaculture*. Chapman and Hall, London.

Eriksson, L.-O. & Alanärä, A. (1990) Timing of feeding behaviour in salmonids. In: J.E. Thorpe & F.A. Huntingford (eds.) *The Importance of Feeding Behaviour for the Efficient Culture of Salmonid Fishes.* World Aquaculture Society, Baton Rouge. pp. 41-48.

Fraser, N.H.C., Metcalfe, N.B. & Thorpe, J.E. (1993) Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proc. R. Soc. Lond* B. **252,** 135-139.

Gibson, R.J. & Keenleyside, M.H.A. (1966). Responses to light of young Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Can.* **23,** 1007-1024.

Grey, R.H. & Tosh, J.R. (1894) *13th Annual Report of the Fishery Board for Scotland,* Part II, Note 2.

Higgins, P.J. & Talbot, C. (1985) Growth and feeding in juvenile Atlantic salmon. In: Cowey C.B., MacKie A.M. & Bell J.G. (eds.), *Nutrition and Feeding in Fish.* Academic Press, London, pp. 243-263.

Hoar, W.S. (1942) Diurnal variations in feeding activity of young salmon and trout. *J. Fish. Res. Bd. Can.* **6,** 90-101.

Jobling, M. (1994) *Fish Bioenergetics.* Chapman and Hall, London.

Jones, J.W. (1959) *The Salmon.* Collins, London.

Keenleyside, M.H.A., & Yamamoto, F.T. (1962) Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). *Behaviour* **19,** 138-169.

Krebs, J.R. & Davies, N.B. (eds.) (1991) *Behavioural Ecology: An Evolutionary Approach.* 3rd edition. Blackwell Scientific Publications, Oxford.

Kristinsson, J.B., Saunders, R.L. & Wiggs, A.J. (1985) Growth dynamics during the development of bimodal length-frequency distribution in juvenile Atlantic salmon (*Salmo salar* L.). *Aquaculture* **45**, 1-20.

Landless, P.J. (1976) Demand-feeding behaviour of rainbow trout. *Aquaculture* **7**, 11-25.

Li, H.W. & Brocksen, R.W. (1977) Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* **11**, 329-341.

McCarthy, I.D., Carter, C.G., & Houlihan, D.F. (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum) *J. Fish Biol.* **41**, 239-256.

Maisse, G., Baglinière, J.L., Landry, G., Caron, F., & Roleau, A. (1988) Identification externe du sexe chez le Saumon atlantique (*Salmo salar.* L.). *Can. J. Zool.* **66**, 2312-2315.

Metcalfe, N.B. (1986) Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. *J. Fish Biol.* **28**, 525-532.

Metcalfe, N.B., Huntingford, F.A. & Thorpe, J.E. (1986) Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Zool.* **64**, 2439-2446.

Metcalfe, N.B., Huntingford, F.A. & Thorpe, J.E. (1988) Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon. *J. Anim. Ecol.* **57**, 463-474.

Metcalfe, N.B. & Thorpe, J.E. (1992) Anorexia and defended energy levels in over-wintering juvenile salmon. *J. Anim. Ecol.* **61,** 175-181.

Müller, K. (1978) Locomotor activity of fish and environmental oscillations. In: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes.* Academic Press, London. pp. 1-20.

Naesje, T.F., Hansen, L.P., & Järvi, T. (1988) Sexual dimorphism in the adipose fin of Atlantic salmon, *Salmo salar* L. *J. Fish Biol.* **33,** 955-956.

Naevdal, G., Leroy, R., & Møller, D. (1981) Variation in growth rate and age at first maturation in rainbow trout. *Fisk. Dir. Skr. Ser. Havunders.* **17,** 71-78.

Noakes, D.L.G & Leatherland, J.F. (1977) Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). *Environ. Biol. Fish.* **2,** 131-136.

Rawlings, C.E. (1989) *Feeding patterns of Atlantic salmon parr.* Unpubl. MSc thesis, Plymouth Polytechnic.

Rawlings, C.E. (1994) *Rhythmic stomach fullness in Atlantic salmon* (Salmo salar L.*) and the influencing variables.* Unpubl. PhD thesis. University of Stirling, Scotland.

Roff, D.A. (1992) *The Evolution of Life Histories.* Chapman & Hall. London.

Rowe D.K. & Thorpe J.E. (1990) Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reductions in feeding and growth during spring months. *Aquaculture* **86,** 291-313.

Rowe, D.K., Thorpe, J.E. & Shanks A.M. (1991) Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* **48,** 405-413.

Schaffer, W.M. (1979) The theory of life-history evolution and its application to Atlantic salmon. *Symp. Zool. Soc. Lond.* **44,** 307-326.

Schaffer, W.M. & Elson, P.F. (1975) The adaptive significance of variations in life history strategy among local populations of Atlantic salmon in North America. *Ecology,* **56,** 577-590.

Simpson, A.L. (1992) Differences in body size and lipid reserves between maturing and non-maturing Atlantic salmon parr, *Salmo salar* L. *Canadian Journal of Zoology* **70,** 1737-1742.

Simpson, A.L. (1993) *Investigation of the Factors Influencing Maturation in Atlantic Salmon,* Salmo salar L., *Parr*. Unpubl. PhD thesis, University of Glasgow, Scotland.

Smith, I.P., Metcalfe, N.B., Huntingford, F.A. & Kadri, S. (1993) Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **117,** 165-178.

Spieler, R.E. & Noeske, T.A. (1984) Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. *Trans. Am. Fish. Soc.* **113,** 528-539.

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Sundararaj, B.I., Nath, P. & Halberg, F. (1982) Circadian meal timing in relation to lighting schedule optimises catfish bodyweight gain. *J. Nutr.* **12,** 1085-1097.

Thorpe, J.E. (1988) Salmon migration. *Sci. Prog. Oxf.* **72,** 345-370.

Thorpe, J.E. (1989) Developmental variation in salmonid populations. *J. Fish Biol.* **35(Suppl. A),** 295-303

Thorpe, J.E. (1994) Reproductive strategies in Atlantic salmon, *Salmo salar*. *Aquacult. Fish. Manage.* **25**, 77-87.

Thurow, F. (1966) Beitrage zur Biologie und Bestandskunde des Atlantischen Lachses (*Salmo salar* L.) in der Ostsee. *Ber. Dt. Wiss. Komm. Meeresforsch.* **18**, 223-379.

Wootton, R.J. (1990) *Ecology of Teleost Fishes.* Chapman & Hall, London.

CHAPTER II

FEEDING RHYTHMS AND COMPETITION IN JUVENILE AND POST-SMOLT ATLANTIC SALMON

INTRODUCTION

Circadian rhythms of activity in fishes have been studied widely, and have been reviewed by Schwassmann (1971), Müller (1978a), Spieler & Kendall (1984), Reebs (1992) and Helfman (1993), the latter concentrating on direct field observations of diel patterns in the wild. Circadian rhythms may be exogenous (under the influence of environmental periodicity) or endogenous (originating and controlled from within the organism). In fish they are generally under endogenous control and synchronised by light (Schwassmann 1971, Nishikawa & Ishibashi 1975, Manteifel *et al.* 1978, Müller 1978a, Kavaliers 1981, Spieler & Noeske 1984, Boujard & Leatherland 1992a, Spieler 1992) with the change in light intensity between light and dark phases being more important as an environmental Zeitgeber than absolute intensity or wavelength of light (Gibson & Keenleyside 1966, Chaston 1968, Molina Borja *et al* 1990, Rawlings 1994). Thus diel patterns of activity in fish can vary with season (Eriksson 1978) and latitude (Müller 1978b) according to photoperiod, although seasonal variation in the diel rhythms of Atlantic salmon parr have been shown also to be strongly influenced by temperature (Fraser *et al.* 1993).

In the 1960's, most studies of diel rhythms in fish measured locomotor activity (Eriksson 1978), but since the 1970's there has been an increasing interest in rhythms in feeding activity (Eriksson & Alanärä 1990, Boujard & Leatherland 1992a, Rawlings 1994), although the exogenous versus endogenous character of feeding rhythms in fishes remains unclear. The adaptive significance of circadian feeding rhythms in fish is likely to be similar to that of other organisms, in that they allow the fish to be prepared physiologically for the acquisition, digestion and assimilation of food. Feeding rhythms are known also in some instances to entrain rhythms of general activity (see Spieler 1992 for review).

In addition to endogenously generated rhythms, choice of feeding time may be influenced by temporal variation in the intensity of competition. Intra-specific differences in the ability of individual fish to compete for resources (especially food and mates) have received much study (see Magurran 1993, for review). These differences become especially important when limited resources increase levels of intraspecific competition. In such situations, poorer competitors have often been observed to adjust their foraging habits in order to avoid competing with dominants.

Such adjustments are often spatial in fish (Fausch 1984, Metcalfe 1986, Hughes 1992), but birds have been observed to make temporal adjustments, with female and less experienced members of flocks feeding after dominant individuals (De Laet 1985, Ramenofsky et al. 1992, Smith 1994), there are however, few if any published accounts of this latter phenomenon in fish (but see IV).

Salmonid parr are territorial and exhibit a wide range of aggressive behaviours when competing for food (from displays to fights) (Kalleberg 1958, Keenleyside & Yamamoto 1962, Bachman 1984), but at smolting, salmon tend to aggregate and so appear to be less aggressive. In IV however, I found that in one-sea-winter salmon, smaller fish were more likely to avoid competitors when approaching food pellets. Similarly, some salmon parr have been observed to 'veer away' from food at the last minute when faced with a competitor (Metcalfe 1989, Huntingford et al. 1993). Size however, is not linked initially with dominance status in parr - dominant fish eventually become larger than subordinates through greater food intake and subsequently faster growth rates (Huntingford et al. 1990, Metcalfe 1994). Given that salmon populations compete for food, one could expect aggressive interactions, and/or size to influence patterns of feeding activity at the individual and/or population level.

There have been a number of studies of daily feeding rhythms in Atlantic salmon that have produced variable results. Some of this variability may be due to differences between life history stages, since studies have used both parr (Hoar 1942, Hirata 1973, Higgins & Talbot 1985, Fraser et al. 1993, Rawlings 1994) and post-smolts or older fish in sea cages (Kadri et al. 1991, Rawlings et al. 1991, Blyth et al. 1993, Smith et al. 1993). Additionally, these studies illustrate that feeding rhythms may vary within life history stages. All of the studies have demonstrated that Atlantic salmon do not necessarily maintain a fixed appetite level throughout the daylight hours (as many commercial feeding schedules had previously assumed) and the authors have concluded generally that the observed feeding patterns were likely to be entrained to diurnal variation in light and/or temperature. Such data are of importance to the development and maintenance of efficient feeding regimes in salmon culture. Spieler (1977) and Parker (1984) recognised the importance of incorporating the rhythmic

nature of feeding in fish when developing feeding practices in aquaculture, this being possible only once such rhythms have been studied and understood.

This chapter describes two studies of feeding rhythms in Atlantic salmon, carried out on parr and post smolts, aimed at determining whether daily rhythms of feeding occur, and if so, how these relate to environmental conditions and competitive interactions.

EXPT. 1: DAILY PATTERNS OF FEEDING AND AGGRESSION IN ATLANTIC SALMON PARR UNDER NATURAL CONDITIONS.

Given the need for increased economic efficiency in the salmon culture industry, as well as concern over pollution of Scotland's oligotrophic freshwater bodies, development of feeding regimes that minimise food wastage among salmon parr is of both commercial and ecological importance (Kadri 1988, Nature Conservancy Council 1991, Gowen 1994, Metcalfe 1994, Thorpe & Cho in press). The daily and seasonal rhythms of salmon parr thus merit detailed investigation so as to allow feeding schedules to be matched to the feeding patterns of the fish.

A crepuscular feeding pattern was observed in summer in salmon parr by Hoar (1942), who concluded that this was controlled primarily by water temperature, with possible secondary influence by light. In contrast, a morning peak in both feeding and locomotor activity was reported by Hirata (1973), although observations were made at 6-hourly intervals in this case. Rawlings (1994) examined diel patterns of stomach fullness at 4-hourly intervals under laboratory and ambient conditions. She found a daily peak in stomach fullness at 1800 hours in spring and summer, no consistent peak in autumn and a small but relatively constant level of stomach fullness in winter. No such rhythm was observed under constant light and temperature and it was concluded that changing light levels were important in synchronising feeding peaks. A crepuscular feeding pattern was found amongst salmon parr in May with very little feeding activity during hours of darkness (Higgins & Talbot 1985), but fish become increasingly nocturnal when water temperatures drop below 10^0C (Fraser *et al.* 1993).

Two studies were made to answer the following questions for parr under ambient light and temperature regimes:

i) Do daily feeding rhythms exist and what form do they take?

ii) Is the daily pattern of feeding similar at different times of year?

iii) How does feeding activity relate to the incidence of aggressive interactions?

Materials and Methods

In early March 1992, fifteen sibling 1 year old hatchery-reared salmon parr (mean length 124.6mm) were identified by size as being due to migrate to sea in the coming spring [i.e. fish from the upper modal group (UMG) of the size distribution (Thorpe, 1977)]. They were placed in a 1m circular tank with a downward-pointing video camera (C-Technics, Oban) mounted overhead such that the entire tank was within the field of view. The tank received water at ambient temperature and was outdoors, so experiencing ambient photoperiod (approx. 13L:19D - 16L:8D). Light levels were recorded by a light meter (Skye Instruments Ltd., Powys) installed at the tank (within the field of view of the camera) and were observed to peak during the noon recording session (see below) on all days. Small quantities of food were dispensed every 10 minutes throughout the daylight hours (totalling approximately 2% of body weight per day) by a vibrator feeder (Aquaculture Engineering, Rochdale). In order to ensure the capture of a feeding bout during each recording session, video recordings were made for 10 minute periods each. Recording was carried out at 2-hour intervals over a one month period from 20 March; logistic constraints meant that data could not be collected over the entire day on any one day, so recording times were varied between days in a pseudorandom sequence so that all daylight hours were covered over the course of the experiment.

For each filmed feeding bout, data were collected on the number of individual fish responding to food (i.e. attacking or attempting to attack food pellets) and the number of aggressive interactions (chasing or nipping) during a 3 minute period commencing 1 minute prior to food delivery. A maximum of 3 interactions between a specific pair of fish were recorded in each feeding bout.

A similar protocol to that described above was used for a second investigation carried out during autumn 1992 on 16 sibling under-yearling UMG parr. The fish were stocked in the tank described above in early October and data were recorded as described above (with the exception of light level as light meter was no longer available) from 22 October until 2 December.

All times were recorded as GMT for the trials.

Results

Feeding

In spring a clear, statistically significant appetite rhythm was apparent (ANOVA comparing the number of fish feeding per delivery of food, by 2 hour period $F_{6,56}$=4.24, P=0.001; Fig.1a). The fish began feeding in the mid-morning, appetite reached a peak in the early afternoon and thereafter it tailed off as the day progressed. This was not the case in autumn when feeding rhythms lacked a consistent pattern between days (ANOVA as above, $F_{4,56}$=0.76, NS). However, testing the distribution of the number of fish feeding at different times of day against a random distribution generated by binomial probabilities (which assumes fish fed independently of one another), showed that the fish fed in synchrony on any particular day (χ^2_5= 91.7, P<0.001 see Fig. 1b). Therefore feeding was concentrated in time, but did not peak at the same time each day.

Aggression

In both spring and autumn aggression levels varied significantly and consistently through the daylight hours (Fig. 2), being highest at 0800 hours and becoming progressively lower throughout the day (ANOVA based on the number of aggressive interactions per 3 minutes, by 2 hour period for spring $F_{6,56}$=5.63 P<0.0005 and autumn $F_{4,56}$=3.51 P=0.013). Comparison of Figs. 1a and 2a shows that feeding and aggression were out of phase, correlation analysis showing a negative relationship between aggression and feeding (Pearson's r=-0.273, n=63, P=0.03; Fig. 3).
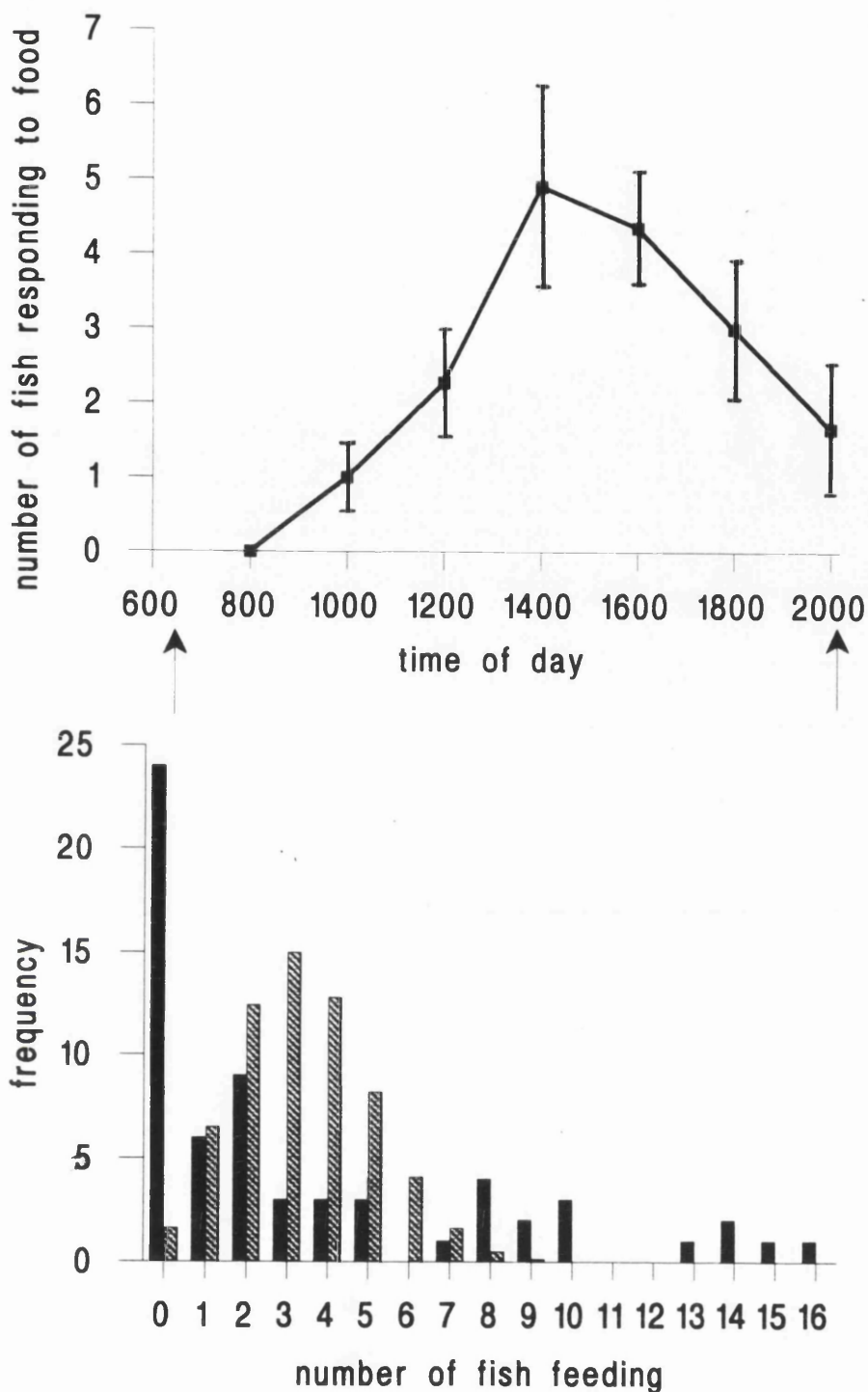
Fig. 1. a) Variation in the number of salmon parr responding to pellets (delivered by a feeder) in relation to the time of day in spring. Number of fish responding presented as means ($\pm$ s.e.) for 2-hourly periods; n ranges from 3 to 11 feeding bouts per mean. Arrows indicate approxiamate times of sunrise and sunset. b) Frequency histogram of observed (dark bars) and expected (striped bars) numbers of fish feeding per bout in autumn; n=53 feeding bouts.
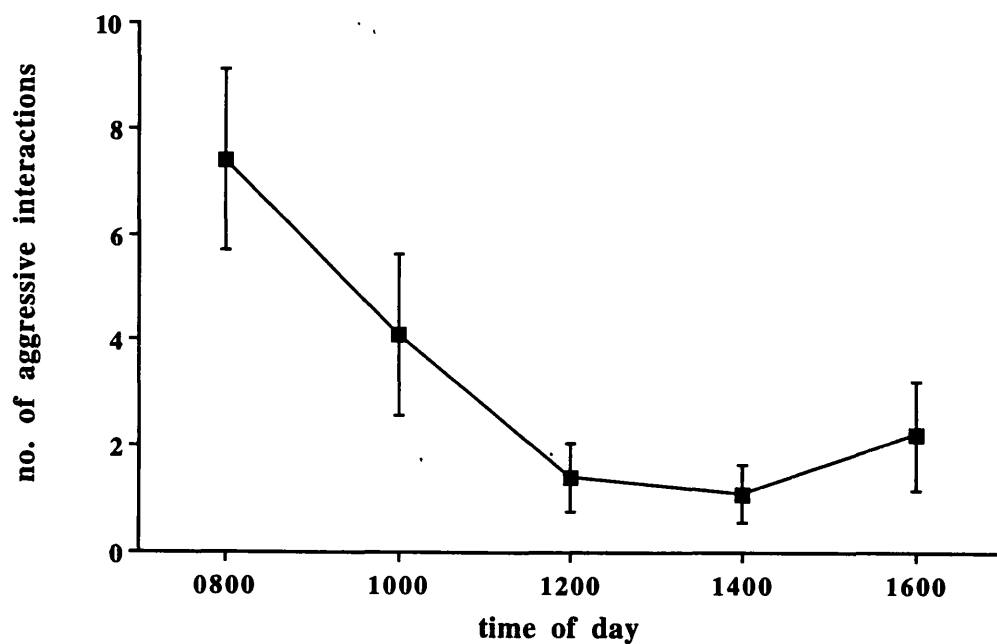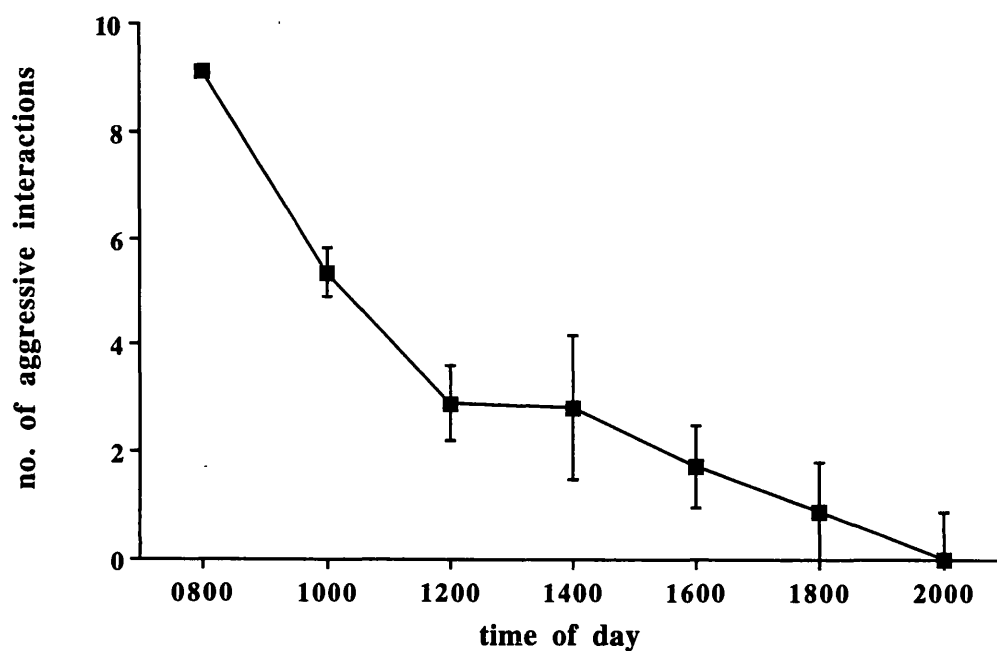
Fig. 2. Variation in the number of aggressive interactions (per 3 minute period) between individuals in relation to time of day in a) spring, and b) autumn. Means presented as in Fig. 1; n ranges from 3 to 11 and 5 to 23 observations per mean for spring and autumn respectively
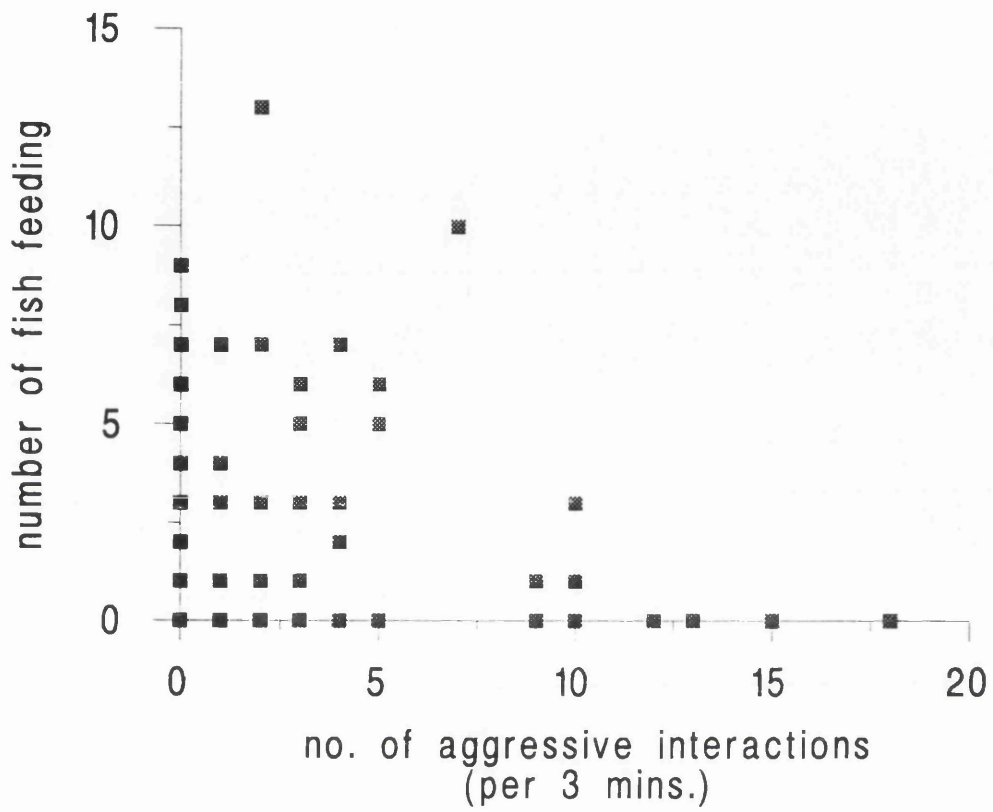
Fig. 3. The relationship in spring between the number of fish feeding and the number of aggressive interactions in a 3 minute period surrounding each feeding bout.

Discussion

With reference to the initial aims of the experiment, daily feeding rhythms with an afternoon peak were observed in spring, while in autumn fish fed synchronously but without a consistent pattern between days. There was also a diurnal rhythm in frequency of aggressive interactions that was similar in both seasons. In spring high levels of aggression seem to have been exclusive to feeding activity. It is important to note that these trials were performed on single groups of fish of different ages. It may be that the difference in feeding patterns observed is not a seasonal effect, but a group effect (e.g. age, social structure etc.). More replicates would be needed to distinguish between these two possibilities. However, Rawlings (1994) also observed an afternoon peak (in stomach fullness of parr) in the spring (and summer) but no consistently significant peak in autumn (or winter). She found that changing light levels (as opposed to absolute light levels or wavelengths) were important in synchronising feeding rhythms and peaks in parr, and suggested that decreasing light levels during the afternoon synchronised feeding during spring and summer and that either the diel change in light or the duration of the daylight period may have affected the occurrence of feeding peaks. However, these hypotheses are untested as yet and hence the underlying causes of the observed feeding rhythms remain unclear. It should be noted that peak light levels in the present study always occurred at 1200, and so the daily feeding peak in spring was certainly occurring under conditions of declining light levels; our results thus provide some support for Rawlings' hypothesis.

In both seasons studied, aggression levels were highest at first light and then declined as the morning progressed. In spring, this aggression was found to be negatively related to feeding activity. This pattern may be due to a breakdown in social order at night when the fish are unable to see each other, the situation being further exacerbated by the homogeneous environment of a plastic tank in which it would be very difficult to establish and maintain a territory (Mikheev *et al.* in press). Fraser *et al.* (1993) observed a substantial reduction in aggressive interactions at night and observed that fish seemed to be ignoring each other during hours of darkness (presumably because they were unable to see). Aggression took place only when fish collided with each other. Similar observations were made during this experiment when

video footage at dawn was recorded at very low light levels; under these conditions fish were distributed randomly in the water column around the tank. However as light levels increased, most fish moved to the centre of the tank, with several aggressive individuals holding station outside this group. Hence it seems that aggression levels are highest in the morning when the fish are re-establishing a social order before they begin feeding.

EXPT 2: DAILY PATTERNS OF FEEDING IN POST SMOLTS UNDER CONSTANT ENVIRONMENTAL CONDITIONS.

Kadri *et al.* (1991) revealed a clear daily feeding rhythm in caged one-sea-winter salmon during summer, with crepuscular peaks in feeding activity and a marked depression in feeding during early afternoon (Fig. 5). The fish had been presented with food pellets regularly throughout the daylight hours, and hence the observed feeding pattern was not entrained to food availability - thus it may have been a direct response to environmental conditions, especially light and/or temperature. A very similar feeding pattern was reported among Atlantic salmon of a similar age in sea cages in Tasmania (Blyth *et al.* 1993), but in a later study carried out at the site used by Kadri *et al.* (1991) in Scotland no consistent daily feeding patterns were found from month to month over the period from autumn to spring (Smith *et al.* 1993). It was concluded that the differences in findings between the two studies were possibly due to the variation in prevailing light conditions between seasons, as Kadri *et al.* (1991) had made observations during a period of high water clarity and little or no cloud cover; therefore the reduced activity in early afternoon may have been in response to high light intensities.

Previous findings have indicated that individual Atlantic salmon in a sea cage may not exhibit the same feeding pattern as that observed at a population level, i.e. individuals do not feed necessarily at every peak in feeding activity of the population (Bjordal *et al.* 1988, Juell & Westerberg 1993). Thus it is possible that individuals have different feeding strategies depending upon social status and/or body size.

The next study was performed in order to answer the following questions:

i) Do daily feeding rhythms persist in post-smolts held under constant temperature, physiochemical conditions and light intensity (during the photophase)?

ii) Do all fish manifest the same pattern of variation regardless of size?
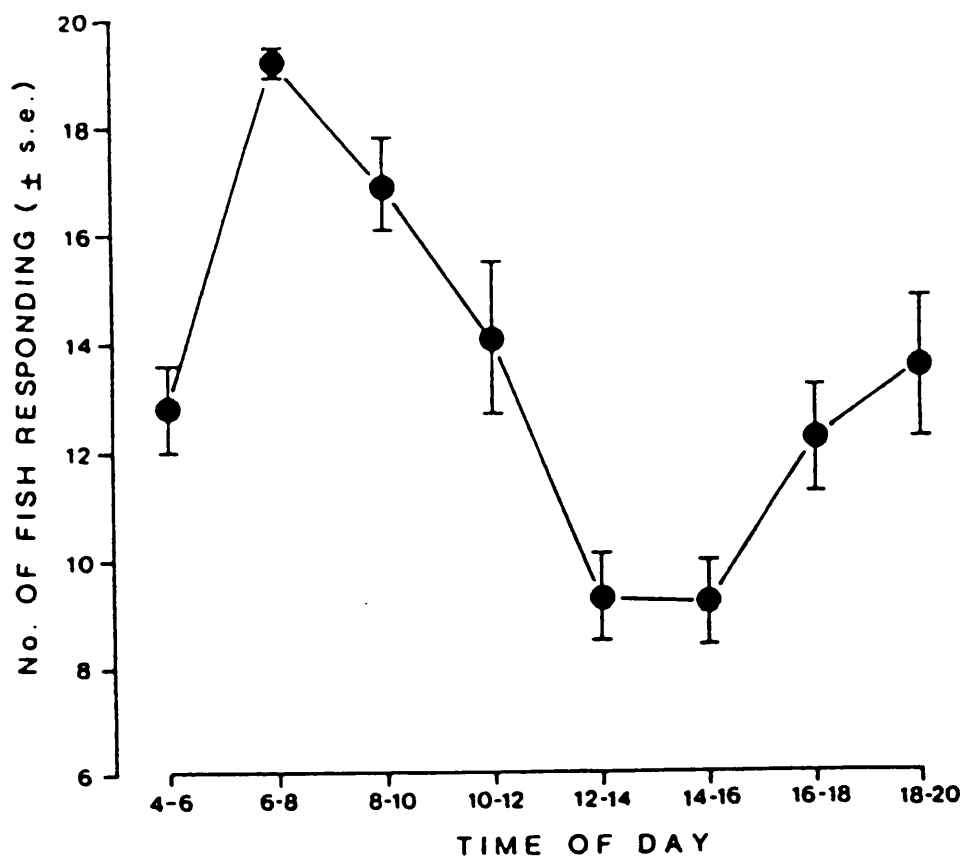
Fig. 5. Variation in the number of one-sea-winter salmon responding to a standardised presentation of five pellets in relation to the time of day. Data collected in June - July in a sea cage. Means presented as in Fig. 1; n ranges from 10 to 25 feed presentations per mean. After Kadri *et al.* (1991).

Materials and Methods

39 sibling Atlantic salmon smolts were transferred from a freshwater hatchery tank to an indoor 2m diameter sea water tank in May 1992. The aquarium was kept under a controlled photoperiod of 12L:12D, the overhead lights (2 x 65w, 18" strip lights, Phillips) turning on and off abruptly at 0800 and 2000 GMT respectively. The water supply passed through a chiller, so that diurnal variation in temperature was negligible (mean temperature= 10.6°C, diurnal variation < 1°C). The food ration (commercial food pellets - BOCM Pauls Ltd., Renfrew) was 2% body weight per day, dispensed in small quantities every 5 minutes throughout the 12 hour light period from a vibrator feeder (Aquaculture Engineering, Rochdale).

A CCTV camera was mounted above the tank pointing downward, viewing an area of approximately $1.5m^2$ below the feeder. It was linked to a video recorder programmed to record for 5 minutes every 2 hours throughout the light phase, hence providing footage of a feeding bout in every recording session. Recording was carried out over a period of four weeks commencing 13 November 1992 (mean fish weight 63.47g).

Data on the total number of fish of different body size feeding in the minute following a delivery of food to the tank were obtained using slow motion video analysis and measurement of fish lengths directly from the monitor screen; the fish were categorised into size classes of large (>20cm, n=8), medium (18-20cm, n=18) & small (<18cm, n=13). Time of day and water temperature were also monitored via a clock and digital thermometer mounted above the tank within the field of view of the camera.

Results

A clear diurnal rhythm was observed in the total number of fish feeding per food delivery (Fig. 6), peaking clearly in the morning and rising again in late afternoon (ANOVA based on 2 hour sampling periods indicated in Fig. 6, $F_{6,93}=5.57$, P<0.001).

When the same data were separated according to fish size categories (Fig. 7), significant patterns of diurnal variation in appetite were found for the small and medium fish (ANOVAS as previous, $F_{6,93}=6.97$, P<0.001, and 2.96, P=0.011 respectively), but not for the larger fish (ANOVA as previous, $F_{6,93}=1.89$, NS). Medium-sized fish, being the largest size-group, fed in a daily pattern similar to that observed at the population level, while small fish showed the highest level of feeding activity at first light and at the end of the light phase, with a marked reduction in feeding in the afternoon. Large fish maintained a relatively consistent level of feeding activity throughout the day. Temporal effects on feeding thus depended upon fish size, with small fish showing a marked diurnal fluctuation while large fish fed at a relatively constant rate (2-way ANOVA comparing fish size against time of day, i.e. interaction term, $F_{6,186}=5.93$ P<0.001).
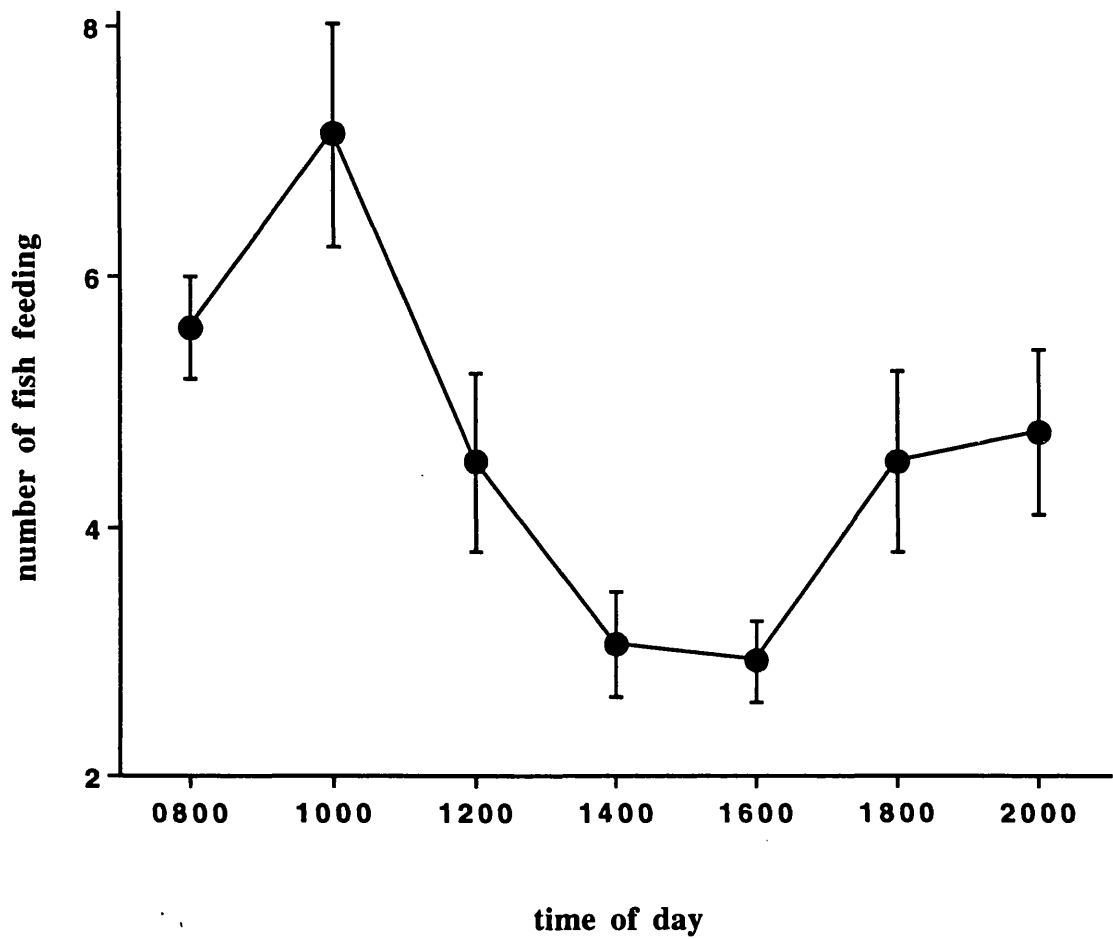
Fig. 6. Variation in the number of post-smolt salmon responding to pellets (delivered by a feeder) in relation to the time of day. Means presented as in Fig. 1; n ranges from 13 to 15 feeding bouts per mean.
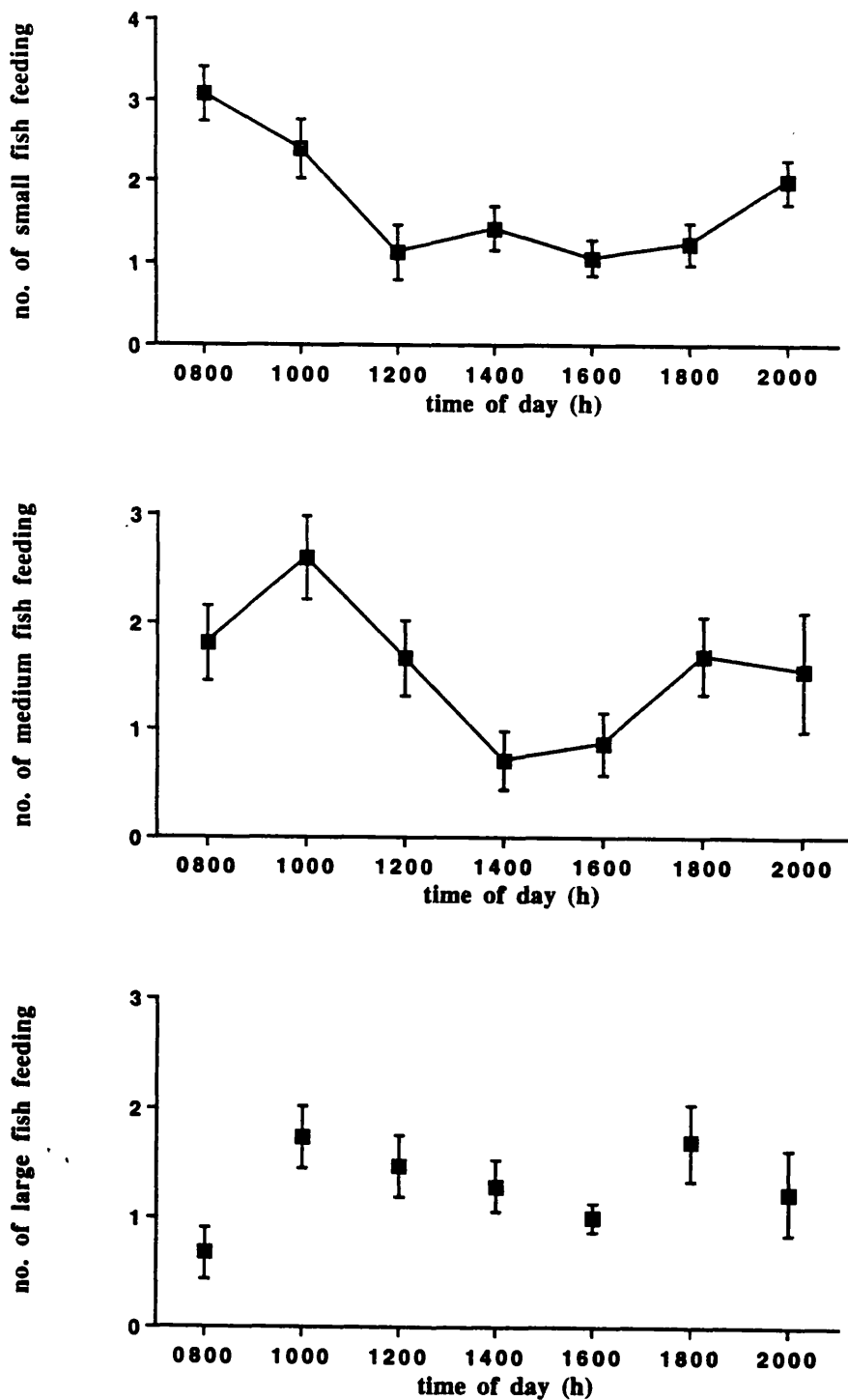
Fig. 7. Variation in the number of a) small (<18cm, n=13), b) medium (18-20cm, n=18) and c) large (>20cm, n=8) post-smolt salmon responding to pellets (delivered by a feeder) in relation to the time of day. Data presented as means (±s.e.), n as in Fig. 6. N.B. Line between points is absent for c) due to lack of significant variation through the day.

Discussion

The feeding rhythm recorded for all fish combined was very similar to that described by Kadri *et al* (1991) and Blyth *et al.* (1993), using large populations of fish in commercial sea cages. Both studies found a daily appetite rhythm, with an early morning peak after a slow start at first light, a trough in the early afternoon, and a second peak in the late afternoon/early evening. All three studies have demonstrated an afternoon drop in appetite. It was previously assumed that this was a response to higher light levels at this time of day, although Hoar (1942) suggested that the afternoon reduction in appetite that he observed in Atlantic salmon parr and juvenile brook charr (*Salvelinus fontinalis* Mitchill) was due to higher temperatures. While environmental variables such as light intensity and temperature levels are likely to have a strong effect upon feeding patterns in salmon, the results of the present study suggest that these factors are not the primary cause of the patterns observed in the present and our previous studies.

Using the same experimental site and equipment as Kadri *et al.* (1991) (but at different times of year), Smith *et al.* (1993) did not observe the same diurnal feeding patterns. Diurnal rhythms of feeding activity are known to change with season in several fish species (Boujard & Leatherland 1992a) and a similarly consistent activity pattern over summer, contrasting with variability in patterns during other periods of the annual cycle, were observed recently in the barbel *Barbus barbus* L. (Baras 1995). As a result of fish farmers' observations of feeding activity during winter periods of short daylength, adult salmon in some Scottish seacages are fed a single daily meal in the morning accordingly (D. Mitchell, pers. comm.).

The similarity between the results of the present study and our previous work suggests that cultured Atlantic salmon in seawater (under relatively stable environmental conditions) display a diurnal rhythm which is initiated by the onset of daylight and subsequent delivery of food, possibly with an 'hour-glass' mechanism (Lees 1966) producing the feeding pattern I have observed. This hypothesis suggests that the fish feed heavily soon after first light, then digest and absorb the food, feeding again once appetite is re-activated following gastric evacuation (Brett 1971, Ware 1972, Grove *et al.* 1978, Vahl 1979, Fletcher 1984, Dos Santos & Jobling 1988).

Small fish fed predominantly at first light, though the reason for this is still not clear. If small fish are only able to eat relatively small quantities of food, they are likely to be hungriest, particularly at first light. However, Kadri *et al.* (1991) and Blyth *et al.* (1993) both observed a feeding peak in their trial populations some time after first light and it may be that smaller fish are avoiding competition with their larger siblings by eating as much as possible before the latter begin feeding. The large fish were the only group which did not exhibit a consistent daily feeding rhythm. This may be because they are able to feed freely throughout the daylight hours, rather than feeding only when possible. This hypothesis is supported to some degree by the findings of McCarthy *et al.* (1992), who found that rainbow trout (*Oncorhynchus mykiss*) with higher rankings in the feeding hierarchy had the least variation in their day to day food intake (see also IV). I know of no similar studies of diel feeding activity that test for differences according to size or social status. Alanärä & Brannas (unpubl.) have found large differences in diel activity between individuals in both rainbow trout and Arctic charr (*Salvelinus alpinus*), but these appear to be unrelated to social status. Helfmann (1981) found size and age-related differences in daily feeding patterns within a fish community in the field, but these seem to be associated with predator-prey interactions (see later). While it is possible that the feeding pattern observed among the smaller individuals of the present study was adaptive such that feeding in the wild occurs only in periods of low light when predation pressure may be less, size differences between fish (which were significant in terms of competition) were not considered sufficiently significant to generate a large difference in vulnerability to predators.

GENERAL DISCUSSION

Boujard & Leatherland (1992b) found crepuscular feeding patterns in demand-fed rainbow trout under photoperiod regimes of 12 and 16 hours of light per 24 hours. In their experiment there was negligible diurnal variation in temperature, and light intensity was constant throughout the 'day', with the exception of a 30 minute twilight phase at both 'dawn' and 'dusk'. However, under a shorter photoperiod (i.e. 8 hours of light per 24), there were no such clear peaks of feeding activity, although the main peak did occur in the morning, presumably due to there being fewer hours in which to feed, digest and feed again. In rainbow trout feeding rhythms have been shown to parallel closely rate of gastric emptying (Grove *et al.* 1978). Thus the photoperiod effect observed by Boujard & Leatherland (1992b) may explain in part the seasonal differences in daily feeding pattern found in Expt. 1 and between the results of Kadri *et al.* (1991) and Smith *et al.* (1993).

Rawlings *et al.* (1991) studying patterns of stomach fullness in post-smolts at a cage site a little further north than (but over same period as) that used by Kadri *et al.* (1991), found a single peak in feeding activity during the afternoon. The difference in results between these studies is possibly due to differences in the age of the fish, as Rawlings (1994) has demonstrated a feeding pattern in Atlantic salmon parr which is very similar to that she observed in post-smolts. Rawlings (1994) suggested that the difference in results between her study and that of Kadri *et al.* (1991) may have been due to the effect of fish being transferred newly to seawater and not yet having developed a feeding pattern suited to the new environment. Also, Blyth (pers. comm.) has found afternoon feeding peaks to occur in Atlantic salmon for the first few months (only) after transfer to sea water. In a fish community of a temperate lake, larger and older individuals were often active later in the evening than smaller fish and in several species, diurnal juveniles changed to nocturnally foraging adults (Helfmann 1981). These differences were attributed to greater predation pressure on smaller, younger fish and a foraging shift to larger prey, which were only active at night. An evolutionarily adaptive change in daily feeding pattern occurring sometime after Atlantic salmon smolts enter sea water is therefore quite possible, since both their prey and their predators change at this time.

Generally there have been large differences in results of studies of feeding rhythms in fishes, even of the same species (Boujard and Leatherland 1992a). In most cases these differences can be attributed to experimental conditions, including variation in fish size and age, food composition, feed ration and regime, method of food dispensation, method and frequency of data collection and of course environmental conditions, all contributing to differences in results between studies. Further, standardised studies are thus needed to tease these sources of variation apart.

REFERENCES

Bachman, R.A. (1984) Foraging behaviour of free-ranging wild and hatchery brown trout in a stream. *Trans. Am. Fish. Soc.* **113,** 1-32.

Baras, E. (1995) Seasonal activities of *Barbus barbus*: effect of temperature on time-budgeting. *J. Fish Biol.* **46,** 806-818.

Bjordal, Å., Fernø, A., Furevik, D. & Huse, I. (1988) Effect on salmon (*Salmo salar*) from different operational procedures in fish farming. *Int. Counc. Explor. Sea* C.M. F:16.

Blyth, P.J., Purser, G.J. & Russell, J.F. (1993) Detection of feeding rhythms in seacaged Atlantic salmon using new feeder technology. In: H. Reinertsen, L.A. Dahle, L. Jorgensen L. & Tvinnereim K. (eds.) *1st International Fish Farming Technology Conference.* Trondheim, Norway. pp. 209-216.

Boujard, T. & Leatherland, J.F. (1992a) Circadian rhythms and feeding time in fishes. *Environ. Biol. Fish.* **35,** 109-131

Boujard, T. & Leatherland, J.F. (1992b) Demand feeding behaviour and diel pattern of feeding activity in *Oncorhynchus mykiss* held under different photoperiod regimes. *J. Fish Biol.* **40,** 535-544.

Brett, J.R. (1971) Satiation time, appetite and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd. Can.* **28,** 409-415.

Chaston, I. (1968) Influence of light on activity of brown trout (*Salmo trutta*). *J. Fish. Res. Bd. Can.* **25,** 1285-1289.

De Laet, J.F. (1985) Dominance and anti-predator behaviour of Great Tits *Parus major*: a field study. *Ibis.* **127,** 372-377.

Dos Santos & Jobling (1988) Gastric emptying in cod, *Gadus morhua*: effects of food particle size and dietary energy content. *J. Fish Biol.* **33,** 511-516.

Eriksson, L.-O. (1978) Nocturnalism versus diurnalism; dualism within fish individuals. . In: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes.* Academic Press, London. pp. 69-90.

Eriksson, L.-O. & Alanärä, A. (1990) Timing of feeding behaviour in salmonids. In: J.E. Thorpe & F.A. Huntingford (eds.) *The Importance of Feeding Behaviour for the Efficient Culture of Salmonid Fishes.* World Aquaculture Society, Baton Rouge. pp. 41-48.

Fausch, K.D. 1984. Profitable stream positions for salmonids: relating specific growth rate to net energy gain. *Can. J. Zool.,* **62,** 441-451.

Fletcher, D.J. (1984) The physiological control of appetite in fish. *Comp. Biochem. Physiol.* **78A,** 617-628.

Fraser, N.H.C., Metcalfe, N.B. & Thorpe, J.E. (1993) Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proc. R. Soc. Lond.* B. **252,** 135-139.

Gibson, R.J. & Keenleyside, M.H.A. (1966). Responses to light of young Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd. Can.* **23,** 1007-1024.

Gowen, R.J. (1994) Managing eutrophication associated with aquaculture development. *J. Appl. Icthyol.* **10,** 242-257.

Grove, D.J., Loizdes, L.G. & Nott, J. (1978) Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *J. Fish Biol.* **12,** 507-516.

Helfman, G.S. (1981) Twilight activities and temporal structure in a freshwater fish community. *Can. J. Fish. Aquat. Sci.* **38**, 1405-1420.

Helfman, G.S. (1993) Fish behaviour by day, night and twilight. In: T.J. Pitcher (ed.) *Behaviour of Teleost Fishes.* 2nd edn. Chapman & Hall. London. pp. 479-512.

Higgins P.J. and Talbot C. (1985) Growth and feeding in juvenile Atlantic salmon. In: Cowey C.B., MacKie A.M. & Bell J.G. (eds.), *Nutrition and Feeding in Fish.* Academic Press, London, pp. 243-263.

Hirata, T. (1973) Diurnal rhythms of metabolic and activity rates in juvenile Atlantic salmon., *Salmo salar*, Linnaeus. *Memoir. Fac. Fish.*, Kagoshima University **22**, 73-93.

Hoar, W.S. (1942) Diurnal variations in feeding activity of young salmon and trout. *J. Fish. Res. Bd. Can.* **6**, 90-101.

Hughes, N.F. (1992) Ranking of feeding positions by drift-feeding Arctic grayling (*Thymallus arcticus*) in dominance hierarchies. *Can. J. Fish. Aquat. Sci.* **47**, 1994-1998.

Huntingford, F.A., Metcalfe, N.B. & Thorpe, J.E. (1993) Social status and feeding in Atlantic salmon *Salmo salar* parr: the effect of visual exposure to a dominant. *Ethology* **94**, 201-206.

Huntingford, F.A., Metcalfe, N.B., Thorpe, J.E., Graham, W.D. & Adams, C.E. (1990) Social dominance and body size in Atlantic salmon parr (*Salmo salar*). *J. Fish Biol.* **36**, 877-881.

Juell, J.E. & Westerberg , H. (1993) An ultrasonic telemetric system for automatic positioning of individual fish used to track Atlantic salmon (*Salmo salar*) in sea cages. *Aquaculture Engineering* **12,** 1-18.

Kadri, S. (1988) *A Screen Filter's Performance in Treating Salmonid Hatchery Effluent.* Unpubl. MSc thesis, Institute of Aquaculture, University of Stirling.

Kadri S., Metcalfe N.B., Huntingford F.A. & Thorpe J.E. (1991) Daily feeding rhythms in Atlantic salmon in sea cages. *Aquaculture* **92,** 219-224

Kalleberg, H. (1958) Observations in a stream tank of territoriality and competition in juvenile salmon and trout. *Rep. Inst. Freshw. Res. Drott.* **39,** 55-98.

Kavaliers, M. (1981) Period lengthening and disruption of socially facilitated circadian activity rhythms of goldfish by lithium. *Physiol. Behav.* **27,** 625-628.

Keenleyside, M.H.A. & Yamamoto, F.T. (1962) Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). *Behaviour* **19,** 138-169.

Lees, A.D. (1966) Photoperiodic timing in insects. *Nature* **210,** 986-989.

McCarthy, I.D., Carter, C.G., & Houlihan, D.F. (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol.* **41,** 239-256.

Magurran, A.E. (1993) Individual differences and alternative behaviours. In: T.J. Pitcher (ed.) *Behaviour of Teleost Fishes.* 2nd edn. Chapman & Hall, London. pp. 441-477.

Manteifel, B.P., Girsa, I.I. and Pavlov, D.S. (1978) On rhythms of fish behaviour. In: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes.* Academic Press, London. pp. 215-224.

Metcalfe, N.B. (1986) Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. *J. Fish Biol.*, **28,** 525-532.

Metcalfe, N.B. (1989) Differential response to a competitor by Atlantic salmon adopting alternative life-history strategies. *Proc. R. Soc.* B. **236,** 21-27.

Metcalfe, N.B. (1994) The role of behaviour in determining salmon growth and development. *Aquacult. Fish. Manage.* **25,** 67-76.

Mikheev, V.N., Huntingford, F.A. & Thorpe, J.E. (In press) Visual complexity and the establishment of territoriality in juvenile Atlantic salmon. *Anim. Behav.*

Molina Borja, M., Perez, E., Pupier, R. & Buisson, B. (1990) Entrainment of the circadian activity rhythm in the juvenile trout, *Salmo trutta* L., by red light. *J. Interdisc. Cycle Res.* **21,** 81-89.

Müller, K. (1978a) Locomotor activity of fish and environmental oscillations. In: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes.* Academic Press, London. pp. 1-20.

Müller, K. (1978b) The flexibility of the circadian system of fish at different latitudes. In: J.E. Thorpe (ed.) *Rhythmic Activity of Fishes.* Academic Press, London. pp. 91-104.

Nature Conservancy Council (1991) *Fish farming and the Scottish Freshwater Environment.* A report prepared for the Nature Conservancy Council by: Institute of Aquaculture, University of Stirling; Institute of Freshwater Ecology, Penicuik, Mid-Lothian & Institute of Terrestrial Ecology, Banchory. NCC Edinburgh.

Nishikawa, M. & Ishibashi, T. (1975) Entrainment of the activity rhythm by the cycle of feeding in the mud-skipper, *Periophthalmus cantonensis. Zool. Mag.* (Tokyo) **84,** 184-189.

Parker, N.C. (1984) Chronobiologic approach to aquaculture. *Trans. Am. Fish. Soc.* **113**, 545-552.

Ramenofsky, M., Gray, J.M. & Johnson, R.B. (1992) Behavioural and physiological adjustments of birds living in winter flocks. *Ornis Scand.* **23**, 371-380.

Rawlings, C.E., Talbot, C., Thorpe, J.E. & Bromage, N. (1991) Feeding rhythms of Atlantic salmon smolts (*Salmo salar* L.) in sea cages in summer. In: *Aquaculture & the Environment*, N. de Pauw and J.Joyce (eds.) *European Aquaculture Society Special Publication.* **14**, 271-272.

Rawlings, C.E. (1994) *Rhythmic stomach fullness in Atlantic salmon* (*Salmo salar* L.) *and the influencing variables.* Unpubl. PhD thesis. University of Stirling, Scotland.

Reebs, S. (1992) Sleep, inactivity and circadian rhythms in fish. In: M.A. Ali (ed.) *Rhythms in Fishes.* NATO ASI Series A: Life Sciences **236**, 127-136.

Schwassmann, H.O. (1971) Biological rhythms. In: W.S. Hoar & D.J. Randall (eds.) *Fish Physiology.* Vol. 2. Academic Press, New York.

Smith, I.P., Metcalfe, N.B., Huntingford, F.A. & Kadri, S. (1993) Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **117**, 165-178.

Smith, R.D. (1994) *Snow Buntings Plectrophenax nivalis: The Behavioural Ecology and Site Use of an Itinerant Flock Species in the Non-Breeding Season.* Unpubl. PhD thesis, University of Glasgow.

Spieler, R.E. (1977) Diel and seasonal changes in response to stimuli: a plague and a promise for mariculture. *Proc. World Maricult. Soc.* **8**, 865-873.

Spieler, R.E. (1992) Feeding-entrained circadian rhythms in fishes. . In: M.A. Ali (ed.) *Rhythms in Fishes*. NATO ASI Series A: Life Sciences **236,** 137-146.

Spieler, R.E. & Kendall, R.L. (1984) Rhythmicity in fishes. *Trans. Am.. Fish. Soc.* **113,** 411-413.

Spieler, R.E. & Noeske, T.A. (1984) Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. *Trans. Am. Fish. Soc.* **113,** 528-539.

Thorpe, J.E. (1977) Bimodal distribution of length of juvenile Atlantic salmon under artificial rearing conditions. *J. Fish Biol.* **11,** 175-184.

Thorpe, J.E. and Cho, C.Y. (in press) Minimising waste through bioenergetically and behaviourally based feeding strategies. *Wat. Sci. Tech.*

Vahl, O. (1979) An hypothesis on the control of food intake in fish. *Aquaculture* **17,** 221-229.

Ware, D.M. (1972) Predation by rainbow trout (*Salmo gairdneri*): the influence of hunger, prey density and prey size. *J. Fish. Res. Bd. Can.* **29,** 1193-1201.

CHAPTER III

THE EFFECTS OF FEEDING REGIME AND INDIVIDUAL VARIABILITY ON DIGESTIVE EFFICIENCY IN POST-SMOLTS.

INTRODUCTION

Digestive efficiency in fish is affected by three main factors: a) the digestibility of the nutrients, b) the activity of the digestive enzymes and c) the length of time the food is exposed to the action of digestive enzymes (Hepher 1988). These primary factors are each affected by a large number of secondary factors, the most important of which are: fish species, age, size, and physiological condition, water temperature [which may not have a great effect upon digestibility, but is positively correlated with digestion rate (Brett *et al.* 1969)], salinity, food composition, feeding level and feeding frequency (see Hepher 1988, De Silva 1989 for reviews).

Several of the above factors may generate differences in digestive efficiency among individuals within a population: Windell *et al.* (1978) found an effect of fish size upon protein digestibility in rainbow trout (*Oncorhynchus mykiss*) at 7°C, but not at higher temperatures. Stress affects the physiological condition of fish and under competitive conditions, subordinate individuals may suffer stress and/or food deprivation, which may in turn affect digestive efficiency (Hepher 1988, De Silva & Anderson 1995). Notably, Abbott & Dill (1989) found that among steelhead trout (*O. mykiss*) fed equal rations, subordinate fish grew less well than dominants. Subordinate fish in a feeding hierarchy are known to have more day to day variation in food intake than their dominant counterparts (McCarthy *et al.* 1993a); such a difference in feeding patterns may itself be a cause of individual variation in digestive efficiency. Feeding regime and food dispersal may also affect the distribution of food among individuals (Thorpe *et al.* 1990) and thus could be expected to have an indirect effect upon individual digestive efficiencies.

Protein is the basic component of animal tissues and thus an essential nutrient for both maintenance and growth. Protein in fish feeds must therefore meet both the qualitative and quantitative requirements of the fish if they are to thrive and grow at an optimal rate. An important qualitative feature of these proteins is that they can be digested efficiently by all the fish in the population to which they are fed. However, it has previously been reported that protein digestive efficiency may vary among individuals within a sibling population of Atlantic salmon, though the reasons for observed differences remained unclear (Carter *et al.* 1993). Intra-individual

differences in digestive efficiency of fish have also been observed from day to day under constant environmental conditions, independently of the amount of food ingested (De Silva & Perera 1983). Thus it is clear that variation in digestive performance exists both between and within individuals, but the reasons for such variaton are poorly understood. The study described below was designed to address these issues and, in particular, to answer the following questions:

- How much do individual Atlantic salmon post-smolts differ in food intake and protein digestion?

- Does feeding regime affect food intake and protein digestion at an individual and population level?

- Is there a relationship between food intake and protein digestive efficiency, and is individual variation in these two factors consistent across feeding regimes

  How do protein digestion and food intake relate to body weight and growth under different feeding regimes

MATERIALS AND METHODS

On 7 May 1993, 40 sibling Atlantic salmon smolts (mean weight 12.75g) were anaesthetised, weighed, measured and individually marked using alcian blue. One week later they were transferred from the University Field Station at Rowardennan to the Dept. of Zoology, University of Glasgow where they were kept in a 2m diameter indoor sea water tank on a 12L:12D photoperiod, the light phase commencing at 0800 GMT. 15 fish died, either through osmoregulatory failure (in the first 3 days following transfer to sea water) or 6-7 weeks post-transfer through failing to begin feeding (i.e. 'failed smoltification', Usher *et al.* 1991, Stradmeyer 1994).

Food was delivered in two ways. From 7 May to 14 December it was dispensed by an automatic vibrator feeder (Aquaculture Engineering) which delivered food to a very localised area in the tank. This dispensed 2% of the bodyweight of the fish per day at a trickle rate (5 minute intervals throughout the 12 hour light phase). This is termed the continuous feeding regime. From 15 December to 24 January the fish were given a meal feeding regime, being fed the same total daily amount but by hand, twice a day (once at 10am and once at 6pm), with food being spread uniformly across the water surface.

Feeding and digestion trials commenced at 0800h 13 November; food labelled with 1% (by weight) chromic oxide was delivered to the tank until 1400h November 15. At this point, food labelled with both 1% chromic oxide and 2% (by weight) x-ray opaque ballotini beads (Jencons Ltd.) was dispensed until the end of the 'day' (i.e. 2000h). Both forms of labelled food were delivered at the same rate and in the same manner as the normal food. Fish were then anaesthetised, weighed, measured and x-rayed (Todd Research 80mV portable X-ray Unit, using an exposure time of 3 seconds; Talbot & Higgins 1983). A sample of faeces was collected from each individual by stripping (Austreng 1978). Fish were observed to feed (apparently as normal) the following morning and so it was assumed that the procedure did not exert any lasting effects upon the fish. The above procedure was then repeated on 3, 10 and 14 December. During the meal feeding phase, chromic oxide labelled food was similarly dispensed by hand as per normal food from 2 days prior to sampling, with chromic oxide and ballotini labelled food introduced at the final meal before sampling.

Sampling under meal feeding conditions took place on 23 & 31 December and 13 & 24 January. Trials on 10 & 31 December and 24 January involved dispensing the ballotini-labelled food in the morning and sampling from 1400h. No faecal samples were collected on 14 December or 24 January.

Individual food intake rate (expressed as percentage of bodyweight consumed per day = I_f) was calculated from X-radiographs as described by Talbot & Higgins (1983), Thorpe *et al.* (1990) and McCarthy *et al.* (1993b). Percentage protein in the faeces and feed (P_{faeces} and P_{feed}) were estimated by assaying for the total nitrogen content of faecal and feed samples using an elemental analyser (Perkin-Elmer 2400). Percentage chromic oxide in faeces and food(C_{faeces} and C_{feed}) were measured using the acid digestion technique described by Furukawa & Tsukahara (1966).

The following formulae were used to derive parameters for use in data analysis:

Protein digestive efficiency [D_p(%)] =

$$100\text{-}(100 \times ((\%C_{feed}/\% \ C_{faeces}) \times (\%P_{faeces}/\% \ P_{feed})))$$

(Maynard & Loosli, 1969):

Relative protein intake rate [I_p(% bodyweight per day)] $= I_f \times D_p$

Coefficient of variation in food intake [CV_i] $= 100 \times$ (s.d./mean)]

where s.d. and mean are the standard deviation and mean of I_f.

Specific growth rate ($G = \%$ gain in weight per day) $= 100 \times$ [(lnW_f-lnW_i)/ t]

where W_f = final weight

W_i = initial weight

t = time elapsed in days

RESULTS

Table 1 shows the degree of individual variation in food intake rate, digestive efficiency and relative protein intake rate for continuous and meal feeding respectively.

Table 1. Means, standard errors, ranges and sample sizes of food intake rate (I_f), protein digestive efficiency (D_p) and relative protein intake rate (I_p) under continuous and meal feeding regimes.

variable	continuous feeding				meal feeding			
	mean	s.e.	range	n	mean	s.e.	range	n
I_f (%bodyweight.d^{-1})	0.76	0.052	0.32-1.38	25	0.79	0.076	0.29-1.76	24
D_p (%)	81	1.35	59-87	22	76	2.12	50-88	21
I_p (%bodyweight.d^{-1})	0.59	0.021	0.27-1.08	22	0.63	0.030	0.20-1.27	20

Paired t-tests showed a significant difference in protein digestive efficiency (t=2.8, n=20, P=0.012) between continuous and meal-fed regimes, with fish showing improved protein digestive efficiency under continuous feeding. Although there were also large individual differences in food intake rate and relative protein intake rate (see Table 1), paired t-tests showed no significant differences between the two regimes for these parameters (t=0.41, n=24, NS & t=0.20, n=19, NS respectively). There was a significant, positive relationship between mean food intake rates under the two regimes (Spearman's rank correlation, r_{sp}=0.420, n=24, P=0.041), indicating that fish that did well under continuous feeding also did well under a meal fed regime (see Fig. 1). Kendall's coefficient of concordance was highly significant for food intake rate under both continuous and meal feeding regimes (W_3=0.6932, n=25 P<0.0005 & W_3=0.4246, n=24, P<0.0005 respectively), indicating a tendency for individuals to maintain their rank in the 'feeding hierarchy' from day to day. There was no significant relationship, between mean food intake rate and coefficient of variation in food intake for the fish population, under either regime (r_{sp}=-0.1097, n=25, NS & r_{sp}=-0.3209, n=24, NS for continuous and meal feeding regimes respectively)
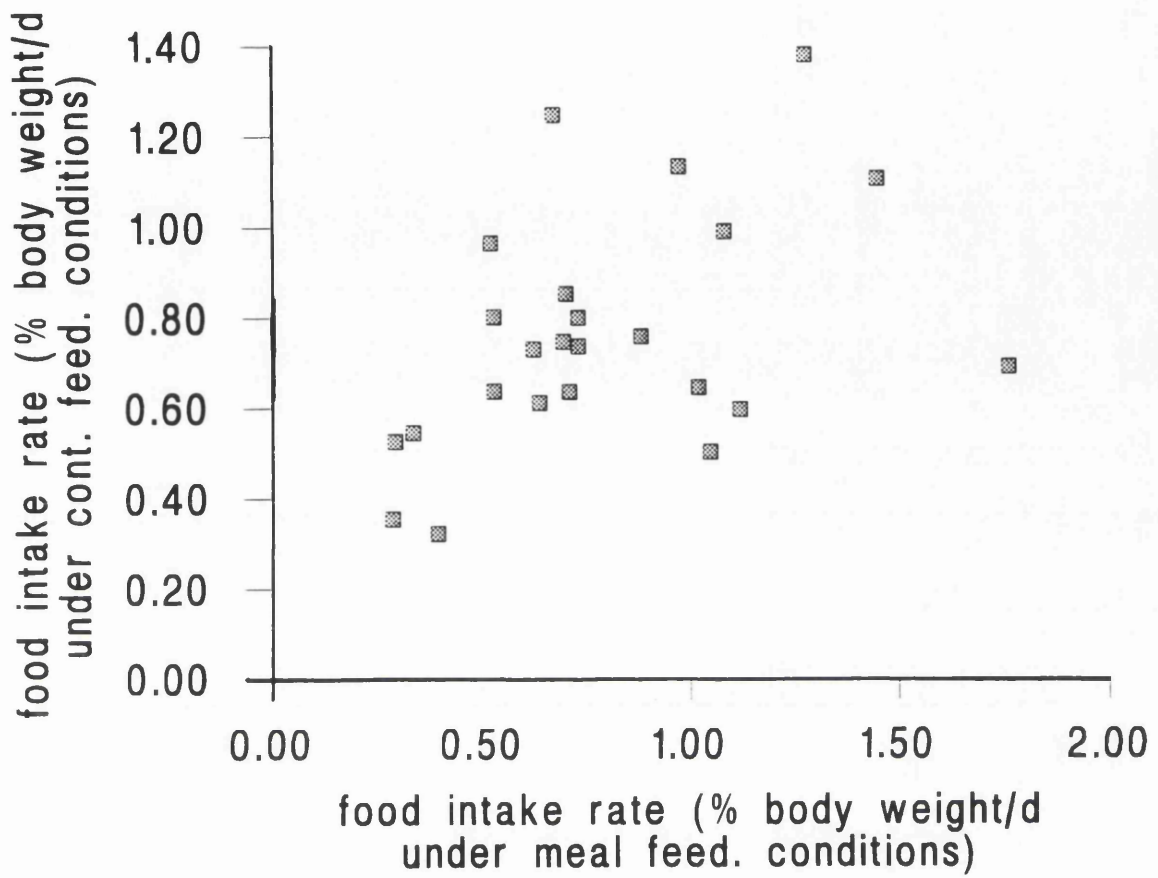
Fig. 1. The relationship between food intake rate (% body weight.d^{-1}) under continuous and meal feeding regimes.

Protein digestive efficiency was significantly negatively correlated with food intake rate for continuous feeding (r_{sp}=-0.473, n=22, P=0.026), but no such relationship was found under meal feeding conditions (r_{sp}=-0.053, n=20, NS). Hence fish that ate more under continuous feeding digested protein less efficiently, while digestion efficiency was independent of food intake rate for meal feeding (Fig. 2).

Specific growth rate was not significantly correlated with relative protein intake rate under meal feeding (r_{sp}=0.266, n=20, NS) but there was a significant positive correlation under continuous feeding (r_{sp}=0.461, n=22, P=0.031; see Fig. 3). Relative protein intake rate was positively correlated with body weight for meal feeding (r_{sp}=0.557, n=20, P=0.011) but the equivalent relationship, while positive, was not significant for continuous feeding (r_{sp}=0.382, n=22, NS; Fig. 4).
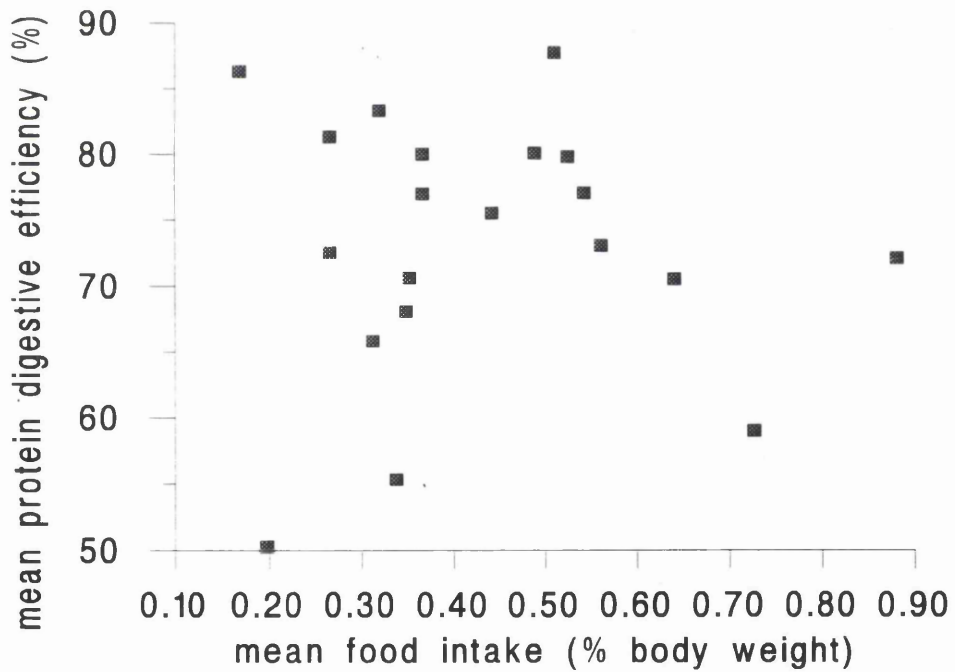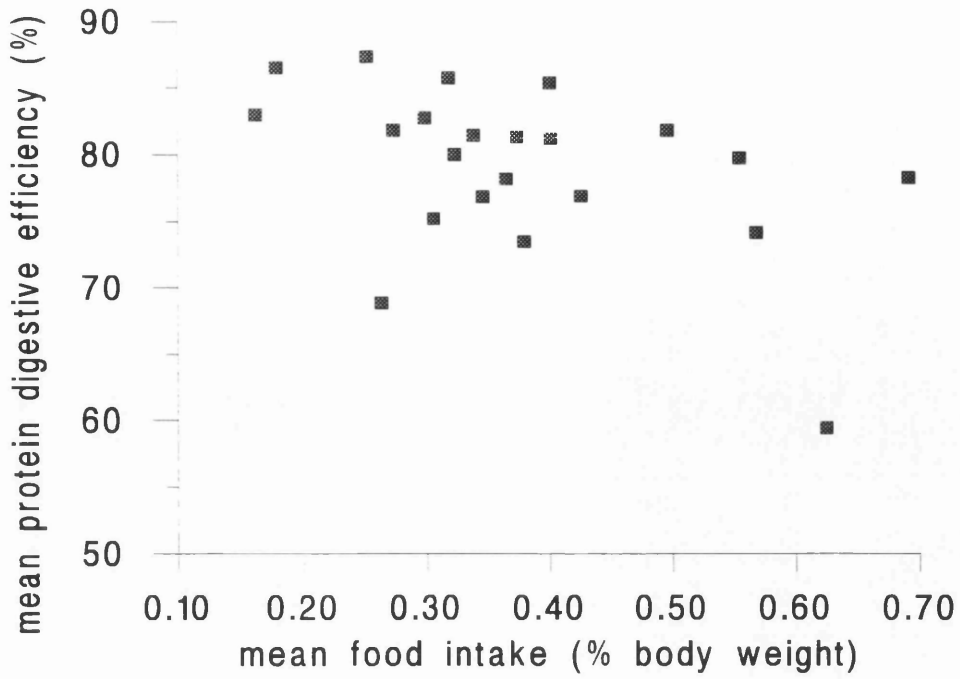
Fig. 2. The relationship between food intake rate (% body weight.d^{-1}) and protein digestion efficiency (%) under a) continuous feeding and b) meal feeding.
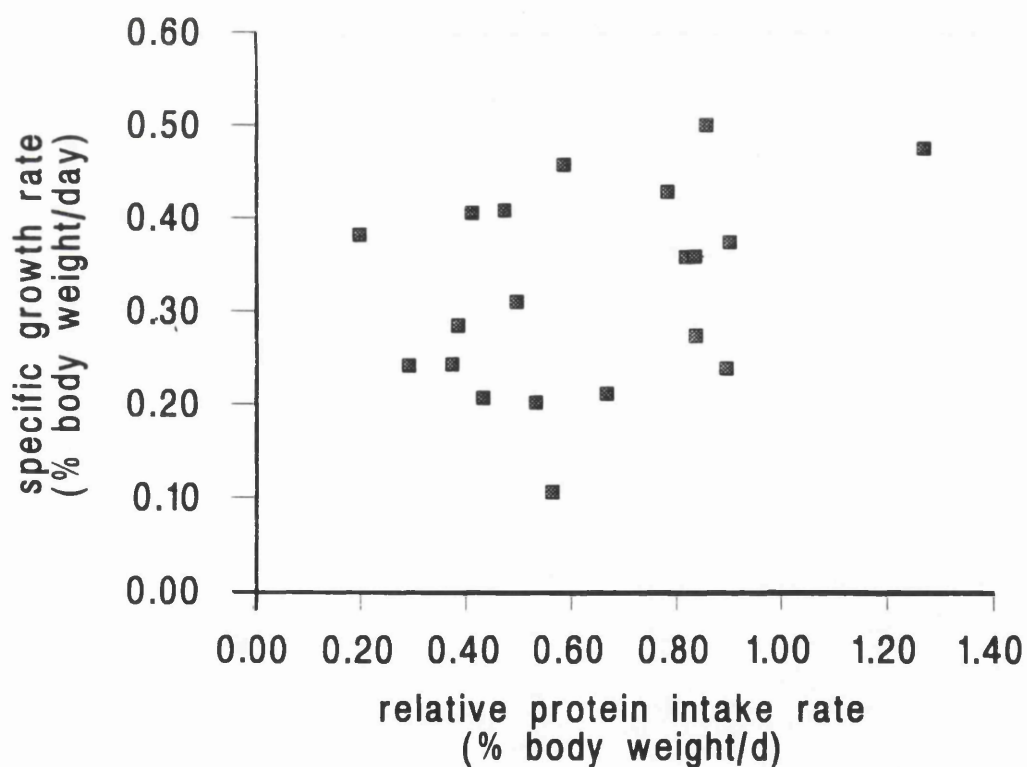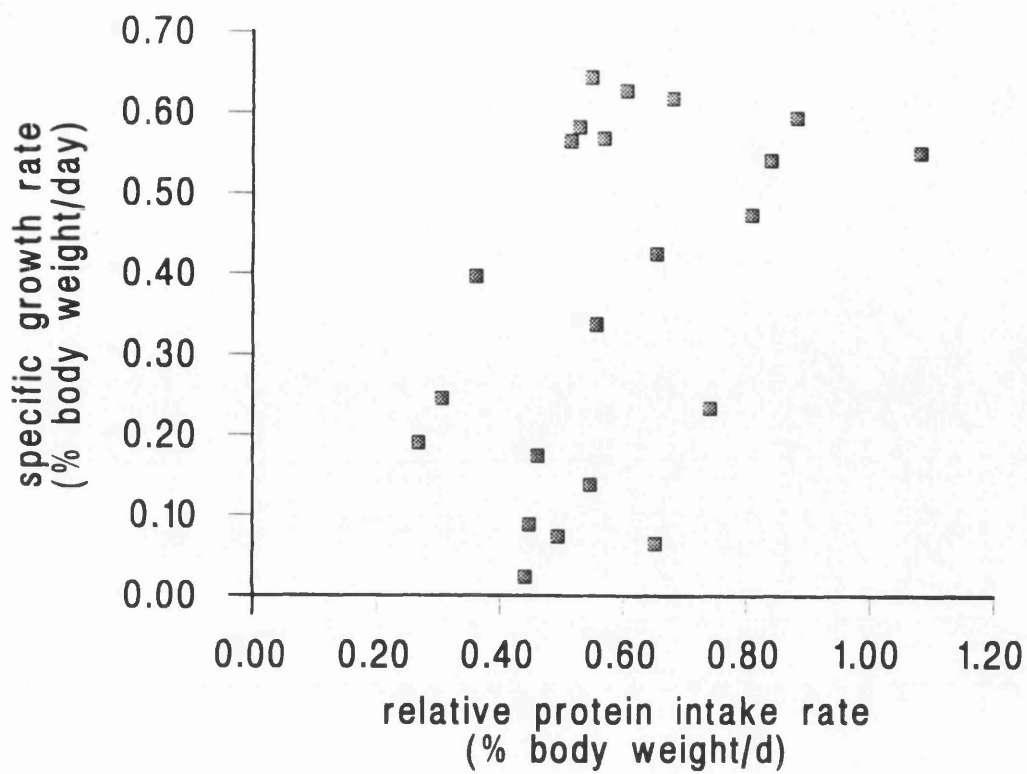
Fig. 3. The relationship between growth (% body weight.d^{-1}) and relative protein intake rate (% body weight.d^{-1}) under a) continuous feeding and b) meal feeding.
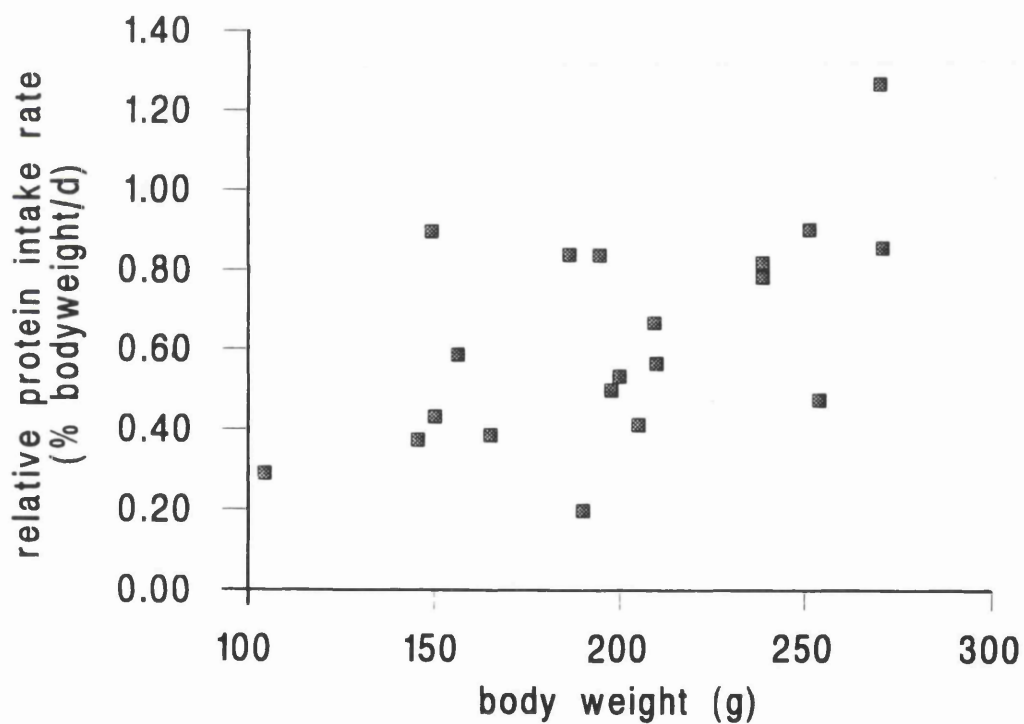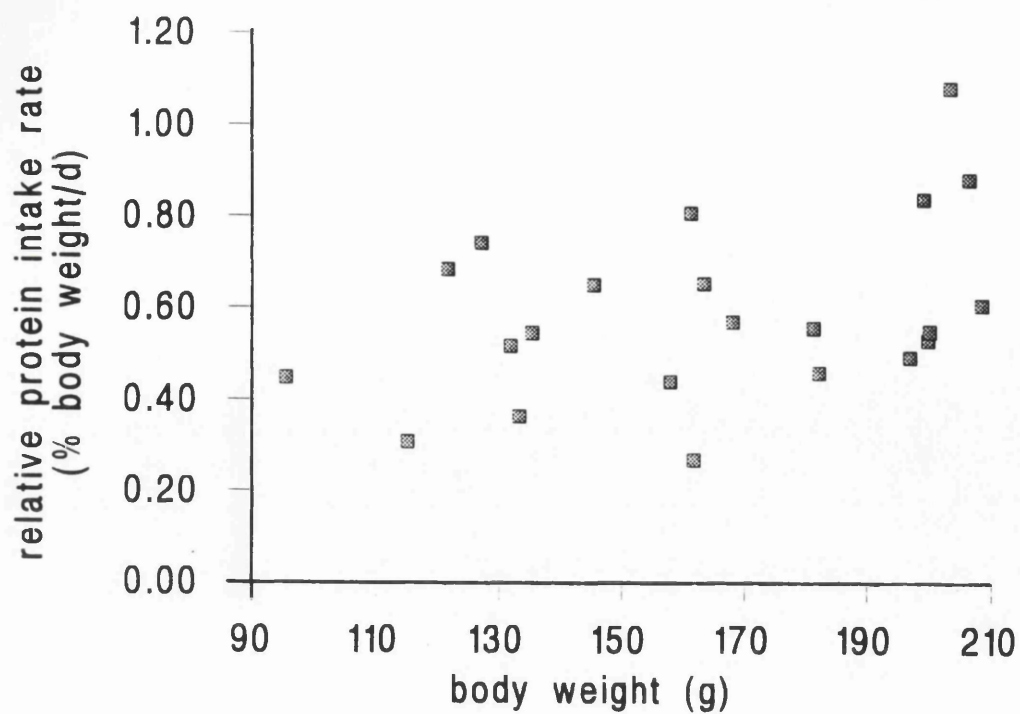
Fig. 4. The relationship between relative protein intake rate (% body weight.d^{-1}) and body weight (g) under a) continuous feeding and b) meal feeding.

DISCUSSION

The positive correlation between food intake rates for the two feeding regimes under scrutiny in the present study suggests that the 'feeding hierarchy' did not alter significantly after feeding regime was altered. However, the two regimes did produce different results with regard to some of the parameters measured (see below).

The stability of the feeding hierarchy indicated by Kendall's coefficient of concordance may explain the lack of any significant relationship between food intake rate and coefficient of variation in food intake. Hence coefficient of variation in food intake for individual fish could not be used as an indicator of 'feeding rank' (*sensu* McCarthy *et al.* 1993a).

Continuous feeding produced higher digestive efficiencies. This may be due to the relatively rapid shunting of food through the gut under meal feeding conditions when fish must feed quickly, reaching satiation in a short time. Food presentation and feeding rate are both known to influence the rate at which food moves through the alimentary canal (De Silva & Andersen 1995), which will in turn affect time of exposure to digestive processes (Jobling 1994) and therefore, presumably digestive efficiency.

Continuous feeding produced a negative relationship between food intake rate and digestive efficiency but no such relationship was found for meal feeding. Several previous studies have demonstrated a decrease in digestive efficiency as feeding level is increased (e.g. Elliot 1976, From & Rasmussen 1984, Henken *et al.* 1985), although such a relationship was not observed in all studies (e.g. Birkett 1969, Kelso 1972, Cui & Wootton 1988). The lack of a relationship observed under meal feeding conditions may be the result of gastric emptying being prolonged, i.e. ingested food is not pushed through the gut by further ingestion, resulting in digestive efficiency being independent of food intake.

Only during continuous feeding was there a positive relationship between food intake rate and growth. Jobling *et al.* (1989) suggested that food intake should be monitored on several occasions over the course of a long-term growth study 'if the expected relationship between food consumption and growth is to be revealed'. It may be that the second stage (meal feeding) of the experiment (although longer than

the first stage) was not long enough to allow accurate estimates of food intake and/or growth, precluding the emergence of a positive relationship between the two.

Under the meal feeding regime there was a positive relationship between relative protein intake rate and body weight, with a weaker relationship between these two parameters under continuous feeding, suggesting a more pronounced feeding hierarchy under meal feeding conditions. This may be because all fish are hungry whenever food is delivered under meal feeding, and hence dominant fish could be expected to feed aggressively at these times. Continuous feeding would however, allow subordinates to feed out of phase with dominants (see II & IV).

Although the present study was carried out on a small scale and over a short term, the data presented above are valuable in providing an initial insight into the causes of individual variability in digestive efficiency.

REFERENCES

Abbott, J.C. and Dill, M. (1989) The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. *Behaviour* **108**, 104-113.

Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastro-intestinal tract. *Aquaculture* **13**, 265-272.

Birkett, L. (1969) The nitrogen balance of plaice, sole and perch. *J. Exp. Biol.* **50**, 375-386.

Brett, J.R., Shelbourn, J.E. & Shoop, C.T. (1969) Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *J. Fish. Res. Bd. Can.* **26**, 2363-2394.

Carter, C.G., Houlihan, D.F., Buchanan, B. & Mitchell, A.I. (1993) Protein-nitrogen flux and protein growth efficiency of individual Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem.* **12**, 300-315.

Cui, Y. & Wooton, R.J. (1988) Bioenergetics of growth of a cyprinid, *Phoxinus phoxinus*: the effect of ration, temperature and body size on food consumption, faecal production and nitrogenous excretion. *J. Fish Biol.* **33**, 431-443.

De Silva, S.S. (1989) Digestibility estimations of natural and artificial diets. In: S.S. De Silva (ed.) *Finfish Nutrition Research in Asia.* Asian Fisheries Society, Manila.

De Silva, S.S. & Anderson, T.A. (1995) *Fish Nutrition in Aquaculture.* Chapman and Hall, London.

De Silva, S.S. and Perera, M.K. (1983) Digestibility of an aquatic macrophyte by the cichlid *Etroplus suratensis* with observations on the relative merits of three

indigenous components as markers and daily changes in protein digestibility. *J. Fish Biol.* **23,** 675-684.

Elliot, J.M. (1976) Energy losses in the waste products of brown trout (*Salmo trutta* L.). *J. Anim. Ecol.* **45,** 561-580.

From, J. & Rasmussen, G. (1984) A growth model, gastric evacuation, and body composition in rainbow trout, *Salmo gairdneri* Richardson, 1836. *Dana* **3,** 61-139.

Furukawa, A. & Tsukahara, H. (1966) On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bull. Jpn .Soc. Sci. Fish.* **32,** 502-506.

Henken, A.M., Kleingeld, D.W. & Tijssen, P.A.T. (1985) The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish (*Clarias gariepinus*) (Burchell, 1822). *Aquaculture* **51,** 1-11.

Hepher, B. (1988) *Nutrition of Pond Fishes.* Cambridge University Press.

Jobling, M. (1994) *Fish Bioenergetics.* Chapman and Hall, London.

Jobling, M., Baardvik, B.M. & Jorgensen, E.H. (1989) Investigation of food-growth relationships of Arcticcharr *Salvelinus alpinus* L. using x-radiography. *Aquaculture* **81,** 367-372.

Kelso, J.R.M. (1972) Conversion, maintenance, and assimilation for walleye, *Stizostedion vitreum vitreum*, as affected by size, diet and temperature. *J. Fish. Res. Bd. Can.* **29,** 1181-1192.

Maynard, L.A. & Loosli, J.K. (1969) *Animal Nutrition.* 6th ed., McGraw-Hill, New York.

McCarthy, I.D., Carter, C.G., and Houlihan, D.F., (1993a) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol.* **41**, 239-256.

McCarthy, I.D., Houlihan, D.F., Carter, C.G. & Mouton, K. (1993b) Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. *Proc. Nutr. Soc.* **52**, 427-436.

Stradmeyer, L. (1994) Survival, growth and feeding of Atlantic salmon, *Salmo salar* L., smolts after transfer to sea water in relation to the failed smolt syndrome. *Aquacult. Fish. Manage.* **25**, 103-112.

Talbot, C. & Higgins, P.J. (1983) A radiographic method for feeding studies on fish using metallic iron powder as a marker. *J. Fish Biol.* **23**, 211-220.

Thorpe, J.E., Talbot, C., Miles, M.S., Rawlings, C. and Keay, D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **90**, 41-47.

Usher, M.L., Talbot, C. & Eddy, F.B. (1991) Effect of transfer to sea water on growth and feeding in Atlantic salmon smolts. *Aquaculture* **94**, 309-326.

Windell, J.T., Foltz, J.W. & Sarokon, J.A. (1978) Effect of fish size, temperature and amount fed on nutrient digestibility of a pelleted diet by rainbow trout *Salmo gairdneri*. *Trans. Am. Fish. Soc.* **107**, 613-616.

CHAPTER IV

SOCIAL INTERACTIONS AND THE DISTRIBUTION OF FOOD AMONG ONE SEA-WINTER ATLANTIC SALMON IN A SEA-CAGE.

INTRODUCTION

Two primary goals of aquaculture are to maximise production efficiency and, in the process, to produce animals of more-or-less uniform size (Noakes & Grant 1992). For fish in intensive husbandry systems, social interactions, and in particular aggressive behaviour, can seriously interfere with the achievement of these goals. Growth depensation, in which large size differences develop among fish that initially were similar in size, has been reported for a number of commercially-important species of fish e.g. rainbow trout, *Oncorhynchus mykiss* (Li & Brocksen 1977, Metcalfe 1986), coho salmon, *Oncorhynchus kitsutch* (Fagerlund *et al.* 1981), cichlids, *Tilapia zillii* (Koebele 1985), Atlantic salmon, *Salmo salar* (Metcalfe *et al.* 1990) and Arctic charr, *Salvelinus alpinus* (Jobling *et al.* 1993). This differential growth depends to a large extent on aggressive interactions among the fish, resulting in certain dominant fish consuming the bulk of the available food (Fernö & Holm 1986, Jobling & Reinsnes 1986, Carter *et al.* 1992; McCarthy *et al.* 1992; Jobling & Baardvik 1994).

Behaviour patterns that enable individuals to monopolise limited resources are likely to promote fitness (Fausch 1984, Metcalfe 1986, Grant 1993, Nakano 1995), so the mechanisms that enable fish to compete fiercely may have been strongly favoured by natural selection. These mechanisms may, therefore, be difficult to eliminate (Ryer & Olla 1995), but if the behavioural mechanisms that generate differential access to resources are understood, it might be possible to circumvent them (Davis & Olla 1987, Huntingford *et al.* 1990, Noakes & Grant 1992).

It might be assumed that by maintaining fish at high density individuals would be prevented from defending specific areas (Kalleberg 1958, Fenderson & Carpenter 1971, Grant, 1993). While this is generally true (e.g. Reftsie & Kittlesen 1976, Noakes & Grant 1992, Jørgensen *et al.* 1993), Fernö & Holm (1986) found that (at approximately 10kg.m^{-3}) a few dominant Atlantic salmon parr could defend certain areas of a tank and thus, possibly, gain preferential access to any food that arrived in these areas. Even when territoriality is not apparent, the fact that fish differ in ability to compete for resources may result in differences in growth rate (Metcalfe *et al.* 1989, Metcalfe 1991), and some fish may be inhibited from feeding by the presence of dominant individuals (Koebele 1985, Huntingford *et al.* 1993). To evaluate the extent

of these effects, it is necessary to monitor the behavioural interactions and food intake of individual fish over a number of feeding sessions. Gathering of behavioural data at an individual level requires that fish are stocked at densities much lower than those usually found in commercial operations. In this chapter I report on the feeding success of individually-marked, one-sea-winter Atlantic salmon monitored by underwater video in a sea cage. I test whether the amount of food obtained by each fish was related to social status and ability to compete.

MATERIAL AND METHODS

Nineteen one-sea-winter Atlantic salmon (from a population of mean weight ca. 1.1 kg) were obtained from Stirling Aquatech Ltd, Oban, West Scotland in May 1989. The fish were immobilised by benzocaine anaesthesia and marked with simple coded tags with different patterns of reflective plastic. This allowed the fish to be recognised individually when viewed by a video camera from above. The fish were then housed in a small (4 x 4 x 4m) experimental sea cage at Dunstaffnage Bay, Oban and fed to excess from an automatic feeder that delivered standard commercial pellets throughout the hours of daylight. The fish were maintained under these conditions for several weeks prior to experimental trials.

Prior to behavioural screening the feeder was turned off and the fish were deprived of food for 12 hours (overnight). Behavioural screening involved throwing one food pellet by hand into the water (at the point where the feeder had been delivering pellets) every 30 seconds until feeding ceased (i.e. person delivering pellets observed no response by the fish to 3 consecutive pellets). During the feeding session, the fish were filmed by means of an underwater camera (C-Technics, Oban) sited just below the surface of the water and pointing downwards. This was connected by a cable to a Sony Video Walkman; a microphone allowed commentary to be recorded onto the video tapes.

The number of pellets presented during individual trials ranged from 102-222, so the video recording per trial ranged from 51 to 111 minutes. Slow motion analysis of the resulting videos (played back on a monitor) allowed me to record, on most occasions, the identity of the fish that took the pellet and the occurrence and outcome of any interactions between fish. Interactions were defined as follows:

1) Avoidance - fish approaching a food pellet turns away to avoid a competitor approaching the same pellet.

2) Beaten - fish attempting to catch a sinking pellet is beaten to it by a competitor without any sign of avoidance.

Five trials were performed on the same group of fish between July 10 and July 20, 1989 inclusive. At the end of the experiment all fish were killed, weighed, measured, dissected and gonads examined in order to assign gender and reproductive status. As fish were not weighed at the start of the trial, all body weight data refer to final

weight; the short duration of the experiment ensured that weight changes would have been minimal.

Data analyses

Relative food intake was calculated by expressing absolute food intake (i.e. total number of pellets eaten by an individual) as a percentage of bodyweight. The significance of correlations between food intake (both relative and absolute) and other parameters (see below) was tested using Spearman's rank correlation. Gender differences in food intake were tested for using Mann Whitney's U-test.

Average rank of first pellet eaten was calculated for each individual by ranking pellets in order presented for each trial and in each case establishing the rank of the first pellet eaten by a given individual. These ranks were then averaged over the five trials for each individual to give an indication of feeding order among fish in the group.

Coefficient of variation in daily food intake was calculated as:

100 x (s.d./mean) of absolute food intake over the 5 trials.

RESULTS

The identities of fish involved in each feeding bout (capturing and attempting to capture pellets) were successfully recorded 92.5% of the time, the remaining pellets either being outside of the field of view of the camera or having been obscured by other fish. Amongst the pellets where fish identities were recorded, 92.5% were eaten.

Only one fish (female), the largest, was found to be maturing; it made no feeding attempts throughout the trials. This fish was excluded from the analyses. A further four fish that made fewer than four feeding attempts throughout the trials were also excluded as they were not considered part of the feeding population.

In terms of the number of pellets eaten, food was distributed very unevenly among the remaining fish (n=14, Fig. 1). There was no significant relationship between number of pellets eaten and either final weight (r_{sp}=0.41, n=14, NS) or gender (U=22, n=6 males and 8 females, NS) of fish. Relative food intake was also unrelated to either of these variables (r_{sp}=-0.06, NS and U=18, NS, respectively).

The most successful fish (i.e. those obtaining the greatest amount of food as a percentage of body weight) were usually among the first to feed in any feeding session, with their companions either obtaining food later, or failing to feed within a given session (Fig. 2). Although overt aggression was observed on very few occasions, a number of fish often failed to obtain a pellet as they avoided their, presumably dominant, companions. These fish tended to be the smallest individuals in the population (Fig. 3). The reduced food intake amongst these fish was therefore due to intimidation and not to lack of appetite.

Fish were often beaten to pellets by competing individuals, and the fish that ate most were beaten most often (Fig. 4a). However, there was no significant relationship between food intake and percentage of feeding attempts in which a particular fish was beaten to a pellet (Fig. 4b), suggesting that there was often a scramble for food amongst fish.

There was a significant negative relationship between the total relative food intake of a fish and the coefficient of variation in daily food intake, showing that fish that consumed the greatest proportion of the food supply had less day to day variation in their daily consumption than did fish that consumed less (Fig. 5).
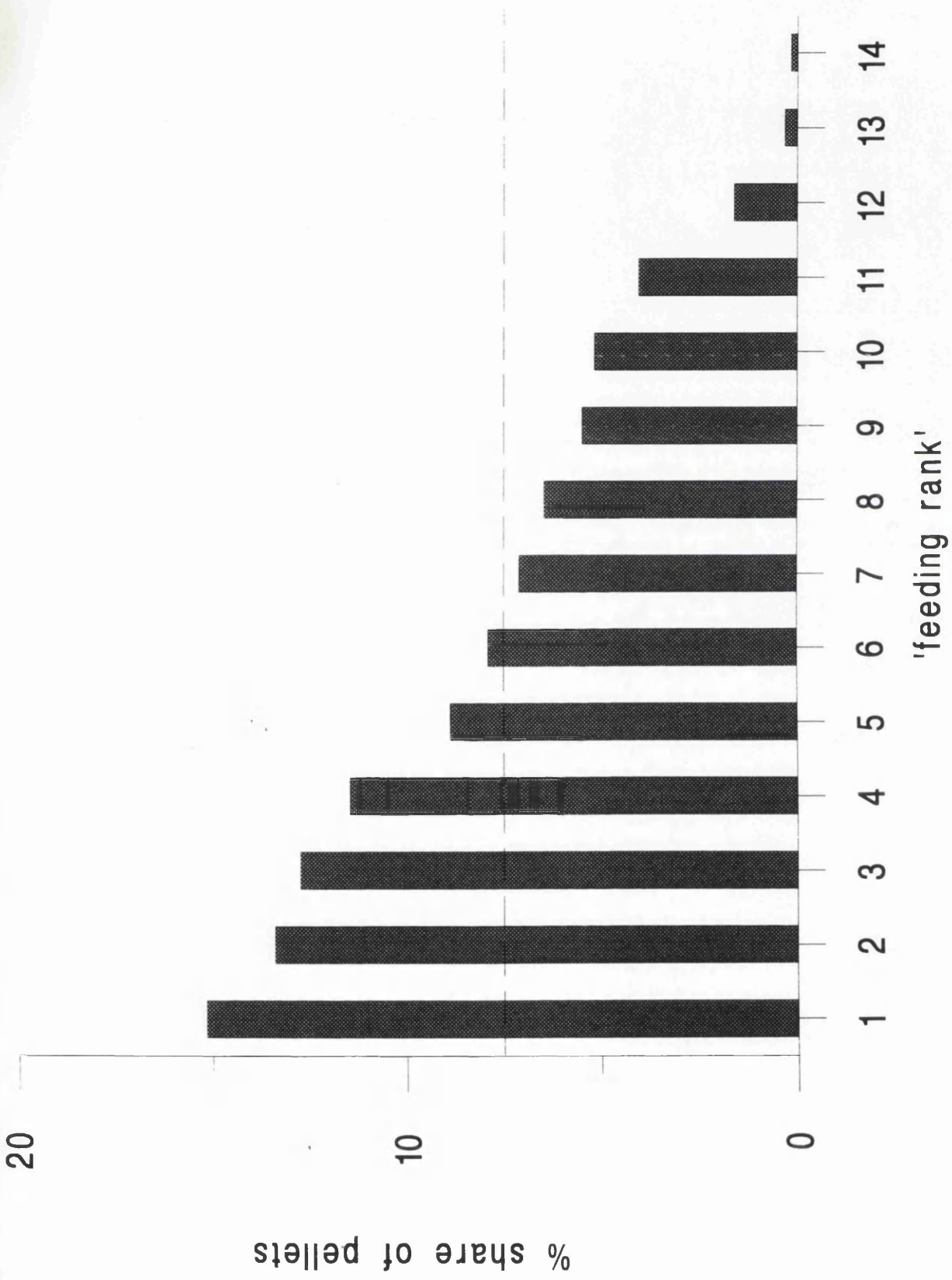
Fig. 1. Percentage share of food consumed by individual fish. Dotted line represents level of food acquisition if shared equally among individuals.
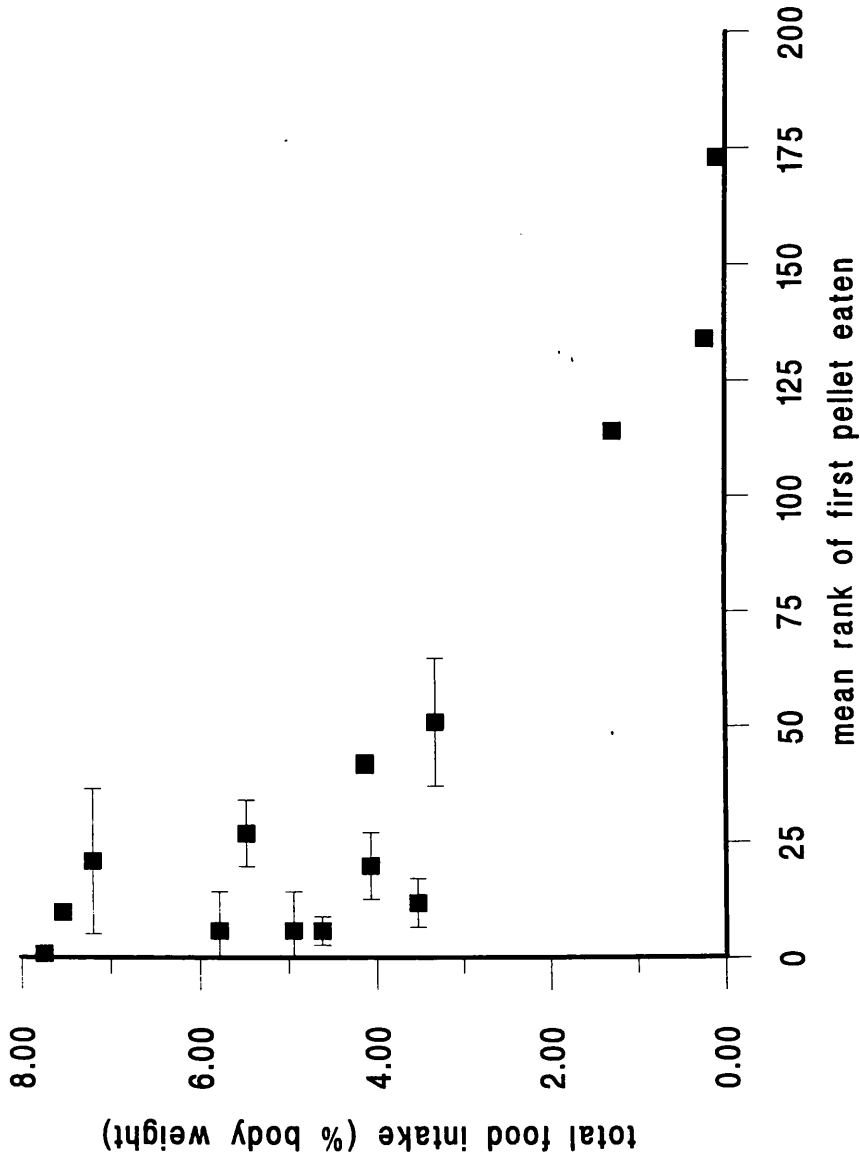
Fig. 2. The relationship between total relative food intake (% body weight) and the average rank of the first pellet eaten (mean ± s.e.) per trial by individual salmon (r_{sp}=-0.78, n=14, P=0.001).
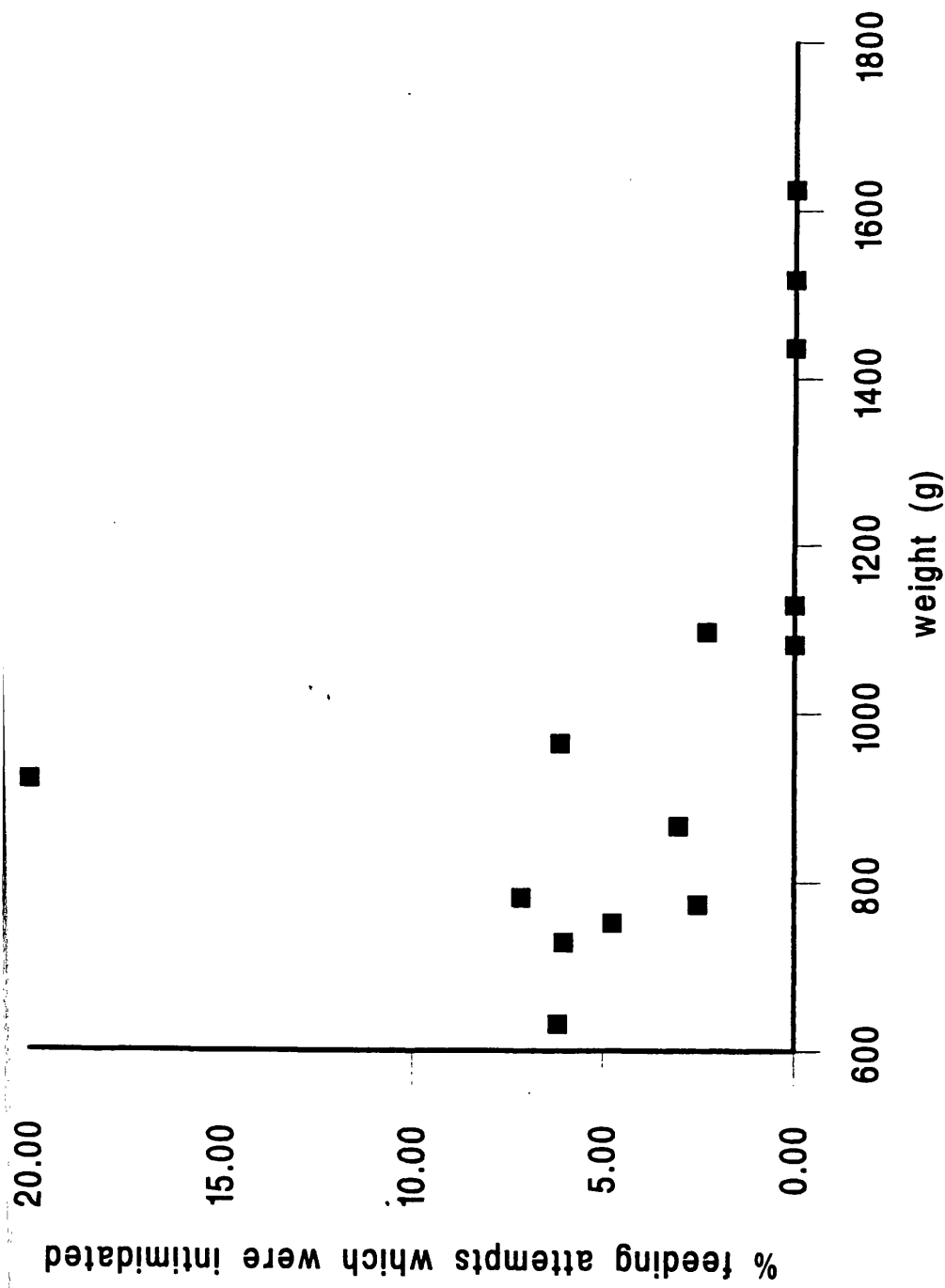
Fig. 3. The relationship between the percentage of feeding attempts which resulted in avoidance of a competitor and body weight for each individual (r_{sp}=-0.74, n=14, P=0.003).

70
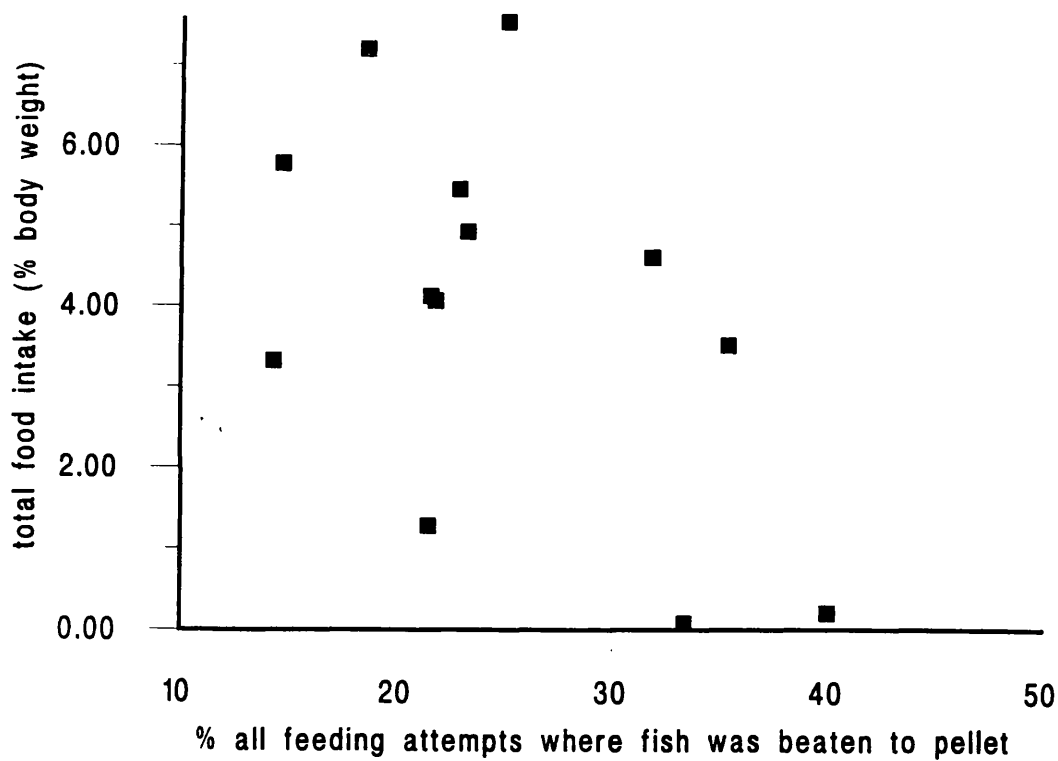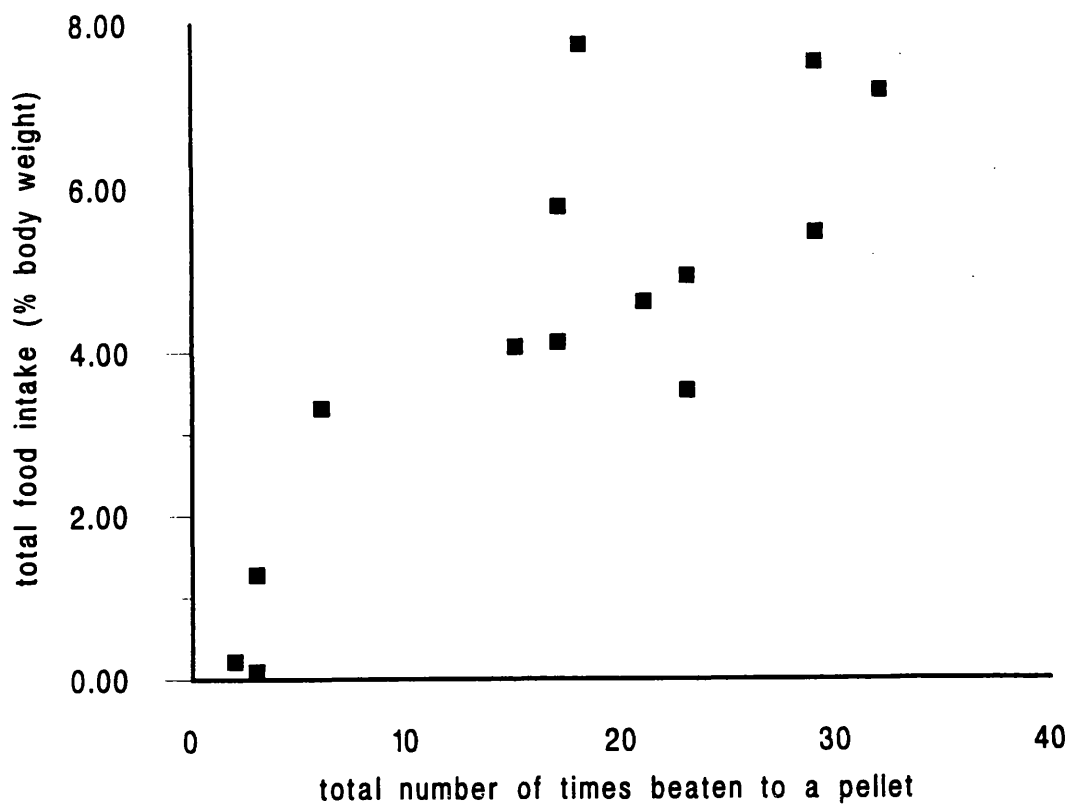
Fig. 4. The relationships between a) total relative food intake (% body weight) and the total number of times an individual was beaten to a pellet (r_{sp}=0.77, n=14, P=0.001) and b) total relative food intake and the percentage of all feeding attempts where an individual was beaten to a pellet (r_{sp}=-0.27, n=14, NS).
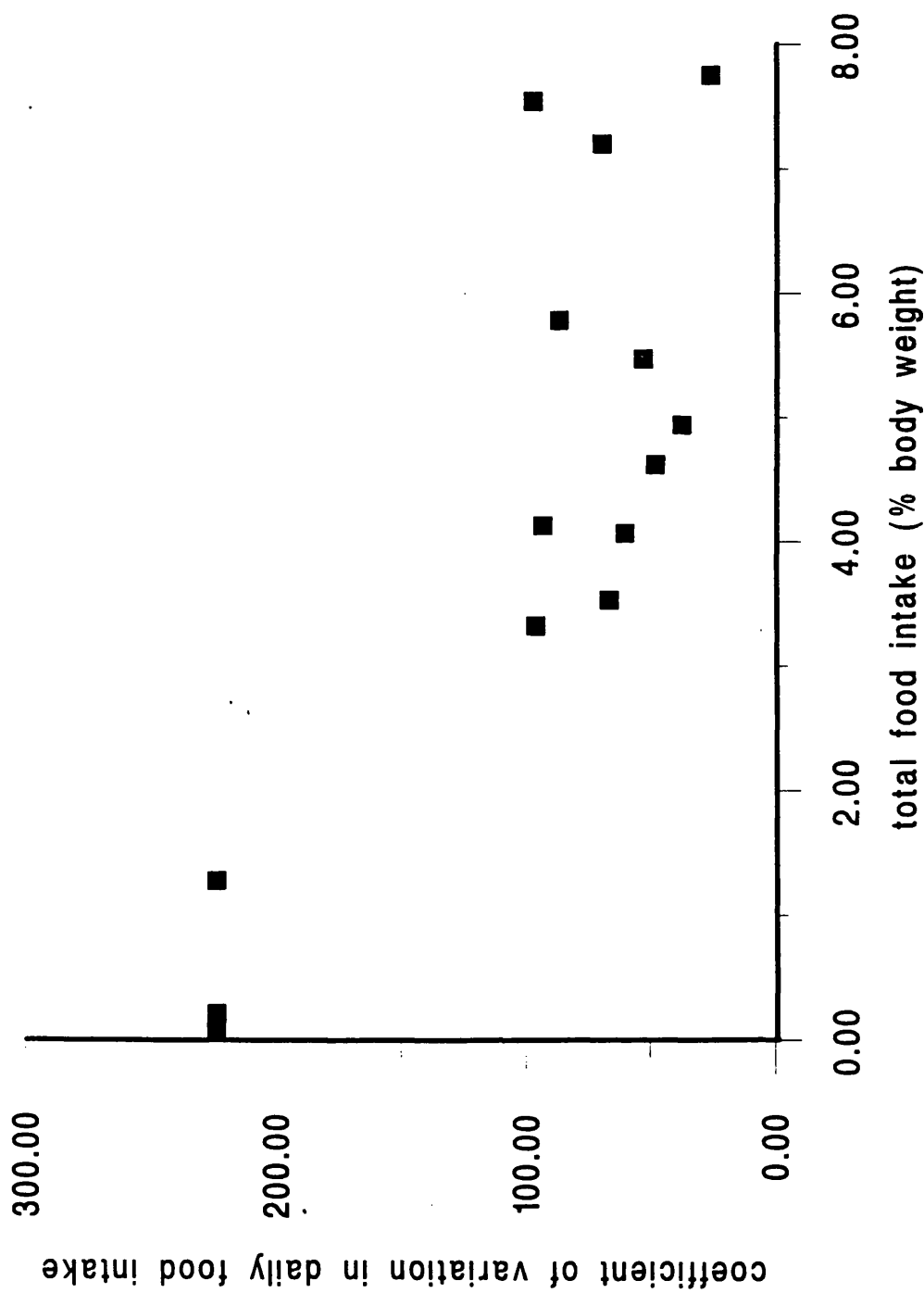
Fig. 5. The relationship between coefficient of variation in food intake per trial and total relative food intake (% body weight) (r_{sp}=-0.60, n=14, P=0.022).

DISCUSSION

In this study I used direct observation of feeding interactions among salmon in a sea cage to obtain information about the mechanisms underlying differential food acquisition. Food was presented in such a way that it could be monopolised, as it was predictably distributed in time and clumped in space (Magnuson 1962, Grant & Kramer 1992). As a consequence, there were marked differences in food acquisition among individual fish (Fig. 1), that were unrelated to size or gender. Huntingford *et al.* (1990) found that size was a poor predictor of status (assessed by monopolisation of food and intimidation of siblings) in Atlantic salmon parr. These findings for Atlantic salmon contrast with results reported for other salmonids [e.g. Jenkins (1969) working with rainbow trout, *Oncorhynchus mykiss*, and brown trout *Salmo trutta*, and Abbott & Dill (1989) working with steelhead, *O. mykiss*].

There was evidence of sequential feeding, with the fish that were most successful overall obtaining food earliest in a given feeding bout (Fig. 2). The observed differential success among individuals appeared to be due to intimidation of subordinates by dominants, rather than to overt aggression. The most successful fish fed first, very near the water surface, with many pellets being contested by several individuals. Other fish were observed to capture pellets in the middle of the cage, once the pellets had passed through the feeding area of the first group of fish. Pellets in the middle of the cage were sometimes contested and most of the avoidance which took place seemed to occur among fish that fed in this area of the cage. A few fish adopted a third strategy of feeding very close to or directly from the bottom of the cage (pers. obs.). Metcalfe (1986) suggested that the optimum strategy for a subordinate rainbow trout may sometimes be to minimise energy expenditure rather than to maximise food intake. This strategy may have been adopted by the most subordinate Atlantic salmon in the present study, in that they fed on pellets which both passed close to them and could be intercepted without incurring competition. While salmonid parr (which are territorial) exhibit a wide range of aggressive behaviours when competing for food (from displays to fights) (Keenleyside & Yamamoto 1962, Bachman 1984), at smolting, salmon tend to aggregate and appear to be less aggressive. Among the actively feeding fish, there was often a scramble for food, such that even relatively successful fish were often beaten to food items (Fig. 4).

Their feeding behaviour was therefore more similar to that of shoaling species (Ryer & Olla 1991, Metcalfe & Thomson 1995).

The negative correlation observed between relative food intake and coefficient of variation in daily food intake (Fig. 5) reflects the unpredictable and erratic day-to-day rate of food consumption by subordinates in the population. Similar relationships were described by Carter *et al.* (1992) in grass carp (*Ctenopharyngodon idella*), Jobling *et al.* (1993), Jobling & Baardvik (1994) in Arctic charr and McCarthy *et al.* (1992) in rainbow trout. All of these authors assumed that a significant negative relationship between proportions of food supply consumed by given individuals and coefficients of variation in daily food intake for the same fish would indicate a strong feeding hierarchy. However, none corrected mean food intake for body size and so failed to give an indication of variation in relative daily consumption in relation to competitive ability. The present study, however, confirms that these previous findings do hold for relative food intake.

In the course of the present trials, feed was delivered from a point source at thirty second intervals. Hopper feeding systems on fish farms often deliver food in a similar way, with food dispensed at the middle of a cage at regular intervals throughout the daylight hours. Research on cichlids, *Cichlasoma nigrofasciatum*, has shown that dominant fish become more aggressive, more sedentary and monopolise the food when resources are predictable (Grand & Grant 1994). Thus if the food presentation was made less predictable in time and space, it would become more difficult for dominant individuals to monopolise. For example, if fish in a cage are fed by hand, with food being spread evenly over the water surface in a few discrete meals per day, there is a much more even distribution of food among individuals than if the same amount of food is delivered automatically by a hopper dispensing food regularly at a single point (Thorpe *et al.* 1990). Methods of food presentation which reduce the advantages of social dominance and ensure relatively even feeding opportunities for all individuals in a population should, therefore, diminish growth depensation amongst cultured fish and the development of such feeding methods should be given priority (Thorpe & Cho in press).

REFERENCES

Abbott, J.C. & Dill, M. (1989) The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. *Behaviour* **108,** 104-113.

Bachman, R.A., (1984) Foraging behaviour of free-ranging wild and hatchery brown trout in a stream. *Trans. Am. Fish. Soc.* **113,** 1-32.

Carter, C.G., Houlihan, D.F., McCarthy, I.D. & Brafield, A.E. (1992) Variation in the food intake of grass carp, *Ctenopharyngodon idella* (Val.), fed singly or in groups. *Aquat. Living. Res.* **5,** 225-228.

Davis, M.W. & Olla, B.L. (1987) Aggression and variation in growth of chum salmon (*Oncorhynchus keta*) juveniles in seawater: effect of limited rations. *Can. J. Fish. Aquat. Sci.* **44,**192-197.

Fagerlund, U.H.M., McBride, J.R., & Stone, E.T. (1981) Stress-related effects of hatchery rearing density on coho salmon. *Trans. Am. Fish. Soc.* **110,** 644-649.

Fausch, K.D. (1984) Profitable stream positions for salmonids: relating specific growth rate to net energy gain. *Can. J. Zool.* **62,** 441-451.

Fenderson, O.C. & Carpenter, M.R. (1971) Effects of crowding on the behaviour of juvenile hatchery and wild landlocked Atlantic salmon (*Salmo salar* L.). *Anim. Behav.* **19,** 439-447.

Fernö, A. & Holm, M. (1986) Aggression and growth of Atlantic salmon parr. I. Different stocking densities and size groups. *FiskDir. Ser. HavUnders.* **18,** 113-122.

Grand, T.C. & Grant, J.W.A. (1994) Spatial predictability of food influences its monopolisation and defence by juvenile convict cichlids. *Anim. Behav.* **47,** 91-100.

Grant, J.W.A. (1993) Whether or not to defend? The influence of resource distribution. *Mar. Behav. Physiol.* **23,** 137-153.

Grant, J.W.A. & Kramer, D.L. (1992) Temporal clumping of food arrival reduces its monopolization and defence by zebrafish, *Brachydanio rerio*. *Anim. Behav.* **44,** 101-110.

Huntingford, F.A., Metcalfe, N.B. & Thorpe, J.E. (1993) Social status and feeding in Atlantic salmon *Salmo salar* parr: the effect of visual exposure to a dominant. *Ethology* **94,** 201-206.

Huntingford, F.A., Metcalfe, N.B., Thorpe J.E., Graham W.E. & Adams C.E. (1990) Social dominance and body size in Atlantic salmon parr, *Salmo salar* L. *J. Fish Biol.* **36,** 877-881.

Jenkins, T.M. (1969) Social structure, position choice and microdistribution of two trout species (*Salmo trutta* and *Salmo gairdneri*) resident in mountain streams. *Anim. Behav. Monogr.* **2,** 57-123.

Jobling, M. & Baardvik, B.M. (1994) The influence of environmental manipulations on inter- and intra-individual variation in food acquisition and growth performance of Arctic charr, *Salvelinus alpinus*. *J. Fish Biol.* **44,** 1069-1087.

Jobling, M. & Reinsnes, T.G. (1986. Physiological and social constraints on growth of Arctic charr *Salvelinus alpinus* L.: an investigation of factors leading to stunting. *J. Fish Biol.* **28,** 379-384

Jobling, M., Jørgensen, E.H., Arnesen, A.M. & Ringø, E. (1993) Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. *Aquacult. Int.* **1,** 20-47

Jørgensen, E.H., Christiansen, J.S. & Jobling, M. (1993) Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). *Aquaculture* **110,** 191-204.

Kalleberg, H. (1958) Observations in a stream tank of territoriality and competition in juvenile salmon and trout, (*Salmo salar* L. and *S. trutta* L.). *Rep. Inst. Freshw. Res. Drott.* **39,** 55-98.

Keenleyside, M.H.A., & Yamamoto, F.T. (1962) Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). *Behaviour,* **19,** 138-169.

Koebele, B.P. (1985) Growth and the size hierarchy effect: an experimental assessment of three proposed mechanisms; activity differences, disproportional food acquisition, physiological stress. *Env. Biol. Fish.* **12,** 181-188.

Li, H.W. & Brocksen, R.W. (1977) Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* **11,** 329-341.

McCarthy, I.D., Carter, C.G., & Houlihan, D.F. (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum) *J. Fish Biol.* **41,** 239-256.

Magnuson, J.J. (1962) An analysis of aggressive behaviour, growth, and competition for food and space in medaka (*Oryzias latipes* (Pisces, Cyprinodontidae)). *Can. J. Zool.* **40,** 313-363.

Metcalfe, N.B. (1986) Intraspecific variation in competitive ability and food intake in salmonids: consequences for energy budgets and growth rates. *J. Fish Biol.* **28,** 525-532.

Metcalfe, N.B. (1991) Competitive ability influences seaward migration age in Atlantic salmon. *Can. J. Zool.* **69,** 815-817.

Metcalfe, N.B., Huntingford, F.A., Graham, W.D. & Thorpe, J.E. (1989) Early social status and the development of life history strategies in Atlantic salmon. *Proc. Roy. Soc. Lond.* B. **236**, 7-19.

Metcalfe, N.B., Huntingford, F.A., Thorpe, J.E. & Adams, C.E. (1990) The effects of social status on life-history variation in juvenile salmon. *Can. J. Zool.* **68**, 2630-2636.

Metcalfe, N.B. & Thomson, B.C. (1995) Fish recognize and prefer to shoal with poor competitors. *Proc. Roy. Soc. Lond.* B. **259**, 207-210

Nakano, S. (1995) Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *J. Anim. Ecol.* **64**, 75-84.

Noakes, D.L.G. & Grant, J.W. (1992) Feeding and social behavior of brook and lake charr. In: J.E. Thorpe & F.A. Huntingford (eds.), The importance of feeding behavior for the efficient culture of salmonid fishes. World Aquaculture Society, Baton Rouge, pp. 13-20.

Reftsie, T. & Kittlesen, A. (1976) Effect of density on growth and survival of artificially reared Atlantic salmon. *Aquaculture* **8**, 319-326.

Ryer, C.H. & Olla, B.L. (1991) Agonistic behaviour in schooling fish - form, function and ontogeny. *Env. Biol. Fish.* **31**, 355-365.

Ryer, C.H. & Olla, B.L. (1995) The influence of food distribution upon the development of aggressive and competitive behaviour in juvenile chum salmon, *Oncorhynchus keta. J. Fish Biol.* **46**, 264-272.

Thorpe, J.E. & Cho, C.Y. (in press) Minimising waste through bioenergetically and behaviourally based feeding strategies. *Wat. Sci. Tech.*

Thorpe, J.E., Talbot, C., Miles, M.S., Rawlings, C. & Keay, D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **90,** 41-47.

CHAPTER V

DIFFERENTIAL PATTERNS OF FEEDING AND RESOURCE ACCUMULATION IN MATURING AND IMMATURE ATLANTIC SALMON.

This chapter forms the basis of a paper submitted to *Aquaculture International* in October 1995.

INTRODUCTION

There is usually a minimum size below which reproduction does not occur for individuals of a given species. Ectotherms such as teleost fish, having indeterminate growth, have no fixed size of first reproduction and therefore exhibit wide variation in age at first maturity (Wootton 1990, Roff 1992). This variation is exhibited between species, between different populations of the same species and even within the same population (Wootton 1990). Intra-population variation in age at maturity has been studied intensively in salmonids, providing evidence that control of the timing of maturation is a performance-dependent process (Thurow 1966, McCormick & Naiman 1984, Chadwick *et al.* 1986, Thorpe 1986, 1989, Rowe & Thorpe 1990, Rowe *et al.* 1991, Simpson 1992). In sea-run Atlantic salmon, fish that will mature first show increasing relative gonad weights by late autumn, approximately one year before they would be ready to spawn (Thorpe 1994). Elevated plasma levels of sex steroid hormones have been detected in February (Scott & Sumpter 1983). As these individuals mature, they grow faster than their immature siblings during the spring and early summer, with the weight difference being largest around June (Aksnes *et al.* 1986). Recently a negative correlation was found between an individual's body reserves in June and subsequent appetite (and presumably therefore rate of accumulation of reserves) in maturing Atlantic salmon, with fish in best condition ceasing to feed earliest (VII, VIII). Thus, it seems that there is a critical level of body reserves that must be attained prior to the onset of maturation-related anorexia.

Little is however known about the mechanism by which these breeding reserves are accumulated rapidly at a time (late spring/early summer) when water temperatures are low and fish would be expected to have a low feeding motivation. In the present chapter I compare feeding responses, weight, length, body condition and body lipid reserves in maturing and immature fish from the autumn of their first year at sea until the following summer, when the maturing fish become anorexic.

MATERIALS AND METHODS

Two separate investigations were carried out:

1. Feeding Motivation

This trial was performed at a fish farm in Glenmore Bay, Argyll, Scotland (McConnell Salmon Ltd.). Approximately 9500 Atlantic salmon smolts were stocked in a 12 x 12 x 8m deep sea cage during the spring of 1993. At four-weekly intervals From 25 November 1993 until 19 May of the following year, samples of 200 fish were taken from the cage according to the following criteria:

1) Feeding fish: 100 fish were captured by throwing a handfuls of food pellets into the water and netting fish that rose towards the surface to feed.

2) Control fish: 100 fish were captured by crowding the entire cage population into a small area and netting fish at random.

All fish caught were given Visible Implant (VI) tags (Northwest Marine Technology Ltd.) to allow for later identification, and they were then returned to the cage. There were no sampling sessions in December, due to logistical problems. On 19 June 1994, maturing fish (identified on the basis of secondary sexual characteristics; see Aksnes *et al.* 1986) were removed from the cage. A sample of immature fish (numbering approximately half that of maturing fish) was also removed. All fish removed (hereafter termed the study population) were subsequently killed. Those with VI tags (n=310) were identified, dissected, sexed (70% male, 30% female) and their gonads weighed. 73% of males and 54% of females were found to be maturing (i.e. belonging to the upper modal group in the bimodal frequency distribution of gonadosomatic index [GSI = gonad wet weight as a percentage of body wet weight], with a GSI greater than 0.10% in males or 0.38% in females).

2. Development of maturational differences

This experiment was conducted at Sheilavaig, South Uist, Scotland, on a fish farm owned by McConnell Salmon Ltd. On 25 September 1993, 1250 Atlantic salmon post-smolts were given VI tags, weighed, measured and stocked in a 9 x 9 x 4m deep sea cage. Samples of 200x fish were weighed and measured monthly thereafter until June 1994. Body lipid levels (i.e. lipid stores associated with the muscle mass) were estimated on each sampling date by placing a Torry Fat Meter (Distell UK Ltd.) on the left flank of each fish directly below the dorsal fin (Kent 1990). Exceptions to this protocol were in November, when all fish were weighed and forklength measured, February, when no lipid estimations were made due to equipment failure, and March and April when no samples were taken due to logistical problems. On 26 July, when all fish were killed, identified, sexed (57.3% male, 42.7% female) and their gonads weighed. 34.4% of male and 27.8% of female (and 31.6% of all) fish were found to be maturing (i.e with a GSI greater than 0.20% in males or 0.42% in females).

Data analysis

Condition factor was calculated as follows (Ricker 1979, Bolger & Connolly 1989):

Condition factor $(C) = 10{,}000 \times W/L^k$

where   W = weight (g)

L = forklength (mm)

k = the slope of the regression of ln(W) on ln(L);

Specific growth rate (SGR = % gain in weight per day) $= 100 \times \dfrac{[\ln W_f - \ln W_i]}{t}$

where W_f = final weight

W_i = initial weight

t = time elapsed in days

In Expt. 1 G-tests were performed in order to test whether the proportions of maturing and immature fish in feeding and control samples deviated significantly from their respective representations in the study population.

In Expt. 2 differences between male and female, and maturing and immature fish, were tested using 2-way ANOVA with P=0.01 defined as the minimum level of significance (in order to avoid Type II errors among the many ANOVA's performed). The relationships between weight and forklength were compared using ANCOVA.

RESULTS

1. Feeding Motivation

The relative number of maturing fish in feeding samples was not significantly different from that expected from the study population for the period November to March (Fig. 1). However, in April and May maturing fish were present in significantly and increasingly higher numbers than expected in the feeding samples (G_1=6.09, n=45 P<0.05 and G_1=17.65, n=33 P<0.001 respectively) (Fig. 1a). Thus by May over 97% of the feeding fish were maturing, whereas mature fish made up only 68% of the study population. In contrast the control samples showed no significant difference from expected proportions of maturing fish throughout the trial period (Fig. 1b).
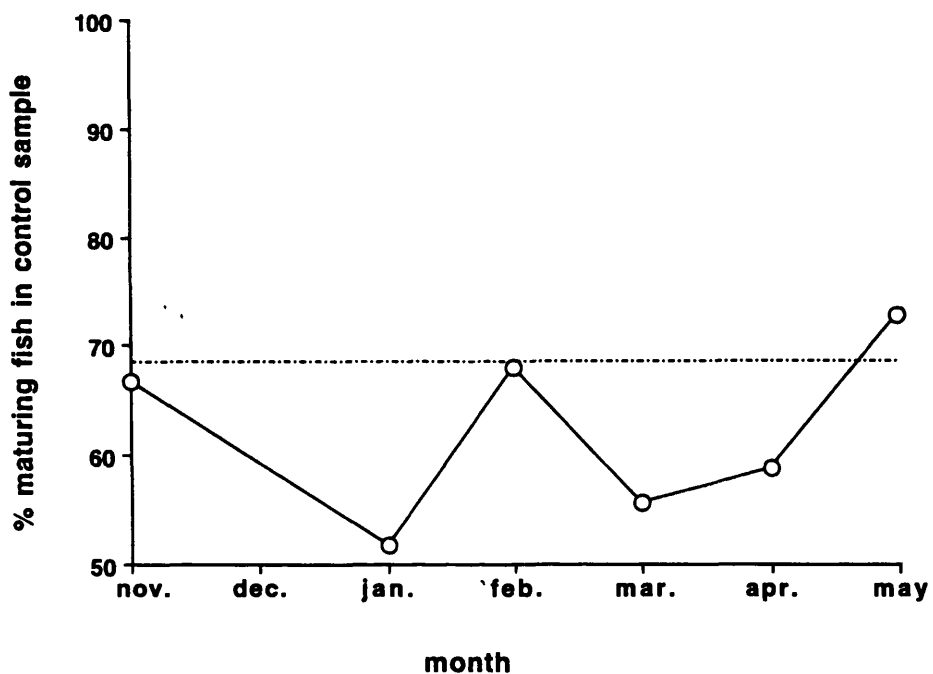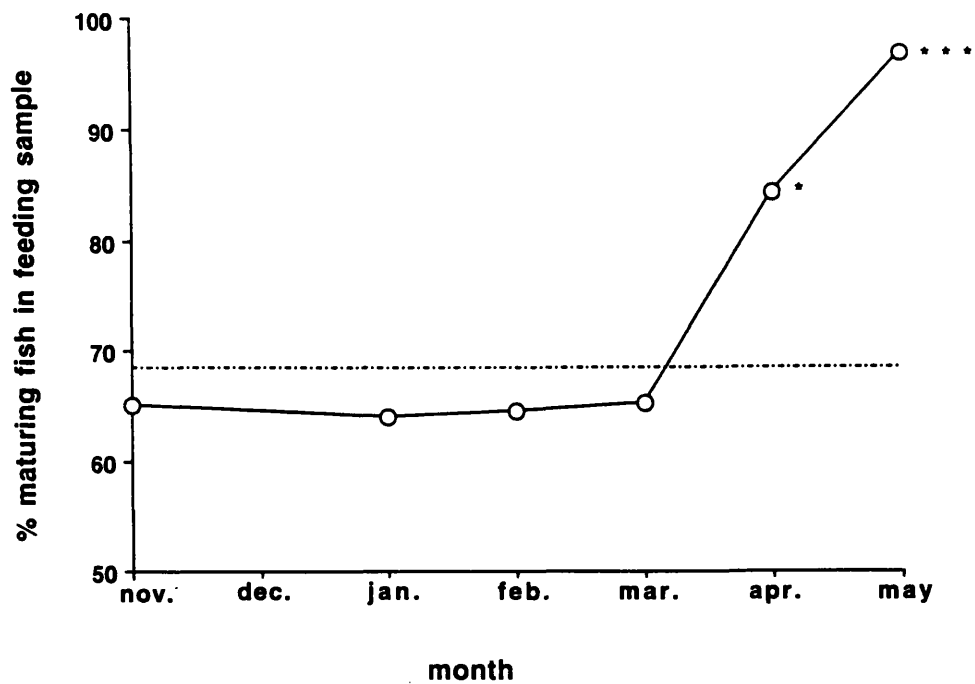
Fig. 1. Proportion of maturing fish in (a) feeding sample and (b) control sample each month in Expt. 1. Dotted line represents proportion of maturing fish in study population; n ranges from 51-83 in each sample. Asterisks denote significant differences from expected values (*=P<0.05, ***=P<0.001).

2. Development of Maturational Differences.

Fig. 2. shows the changes in mean (±S.E.) weight and forklength for subsamples of maturing and immature fish sampled over the course of Expt. 2. There was no significant difference between maturing and immature fish in September, but a month later maturing fish were significantly heavier. By November maturing fish were significantly longer than the immatures. Thereafter mature fish were significantly heavier and longer in all months except December. The difference in weight between the two groups became progressively larger during the spring, but by July body weight was converging as growth slowed in the maturing fish whereas immature fish continued to gain weight.

Fig. 3 illustrates the equivalent changes in body lipids and condition factor for maturing and immature fish. As for weight and forklength, body lipids and condition factor were not significantly different between maturing and immature fish at the start of the trial in September, but one month later maturing fish had higher body lipid levels and condition factors than their immature siblings. Percentage body lipid rose sharply in early winter (a month earlier and to a greater extent in maturing fish) and then increased steadily during the spring in both groups of fish. During late spring/early summer, % body lipid continued to show the same rate of increase in immature fish, while in maturing fish it rose sharply to peak in June and then dropped rapidly in July. This loss of lipid in maturing fish coincided with a period of rapid lipid deposition in immature fish, so that at the end of the experiment in July the two types of fish were tending toward similar lipid levels (although these were still significantly higher in maturing fish).

Condition factor rose sharply during September and November in both groups, but then remained steady throughout the winter. The condition factor of immature fish dropped steadily from mid-winter to the end of the experiment in July, whereas that of maturing fish rose to a peak in June followed by a rapid decline in July, a similar pattern to that of body lipid. As condition factor differed significantly between maturing and immature fish for much of the study, I also investigated the dynamics of the relationship between the two components of condition factor (weight and forklength) and how these vary between maturing and immature fish in late spring/early summer (when the difference between the two groups was greatest).
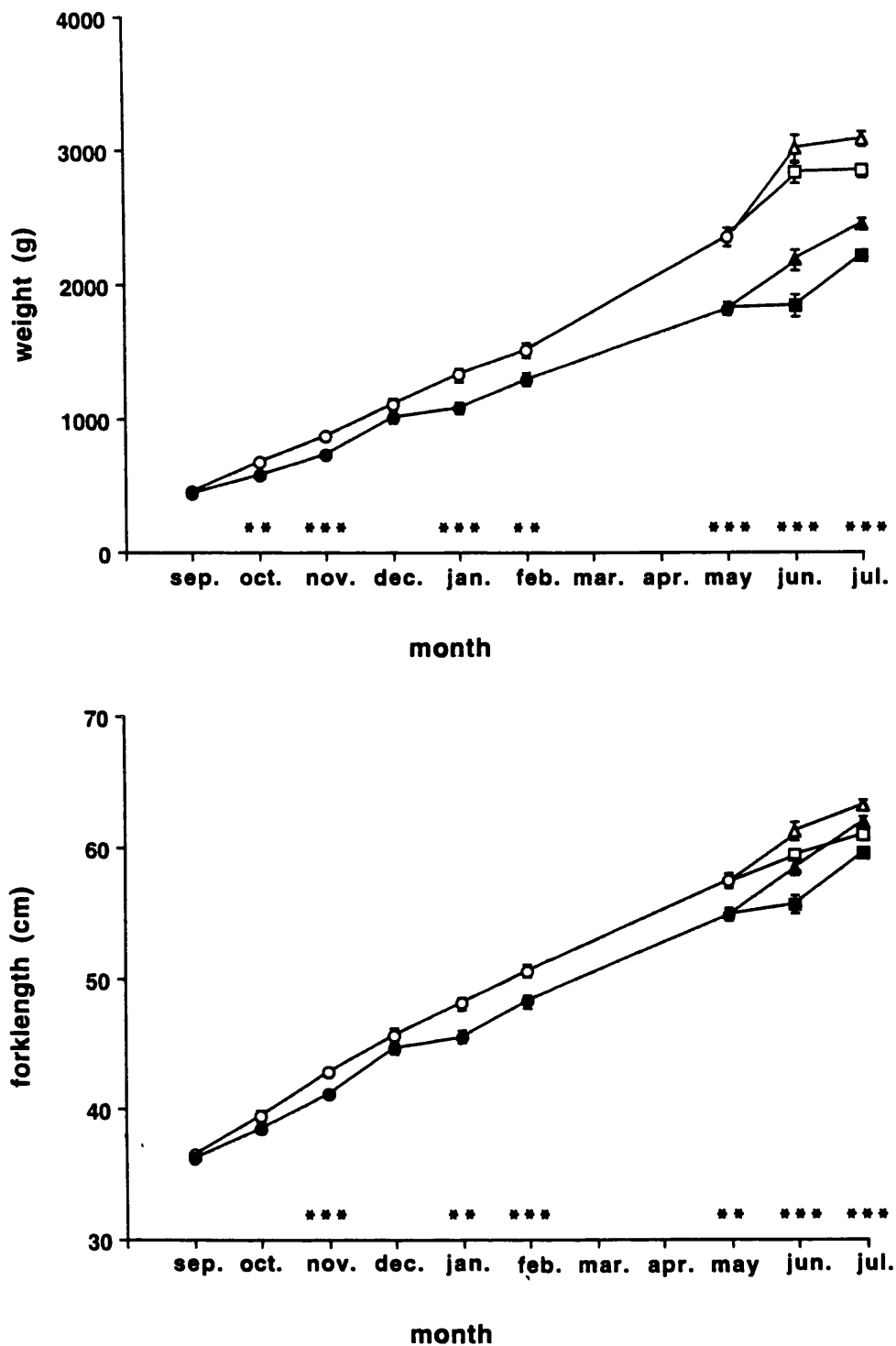
Fig. 2. Changes in (a) body weight and (b) forklength by month for maturing and immature fish in Expt. 2. Data presented as means $\pm$ S.E. Asterisks denote significant differences between maturing and immature fish (ANOVA, ** = P<0.01, *** = P<0.001; n ranges from 113-649 each month). Open and closed symbols represent maturing and immature fish respectively; females are represented by squares, males by triangles and both sexes by circles (sexes are combined when not significantly different).
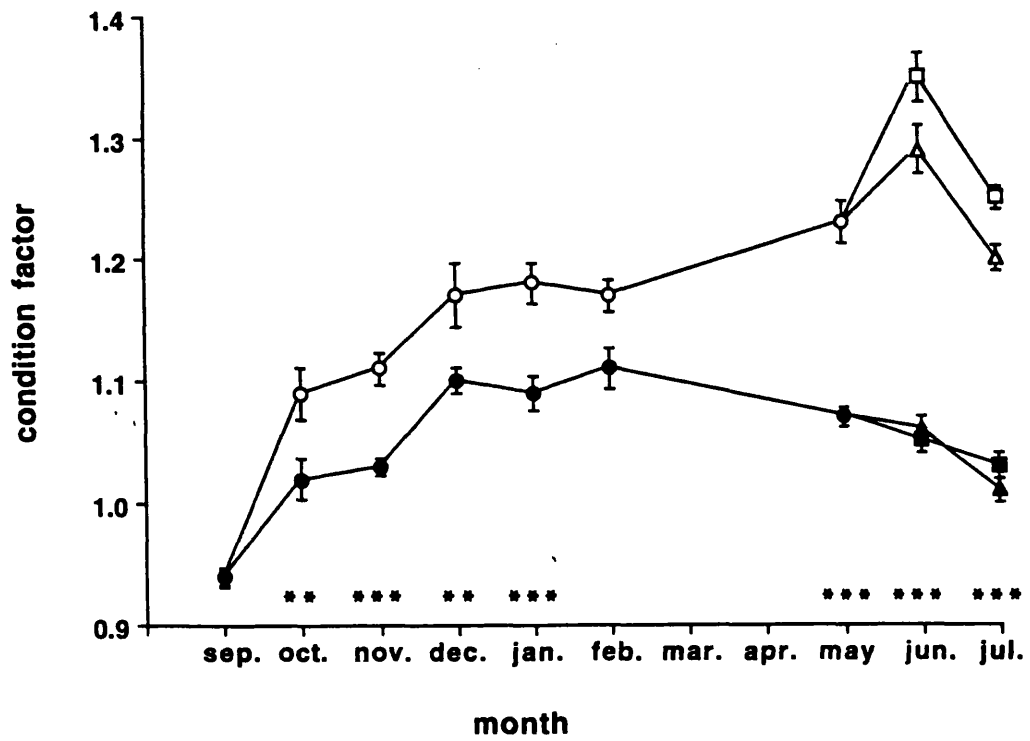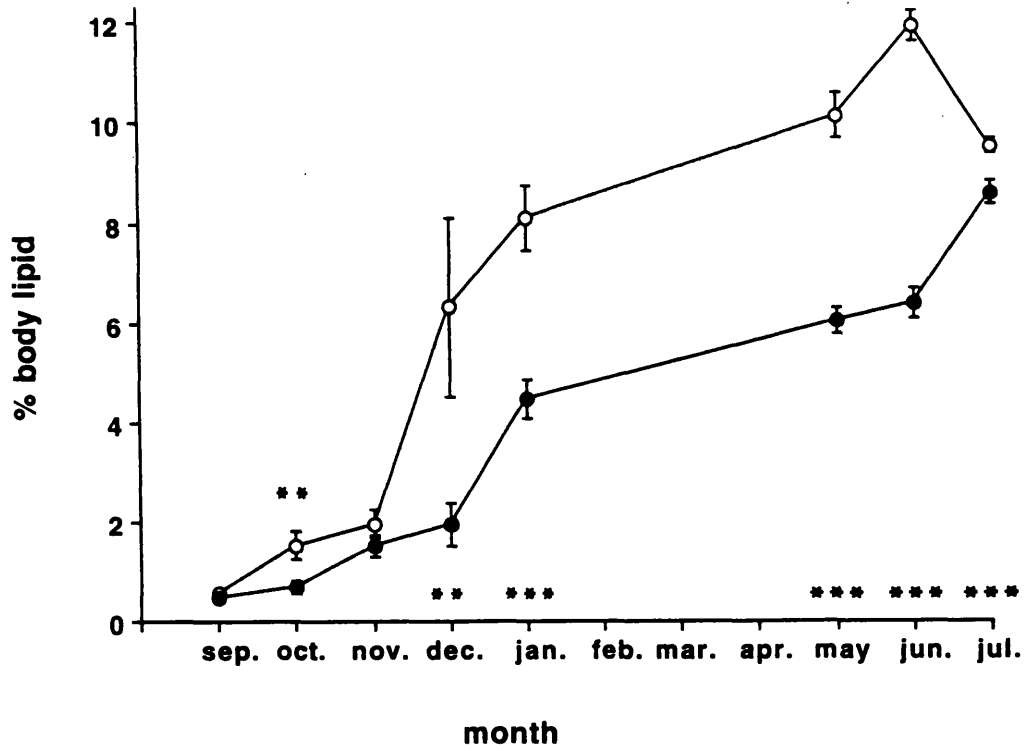
Fig. 3. Changes in (a) body lipids and (b) condition factor by month for maturing and immature fish in Expt. 2. Sample sizes and data presentation as in Fig. 2.

Covariance analysis revealed no significant differences between maturing and immature fish in either slope or elevation of the regression of ln (weight) on ln (forklength) in February but significant differences from May to July (Table 1, Fig. 4). Maturing fish thus become heavier for a given forklength than immature fish sometime in the spring.

Table 1. Analysis of covariance for regressions of ln(weight) on ln(forklength) for maturing and immature fish in Expt. 2.

Date	Comparison of slopes			Comparison of elevations		
	F	d.f.	P	F	d.f.	P
21 Feb	0.34	1,135	NS	0.19	1,136	NS
10 May	2.19	1,153	NS	65.71	1,154	<0.001
21 June	5.35	1,155	0.022	-	-	-
28 July	23.13	1,644	<0.001	-	-	-

Though patterns of growth were somewhat variable over the study period, maturing fish grew faster throughout the trial period up until June/July, when their growth rates dropped dramatically. May (when maturing fish dominate in the feeding sample; see Fig. 1) is the period when they are growing most rapidly compared with immature fish (Fig. 5).

The ratio of growth in weight to that in length ($SGR(w)/SGR(l)$) is a reflection of the allometry of growth. Immature fish maintained a relatively constant $SGR(w)/SGR(l)$ ratio from late winter to summer (Fig. 6). In contrast maturing fish had higher ratios in spring, but lower ratios by June-July; they were thus putting on weight rather than length earlier in the year but losing it by the summer.

ANOVA revealed two-way interactions between gender and reproductive status for bodyweight in October ($P=0.007$) and both bodyweight ($P=0.008$) and condition factor ($P=0.008$) in November. Significant gender differences (all $P<0.01$) emerged in the last two months of sampling. Males had higher mean weights and lengths than females in June and July among both maturing and immature fish, and by July immature males were as long as maturing females (Fig. 2). However, maturing females maintained higher condition factors than maturing males over this period, and amongst immature fish, while mean condition factors were very similar for males and

females, in June female condition factor was higher by July (Fig. 3). No significant gender differences or two-way interactions were observed for % body lipid or growth rates throughout the trial period.
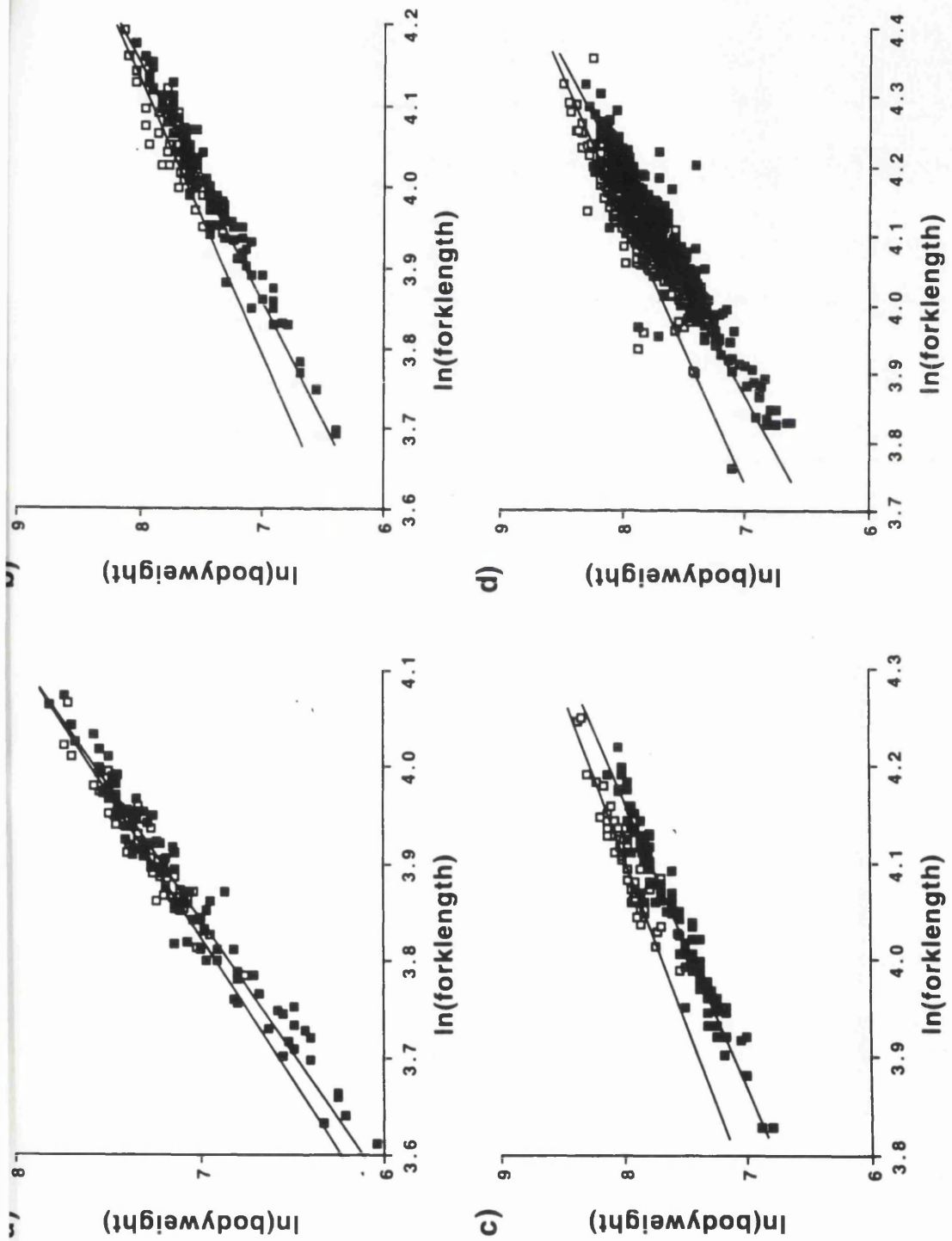
Fig. 4. Relationships between ln(body weight) and ln(forklength) for maturing and immature fish in (a) February, (b) May, (c) June and (d) July in

Expt. 2. Open squares represent maturing fish, closed squares represent immature fish.

92
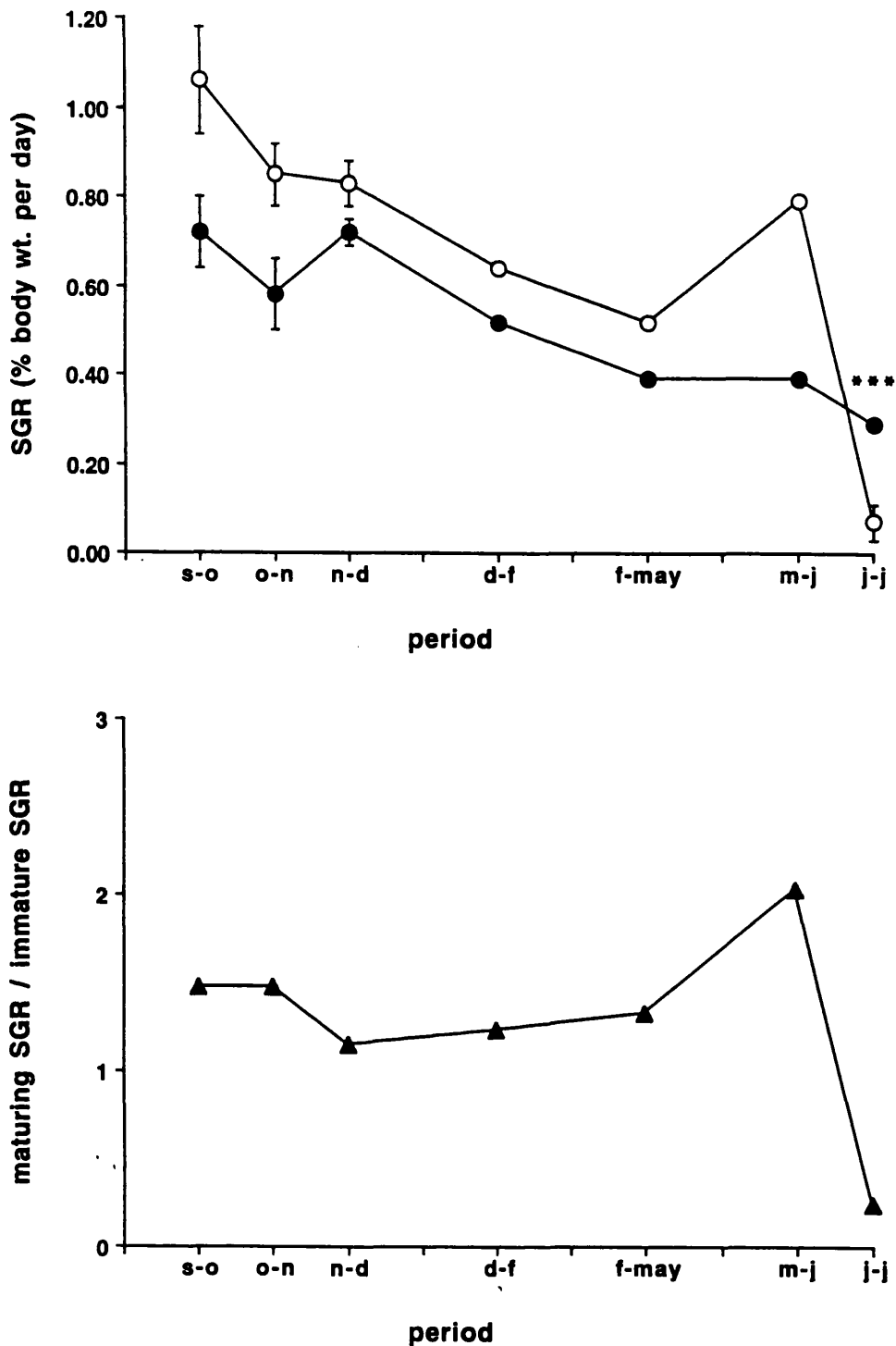
Fig. 5. (a) Changes in weight specific growth of maturing and immature fish over time in Expt. 2. Sample sizes and data presentation as in Fig. 2. (b) Changes in ratio of SGR(maturing fish) / SGR(immature fish) by month. Growth rates for December - June were calculated from changes in sample mean weights and lengths, hence no standard errors or statistical analyses are available for this period.
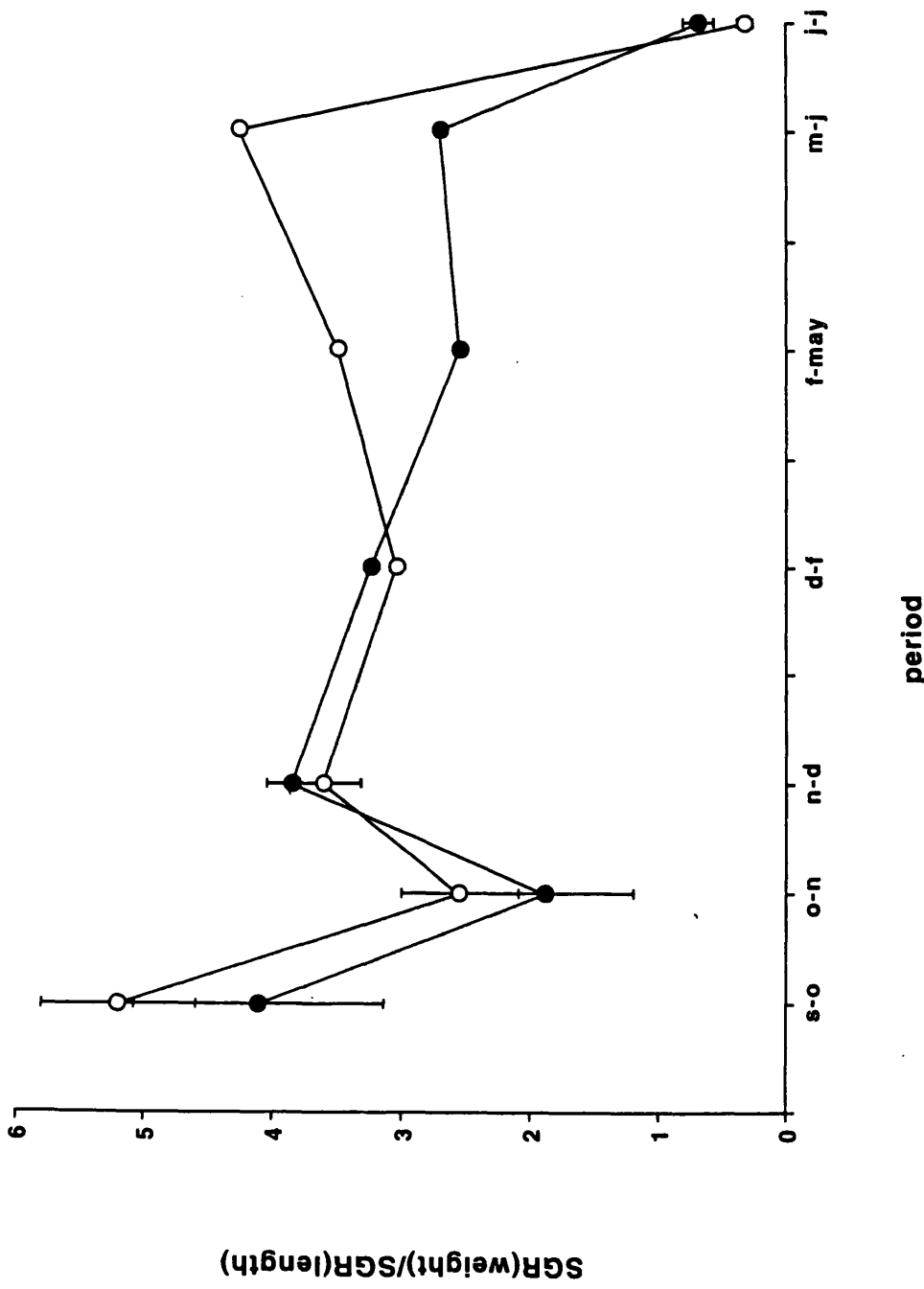
Fig. 6. Changes in SGR(weight)/SGR(length) by month for maturing and immature fish in Expt. 2. Growth rates for December - June were calculated from changes in sample mean weights and lengths, hence no standard errors or statistical analyses are available for this period. Sample sizes and data presentation as in Fig. 2.

94

DISCUSSION

Feeding motivation

My results demonstrate an apparent appetite surge in maturing fish in April/May (approximately two months before the onset of maturation-related anorexia). Increased feeding motivation is thus likely to be the behavioural mechanism by which the observed increased differentials in growth and weight between maturing and immature fish arise (Hunt *et al.* 1982, Aksnes *et al.* 1986, this study). This spring/summer period of increased feeding motivation and subsequent accelerated growth in maturing Atlantic salmon appears to facilitate the acquisition of a critical level of nutrient reserves before upriver migration to the spawning grounds (VIII).

Replenishment of lipid depots (Rowe *et al.* 1991) and build up of lean mass reserves (VIII) are important for maintenance of the maturation process, and maturing salmon may have adapted to seasonal variation in ocean productivity levels by delaying a surge in feeding intensity (to facilitate accumulation of nutrient reserves) until April/May. A similar adaptive mechanism has been demonstrated in autumn in Atlantic salmon parr that will migrate downstream to sea in the following spring. These fish have a surge in appetite and growth in the autumn (at a time of declining temperatures), probably in order to build up reserves and increase body size prior to the reduced temperatures and daylengths of winter. This 'fast development strategy' adopted by fish that will enter the sea during the following spring contrasts with the 'slow development strategy' of fish that will remain in fresh water for at least a further year. The latter become anorexic and cease growing (Kristinsson *et al.* 1985, Metcalfe *et al.* 1986, 1988). Winter is a season of low productivity when attempts to acquire and store resources would be relatively costly in terms of energy expended and increased vulnerability to predation due to greater foraging activity. Simpson (1993) showed a surge and peak in appetite in Atlantic salmon parr in May (independent of food supply) and suggested that this may be due to water temperatures increasing and allowing greater activity (and therefore feeding) for the first time after winter. Fish would thus have an opportunity to replace fat reserves lost during the winter (Gardiner & Geddes 1980, Higgins & Talbot 1985, Cunjak 1988, Rowe *et al* 1991, Metcalfe & Thorpe 1992). Water temperatures at the experimental

site of the present study did not however, show a marked increase in April/May; there was a steady increase of 1-2°C per month from the beginning of the year until May, a pattern which does not follow that of feeding motivation of maturing fish in my study. Elliot (1976) found that maximum gross growth efficiency was obtained with temperatures of 8-11°C in trout and it may be that the April/May period of increased feeding motivation coincided with similarly optimal growth conditions for salmon.

The observed differences in feeding behaviour between maturing and immature fish may have potential as a grading criterion for the removal of maturing fish from sea cages in the early summer. Maturing fish are harvested at this time because they will lose market value once they become anorexic, lose weight and acquire their characteristic 'dirty' coloration (Aksnes et al. 1986). Presently the separation process is carried out manually by fish farmers, with each fish being graded on the basis of external appearance. This type of grading operation is labour intensive and often fails to remove all maturing fish from a population. Grading salmon on the basis of behaviour could be performed with minimal handling of the fish and with less effort than present grading systems.

Development of maturational differences.

The results illustrated by Figs 2 and 3 indicate that our trials commenced just prior to the divergence of the maturing and immature salmon with respect to body size and body lipid levels. Differences between immature and maturing fish in mean weight increase between February and June (when the mean weight difference was largest), after which growth almost ceased in maturing fish while immature fish continued to gain weight. These observations and their timing are in agreement with the findings of previous studies of Atlantic salmon in sea cages (Hunt et al. 1982, Aksnes et al. 1986).

Body lipid levels in maturing fish increased rapidly in early winter; these fish then maintained higher levels than immature fish, although showing similar seasonal trends until the spring. There was a sharp rise in lipid levels in maturing fish in May and subsequent fall after June. This phenomenon coincided with a rise and fall in condition factor of maturing fish over a period when immature fish were steadily losing condition yet accumulating lipid reserves. Aksnes et al. (1986) showed similar

differences in condition factor among Atlantic salmon in sea cages, again finding a peak in condition of maturing fish in June/July. The divergent trends in condition factor and body lipids in immature fish highlight the fact that the frequent assumption of a close link between condition factor and levels of lipid reserves is often erroneous. Poor correlations between the two variables have previously been reported by Simpson *et al.* (1992) and (VIII).

Maturing Atlantic salmon need to replenish lipid reserves in spring - these having been depleted during the period of low temperatures and decreased appetite in winter (Smith *et al.* 1993) - if reproduction is to proceed (Rowe *et al.* 1991, Taranger 1993). Maturing adult Atlantic salmon migrate upriver to spawning grounds and simultaneously invest in gonadal growth at a time when they are not feeding: the maturation process is thus energetically very costly (Tillik 1932, Jonsson *et al.* 1991). Previous work has suggested that maturing individuals must accumulate sufficient reserves to surpass a critical threshold before becoming anorexic (and presumably beginning upriver migration) (VII, VIII). This process is reflected in the trends illustrated by Fig. 3, which shows that maturing fish rapidly increase condition factor and body lipids in late spring as they approach threshold levels. Once these are reached (in June for many fish in our trial), the fish become anorexic and so undergo marked declines in both parameters.

The build up of protein reserves and development of gonadal material are also reflected in Fig. 4, which shows that maturing fish were heavier for a given forklength in May, June and July. Similar findings were reported by Hunt *et al.* (1982) from April/May 'until the deteriorative changes associated with anorexia eventually reversed the status of the two groups'. Although I did not observe higher weights for a given length in immature fish before the end of our trials, the cessation of growth observed in maturing fish by July suggests that relative weight of immature fish may well have exceeded that of maturing fish had the trial continued. Hunt *et al.* (1982) suggested that greater weights observed in maturing fish were due to the secondary (anabolic) effect of low levels of sex-steroids in the plasma, the latter being well known as promoters of muscle growth. Using androgen implants in immature salmon, Berglund *et al.* (1992) obtained results suggesting a causal relationship between androgens and the accelerated growth pattern observed in maturing male Atlantic salmon parr (Rowe & Thorpe 1990, Berglund 1992, Simpson 1992). It would thus

seem that, in addition to their other functions, hormones associated with the maturation process serve as facilitators of the build up of required protein reserves mentioned above.

The gender differences in weight, length and condition factor require further investigation in order to clarify the patterns and processes occurring, as I do not know of any comparable results from other studies. Immature males and females appeared to diverge in weight and length from June, a time at which they were beginning to experience less feeding competition from maturing fish (see later); this may be due to differing strategies between males and females in the lead up to the onset of the maturation cycle in the autumn. Previous studies have not however, reported any such differences among immature fish. Maturing males also differed from maturing females in weight, length and condition factor in June and July. A sharp increase in serum levels of sex-specific hormones associated with maturation (11-oxotestosterone in males and 17β-oestradiol in females) was observed in maturing fish at this time by McLay *et al.* (1992) and may well be associated with the differences I have reported.

I observed significantly different length-weight relationships between maturing and immature fish in June and July, most probably due to the effects of the asynchronous onset of anorexia in the maturing component of the population, as the subsequent weight losses in anorexic individuals could be expected to reduce the slope parameter of a weight/length regression.

My observations of higher SGR(w)/SGR(l) ratios in maturing fish corroborated those of Hunt *et al.* (1982), who found disproportionate growth in weight in maturing fish from mid/late March to May/June, which I observed to be the period of greatest differential feeding responses and growth between maturing and immature fish. Berglund *et al.* (1992) also found androgen implants administered to immature salmon parr produced more pronounced growth in weight than in length.

It is possible that maturing fish feed aggressively (and may attempt to monopolise food) at this time of year. During these trials, food was spread evenly across the surface of the cage and so monopoly of food would have proved difficult and uneconomical. In an earlier study however (VII), I observed an increasing appetite of immature fish from mid-June onwards, at the same time as that of maturing fish was decreasing. McLay *et al.* (1992) and Gjerde *et al.* (1994) observed similar patterns of growth rates in maturing and immature Atlantic salmon over the spring/summer

period. Such data however, provide only circumstantial evidence for immature fish being out-competed for food by those maturing, and further work is required to properly test this hypothesis.

In immature fish I observed decreasing SGR(w)/SGR(l) ratios even after maturing fish had become largely anorexic. Moreover, SGR(w)/SGR(l) levels appear to have been much lower during the spring/summer period than they were in the previous September/October (Fig. 5). The trends in weight, length, body lipids and condition factor seen in Figs 2 and 3 suggest that immature fish were building up lipid reserves, while condition factor gradually declined. Thus it seems that in the spring immature fish invest more heavily in skeletal growth than muscle growth. Saif-Abdullah (1981) observed a similar pattern in sea caged immature Atlantic salmon up until late June, after which the trend reversed and growth in weight became more important than growth in length. Hence it seems that these growth patterns are entrained to changes in daylength, with muscle growth becoming important as daylength decreases and the onset of the next maturation cycle approaches. Such a strategy would make individuals much larger at maturity than their earlier-maturing siblings, and so they would reap the benefits of higher fecundity (Wootton 1984). Investment in skeletal growth in preference to protein storage (relative to maturing fish) would explain the steady decline in condition factor I observed in these fish through the spring and early summer, even once maturing fish had ceased feeding.

A similar relationship between feeding, nutrient reserves and maturation/breeding and various strategies to facilitate the accumulation of reserves have been demonstrated in other vertebrates. Gonad maturation in amphibians (Fitzpatrick 1976) and reptiles (Derickson 1976) has been shown to depend upon the development of a fat body in association with each individual gonad. Female mallard ducks (*Anas platyrhynchos*) obtain the protein required for egg production from intensive foraging for protein-rich food items (Krapu 1981). Fecundity is positively correlated with food supply, diet and body condition in female lesser black-backed gulls (*Larus fuscus*) (Houston *et al.* 1983, Bolton *et al.* 1993) and Tengmalm's owl (*Aeogolius funereus*)(Korpimaki & Hakkarainen 1991). In migratory birds (as in Atlantic salmon), additional energy reserves are required prior to departure for the breeding grounds. For example the Grey Catbird (*Dumetella carolinensis*) increases its flight muscles by up to 35% (Marsh 1984). Davidson & Evans (1986) found that the degree

of increase in muscle mass of migratory shorebirds prior to departure for their breeding grounds was greater than that required for the journey alone and so concluded that the surplus protein was stored for use at the migratory destination.

I have demonstrated here how observed changes in relative food intake lead to differences in growth, lipid deposition and body condition between immature and maturing adult Atlantic salmon. This is an example of a dramatic endogenous modulation of appetite as part of a complex control mechanism that ensures that fish will only reproduce if they are in adequate condition and that they maximise condition prior to the final stages of their reproductive cycle when they will become anorexic.

REFERENCES

Aksnes, A., Gjerde, B. & Roald, S.O. (1986) Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **53**, 7-20.

Berglund, I. (1992) Growth and early sexual maturation in Baltic salmon (*Salmo salar*) parr. *Can. J. Zool.* **70**, 205-211.

Berglund, I., Mayer, I. & Borg, B. (1992) Effects of sexual maturation, castration, and androgen implants on growth in one- and two-year-old parr in a Baltic Atlantic salmon (*Salmo salar* L.) stock. *J. Fish Biol.* **40**, 281-292.

Bolger, T., and Connolly, P.L. (1989) The selection of suitable indices for the measurement and analysis of fish condition. *J. Fish Biol.* **34**, 171-182.

Bolton, M., Monaghan, P. & Houston, D.C. (1993) Proximate determination of clutch size in lesser black-backed gulls: the roles of food supply and body condition. *Can. J. Zool.* **71**, 273-279.

Chadwick, E.M.P., Randall, R.G. & Leger, C. (1986) Ovarian development of Atlantic salmon (*Salmo salar*) smolts and age at first maturity. In: D.J. Meerburg (ed.) *Salmonid Maturation. Can. Spec. Publ. Fish. Aquat. Sci.* **89**, 15-23.

Cunjak, R.A. (1988) Physiological consequences of overwintering in streams: the cost of acclimitization? *Can. J. Fish. Aquat. Sci.* **45**, 443-452.

Davidson, N.C. and Evans, P.R. (1986) Prebreeding accumulation of fat and muscle protein by arctic-breeding shorebirds. In: H.Ouellet (ed.) *Acta XIX Congressus Internationalis Ornithologica* Vol. 1 University of Ottowa Press, Ottowa, Ontario. pp. 342-351.

Derickson, W.K. (1976) Lipid storage and utilization in reptiles. *Amer. Zool.* **16,** 711-723.

Elliott, J.M. (1976) The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. *J. Anim. Ecol.* **45,** 923-948.

Fitzpatrick, L.C. (1976) Life history patterns of storage and utilization of lipids for energy in amphibians. *Amer. Zool.* **16,** 725-732.

Gardiner, W.R. & Geddes, P. (1980) The influence of body composition on the survival of juvenile salmon. *Hydrobiologia* **154,** 3-11

Gjerde, B., Simianer, H., & Refstie, T. (1994) Estimates of genetic and phenotypic parameters for body weight, growth rate and sexual maturity in Atlantic salmon. *Livest. Prod. Sci.* **38,** 133-143.

Higgins, P.J. & Talbot, C. (1985) Growth and feeding in juvenile Atlantic salmon. In: C.B. Cowey, A.M. MacKie and J.G. Bell (eds.) *Nutrition and feeding in fish.* Academic Press, London. pp. 243-263.

Houston, D.C., Jones, P.J. & Sibley, R.M. (1983) The effect of female body condition on egg laying in Lesser Black-backed Gulls. *J. Zool. (London)* **200,** 509-520.

Hunt, S.M.V., Simpson, T.H., & Wright, R.S. (1982) Seasonal changes in the levels of 11-oxotestosterone and testosterone in the serum of male salmon, *Salmo salar* L., and their relationship to growth and the maturation cycle. *J. Fish Biol.* **20,** 105-119.

Jonsson, N., Jonsson, B. & Hansen, L.P. (1991) Energetic cost of spawning in male and female Atlantic salmon (*Salmo salar* L.). *J. Fish Biol.* **39,** 739-744

Kent, M. (1990) Hand-held instrument for fat/water determination in whole fish. *Food Cont.* **2**, 47-53.

Korpimaki, E. & Hakkarainen, H. (1991) Fluctuating food supply affects the clutch size of Tengmalm's Owl independent of laying date. *Oecologia* **85**, 543-552.

Krapu, G.L. (1981) The role of nutrient reserves in Mallard reproduction. *Auk* **98**, 29-38.

Kristinsson, J.B., Saunders, R.L. & Wiggs, A.J. (1985) Growth dynamics during the development of bimodal length-frequency distribution in juvenile Atlantic salmon (*Salmo salar* L.). *Aquaculture* **45**, 1-20.

Marsh, R.L. (1984) Adaptations of the Grey Catbird, *Dumatella carolinensis*, to long distance migration: flight muscle hypertrophy associated with elevated body mass. *Physiol. Zool.* **57**, 105-117.

McCormick, S.D. & Naiman, R.J. (1984) Some determinants of maturation in brook trout, *Salvelinus fontinalis*. *Aquaculture* **43**, 269-278.

McLay, H.A., Youngson, A.F., Wright, R.S. & Johnstone, R. (1992) Effects of rearing density on sexual maturation and growth in sea-cage reared Atlantic salmon, *Salmo salar* L. *Aquacult. Fish. Manage.* **23**, 353-365.

Metcalfe, N.B., Huntingford, F.A. & Thorpe, J.E. (1986) Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Zool.* **64**, 2439-2446.

Metcalfe, N.B., Huntingford, F.A. & Thorpe, J.E. (1988) Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon. *J. Anim. Ecol.* **57**, 463-474.

Metcalfe, N.B. & Thorpe, J.E. (1992) Anorexia and defended energy levels in over-wintering juvenile salmon. *J. Anim. Ecol.* **61,**175-181.

Ricker, W.E. (1979) Growth rates and models. In: . W.S. Hoar, D.J. Randall & J.R. Brett (eds) *Fish Physiology* Vol. 8. Academic Press, New York pp. 677-743.

Roff, D.A. (1992) *The Evolution of Life Histories, Theory and Analysis.* Chapman & Hall, London.

Rowe D.K. & Thorpe J.E. (1990) Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reductions in feeding and growth during spring months. *Aquaculture* **86,** 291-313.

Rowe, D.K., Thorpe, J.E. & Shanks A.M. (1991) Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* **48,** 405-413.

Saif-Abdullah, M. (1981) *Changes in Growth and Gonadal Hormone Status in the Sea water Life of Atlantic Salmon (Salmo salar L.)* Unpubl. PhD thesis. University of Aberdeen.

Scott, A.P. & Sumpter, J.P. (1983) A comparison of the female reproductive cycles of autumn-spawning and winter-spawning strains of rainbow trout (*Salmo gairdneri* Richardson). *Gen. Comp. Endocrinol.* **52,** 79-85.

Simpson, A.L. (1992) Differences in body size and lipid reserves between maturing and non-maturing Atlantic salmon parr, *Salmo salar* L. *Can. J. Zool.* **70,** 1737-1742.

Simpson, A.L., (1993*). Investigation of the Factors Influencing Maturation in Atlantic Salmon, Salmo salar L., Parr.* Unpubl. PhD thesis, University of Glasgow, Scotland.

Simpson, A.L., Metcalfe, N.B. & Thorpe, J.E. (1992) A simple non-destructive biometric method for estimating fat levels in Atlantic salmon, Salmo salar L., parr. *Aquacult. Fish. Manage.* **23**, 23-29.

Smith, I.P., Metcalfe, N.B., Huntingford, F.A. & Kadri, S. (1993) Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **117**, 165-178.

Taranger, G.L. (1993) Sexual maturation in Atlantic salmon, *Salmo salar* L.; aspects of environmental and hormonal control. Unpubl. PhD thesis, University of Bergen, Norway.

Thorpe, J.E. (1986) Age at first maturity in Atlantic salmon *Salmo salar*. In: D.J. Meerburg (ed.) *Salmonid Maturation. Can. Spec. Publ. Fish. Aquat. Sci.* **89**, 7-14.

Thorpe, J.E. (1989) Developmental variation in salmonid populations. *J. Fish Biol.*, **35(Suppl. A)**, 295-303

Thorpe, J.E. (1994) Reproductive strategies in Atlantic salmon, *Salmo salar* L. *Aquaculture & Fisheries Management* **25**, 77-87.

Thurow, F. (1966) Beitrage zur Biologie und Bestandskunde des Atlantischen Lachses (*Salmo salar* L.) in der Ostsee. *Ber. Dt. Wiss. Komm. Meeresforsch.* **18**, 223-379.

Tillik, Z.E. (1932) Changes in the chemical composition of the flesh of the Svir salmon in relation to spawning migration. *Izv. vses. Inst. ozern. rechn. ryb.* **14**, 133-148. Cited in *Comm. Fish. Abstr.* (1965) **18**, 12-13.

Wootton, R.J. (1984) Introduction: tactics and strategies in fish reproduction. In: G.W. Potts & R.J. Wootton (eds.) *Fish Reproduction: Strategies & Tactics.* Academic Press, London pp. 1-12.

Wootton, R.J. (1990) *Ecology of Teleost Fishes* Chapman & Hall, London.

CHAPTER VI

EARLY MORPHOLOGICAL PREDICTORS OF MATURITY IN ADULT ATLANTIC SALMON.

This chapter forms the basis of a paper submitted to *Aquaculture* in March 1995.

INTRODUCTION

Among one-sea-winter Atlantic salmon, a proportion of any given population will mature prior to the second winter at sea. The maturing fish will grow faster and undergo morphological changes in the spring prior to the spawning season (Hunt *et al.* 1982, Youngson *et al.* 1988, McLay *et al.* 1992, V). The changes in morphology of maturing fish may be of use in aquaculture as a marker of reproductive status at the individual level, and thus they merit investigation.

Salmonids are generally regarded as monomorphic until they reach maturity, after which they develop sexually dimorphic characteristics (Bodington 1987). In adult Atlantic salmon, such dimorphism has been recorded for body length (Jones 1959), adipose fin length (Naesje *et al.* 1988) and head and jaw morphology (Bodington 1987, Maisse *et al.* 1988), all of which are relatively larger in males. Sexual differences in head morphology have also been shown in immature Atlantic salmon parr, though these are not large enough to predict gender accurately on the basis of secondary characteristics (Simpson 1993). In adult Atlantic salmon, fish that will mature show increasing relative gonad weights by late autumn, approximately one year before they would be ready to spawn (Thorpe 1994). Elevated plasma levels of sex steroid hormones have been detected in February (Hunt *et al.* 1982, Scott & Sumpter 1983, Youngson *et al.* 1988), but sexual dimorphism in external morphology is not apparent until late spring/early summer (Bodington 1987). Morphological differences between maturing and immature fish would be expected to coincide with the appearance of sexual dimorphism.

For salmon farmers, knowledge of the gender of individuals amongst their stock is not an important issue, as male and female salmon grow at broadly similar rates and command the same market prices. Differential growth rates and prices do however exist between immature and mature salmon, and hence it is important to be able to predict what proportion of a population will mature in any given year and to be able to separate mature from immature fish as early as possible. This would also prevent potential monopolisation of food by maturing fish (V) and ease the harvest of such fish before they cease growth (VII, VIII) and suffer a decrease in flesh quality (Aksnes *et al.* 1986).

Simpson (1993) was able to discriminate mature male parr from immature individuals on the basis of head morphology with 93% accuracy in the autumn of spawning, while Bodington (1987) working on adult salmon found significant correlations between gonadosomatic index (GSI, gonad weight as a percentage of total weight) and several biometric measurements for maturing fish of both sexes in late spring, approximately 6 months prior to spawning. In this paper I describe an investigation of the external morphometric correlates of maturation in Atlantic salmon and how these change with time. The data are used to determine if simple morphometric measurements, combined in a standard multivariate analysis, can predict maturation and hence be used in aquaculture.

MATERIALS AND METHODS.

Two separate investigations were carried out using genetically distinct Scottish strains of Atlantic salmon:

Experiment 1.

One hundred one-sea-winter Atlantic salmon (mean weight = 1.70 kg) were transferred from a production density tank to a 5m circular sea water tank at Otter Ferry Fish Farm, Argyll, Scotland on 11 May, 1993. On May 18, all fish were marked individually using Visible Implant (VI) tags (Northwest Marine Technology) inserted into the adipose eyelid. On the same day, and fortnightly from then on, the forklength (to the nearest mm), weight (to the nearest 10g) and more detailed biometric measurements (following Bodington 1987) were collected directly from each individual (Fig. 1) using vernier callipers. Details of rearing conditions can be found in VII. 20 fish either died early in the trial due to anaesthetic overdose or lost their VI tags during the course of the trial. These individuals were excluded from the analysis. The trial finished on 11 August, when the 80 remaining fish were all killed, identified, sexed (14% male, 86% female) and their gonads weighed. 91% (10/11) of male and 74% (51/69) of female fish were found to be maturing, defined as the upper modal group in the bimodal frequency distribution of gonadosomatic index (GSI = gonad wet weight as a percentage of body wet weight), with a GSI greater than 1.1% in males or 1.8% in females (Fig 2). The reproductive status of the small number of inter-modal individuals was determined by examining their growth patterns during the trial (V, VIII).

Experiment 2.

On 25 September 1993, 1250 Atlantic salmon post-smolts (mean weight = 0.45 kg) were retrieved from a production cage, marked with VI tags, weighed, measured (forklength only) and stocked in a 9 x 9 x 4m deep sea cage at Sheilavaig, South Uist, Scotland, in a fish farm owned by McConnell Salmon Ltd. Fish were fed standard commercial feed pellets (BOCM Pauls Ltd.) by hand to satiation 1-3 times per day according to daylength. Samples of 200 fish were collected (by handnet), weighed and

measured monthly thereafter until June 1994. The only exceptions to this protocol were in November (when all fish were weighed and measured) and March and April (when no samples were taken due to logistical problems). During the course of the trial, 601 fish either succumbed to the effects of an earlier pancreatic infection and died or lost their VI tags and were thus excluded from the analysis. The trial finished on 26 July, when all 649 remaining fish were killed, identified, sexed (58% male, 42% female) and their gonads weighed. 33% (125/377) of male and 27% (73/272) of female fish were found to be maturing (i.e with a GSI greater than 0.29% in males or 0.64% in females, see Fig. 2). Reproductive status of inter-modal individuals was determined as described for Expt. 1.
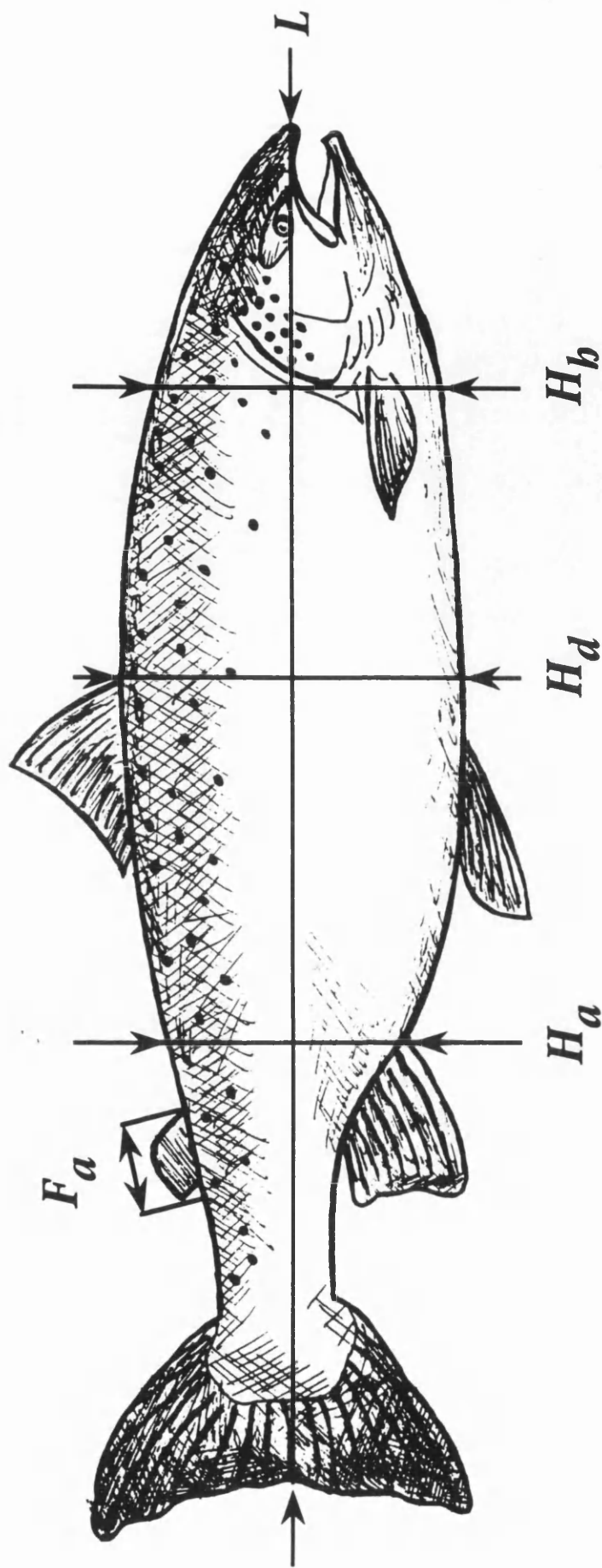
Fig. 1. The positions of biometric measurements taken. F_a=Adipose fin length, L=forklength, H_h=head height (taken just behind the operculum), H_d=dorsal height (taken at the point where the dorsal fin arises from the body), H_a=anal height (taken at the point where the anal fin arises from the body).
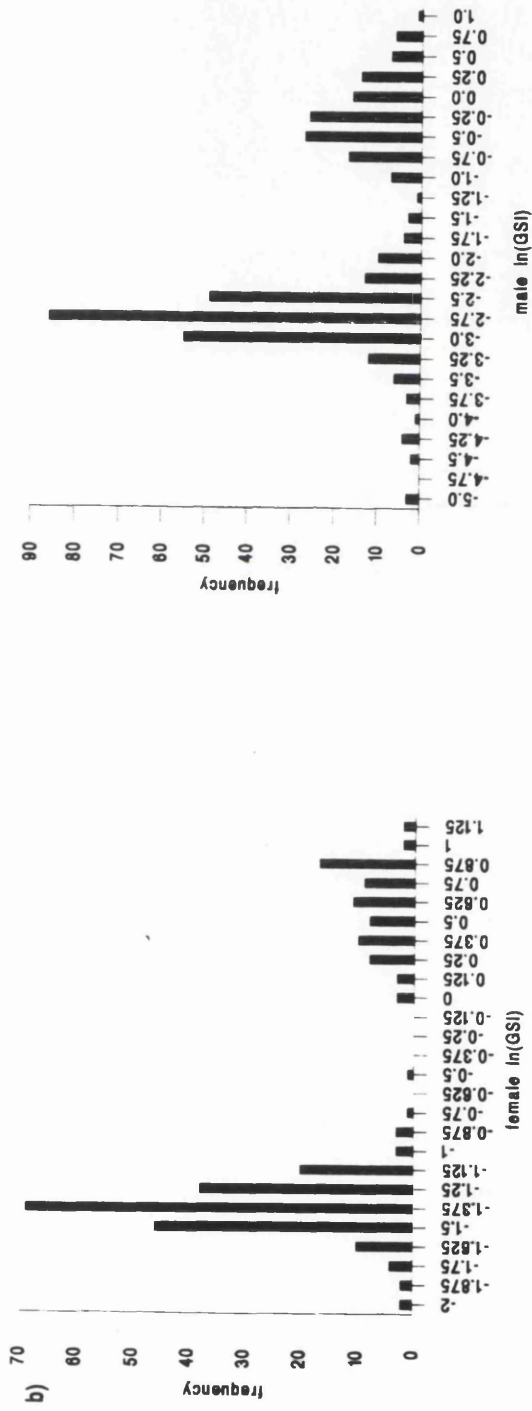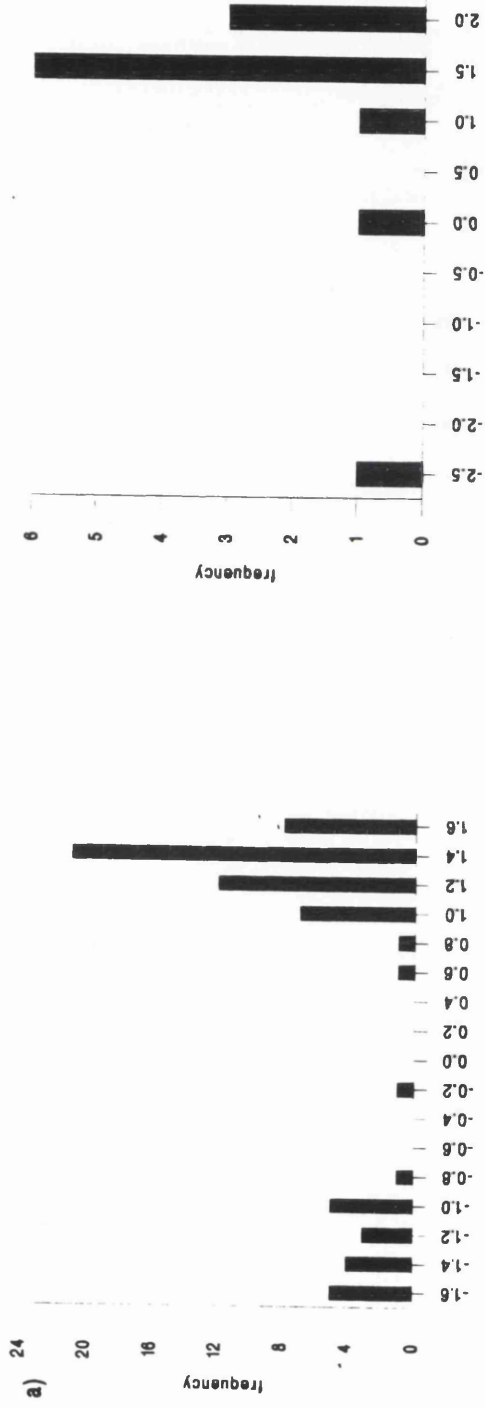
Fig. 2. Frequency histograms of gonadosomatic index (GSI=gonad weight as a percentage of body weight) for males and females from a) Expt. 1 and b) Expt. 2.

Data analysis

Condition factor was calculated as follows (Ricker 1979, Bolger & Connolly 1989):

condition factor (C) = 10,000 x W/L^k

where   W = weight (g)

L = forklength (mm)

k = the slope of the regression of ln(W) on ln(L);

Biometric measurements (except weight and forklength) were standardised for body size by using a variation of Ricker's formula:

$X' = X/L^k$

where X' = the standardised measurement

X = the body measurement in question

L=forklength

k= slope of regression of ln(X) on ln(L)

Stepwise discriminant analysis (SPSS for Windows, see Norusis 1993) was used to identify the external correlates of maturation on each sampling date in Experiment 1 (in which more detailed biometric data were collected). The results of these analyses were then used to identify those variables which provided the strongest and most consistent predictors of maturation. The discriminant analysis was then repeated for these variables alone in both experiments.

RESULTS

Stepwise discriminant analysis showed forklength and condition factor to be the only significant predictors of reproductive status at the earliest sampling point in Expt. 1 (18 May), when the reproductive status of 88.75% of individual fish was predicted successfully. Therefore these two parameters were used in discriminant analyses for each sampling date in Expt. 1 and from May onwards in Expt. 2. (Table 1; less than 60% of fish were classified correctly by discriminant analysis at the prior sampling date in February).

Table 1. Results of stepwise discriminant analysis predicting reproductive status from forklength and condition factor. var. ent.=variables entered by stepwise procedure; dir. for mat.=direction (positive or negative) of variable for maturing fish; % corr. class.=percentage of cases correctly classified; n_m=sample size (maturing); n_i=sample size (immature). *neither variable was entered, % correctly classified based on analysis where both variables were forced into the discriminant equation.

Experiment 1.

date	var. ent.	dir. for mat.	% corr. class	n_m	n_i
18 May	cf, fkl	+	88.75	61	19
9 June	cf, fkl	+	93.67	61	19
24 June	cf, fkl	+	95.00	61	19
8 July	cf, fkl	+	92.50	61	19
21 July	cf, fkl	+	90.00	60	19
5 Aug.	-	+	53.75*	61	19

Experiment 2.

date	var. ent.	dir. for mat.	% corr. class	n_m	n_i
10 May	cf.	+	88.54	39	118
21 June	cf, fkl	+	93.71	52	107
28 July	cf, fkl	+	89.81	20	443
					5

116

Forklength and condition factor predicted reproductive status correctly in 89-95% of the fish at the two sites between May and July, but neither variable proved significant for discrimination by 5 August, when only 53% of individuals were classified correctly. The relationship between forklength and condition factor at the time of strongest prediction levels and at the end of each trial for maturing and immature fish is shown in Fig. 3. When comparing the discriminatory power of these two variables with that of condition factor alone, the combination of forklength and condition factor was found to improve prediction level on all sample dates, with the difference in discrminatory power ranging from 2-9%.

Anal height and dorsal height also featured prominently in the original discriminant analyses (see 'Materials and methods' section) and thus I performed another set of discriminant analyses using these measurements alone for the three dates in Expt. 1 on which both measurements were taken (Table 2).

Table 2. Results of stepwise discriminant analysis predicting reproductive status from anal and dorsal heights (H_a and H_d respectively). See Table 1 for definition of column headings. n_m=61, n_i=19.

date	var. ent.	dir.	for mat.
24 June	H_a	+	90.00
8 July	H_a	+	86.25
5 Aug.	H_a	+	96.25
	H_d	-	

Anal height alone was a significant predictor of reproductive status in June and July, giving correct classification for approximately 90% of fish in both cases. On 5 August both anal and dorsal height were entered to give a discriminant function which classified 96.25% of the fish correctly, at a time when forklength and condition factor no longer had any discriminatory power. Head height and adipose fin length did not prove to be strong predictors of maturation, presumably because these measurements are closely associated with sexual dimorphism (Bodington 1987, Naesje et al. 1988) and could thus not serve as general predictors of maturation for both sexes.

Having derived apparently useful discriminant functions for the purpose of assessing the reproductive status of fish on the basis of secondary characteristics, I then applied functions derived from forklength and condition factor of fish at one site to fish at the other site (Table 3).

Table 3. Results of application of discriminant function derived from one experiment to data obtained in the other. See Table 1 for sample sizes and definition of column heading.

(a)

Discriminant function from Expt. 1 applied to Expt. 2		
Expt. 1	Expt. 2	
date	date	% corr. class.
18 May	10 May	83.44
24 June	21 June	81.76
21 July	28 July	34.41

(b)

Discriminant function from Expt. 2 applied to Expt. 1		
Expt. 2	Expt. 1	
date	date	% corr. class.
10 May	18 May	23.75
21 June	24 June	78.75
28 July	21 July	23.75

The functions derived from Expt. 1 were moderately good predictors for individuals in Expt. 2 in May and June but poor in July (see Table 3a). Functions derived from Expt. 2 gave poor predictions of reproductive status for fish in Expt. 1 in May and July (24% classified correctly), but were better in June (see Table 3b).
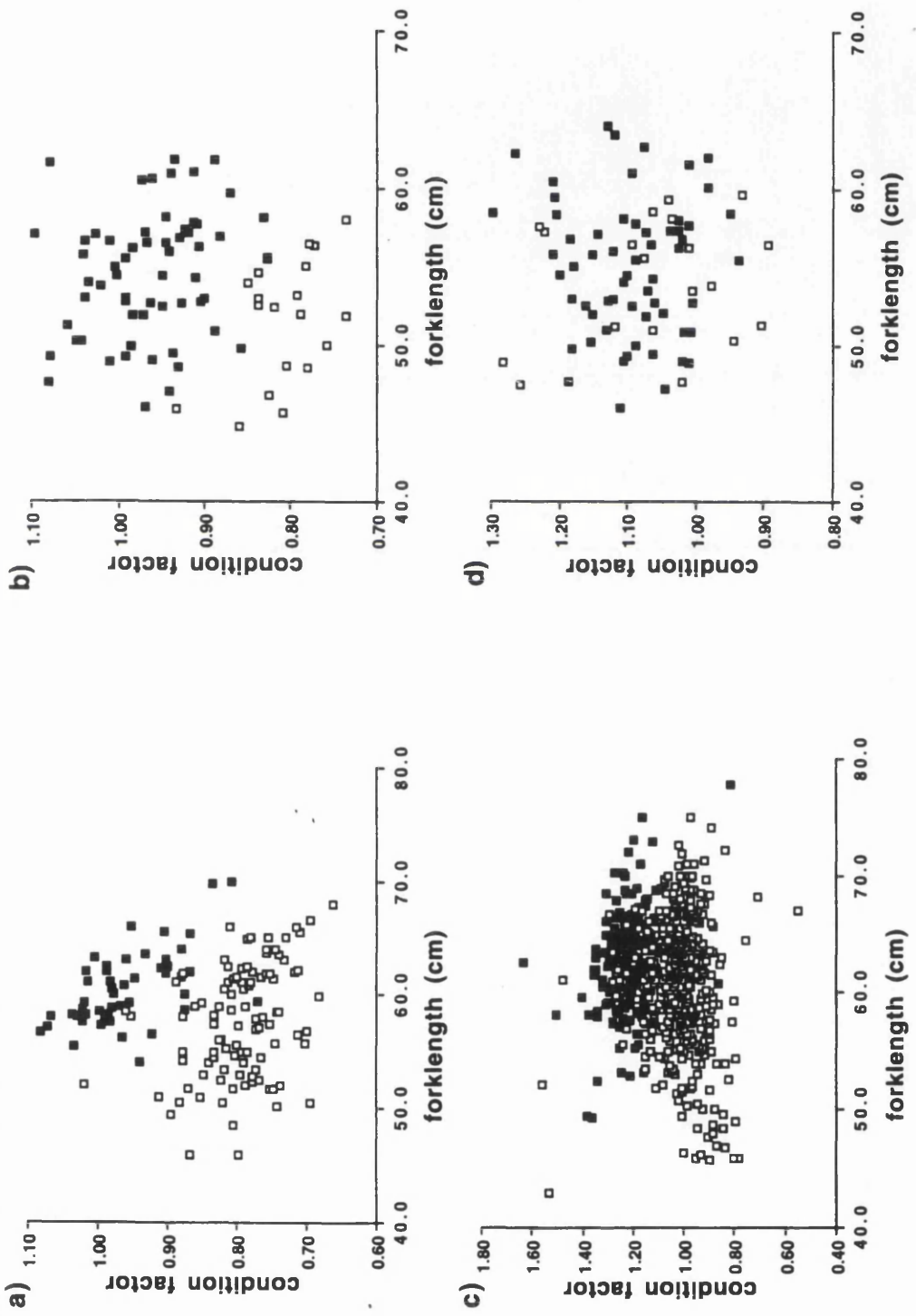
Fig. 3. The relationship between forklength and condition factor on selected dates through the trials for maturing (*m*) and immature (*i*) fish: a) June 21 Expt. 2, n_m=52, n_i=107; b) June 24 Expt. 1, n_m=61, n_i=19; c) July 28 Expt. 2, n_m=205, n_i=443; d) August 5 Expt. 1, n_m=61, n_i=19. Closed squares represent maturing fish, open squares represent immature fish.

119

DISCUSSION

Forklength and condition factor are strong predictors of reproductive status of individuals from at least May until late July, with the discriminant analysis being most accurate in late June in both studies (reproductive status correctly predicted for 93.7 and 95.0% of individuals respectively). Data from V indicate that late June would also have been the optimal time for maturing fish to be harvested, as they were heaviest and in best condition at this time, shortly before becoming anorexic and rapidly losing weight. On August 5, forklength and condition factor were no longer significant predictors of reproductive status and the function produced predictions barely better than chance. This is assumed to be due to the anorexia of maturing individuals and concomitant increased growth in immature fish (VII, VIII).

Anal height alone was also a strong predictor of reproductive status for fish in Expt. 1 in both June and July (dorsal height having been excluded from the function), with maturing fish having deeper bodies. However it did not prove as accurate a predictor as forklength and condition factor at any of the sampling points concerned. By August however, anal and dorsal height together gave a very strong prediction of maturity. This comes, however, at a time when reproductive status can be very easily and accurately assessed by eye due to changes in skin and fin coloration as well as body dimensions of maturing fish. Bodington (1987) found correlations between GSI and body depth parameters (head, dorsal and anal heights, but especially the latter) for both male and female adult Atlantic salmon in April and May, indicating that anal height could thus be expected to be a strong predictor of reproductive status in one-sea-winter salmon from this time of year onward.

Applying discriminant functions between stocks met with less success in our trials. There are many possible reasons for this, one being morphological differences between stocks, which have already been widely studied in salmonids (sockeye salmon (*Oncorhynchus nerka*) Foerster 1968; pink salmon (*Oncorhynchus gorbuscha*) Berg 1979; brown trout (*Salmo trutta*) Yevsin 1980; Pacific salmon (*Oncorhynchus* spp.) Winans 1984; Atlantic salmon Schaffer *et al.* 1975, Thorpe *et al.* 1981 and Bodington 1987). The two trials described in the present paper were not only carried out on different stocks but also used two sites that differed in their culture conditions and feeding regimes, both of which are known to produce morphological differences in

salmonids from the same stock (Il'enkova & Kazakov 1981, Romanov 1984, Bodington 1987, Currens *et al.* 1989). Hence although the same variables allowed predictions of reproductive status for both trials in the present study, such functions could not necessarily discriminate well in a population of mixed stock. It would thus not be possible to produce a simple mathematical means of discriminating maturing from immature fish that would be robust between stocks, sites, cohorts or rearing conditions. However, suitable parameters to serve as a guide for visual discrimination of reproductive status have been identified.

REFERENCES

Aksnes, A., Gjerde, B. & Roald, S.O. (1986) Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **53**, 7-20.

.

Berg, R.E. (1979) External morphology of the pink salmon, (*Oncorhynchus gorbuscha*), introduced into Lake Superior. *J. Fish. Res. Bd. Can.* **36**, 1283-1287.

Bodington, P. (1987) *A Study on the Feasibility of Externally Sexing Farmed Atlantic Salmon (Salmo Salar) prior to the Development of Secondary Sexual Characteristics*. Unpubl. MSc. thesis, University of Stirling, Scotland.

Bolger, T., & Connolly, P.L. (1989) The selection of suitable indices for the measurement and analysis of fish condition. *J. Fish Biol.* **34**, 171-182.

Currens, K.P., Sharpe, C.S., Hjort, R., Schreck, C.B. & Hiram, W.L. (1989) Effects of different feeding regimes on the morphometrics of chinook salmon (*Onchorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). *Copeia.* **3**, 689-695.

Foerster, R.E. (1968) The sockeye salmon, *Oncorhynchus nerka*. *Bull. Fish. Res. Can.* **162**, 1-422.

Hunt, S.M.V., Simpson, T.H., & Wright, R.S. (1982) Seasonal changes in the levels of 11-oxotestosterone and testosterone in the serum of male salmon, *Salmo salar* L., and their relationship to growth and the maturation cycle. *J. Fish Biol.* **20**, 105-119.

Il'enkova, S.A. & Kazakov, R.V. (1981) Morfologicheskaya kharakteristika zavodskoi molodi prokhodnykh lososevykh ryb roda Salmo. Soobshchenie I. Otsenka fenotipicheskoi izmenchivosti segoletok atlanticheskogo lososya *Salmo salar* L. *Biol. Sel. Ryb* **174**, 15-23. [In Russian]

Jones, J.W. (1959) *The Salmon*. Collins, London.

Maisse, G., Baglinière, J.L., Landry, G., Caron, F., & Roleau, A. (1988) Identification externe du sexe chez le Saumon atlantique (*Salmo salar.* L.). *Can. J. Zool.* **66,** 2312-2315.

McLay, H.A., Youngson, A.F., Wright, R.S. & Johnstone, R. (1992) Effects of rearing density on sexual maturation and growth in sea-cage reared Atlantic salmon, *Salmo salar* L. Aquacult. Fish. Manage., **23,** 353-365.

Naesje, T.F., Hansen, L.P., & Järvi, T. (1988) Sexual dimorphism in the adipose fin of Atlantic salmon, *Salmo salar* L. *J. Fish Biol.* **33,** 955-956.

Norusis, M.J. (1993) *SPSS for Windows Professional Statistics Manual.* SPSS Inc., Chicago.

Ricker, W.E. (1979) Growth rates and models. In: W.S. Hoar, D.J. Randall & E.M. Donaldson (eds.) *Fish Physiology.* Vol. 8. Academic Press, New York, pp. **677-743**

Romanov, N.S. (1984) Effect of culture conditions on skull morphology in smolts of the masu salmon *Oncorhynchus masou* (Breevoort). *Aquaculture* **41,** 147-153.

Schaffer, W.M. & Elson, P.F. (1975) The adaptive significance of variations in life history strategy among local populations of Atlantic salmon in North America. *Ecology* **56,** 577-590.

Scott, A.P. & Sumpter, J.P. (1983) The control of trout reproduction: basic and applied research on hormones. In: J.C. Rankin, T.J. Pitcher & R.T. Duggan (Editors). *Control Processes in Fish Physiology*, Croom Helm, London. pp. 200-220.

Simpson, A.L. (1993) *Investigation of the Factors Influencing Maturation in Atlantic Salmon, Salmo salar L., Parr.* Unpubl. PhD thesis, University of Glasgow, Scotland.

Thorpe, J.E. (1994) Reproductive strategies in Atlantic salmon, *Salmo salar* L. *Aquacult. Fish. Manage.* **25,** 77-87.

Thorpe, J.E. & Mitchell, K.A. (1981) Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness and current management. *Can. J. Fish. Aquat. Sci.* **38,** 1576-1590.

Winans, G.A. (1984) Multivariate morphometric variability in Pacific salmon: technical demonstration. *Can. J. Fish. Aquat. Sci.* **41,** 147-153.

Yevsin, V.N. (1980) Morphometric features of the brown trout, *Salmo trutta morpha fario*, of the Pulong'a River. *J. Ichthyol.* **20,** 16-23.

Youngson, A.F., McLay, H.A., Wright, R.S. & Johnstone, R. (1988) Steroid hormone levels and patterns of growth in the early part of the reproductive cycle of adult Atlantic salmon (*Salmo salar* L.) *Aquaculture* **69,** 145-157.

CHAPTER VII

ANOREXIA IN ONE-SEA-WINTER ATLANTIC SALMON DURING SUMMER, ASSOCIATED WITH SEXUAL MATURATION

INTRODUCTION

Atlantic salmon (*Salmo salar*) that mature after one winter at sea present a husbandry management problem for fish farmers. They grow faster than their immature counterparts during the spring, and then stop growing at some stage in the summer (Aksnes *et al.* 1986). They must then be harvested, before they lose condition and acquire their characteristic 'dirty' coloration. The fish which remain immature on the other hand, lose condition during spring or early summer, and then recommence growth during late summer or early autumn (Vestfossen *et al.* 1991). The loss of condition by immature fish during the early part of the year may be due to the aggressive feeding observed in maturing fish at this time of year (Tuene *et al.* unpubl.). Given the problems of this division in one sea-winter salmon populations during spring and early summer, I need to identify the causes and timing of reduced growth with a view to making early predictions of which individuals are likely to mature, thus allowing farmers to manage their stocks more effectively. There is very little published information available on this subject, and so there is a need for simple studies to provide a baseline for future work. The present paper describes an experiment that investigated the occurrence and timing of the cessation of feeding in maturing fish, carried out at a site in the west of Scotland.

MATERIALS AND METHODS

Ninety-six one sea-winter Atlantic salmon were stocked in an outdoor, 5 m circular tank on 18 May 1993 at Otter Ferry Salmon farm. The fish were selected on the basis of visible secondary sexual characters such that the group was composed of ~80% maturing fish. The fish were tagged using visible implant (VI) tags to allow individual identification. Thirty individuals from among this group were selected to be monitored for appetite through the summer. On 9 June, and at fortnightly intervals thereafter until 5 August, appetite was monitored using X-radiography and food labelled with Ballotini glass beads, as described by Talbot & Higgins (1983), Thorpe *et al.* (1990) and McCarthy *et al.* (1993). On the morning of each sampling day, the fish were fed with labelled food by hand to satiation, and by a trickle feeder (Dansk Ørredfoder A/S) thereafter for 4 h, at which point the feeder was turned off and sampling began. All 96 fish were retrieved, anaesthetised individually, identified and weighed. Fish selected for appetite monitoring were X-radiographed (Todd Research 80 mV portable X-ray Unit), using an exposure time of 4 sec. The quantity of food eaten by individuals was calculated for each sample day and transformed to a percentage of body weight to give an indicator of relative appetite. Water temperatures were recorded on each sampling day (Fig.1). The fish were killed at the end of the experiment to determine their maturity status
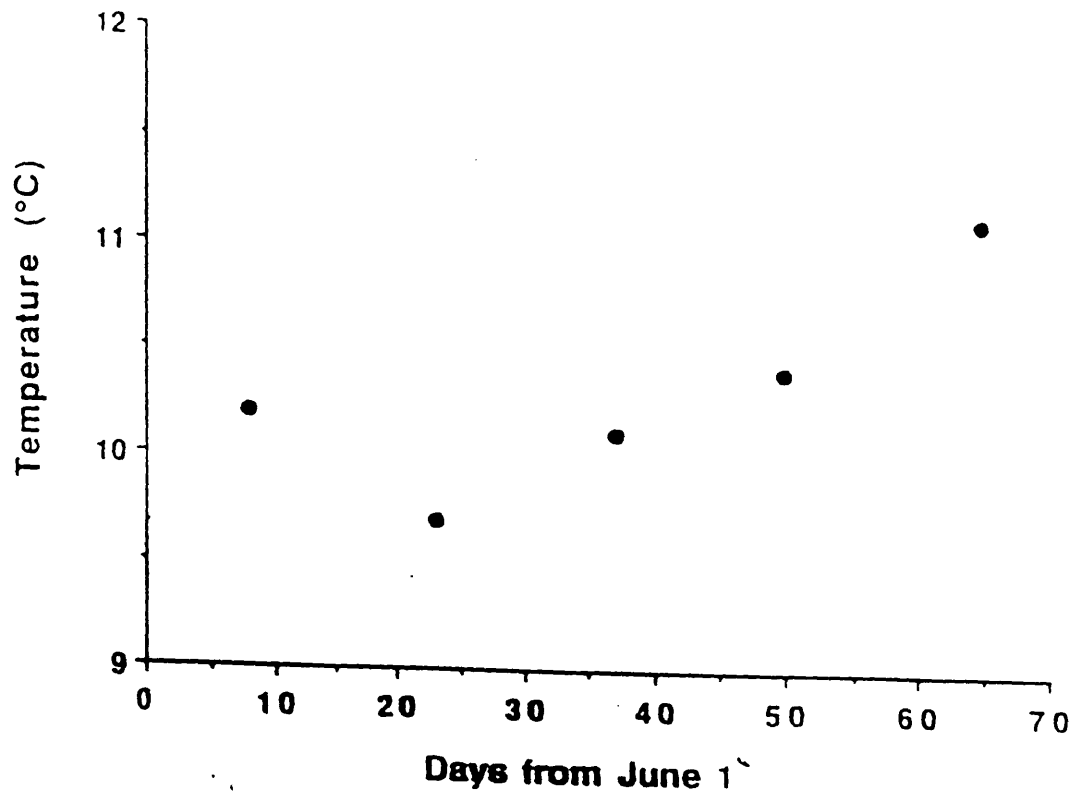
Fig.1. Water temperatures at the experimental site on sampling dates.

RESULTS

Amongst the sampled fish, 26 were maturing and 4 immature. One maturing fish died during the second sampling session, and so was excluded from the analysis. Eleven maturing fish did not feed throughout the sampling days. These fish were excluded from those analyses used to illustrate change in appetite through the summer. For the remaining 18 fish, intake was expressed as percentages of an individual's maximum appetite (intake) throughout the sampling period. Maturing fish showed a decrease from maximum appetite on 9 June to little or no feeding one month later (Fig. 2), while immature fish showed an opposite trend (Fig. 3). Mann-Whitney U-tests showed significant differences in appetite between maturing fish and immature fish on 9 June ($P<0.05$), 21 July and 5 August ($P<0.001$), but no differences on 24 June and 8 July. The frequency distribution of appetite within the maturing and immature subpopulations at the beginning and end of the sampling is given in Fig. 4. The water temperature during the study was lowest on 6 June (8.8°C) and then increased to 11.2°C by 5 August (Fig.1).
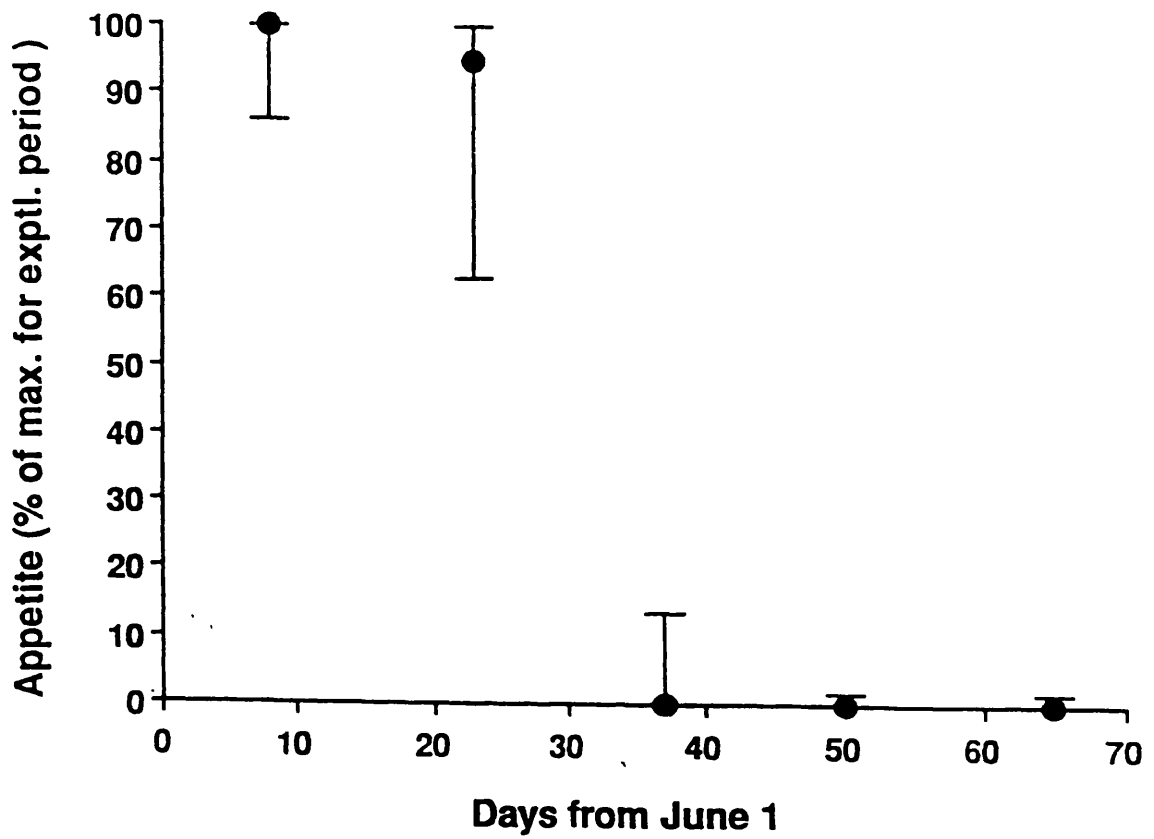
Fig.2. Variation in appetite over summer in maturing fish. Data presented as medians (± semi-interquartile ranges), n=15.
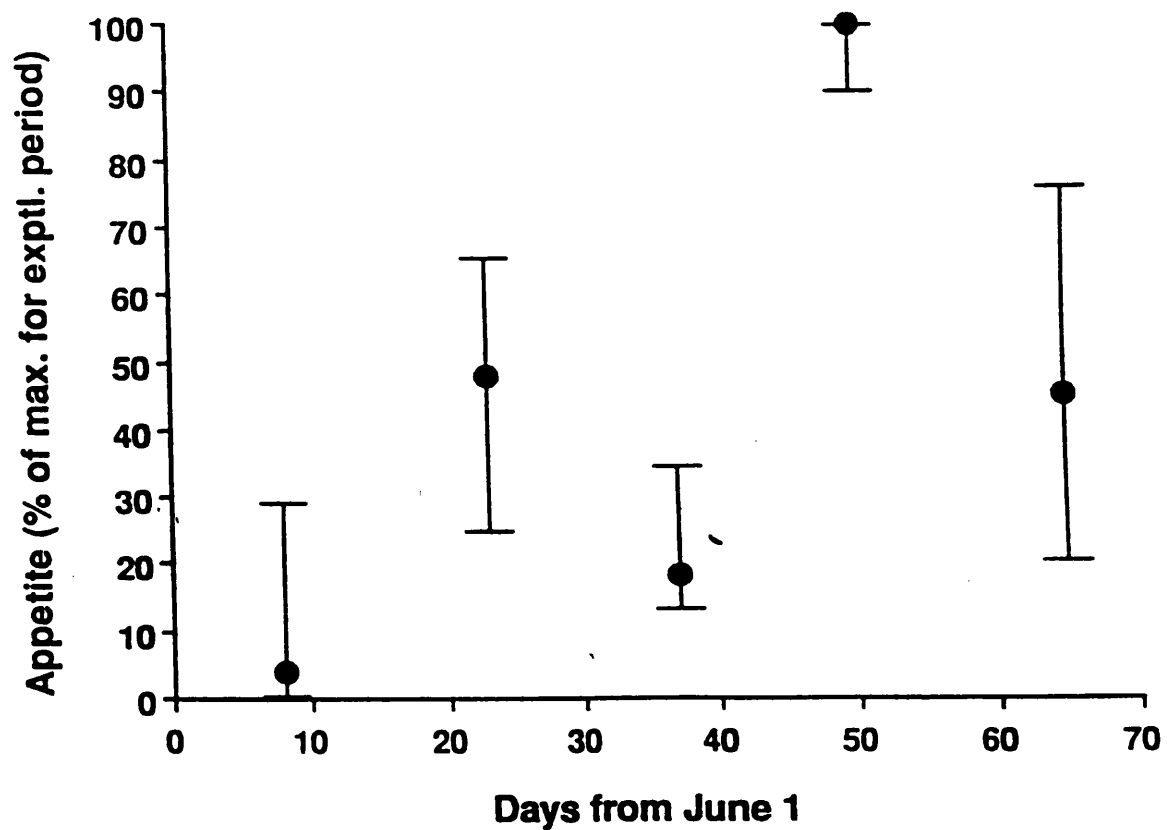
Fig. 3. Variation in appetite over summer in one-sea-winter immature salmon. Data presented as medians (± semi-interquartile ranges), n=4.
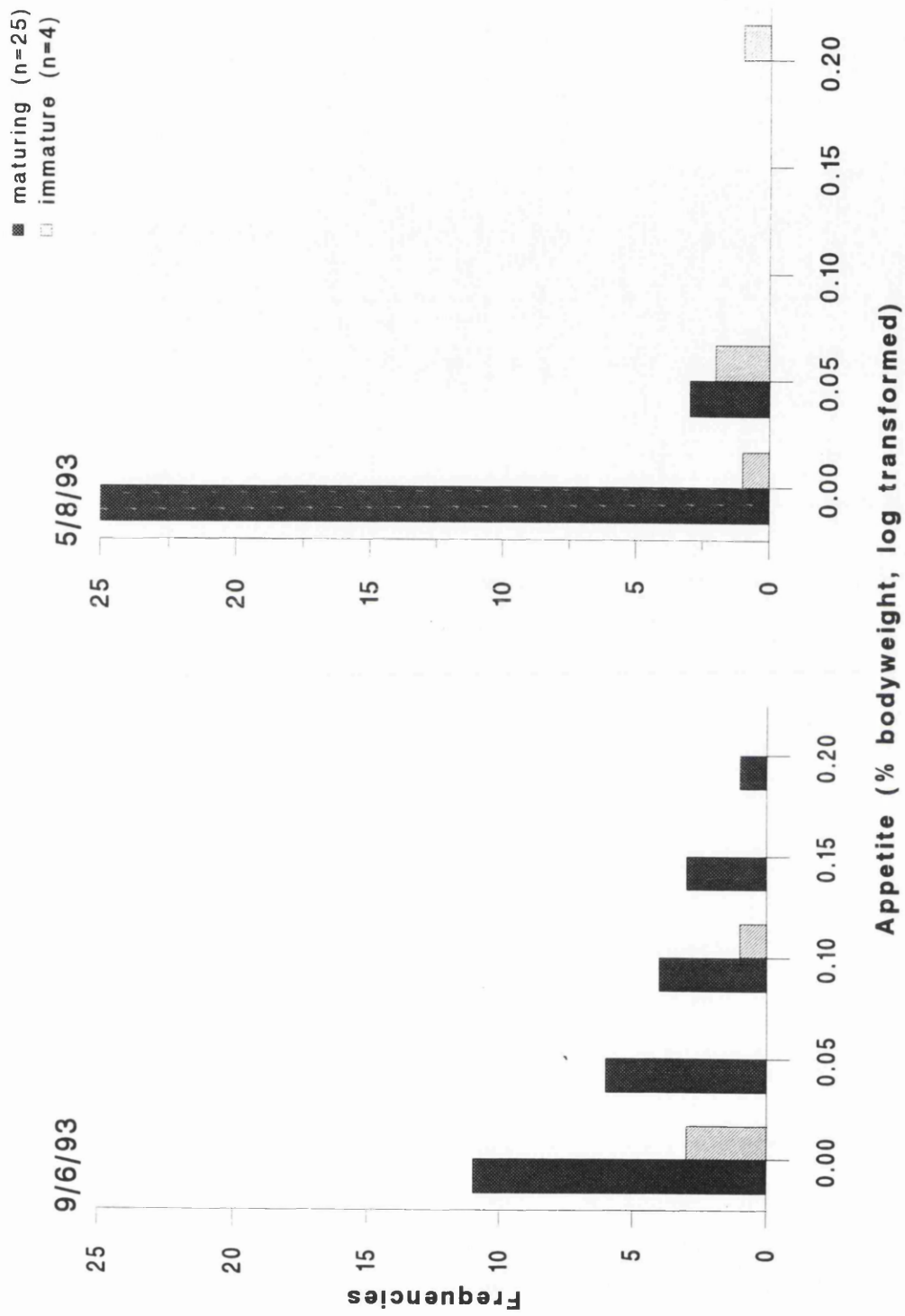
Fig.4. Appetite frequency distributions on the first and last sampling days.

132

DISCUSSION

In the wild, upriver migrating adult Atlantic salmon do not feed, and in caged mature fish a similar drop in appetite parallels this behaviour. Recent studies have suggested that fish may be able to monitor their energy stores internally, and so control their appetite and food intake accordingly (Thorpe 1986, Metcalfe & Thorpe 1992, Jobling & Miglavs 1993). In the case of maturing Atlantic salmon it may be that individuals use a genetically determined threshold fat level as a cue for the cessation of feeding. In the wild, this would provide them with a means of ensuring that they had sufficient energy stores to complete their upriver migration and to meet the costs of spawning (see Jonsson *et al.* 1991).

During the summer the reduction of appetite in maturing fish appears to be independent of ambient water temperatures, while immature fish increase appetite as temperature rises. The number of anorexic maturing fish increased through the summer, until only a few individuals were eating (and they were eating a very small amount) by late summer (Fig. 4). This shows that the maturing population does not cease feeding simultaneously, and since they do not mature simultaneously either, this reinforces the suggestion that anorexia is dependent on an internal developmental cue.

REFERENCES

Aksnes, A., Gjerde, B. and Roald, S.O. (1986) Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **53**, 7-20.

Jobling, M. and Miglavs, I. (1993) The size of lipid depots - a factor contributing to the control of food intake in Arctic charr, *Salvelinus alpinus* ? *J. Fish Biol.* **43**, 487-489.

Jonsson, N., Jonsson, B. and Hansen, L.P. (1991) Energetic cost of spawning in male and female Atlantic salmon (*Salmo salar* L.). *J. Fish Biol.* **39**, 739-744.

McCarthy, I.D., Houlihan, D.F., Carter, C.G. and Mouton, K. (1993) Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. *Proc. Nutr. Soc.* **52**, 427-436.

Metcalfe, N.B. and Thorpe, J.E. (1992) Anorexia and defended energy levels in overwintering juvenile salmon. *J. Anim. Ecol.* **61**,175-181.

Thorpe, J.E. (1986) Age at first maturity in Atlantic salmon, *Salmo salar* L.: freshwater period influences and conflicts with smolting. In: D.J. Meerburg (ed.) *Salmonid Maturation. Can. Spec. Publ. Fish. Aquat. Sci.* **89**, 7-14.

Thorpe, J.E., Talbot, C., Miles, M.S., Rawlings, C.E. & Keay, D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **90**, 41-47.

Vestfossen, K., Taranger, G.L., Stefansson, S.O. and Hansen, T. (1991) Growth and sexual maturation in reared Atlantic salmon (Salmo salar). In: A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe (eds) *Reproductive Physiology of Fish.* Sheffield: FishSymp 91, p.292.

CHAPTER VIII

WHAT CONTROLS THE ONSET OF ANOREXIA IN MATURING ADULT FEMALE ATLANTIC SALMON?

INTRODUCTION

Reproduction amongst fish at higher latitudes is generally a seasonal event, the timing of gonad maturation being controlled by physiological mechanisms with two likely components, an endogenous cycle of gonad development and a mechanism that synchronises this cycle with environmental cues (Wootton 1990), particularly temperature and daylength (Wootton 1982, Lam 1983, Bye 1984, Adams & Thorpe 1989, Scott 1990)

In Atlantic salmon, *Salmo salar* L., spawning generally takes place in autumn. Siblings within a population will not all mature in a given year; the year in which an individual will mature is probably controlled by a condition-dependent switch (Thorpe 1986, 1994). Previous studies have suggested that various cues may be important; these include age-specific body size (McCormick & Naiman 1984), rate of acquisition of surplus energy (Thorpe 1989), absolute energy stores (Thurow 1966, Rowe & Thorpe 1990, Rowe *et al.* 1991) and stage of gonad development (Chadwick *et al.* 1986). Thus, it would appear that an individual must exceed threshold values of one or more of these parameters by a critical time of year to mature.

When an individual does mature, it will return from the marine feeding grounds and enter its home river and migrate to the spawning grounds (Boece 1527, Thorpe 1988). In Scotland, this usually occurs in the summer, (although in some rivers this may occur as early as spring (Mills & Graesser 1981) but may be delayed till early winter (Mills 1989)). Migration and spawning are energetically costly. Individuals can lose 99% of their lipid reserves, 72% of protein and 63% of glycogen during the migration alone (Tillik 1932) and that the energetic cost of spawning itself is over 50% of the fish's remaining energy content (Jonsson *et al.* 1991). Consequently the proportion of adults surviving to spawn again is often small (Mills 1971, Schaffer & Elson 1975).

In spite of the costs, these fish do not feed during the period of several months that they spend in the river system (Grey & Tosh 1894, Calderwood 1907) and have recently been shown to cease feeding before entering freshwater (VII). However, there is surprisingly little information on this dramatic and paradoxical phenomenon of anorexia in adult salmon, as wild salmon are so difficult to track and study at sea. Fish farms provide more favourable conditions for the study of ocean-going salmon, and

there is much anecdotal knowledge amongst fish farmers regarding fish feeding behaviour. However, little quantitative information is available. Maturing fish feed much more actively than immature fish through the spring and early summer prior to spawning (Tuene pers. comm.) until their appetite drops dramatically, at a time that in nature they would be undertaking their upriver migration (VII).

In a pilot study at Kames Fish Farm, Oban, Argyll in 1992 I obtained data on the growth of maturing individual salmon in relation to their condition factor and found a significant negative correlation between the two (Fig. 1). If cessation of growth can be assumed to relate directly to cessation of feeding, these data indicate two key points :

- The cessation of feeding amongst maturing individuals is asynchronous. This was confirmed in VII.

- Cessation of feeding appears to occur when salmon achieve a given condition factor.

These findings suggest an adaptive response whereby fish only terminate feeding when they have acquired the reserves necessary for migration and reproduction. However, it is not clear what condition factor measures, since it is known to have a weak association with lipid levels (Pinder & Eales 1969, Herbinger & Friars 1991, Simpson *et al.* 1992) and may also be correlated with gonad development and mass of muscle protein, both of which may be increased prior to migration.

This paper reports on a study designed to clarify the relationships between body reserves and the onset of anorexia in maturing sea-run salmon. Specifically our aims were:

1)-to investigate whether condition factor in maturing fish was a measure of the extent of body lipids, gonad development and/or lean mass.

2)-to separate the effects of gonad development, body lipids and lean mass upon appetite.

3)-to examine the relationship between body lipids, gonad development and lean mass in midsummer and subsequent rates of change in these variables and in particular to test whether there is a threshold level of body reserves at which the fish cease to feed.
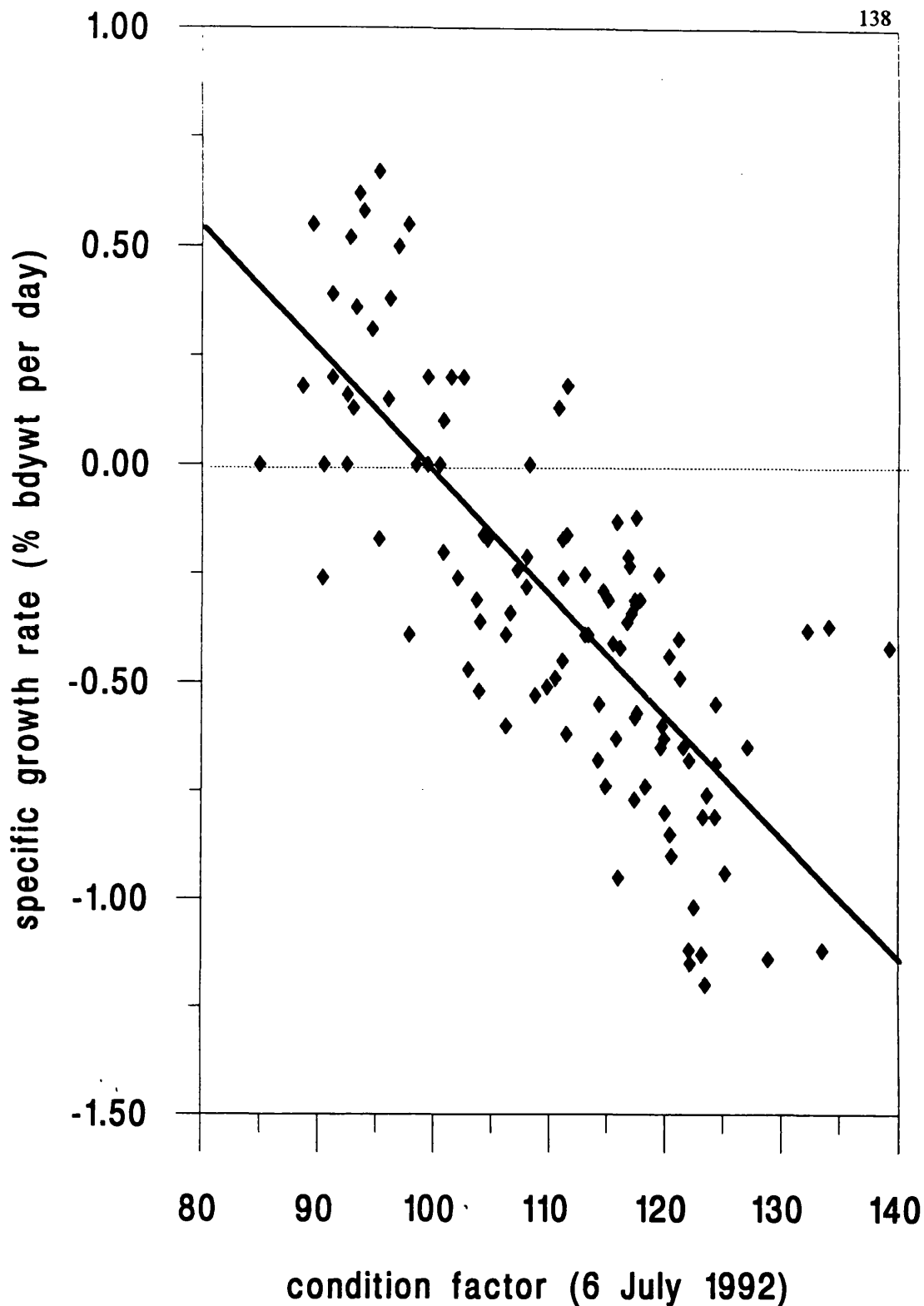
Fig. 1. The relationship between midsummer growth rate (% per day 6 July - 3 August) and condition factor (W/L^k where: W = weight, L = forklength & k = the slope of the regression of ln(W) on ln(L)) on 6 July for maturing Atlantic salmon in sea cages. (r^2=0.60, n=109, P<0.001). Dotted line represents zero growth, below which all values represent a weight loss.

MATERIALS AND METHODS.

One hundred one-sea-winter Atlantic salmon (mean weight = 1.70 kg) were stocked in a 5 m circular sea water tank at Otter Ferry Fish Farm, Argyll, Scotland on 11 May, 1993. The fish had been selected such that approximately 80% of the population could be expected to mature on the basis of secondary sexual characteristics (coloration and shape). The fish were hand fed to excess twice per day and 'trickle fed' all day by automatic feeder throughout the 3 month trial period using BP Nutrition Size 5 salmon pellets.

On May 18, all fish were marked individually using Visible Implant (VI) tags (Northwest Marine Technology). On the same day, and fortnightly from then on, the forklength (to the nearest mm), weight (to the nearest 10g) and more detailed biometric measurements were collected from each individual (Fig. 2) to allow non-invasive estimates of body lipid reserves and gonadosomatic index (GSI, gonad weight as a percentage of total weight) to be calculated (using the same techniques as Simpson *et al* 1992).

A random selection of 30 individuals was used in fortnightly monitoring of appetite by x-radiography using the normal commercial food labelled with Ballotini glass beads as described by Talbot & Higgins (1983), Thorpe *et al* (1990) and McCarthy *et al* (1993). Details of the methods are described in VII. Labelled food was delivered to satiation by hand initially and thereafter for four hours by automatic feeder, on the morning of each sample day, after which the 30 fish were x-radiographed. The quantity of food (g) eaten by each individual was calculated for each sample day and expressed as a percentage of body weight. The relationship between food intake and growth rate for these x-rayed fish was then used to estimate the food intake of the remaining fish (see below).

The trial finished on 11 August, when the fish were all killed, identified, sexed (13.7% male 86.3% female) and their gonads weighed. Since the proportion of males was so small and the sexes cannot be combined due to differences in gonad size, all subsequent analyses were for females alone. 73.9% of the female fish were found to be maturing (i.e. belonging to the upper modal group of gonad weight distribution, with a gonadosomatic index greater than 1.8%). Body lipid levels (i.e. lipid stores associated with the muscle mass) were estimated on the same day by placing a Torry
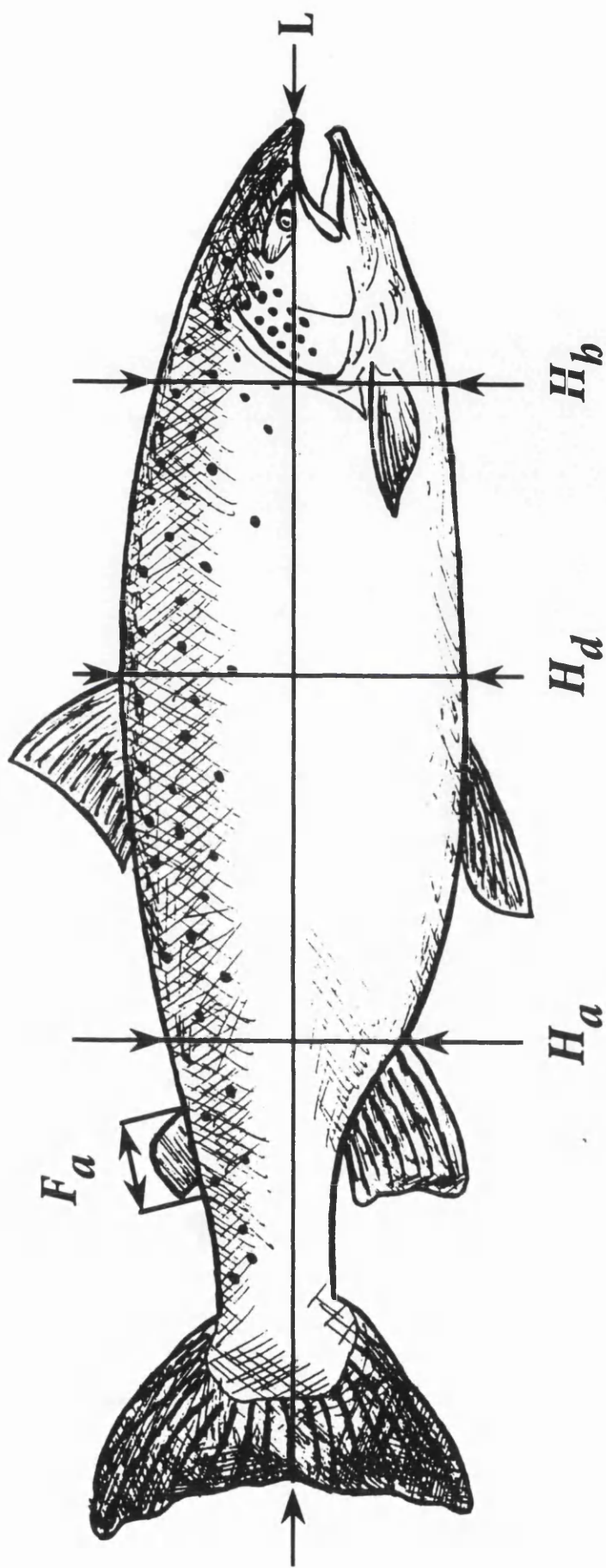
Fig. 2. The positions of biometric measurements taken. F_a=Adipose fin length, L=forklength, H_h=head height (taken just behind the operculum), H_d=dorsal height (taken at the point where the dorsal fin arises from the body), H_a=anal height (taken at the point where the anal fin arises from the body). N.B. Measurements were also made of head length, dorsal width and anal width, but these were not found to contribute significantly to models described below.

140

Fat Meter (Distell UK Ltd.) at four positions on the left flank of each fish and using the mean of these four fat readings as an index of percentage body lipid as described by Kent (1990). The following parameters were calculated using the data collected:

condition factor (C_f) = W/L^k [Ricker (1979), Bolger & Connolly (1989)].

where   W = weight

L = forklength

k = the slope of the regression of ln(W) on ln(L);

specific growth rate (G = % gain in weight per day) = 100 x $\dfrac{[\ln W_f - \ln W_i]}{t}$

where W_f = final weight

W_i = initial weight

t = time elapsed in days

Condition factor alone explained only 23% of the variance in percentage body lipid (n=69, P<0.001) and less than 3% of the variance in GSI (n=69, ns). However, multiple regression analyses of body lipid levels and gonadosomatic index on combinations of the other morphometric measurements (Fig. 2) in addition to weight, forklength and condition factor gave good predictions. The body measurements were standardised for forklength by using a variation of Ricker's formula:

X' = X/L^k

where X' = the standardised measurement

X = the body measurement in question

L=forklength

k= slope of regression of ln(X) on ln(L)

The equations for these models are as follows:

estimated % body lipid (L_e) = 4.61W + 8.71H_a-12.86 r^2=0.730, n=69, P<0.001

where   W = weight

estimated relative gonadosomatic index = $44.3F_a + 13.8C_f - 21.0H_d + 14.4H_a + 0.42$

$r^2=0.717$, n=69, P<0.001

See Fig. 2 for definitions of H_a, F_a & H_d.

Using the above models, estimated % body lipid and estimated relative gonadosomatic index were calculated for June 24 (the first date on which all the relevant morphometric measurements were taken).

I also calculated an index of 'estimated relative lean mass' defined as the residual C_f not accounted for by body lipids. (It was not necessary to control for GSI since this was not significantly related to condition factor). Estimated relative lean mass was calculated by subtracting expected C_f (as defined by a regression equation predicting condition factor from estimated % body lipid; $C_f=0.87 + 0.0135L_e$, $r^2=0.15$, n=51, P=0.003) from observed C_f. Fish with positive values for estimated relative lean mass thus have relatively high levels of lean reserves while those with negative values have relatively low levels of lean reserves.

There was a strong positive correlation between fish size (forklength) and % body lipid ($r^2=0.70$, n=51, P<0.001), hence 'estimated relative lipid' was calculated to control for fish size. Estimated relative lipid is the residual % body lipid from the regression of estimated % body lipid on forklength..

These calculations produced three independent parameters (estimated relative lipid, estimated relative lean mass and estimated relative gonadosomatic index), which were then used in subsequent analyses of the effects of body constituents on appetite.

RESULTS.

There was marked variation in the timing of onset of anorexia. Some individuals ceased feeding before the x-raying began on 9 June, and some were still feeding on August 5 when the last x-ray sample was taken (Fig. 3). Individuals that had high condition factors initially (18 May) had the lowest growth rates over subsequent weeks (18 May-5 Aug): G(May 18-Aug 5) = 1.41 - 1.58C_f(May 18); r^2=0.24, n=50, P<0.001, as found with the pilot data in 1992 (Fig. 1).

There was a strong curvilinear relationship between appetite and growth rate in the x-rayed sample (Fig. 4). The y intercept (i.e. zero appetite) of the regression equation was -0.113, indicating that once fish had stopped feeding they would lose weight at a mean rate of 0.113$\pm$0.016 (mean $\pm$S.D.) % per day. The frequency distribution of weight changes for the complete sample of maturing females (including fish not x-rayed) was bimodal, with the lower modal group corresponding to fish experiencing weight losses of 0.08-0.15% per day (Fig. 5). The mean weight loss of this group was 0.116$\pm$0.022% (mean$\pm$S.D.) per day, not significantly different to the 0..113% per day weight loss of known non-feeding fish, suggesting that all fish in this group had stopped feeding. Therefore, fish were classified as having ceased feeding when their daily weight loss exceeded 0.08%. Appetite for all maturing females could then be estimated from their growth rate using a regression relationship based on Fig. 4:

estimated food intake = 0.239 + 2.02G + 1.06G^2 r^2=0.96, n=17, P<0.001

where estimated food intake = rate of food intake (% bodyweight per hour); values of less than zero were recorded as zero and the resulting data arcsine transformed. Fish that had already ceased feeding prior to the beginning of the study (i.e had an overall estimated food intake < 0.005 % bodyweight per hour, n=29 fish) were excluded from further analyses, since they would already have depleted some of their body reserves, so adding noise to any relationships between the extent of body reserves and the timing of the onset of anorexia.
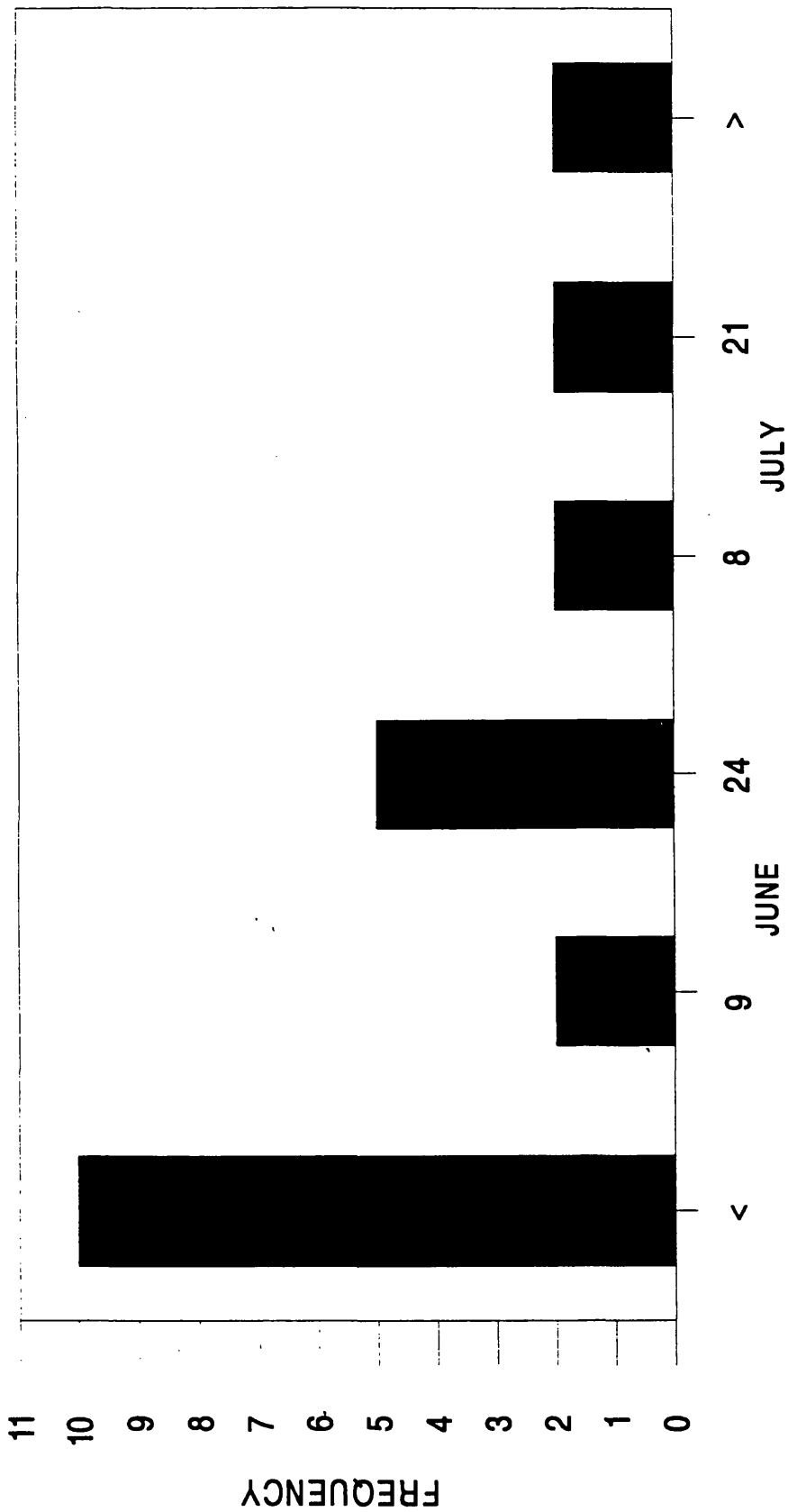
Fig. 3. Frequency histogram of last day of recorded feeding for maturing salmon whose appetite was monitored at fortnightly intervals. First and last columns show fish which had ceased feeding before June 9 and were still feeding on August 5 respectively.

144
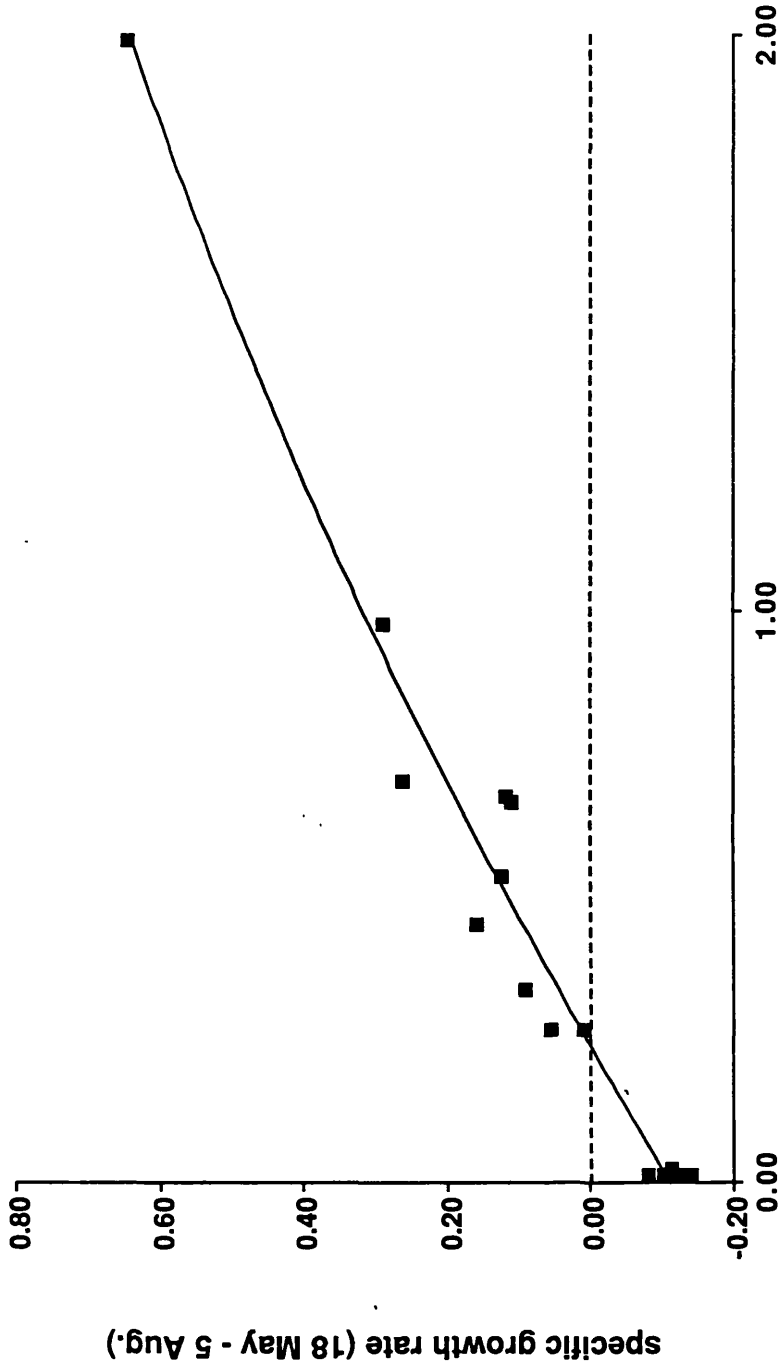
Fig. 4. The relationship between measured appetite (total eaten on sample days, expressed as a percentage of bodyweight) and growth rate (% per day) of maturing female salmon over the trial period. $r^2=0.96$, n=17, P<0.001. Dotted line represents zero growth, below which all values represent a weight loss.

Fig. 5. Frequency distribution of specific growth rates(% per day) for maturing female salmon. Note the bimodal pattern, with the lower mode corresponding to fish that have ceased feeding.

146

I found significant negative relationships between estimated food intake over the period June 24 - August 5 and estimated relative lipid, estimated relative lean mass and estimated relative gonadosomatic index on 24 June (Fig. 6). Loss of appetite thus coincided with high body lipid and lean reserve levels and well-developed gonads. The observed differential drop in appetite suggests that it is determined to some extent by these three parameters. However, there are significant correlations between estimated relative lipid and estimated relative gonadosomatic index, estimated relative lipid and estimated relative lean mass, and estimated relative lean mass and estimated relative gonadosomatic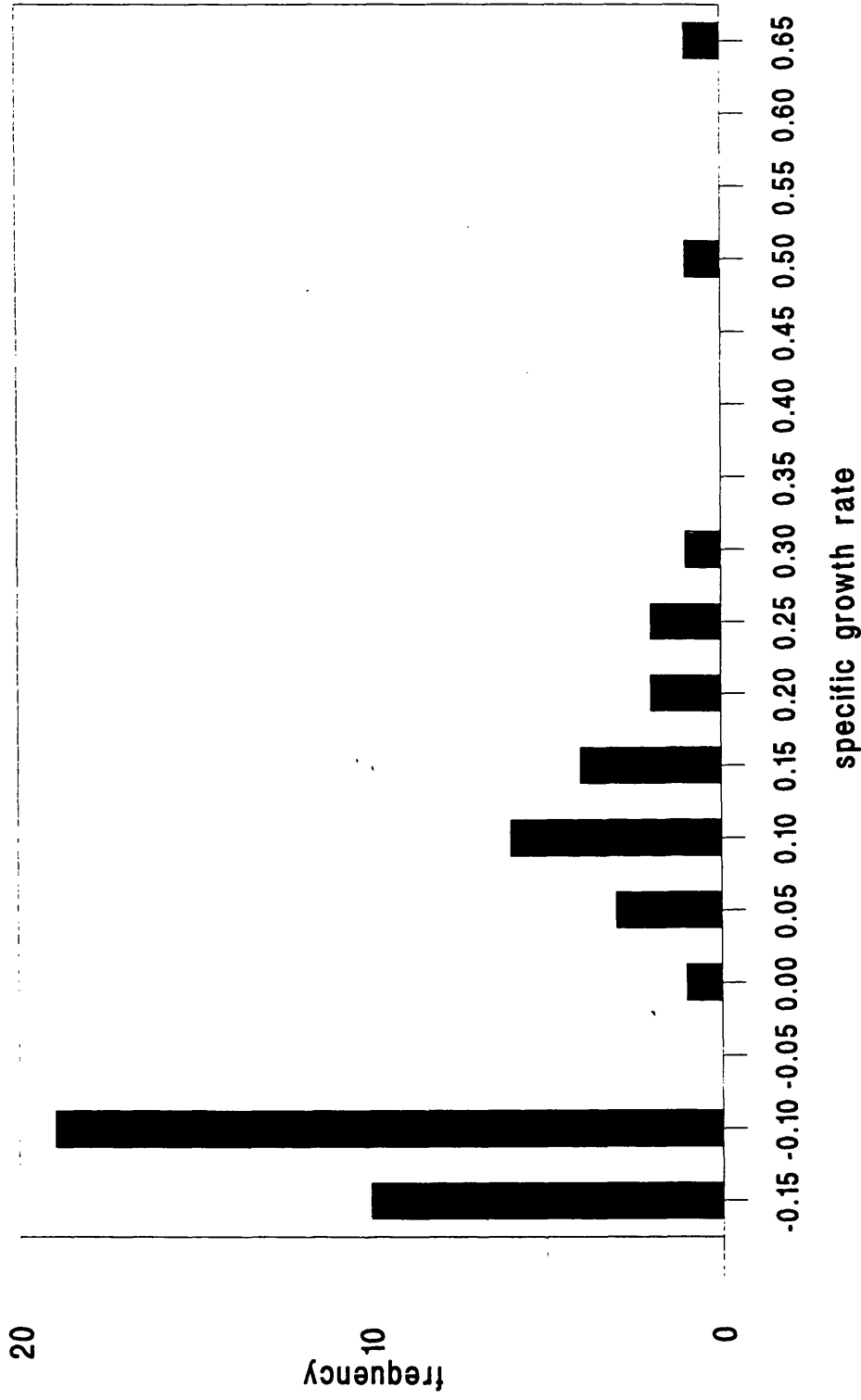 index (Fig. 7). I then compared the values of estimated relative lipid, estimated relative lean mass and estimated relative gonadosomatic index on 24 June with their counterparts on 5 August (Fig. 8). In all three cases there was a significant negative relationship, indicating that fish with low levels of these body reserves continued to build them up rapidly, while those with well developed gonads, fat reserves and/or lean mass reduced and eventually ceased investment in them. It was thus necessary to tease apart these parameters and thereby determine their relative importance with regard to variation in appetite. A stepwise regression of estimated intake (24 June - 5 August) on these three predictors showed lean mass to be the strongest predictor of appetite, explaining 50% of the total variation, while gonad and body lipid reserves explained an insignificant proportion of the remaining variation (Table 1).

Table 1. Results of stepwise regression of estimated food intake (%bodyweight .h^{-1} during the period 24 June-5 August) by maturing female salmon (n=21) on estimated relative lean mass, estimated relative lipid and estimated relative gonadosomatic index on 24 June.

predictor	r^2	P
estimated relative lean mass	0.50	<0.001
estimated relative gonadosomatic index	-	NS
estimated relative lipid	-	NS
overall	0.50	<0.001
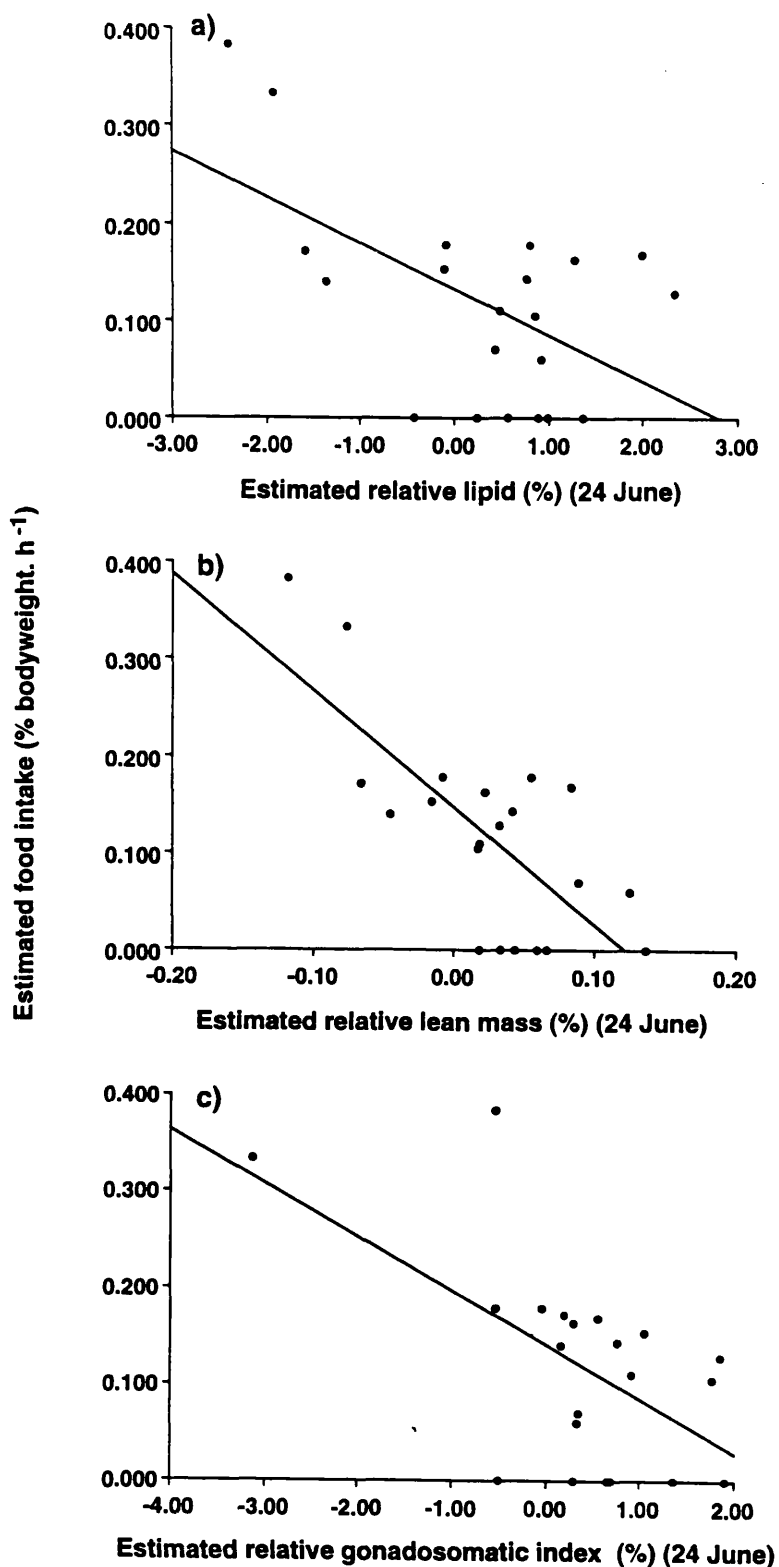
Fig. 6. Relationships between estimated mean rate of food intake (% bodyweight, arc sine transformed) of maturing female salmon (n=21) over the period 24 June - 5 August and a) estimated relative lipid on 24 June (r^2=0.27, P=0.009). b) estimated relative lean mass on 24 June (r^2=0.50, P<0.001). c) estimated relative gonadosomatic index on 24 June (r^2=0.29, P=0.007).
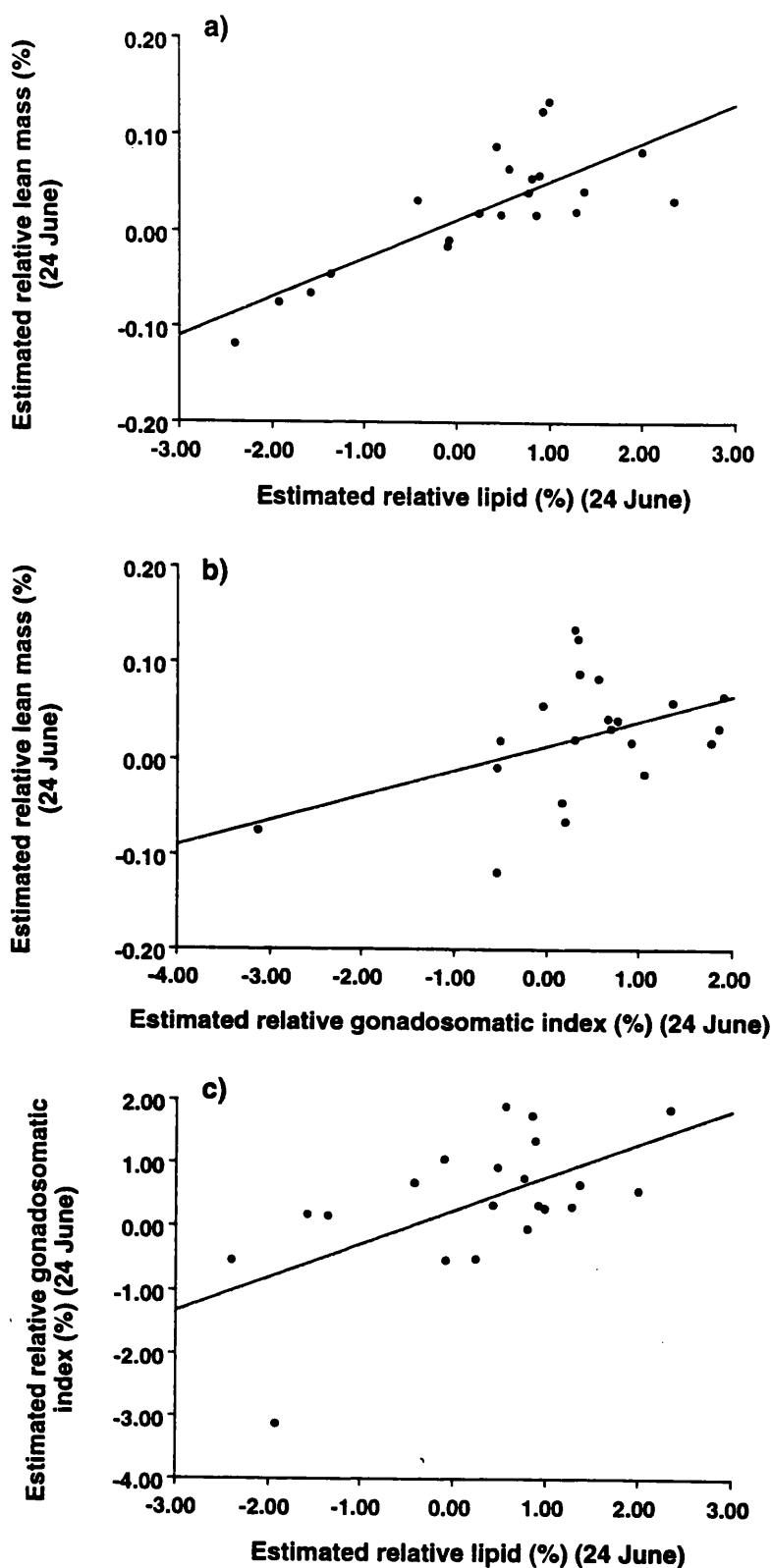
Fig. 7. Relationships between different components of body composition in maturing female salmon (n=21) on 24 June a) estimated relative lean mass and estimated relative lipid (r^2=0.61, P<0.001) b) estimated relative lean mass and estimated relative gonadosomatic index (r^2=0.16, P=0.043) c) estimated relative gonadosomatic index and estimated relative lipid (r^2=0.33, P=0.004).

Fig. 8. Relationships between a) estimated relative lipid (r^2=0.51, P<0.001) b) estimated relative lean mass (r^2=0.23, P=0.016) c) estimated relative gonadosomatic index (r^2=0.18, P=0.031) on June 24 and their respective changes during the period 24 June - 5 August in maturing female salmon (n=21).

DISCUSSION.

The timing of cessation of feeding in maturing Atlantic salmon varied widely amongst individuals. The spread of dates on which fish were last observed to feed ranged over more than 2 months. Such wide variation suggests th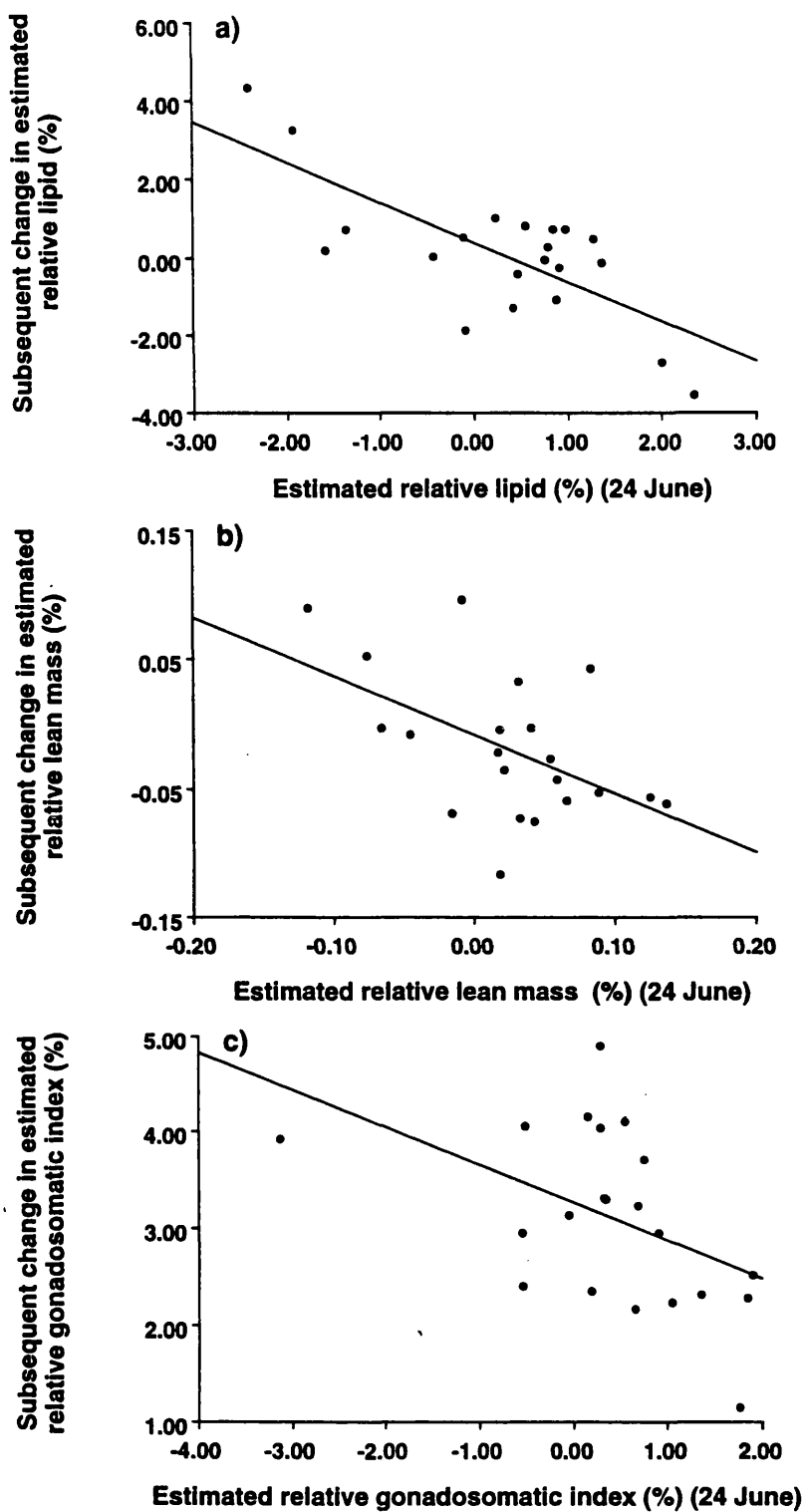e timing of the onset of anorexia is not dependent simply on an environmental cue determining when fish stop feeding and supports the hypothesis of an endogenous mechanism, such as accumulation of a certain level of body reserves.

The strong relationship observed between appetite and specific growth rate (Fig.4) clearly indicates that the observed weight losses are due primarily to a reduction in appetite (as opposed to an increased use of energy reserves to fuel the development of gonads as the spawning season draws closer). Fish that had ceased feeding both before and during the experiment all lost weight at a rate of approximately 0.1% per day, regardless of individual size, weight or condition. These fish continued to swim actively around the tank throughout the study, and so this rate of weight loss may be similar to that of wild fish on their return ocean migration. However metabolic costs may on average be lower once the fish enter their spawning rivers, since they may spend long periods of time motionless in pools conserving energy prior to the final ascent to the spawning grounds (Hawkins & Smith 1986, Laughton 1989,1991, Webb 1989, Berman & Quinn 1991).

I found negative relationships between food intake and estimates of body fat, lean mass and gonad size, suggesting that appetite drops as all three measures increase. The relationships between these three parameters and their respective changes in subsequent weeks indicate that those fish with lower levels of these body reserves increased them at a faster rate than those with higher levels of accumulated reserves. The decline in rate of investment in these reserves as they reach higher levels suggests that there is a genetically-determined target level of body fat, lean mass and gonad development, above which such investment ceases and reserves begin to be utilised. However, the three parameters are inter-correlated and multivariate analyses suggest that gonads and body fat are less important in determining the onset of anorexia, with lean mass the most important. Metcalfe & Thorpe (1992) showed that in overwintering juvenile Atlantic salmon anorexia is regulated by lipid reserves. However at this stage of the life cycle accumulated lipid reserves are needed simply to

stay alive (while the fish remain largely dormant under stones in the stream bed), and gonad and lean reserves are relatively unimportant.

The major component of the increase in lean mass will be muscle protein, which may be needed both for the extra muscular work exerted in a long distance migration and as a protein reserve used to produce eggs (Bradford 1993, Jonsson *et al* 1991, Tillik 1932). However, some of the variation in our measure of 'lean mass' may be due to visceral fat, which is not directly measured by the Torry fat meter. Visceral and carcass fat levels are usually highly correlated, but visceral fat is more mobile and is used as an immediate energy reserve, and has been implicated in the maintenance of maturation in male salmon parr (Rowe & Thorpe 1990); both visceral and carcass fat levels have been shown to be negatively correlated with appetite in Arctic charr *Salvelinus alpinus* L. (Miglavs & Jobling 1993).

A build up of reserves prior to migration to breeding grounds can also be found in other animals, particularly birds, which tend to accumulate large fat stores and increase the size of their flight muscles correspondingly (in order to generate sufficient power to cope with the larger body mass) before leaving their winter feeding grounds (Phillips *et al.* 1985). While not strictly anorexic during migration, arctic-breeding geese may have little scope for feeding upon initial arrival at the breeding grounds, and Ryder (1972) found that successful breeding in the Ross Goose (*Chen rossii*) was dependent on reserves the female had accumulated prior to arrival in the arctic. Similarly Ankney & MacInnes (1978) found that clutch size in Lesser Snow Geese (*Chen caerulescens*) was determined by the size of their remaining nutrient reserves (protein, fat and calcium) on arrival at the breeding grounds.

These findings provide a platform for further work on their evolutionary implications. The control mechanism must reflect the energy requirements for upriver migration and spawning in order to facilitate the accumulation of the necessary reserves before the onset of anorexia, since different stocks have spawning grounds at varying distances from the sea. This threshold level might therefore be expected to vary between populations, such that salmon from shorter river systems would have a low threshold of energy requirements, while fish from very long systems would require large reserves of energy and might even trade gonadal investment for energy reserves in order to complete the migration successfully. This hypothesis is supported by the findings of Beacham & Murray (1993), working on Pacific salmon

(*Oncorhynchus* spp.), who showed that fish spawning in the upper portions of long river systems had a reduced fecundity and egg size compared with coastal spawning populations. Foerster (1968) with sockeye salmon (*Oncorhynchus nerka*) and Schaffer & Elson (1975) and Thorpe & Mitchell (1981) with Atlantic salmon found that age and size at first spawning tended to increase with river length and relative harshness of the upriver migration, possibly due to the need for extra time to build up the large energy reserves required for reproduction under such conditions. It would therefore be interesting to examine the differences between stocks from known spawning areas with respect to relative gonad development and energy accumulation prior to the onset of anorexia.

REFERENCES.

Adams, C.E. & Thorpe, J.E. (1989) Photoperiod and temperature effects on early development and reproductive investment in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **79**, 199-221.

Ankney, C.D. & MacInnes C.D. (1978) Nutrient reserves and reproductive performance of female Lesser Snow Geese. *Auk* **95**, 451-471.

Beacham, T.D. & Murray, C.B. (1993) Fecundity and size variation in North American Pacific salmon (*Oncorhynchus*). *J. Fish Biol.* **42**, 485-508.

Berman, C.H. & Quinn, T.P. (1991) Behavioural thermoregulation and homing by spring chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in the Yakima River. *J. Fish Biol.* **39**, 310-312.

Boece, H.(1527) *A History of Scotland (Scotorum Historiae: Scotorum Regni Descripto, folio XII)* Paris, cited in Mills (1989).

Bolger, T. & Connolly, P.L. (1989) The selection of suitable indices for the measurement and analysis of fish condition. *J. Fish Biol.* **34**, 171-182.

Bradford, R.G. (1993) Differential utilization of storage lipids and storage proteins by Northwest Atlantic herring (*Clupea harengus harengus*). *J. Fish Biol.* **43**, 811-824.

Bye, V.J. (1984) The role of environmental factors in the timing of reproductive cycles. In: G.W. Potts and R.J. Wootton (eds.) *Fish Reproduction: Strategies and Tactics*. Academic Press, London pp. 187-205.

Calderwood, W.L.(1907) *The Life of the Salmon*. Edward Arnold, London, cited in Mills (1989).

Chadwick, E.M.P., Randall, R.G. & Leger, C. (1986) Ovarian development of Atlantic salmon (*Salmo salar*) smolts and age at first maturity. In: D.J. Meerburg (ed.) *Salmonid maturation, Can. Spec. Publ. Fish. Aquat. Sci.* **89**, 15-23.

Foerster, R.E. (1968) The sockeye salmon, *Oncorhynchus nerka. Bull. Fish. Res. Bd. Can.* **162**, 1-422.

Grey, R.H. & Tosh, J.R.(1894) *13th Annual Report of the Fishery Board for Scotland*, Part II, Note 2.

Hawkins, A.D. & Smith, G.W. (1986) Radio-tracking observations on Atlantic salmon ascending the Aberdeenshire Dee. *Scottish Fisheries Research Report* **36**. Department of Agriculture and Fisheries for Scotland.

Herbinger, C.M. & Friars, G.W. (1991) Correlation between condition factor and total lipid content in Atlantic salmon, *Salmo salar* L., parr. *Aquacult. Fish. Manage.* **22**, 527-529.

Jonsson, N., Jonsson, B. & Hansen, L.P. (1991) Energetic cost of spawning in male and female Atlantic salmon (*Salmo salar* L.). *J. Fish Biol.*, **39**, 739-744

Kent, M. (1990) Hand-held instrument for fat/water determination in whole fish. *Food Control*, **2**, 47-53.

Lam, T.J. (1983) Environmental influences on gonadal activity in fish. In: W.S. Hoar, D.J. Randall & E.M. Donaldson (eds.), *Fish Physiology*, Vol. 9B Academic Press, London pp.65-116.

Laughton, R. (1989) The movements of adult Atlantic salmon in the River Tay. *Scottish Fisheries Research Report.* **41.** Department of Agriculture and Fisheries for Scotland.

Laughton, R. (1991) The movements of adult Atlantic salmon (*Salmo salar* L.) in the River Spey as determined by radio telemetry during 1988 and 1989. *Scottish Fisheries Research Report*. **50.** Scottish Office Agriculture and Fisheries Department.

McCarthy, I.D., Houlihan, D.F., Carter, C.G. & Mouton, K. (1993) Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. *Proc. Nutr. Soc.* **52,** 427-436.

McCormick, S.D. & Naiman, R.J. (1984) Some determinants of maturation in brook trout, *Salvelinus fontinalis. Aquaculture* **43,** 269-278.

Metcalfe, N.B. & Thorpe, J.E. (1992) Anorexia and defended energy levels in over-wintering juvenile salmon. *J. Anim. Ecol.* **61,** 175-181.

Miglavs, I. & Jobling, M.(1993) The size of lipid depots-a factor contributing to the control of food intake in Arctic charr, *Salvelinus alpinus. J. Fish Biol.* **43, 487-489**

Mills, D.H. (1971) *Salmon & Trout: A Resource, its Ecology, Conservation & Management.* Oliver & Boyd, Edinburgh.

Mills, D.H. (1989) *Ecology & Management of Atlantic Salmon.* Chapman & Hall, London.

Mills, D.H. & Graesser, N.W. (1981) *The Salmon Rivers of Scotland* Cassell, London.

Phillips, J.G., Butler, P.J. & Sharp, P.J. (1985) *Physiological Strategies in Avian Biology.* Blackie, London.

Pinder, L.J. & Eales, J.G. (1969) Seasonal buoyancy changes in Atlantic salmon (*Salmo salar* L.) parr and smolts. *J. Fish. Res. Bd. Can.* **26,** 2093-2100.

Ricker, W.E. (1979) Growth rates and models. In: W.S. Hoar, D.J. Randall & E.M. Donaldson (eds), *Fish Physiology*, Vol. 8. Academic Press, New York pp. 677-743.

Rowe D.K. & Thorpe J.E. (1990) Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reductions in feeding and growth during spring months. *Aquaculture* **86,** 291-313.

Rowe, D.K., Thorpe, J.E. & Shanks A.M. (1991) Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* **48,** 405-413.

Ryder, J.P. (1972) Biology of nesting Ross's Geese. *Ardea* **60,** 185-215.

Schaffer, W.M. & Elson, P.F. (1975) The adaptive significance of variations in life history strategy among local populations of Atlantic salmon in North America. *Ecology,* **56,** 577-590.

Scott, A.P. (1990) Salmonids. In: A.G. Munro, A.P. Scott, & T.J. Lam (eds.) *Reproductive Seasonality in Teleosts: Environmental Influences.* CRC-Press Boca Raton, Florida pp. 33-52.

Simpson, A.L., Metcalfe, N.B. & Thorpe, J.E. (1992) A simple non-destructive biometric method for estimating fat levels in Atlantic salmon, *Salmo salar* L., parr. *Aquacult. Fish. Manage.* **23,** 23-29.

Talbot, C. & Higgins, P.J. (1983) A radiographic method for feeding studies on fish using metallic iron powder as a marker. *J. Fish Biol.,* **23,** 211-220.

Thorpe, J.E. (1986) Age at first maturity in Atlantic salmon *Salmo salar.* In: D.J. Meerburg (ed.) *Salmonid maturation, Can. Spec. Publ. Fish. Aquat. Sci.* **89,** 7-14.

Thorpe, J.E. (1988) Salmon migration. *Sci. Progr. Oxf.* **72,** 345-370.

Thorpe, J.E. (1989) Developmental variation in salmonid populations. *J. Fish Biol.*, **35(Suppl. A)**, 295-303

Thorpe, J.E. (1994) Reproductive strategies in Atlantic salmon, *Salmo salar. Aquacult. Fish. Manage.* **25,** 77-87.

Thorpe, J.E., Talbot, C., Miles, M.S., Rawlings, C. & Keay, D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture,* **90,** 41-47

Thorpe, J.E. & Mitchell, K.A. (1981) Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness and current management. *Can. J. Fish. Aquat. Sci.* **38,** 1576-1590.

Thurow, F. (1966) Beitrage zur Biologie und Bestandskunde des Atlantischen Lachses (*Salmo salar* L.) in der Ostsee. *Ber. Dt. Wiss. Komm. Meeresforsch.* **18,** 223-379.

Tillik, Z.E. (1932) Changes in the chemical composition of the flesh of the Svir salmon in relation to spawning migration. *Izv. vses. Inst. ozern. rechn. ryb.* **14,** 133-148. Cited in *Comm. Fish. Abstr.* (1965) **18,** 12-13.

Webb, J. (1989) The movements of adult Atlantic salmon in the River Tay. *Scottish Fisheries Research Report.* **44.** Department of Agriculture and Fisheries for Scotland.

Wootton, R.J. (1982) Environmental factors in fish reproduction. In: C.J.J. Richter & H.J.Th. Goos (eds.), *Reproductive Physiology of Fish.* Pudoc, Wageningen. pp. 210-219.

Wootton, R.J. (1990) *Ecology of Teleost Fishes.* Chapman & Hall, London.

CHAPTER IX

GENERAL DISCUSSION

Main findings

The stated aims of this study were to investigate the relationships between a) temporal factors and appetite, and b) maturity and appetite. In pursuit of these original aims, the main findings of the study are as follows:

1) *Temporal factors and appetite.*

For salmon parr under ambient temperature and light conditions, a clear diurnal feeding pattern was observed in spring, with an appetite peak in early afternoon (II). In autumn, parr fed in synchrony but their diurnal feeding pattern was not consistent from day to day. Aggression patterns were similar between these two seasons, with highest levels of aggression at first light and a progressive reduction thereafter. In spring, feeding was therefore out of phase with aggressive interactions. In salmon post-smolts under constant photoperiod and water temperature, a consistent daily feeding pattern also emerged (II). There was, however, a fish-size effect upon diurnal feeding pattern, with small fish showing feeding peaks out of phase with larger individuals and the largest fish showing no diurnal variation in feeding activity. Among a small group of one sea-winter salmon in a sea cage, fish among the higher ranks of the feeding hierarchy were found to feed earlier than their lower ranking counterparts (IV). Smaller fish avoided competitors when approaching pellets, but fish of a similar status appeared to engage in scramble competition when feeding.

Thus (II) contributed to the body of knowledge concerning daily patterns of rhythmicity in fish and how these relate to seasonal cycles. Results reported by Kadri *et al.* (1991) were confirmed and those of Smith *et al.* (1993) and Rawlings *et al.* (1991) given some degree of support by the findings of the present study, as the daily feeding pattern observed in the former study was shown to occur independently of fluctuations in temperature or light intensity. Differences in results between Kadri *et al.* (1991) and the latter studies appear to have been functions of season and life history stage. Individual fish within a population may well be following independent feeding patterns, perhaps in relation to aggressive interactions and position in the social hierarchy. The findings of the present study suggest that daily feeding patterns in salmonids may be controlled by an hour-glass mechanism (Lees 1966), initiated at first light and dependent upon feeding level, social interactions and rate of digestion.

Food intake varied widely among post-smolts, but not between continuous and meal feeding regimes. Digestive efficiency was found to vary significantly both among individuals and between feeding regimes (III). When fed small amounts at frequent intervals, fish that ate more were less efficient in digestion, but when fed two meals per day, digestive efficiency was independent of food intake. Food intake (adjusted for digestive efficiency) was significantly greater for larger fish under the meal feeding regime only, with a similar but weaker relationship under the continuous regime. Individual differences in digestive ability were thus documented in the present study, confirming the only previous report of inter-individual variation of this nature in fishes (Carter *et al.* 1993). A relationship between variations in digestive efficiency and feeding regime were demonstrated clearly, while an indication of links between social status/size of fish and digestive efficiency emerged but requires further study for clarification.

2) *Maturation and appetite.*

In sea cage populations of Atlantic salmon, maturing fish were larger and in better body condition than non-maturing fish from October (the onset of maturation) until the end of the trial in July (V). They also had a greater motivation to feed than non-maturing fish from April. The period of greatest differentials in growth rate, body lipids and condition factor between the two groups of fish coincided with differential feeding responses. A combination of forklength and condition factor proved to be the strongest and most consistent predictor of maturation during late spring/summer for one sea-winter Atlantic salmon (VI). The same predictors proved robust for a second stock of fish subject to different rearing conditions.

The results of the present project confirmed earlier studies relating reproductive status in Atlantic salmon to body size and energy reserves (Rowe & Thorpe 1990, Rowe *et al.* 1991, Berglund 1992, Simpson 1992), and have demonstrated clearly that this differential is apparent at least a year prior to spawning. The patterns of growth and accumulation of reserves during the year prior to breeding were characterised for sea-run salmon, including evidence for a previously undocumented increase in appetite in maturing fish only. The evidence is circumstantial, since immature fish may have lost appetite at this time, but it does agree with the impression gained by fish farmers (pers. comm.). In general, these patterns were similar for male and female

salmon; if the increased energy reserves represent preparation for upstream migrations, this similarity suggests broadly similar energy requirements for the completion of upriver migration to spawning grounds in males and females (Jonsson *et al.* 1991). The similarity in growth patterns between genders also allowed the derivation of general morphometric predictors of maturity for use by fish farmers.

During a period of increasing water temperatures from May to August, maturing one-sea-winter Atlantic salmon showed decreasing appetite from early June, to little or no food intake by early July (VII). By contrast, non-maturing siblings increased food intake steadily from early June throughout the period. The onset of anorexia was asynchronous, the number of anorexic maturing fish increasing throughout the summer, suggesting that anorexia is dependent on an internal cue rather than some external timing mechanism. The onset of anorexia among maturing one sea-winter female salmon was significantly correlated with estimates of fat reserves, lean mass and gonad size, lean mass being most important (VIII). Maturing female salmon thus appear to continue feeding until their lean mass has reached threshold levels.

The drop in appetite and loss of body condition known to occur in salmon prior to breeding (Aksnes *et al.* 1986) was characterised in detail in this study. In addition, a preliminary examination was made of the mechanism by which both the maintenance of increased appetite (when fish are feeding heavily in order to accumulate a threshold level of body reserves) and subsequent appetite loss (once reserves have reached this critical level) are controlled.

Relevance to behavioural ecology and life history theory.

This study has documented regular, seasonally-dependent diurnal feeding patterns in salmon smolts. These appear to be controlled by an hour glass mechanism, triggered by first light each day. Satiation and digestion appeared to be the main factors in the hour glass cycle. However in juvenile salmon in freshwater, aggressive interactions which commenced at dawn seemed to limit feeding responses until later in the day when levels of aggression had become relatively lower. Note that this feeding/aggression pattern is unlikely to occur in the wild as the experimental conditions precluded the establishment and maintenance of the territories (Mikheev *et al.* in press).

It was also demonstrated that individuals within a population may have different patterns of feeding according to size, status and competitive interactions. The smallest fish in a population of salmon smolts appeared to feed at times when larger fish showed a lowered feeding response, while in sea cage conditions a small group of one sea-winter salmon showed a fairly clear feeding order, with fish which were most successful overall feeding first.

There were adaptive changes in appetite found in association with life history strategy which (in addition to providing an energy source for gonadal investment) allowed maturing individuals to accumulate nutrient reserves and to have them available at an appropriate time for breeding related activity (i.e. spawning migration).

While following the same life history pattern in a given year, maturing individuals were asynchronous in their progress toward maturation and subsequent reproduction. The stage of progress for any given individual depended upon performance in building up of energy reserves.

Applicability to the aquaculture industry.

It has been shown here clearly that daily feeding patterns in fish must be considered when developing feeding strategies for aquaculture that will optimise efficiency and minimise pollution. Seasonal effects upon these feeding patterns must be taken into account as well as reduction of the potential for social hierarchies. In theory, this is done best by making the food source difficult to monopolise i.e by distributing food unpredictably in both time and space (Thorpe *et al.*, 1990, Grant & Kramer 1992, Grant 1993, Grand & Grant 1994). In practice, distributing food in such a manner can be done best by hand feeding fish at approximate meal times and spreading food evenly over the water surface. However, my findings indicate that meal-feeding may require more than just two meals a day in order to allow sub-dominant and subordinate fish to feed once dominants are satiated. Although digestive efficiency was significantly greater under a continuous feeding regime, the differential was less than 10% and hence not large enough to merit serious consideration by fish farmers (De Silva & Anderson 1995). Fish which ate more under continuous feeding digested their food less efficiently, while meal feeding did not produce such a relationship. Similarly, variable relationships between food intake and digestion have been shown in

a number of studies (Birkett 1969, Kelso 1972, Elliot 1976, From & Rasmussen 1984, Henken *et al.* 1985, Cui & Wootton 1988). In situations where food intake-dependent reductions in digestive efficiency is likely to occur, feeding fish to satiation (as is common practice) is not recommended, as faecal products are likely to contain a high proportion of undigested nutrients.

Using the findings of the present study, it may be possible now to make early predictions of which individuals are maturing in a cage population, on the basis of morphology and/or feeding response. In addition, those formulating fish feeds and feeding strategies now have a basis upon which it might be possible to delay the onset of anorexia by preventing the build-up of lean mass reserves (the most important component of which remains unclarified).

REFERENCES

Aksnes, A., Gjerde, B. & Roald, S.O. (1986) Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **53**, 7-20.

Berglund, I. (1992) Growth and early sexual maturation in Baltic salmon (*Salmo salar*) parr. *Can. J. Zool.* **70**, 205-211.

Birkett, L. (1969) The nitrogen balance of plaice, sole and perch. *J. Exp. Biol.* **50**, 375-386.

Carter, C.G., Houlihan,D.F., Buchanan, B. & Mitchell, A.I. (1993) Protein-nitrogen flux and protein growth efficiency of individual Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem.* **12**, 300-315.

Cui, Y. & Wooton, R.J. (1988) Bioenergetics of growth of a cyprinid, *Phoxinus phoxinus*: the effect of ration, temperature and body size on food consumption, faecal production and nitrogenous excretion. *J. Fish Biol.* **33**, 431-443.

De Silva, S.S. & Anderson, T.A. (1995) *Fish Nutrition in Aquaculture*. Chapman and Hall, London.

Elliot, J.M. (1976) Energy losses in the waste products of brown trout (*Salmo trutta* L.). *J. Anim. Ecol.* **45**, 561-580.

From, J. & Rasmussen, G. (1984) A growth model, gastric evacuation, and body composition in rainbow trout, *Salmo gairdneri* Richardson, 1836. *Dana* **3**, 61-139.

Grand, T.C. and Grant, J.W.A. (1994) Spatial predictability of food influences its monopolisation and defence by juvenile convict cichlids. *Anim. Behav.* **47**, 91-100.

Grant, J.W.A. (1993) Whether or not to defend? The influence of resource distribution. *Mar. Behav. Physiol.*, **23**, 137-153.

Grant, J.W.A. and Kramer, D.L. (1992) Temporal clumping of food arrival reduces its monopolization and defence by zebrafish, *Brachydanio rerio. Anim. Behav.* **44**, 101-110.

Henken, A.M., Kleingeld, D.W. & Tijssen, P.A.T. (1985) The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish (*Clarias gariepinus*) (Burchell, 1822). *Aquaculture* **51**, 1-11.

Jonsson, N., Jonsson, B. & Hansen, L.P. (1991) Energetic cost of spawning in male and female Atlantic salmon (Salmo salar L.). *J. Fish Biol.* **39**, 739-744

Kadri S., Metcalfe N.B., Huntingford F.A. & Thorpe J.E. (1991) Daily feeding rhythms in Atlantic salmon in sea cages. *Aquaculture* **92**, 219-224

Kelso, J.R.M. (1972) Conversion, maintenance, and assimilation for walleye, *Stizostedion vitreum vitreum*, as affected by size, diet and temperature. *J. Fish. Res. Bd. Can.* **29**, 1181-1192.

Lees, A.D. (1966) Photoperiodic timing in insects. *Nature* **210**, 986-989.

Mikheev, V.N., Huntingford, F.A. & Thorpe, J.E. (In press) Visual complexity and the establishment of territoriality in juvenile Atlantic salmon. *Anim. Behav.*

Rawlings, C.E., Talbot, C., Thorpe, J.E. & Bromage, N. (1991) Feeding rhythms of Atlantic salmon smolts (*Salmo salar* L.) in sea cages in summer. In: N. de Pauw and J.Joyce (eds.) *Aquaculture & the Environment, Eur. Aquacult. Soc. Spec. Publ.* **14**, 271-272.

Rowe D.K. & Thorpe J.E. (1990) Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reductions in feeding and growth during spring months. *Aquaculture* **86**, 291-313.

Rowe, D.K., Thorpe, J.E. & Shanks A.M. (1991) Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* **48**, 405-413.

Simpson, A.L. (1992) Differences in body size and lipid reserves between maturing and non-maturing Atlantic salmon parr, *Salmo salar* L. *Can. J. Zool.* **70**, 1737-1742.

Smith, I.P., Metcalfe, N.B., Huntingford, F.A. & Kadri, S. (1993) Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **117**, 165-178.

Thorpe, J.E., Talbot, C., Miles, M.S., Rawlings, C. and Keay, D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **90**, 41-47.