ENVIRONMENTAL INFLUENCES ON GROWTH, MATURATION AND SMOLTIFICATION IN ATLANTIC SALMON PARR, *SALMO SALAR*.

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

March 2003

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Abstract

The maturation of Atlantic salmon (*Salmo salar*) parr, and its effects on growth and smoltification, causes significant economic losses to commercial aquaculture. The current thesis investigates the role of environmental factors on freshwater development, with the aim of providing information which would help reduce the currently observed levels of maturation in farmed salmon.

The effects of short day "winter" photoperiods were investigated by exposing three replicated groups of fish to an 8 week "winter" photoperiod (LD10:14) commencing in May, August or September, in an otherwise continuous light (LD24:0) regime. A further group was held on LD24:0 throughout. 200 to 300 individuals were PIT tagged in each group in order to follow the growth of fish undergoing different developmental strategies, with the retrospective analysis of such development also possible. The highest incidence of maturation (>20%) was observed in the May winter photoperiod group, with low levels recorded in the August and September fish (<4%), suggesting that maturation may be influenced during a "critical" period in early development. Maturation levels were intermediate (<9%) in the continuous light group indicating that seasonally-changing photoperiodic cues are not necessarily required for gonadal development. The size of mature fish was initially the same as both immature parr and smolts, although the growth of mature individuals subsequently declined, and at the conclusion of the experiment they were significantly smaller. The August photoperiod resulted in the highest incidence of smoltification, with all other treatments resulting in low levels.

In a second experiment, PIT tagged fish were reared under an 8 or 12 week "winter" photoperiod (LD10:14) starting in May or June, in an otherwise continuous light (LD24:0) regime. The highest incidence of maturation (>11%) was found in the 12

week May fish, with intermediate levels in the 8 week May and 8 week June groups (<8%). Low levels were found within the 12 week June group (<0.6%) and it is suggested that a critical period when maturation is influenced may occur during a specific, short period in early development. Throughout the experiments, mature individuals maintained the same size as their immature siblings. The 12 week June photoperiod appeared to result in the highest level of smoltification, although those exposed to the 12 week May photoperiod showed the greatest seawater survival.

In both photoperiod experiments, fish showing some signs of smoltification were also found to be undergoing gonadal development, indicating that maturation and smoltification are not completely mutually exclusive processes.

Possible nutritional effects were considered using different dietary lipid inclusions (either 12.5% or 25%) and variable rations of feed (either full, 2/3 or 1/3 rations). Different dietary lipid inclusions had no effect on growth, although the whole body fat content of individuals was affected, with a switch in dietary fat content during development resulting in a rapid change in body composition. Fish size increased with ration and, although at the lowest ration of feed whole body fat levels were reduced, they were maintained at a set level under the high and intermediate rations, implying a lipostatic control of growth. Maturation levels were low throughout the nutrition experiments, suggesting that genetic influences may have been important. Dietary lipid level had a negligible effect on smoltification, although increases in ration resulted in a greater incidence of smoltification. Using a 0+ photoperiod regime (i.e. LD24:0 applied from March until December, with the exception of an 8 week period of LD17:7 applied from August), smolting individuals showed a reduction in smolt status when compared to those developed under a natural photoperiod. It is suggested that such regimes restrict the mobilisation of long-term energy stores, with the

subsequent development of seawater tolerance affected. However, it was noted that the 0+ regime had increased the incidence of smolts.

In summary, it has been shown that environmental factors such as photoperiod, nutrition and temperature can play an important role in the developmental strategies taken by juvenile Atlantic salmon. Such factors are likely to greatly influence the attainment of size and/or nutritional thresholds necessary for various developmental strategies, in particular if such thresholds occur during seasonally-sensitive "critical" periods when development can be influenced. Furthermore, the life history strategy undertaken by an individual may be affected by endogenous rhythms, cued by seasonally-changing environmental factors. However, there are clear indications that the underlying genetic control of maturation may also be of importance.

Keywords: Atlantic salmon, maturation, smoltification, growth, photoperiod, nutrition.

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Acknowledgements

I would particularly like to thank my supervisor, Prof. Niall Bromage, for his support, guidance and advice throughout the completion of my research. Sadly, Niall passed away suddenly shortly after the submission of this thesis. During my Ph.D. I established a great respect for Niall in terms of both his academic achievements and as a friend. I hope that the research completed for my Ph.D., as well as that which I conduct in the future, do justice to Niall's guidance.

I would also like to thank Dr Mark Porter for his help and supervision. In addition, I would like to acknowledge the financial support provided by Marine Harvest Scotland, and the support and direction given by both David Mitchell and Alastair Smart (MHS).

I would like to express great thanks to Billy Robertson, for his help and expertise in P.I.T tagging. Furthermore, I would like to thank Gordon Beaton as well as Tony, Norman, Neil, Cathy and Shona from the MHS hatchery, Invergarry. Great thanks also to Andy McLean, John Richmond and Ian Cameron from the MHS salmon farm, Kinlochmoidart. In addition, I would like to thank Chris Wallace, Ian McDonald, Billy Elder, Davy MacGillvray and Kenny Gordon, from MHS, for their help in the collection of samples.

I would like to thank past and present employees of the Buckieburn Freshwater Research Facility, including Niall Auchinachie, John Gardiner, Stuart Hall and Alastair McPhee, as well as Rob Murray and the staff at Howietoun hatchery.

Special thanks are long overdue to my colleagues in the Reproduction research group: Ben North, John "J.T." Taylor and Andrew "Merve" Davie, who have made days on the farm and in the lab ones to remember. I would also like to thank the Nutrition research group, in particular Allan Porter, for his help with lipid determination. Additionally, thanks to Catherine Howie, for her patience with my unrelenting questions on statistical analysis.

I would like to thank my colleagues in the Institute of Aquaculture for their support during my research, also friends who have provided words of support over the past three years.

Finally, I would like to express special thanks to my family for their seemingly endless support throughout my entire academic life, which by now is entering epic proportions.

Declaration

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged the work described in this thesis has been conducted independently and has not been submitted for any other degree.

Signature of candidate:

Signature of supervisor:

Date: 7/3/04

Glossary of common and scientific names used within this thesis.

Atlantic and Baltic salmon	*Salmo salar*
brown and sea trout	*Salmo trutta*
rainbow and steelhead trout	*Oncorhynchus mykiss*
pink salmon	*Oncorhynchus gorbuscha*
sockeye salmon	*Oncorhynchus nerka*
coho salmon	*Oncorhynchus kisutch*
chinook salmon	*Oncorhynchus tshawytscha*
masu salmon	*Oncorhynchus masou*
amago salmon	*Oncorhynchus masou* (landlocked form)
chum salmon	*Oncorhynchus keta*
brook trout	*Salvelinus fontinalis*
Arctic charr	*Salvelinus alpinus*
Atlantic halibut	*Hippoglossus hippoglossus*
Atlantic cod	*Gadus morhua*
sea bass	*Dicentrarchus labrax*
stickleback	*Gasterosteus aculeatus*

Dedicated to the memory of

Professor Niall R. Bromage.

Chapter 1: General introduction.

The salmonidae are a teleost group that are naturally pre-adapted to cold, oxygen-rich waters, and although they historically became naturally habituated in the northern hemisphere (Netboy, 1974) examples have now become native to the majority of the worlds continents with the exception of Antarctica.

The salmonidae comprise three main genera: the Atlantic salmon (*Salmo sp*), the Pacific salmon (*Oncorhynchus sp*) and the chars (*Salvelinus sp*). However, within European waters one of the most important species is the Atlantic salmon, *Salmo salar*, and it is this species on which the investigations of the current thesis are based.

Although parr maturation represents an important life history strategy in wild populations, during the freshwater production of Atlantic salmon maturation results in a significant economic loss to productivity, with environmental factors thought to play an important role in the developmental strategies undertaken by individuals during this juvenile stage (Thorpe, 1987a, 1989; Bromage *et al.*, 1993; Duston and Saunders, 1992). Therefore the current investigation was designed in order to address the environmental influences affecting growth, maturation and smoltification in juvenile Atlantic salmon with a view to providing commercially useful information that could be incorporated into production strategies aimed at maximising freshwater productivity.

1.1. The Atlantic salmon: distribution and economic importance

The Atlantic salmon has a native range spanning from northern Spain, up throughout mainland Europe and the British Isles, reaching northern Scandinavia and parts of the former USSR. It is also distributed across the north Atlantic ocean to north east America and eastern Canada (Jones, 1959; Netboy, 1974; MacCrimmon and Gots, 1979). Furthermore, limited attempts have previously been made to naturalise it in non-native regions with success only being found in North America, Argentina, the Faeroes Isles and New Zealand (MacCrimmon and Gots, 1979), although in recent years concerns regarding the introduction of non-native species have meant that such attempts are now inhibited by regulation. However, although the introduction of Atlantic salmon to non-native regions is restricted its commercial aquaculture is now increasingly common throughout much of the world. It should be noted though that reference is often made to the Baltic salmon (e.g. Lundqvist and Fridberg, 1982; Mayer *et al.*, 1990; Berglund, 1992). This does not refer to a separate species of salmonid but a physically isolated stock of *Salmo salar* that is found within the Baltic Sea (MacCrimmon and Gots, 1979).

The Atlantic salmon has long been considered a viable species for commercial aquaculture and its production is now of considerable economic and social importance throughout much of Europe, Canada and South America. Within European waters the majority of production occurs in northern Europe, primarily throughout Scotland and much of Scandinavia particularly Norway. For example in Scotland during the year 2000, Atlantic salmon farming employed over 1800 staff, producing 45.6 million smolts with a total harvest production of nearly 130,000 tonnes (FRS Annual production survey, 2000).

1.2. The Atlantic salmon life cycle

The life cycle of the Atlantic salmon shows considerable plasticity allowing the reproductive success of individuals to be maximised relative to the yearly conditions that present themselves.

Maturing adult Atlantic salmon migrate from the oceans to their natal river from autumn through until late spring, where the female creates a gravel nest or "redd" into which eggs are deposited and fertilised (Fig. 1.1). After the eggs hatch the "alevins" then utilise their yolk sacs, remaining in or close to the gravel river bed. Subsequently, the alevins develop into free swimming fry and with time they develop the classical appearance of parr with distinct parr marks and a darkened coloration. These parr may then reside in fresh water for up to 8 years with males often able to mature during this juvenile stage. During springtime parr may undergo physiological, morphological and behavioural changes that result in smoltification and migration from the cryptic, territorial life of the river environment to the pelagic shoaling life of the sea. Such migrations are typically completed during late spring or early summer with individuals displaying a silvered and streamlined body form.

Individuals will then remain in the sea for up to 5 years before returning to their natal stream to reproduce, with a small proportion returning as grilse after just one year at sea. After spawning the Atlantic salmon, unlike the Pacific salmon, may be able to return to the sea for a further oceanic period and subsequent spawning migration although it is likely that only a small number of these post-spawning "kelts" will survive and return to the sea (see Jones, 1959; Netboy, 1974; Laird and Needham, 1988; Stickney, 1991 for detailed reviews of the Atlantic salmon life cycle).

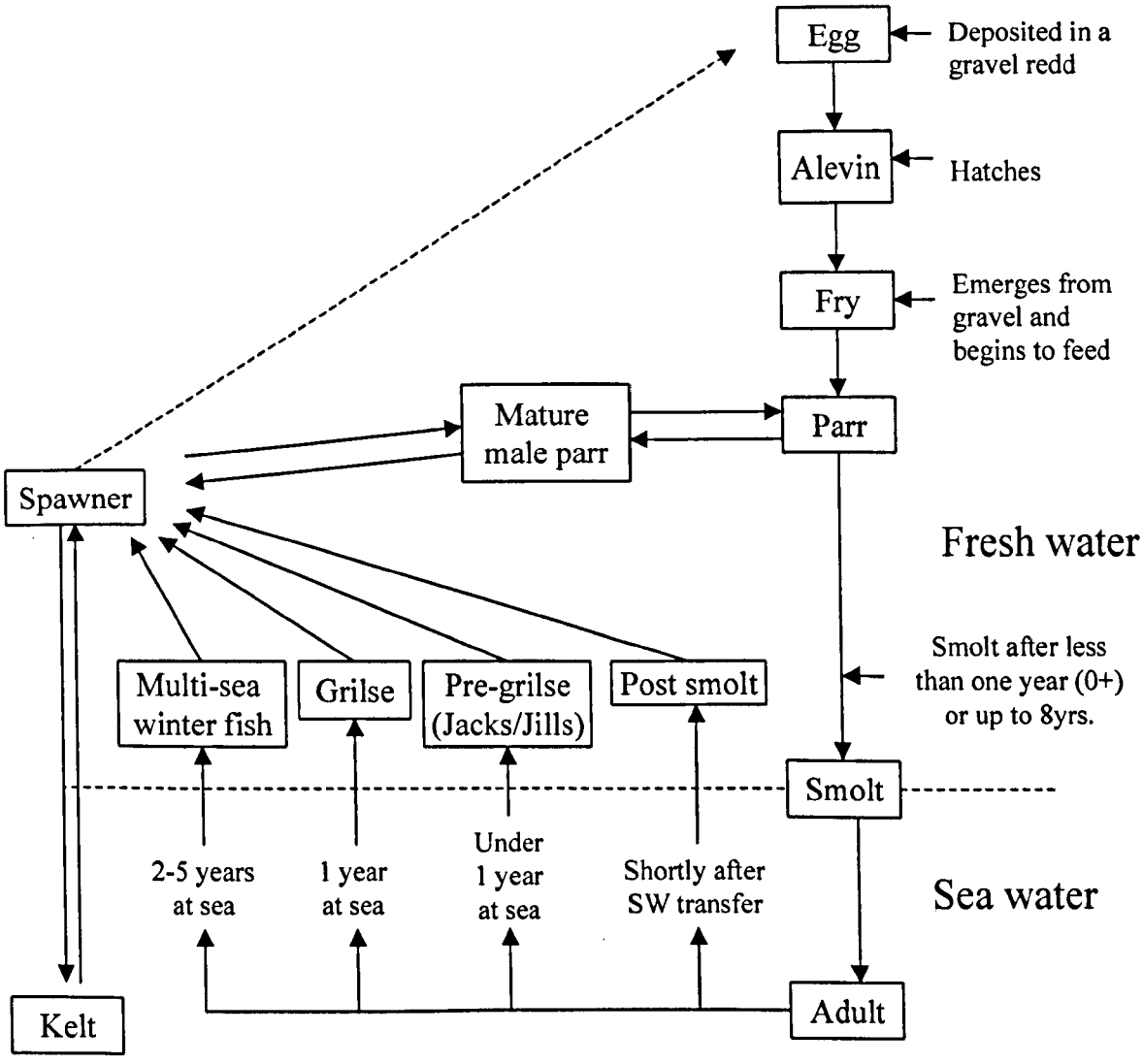


Fig. 1.1 The life cycle of the Atlantic salmon, *Salmo salar* (adapted from Laird and Needham, 1988).

However, it is important to note that in commercial populations production regimes often result in early maturation and smoltification. In particular, the timing of smoltification is manipulated to permit the out-of-season transfer of fish to sea water with individuals transferred after a freshwater period of less than one year (0+), after one year (1+), between one and two years (1.5+), and less frequently after 2 years (2+). However, with such advanced regimes the incidence of parr maturation often increases with maturation shortly after seawater transfer (i.e. post-smolt maturation) or after a period in sea water but prior to the grilse maturation (i.e. as "jacks or "jills") also possible.

Plasticity in the life history strategy of Atlantic salmon therefore arises from the differential timing of maturation or smoltification migrations that individuals within a particular year class perform. It is also evident that within natural populations such life history variations may result in the size of the spawning population being much larger in a given year than might be expected from the particular year class size (Saunders and Schom, 1985).

Within commercial production different aspects of both the marine and freshwater stages of the Atlantic salmon life cycle are manipulated. Typically, superior growth rates are achieved in sea water (c.f. Gjedrem and Gunnes, 1978; Thrush *et al.*, 1994; Duncan *et al.*, 1998) and as such freshwater production focuses on attaining good levels of smoltification, often at times other than during the natural spring period. Seawater production focuses on growing fish to a harvestable size as early and cost effectively as possible. However, it is important to mention that maturation in either

fresh- and sea water, other than when the fish are used as broodstock, will have a detrimental effect on productivity.

1.3. Growth

Growth involves the formation and interaction of many complex physiological and biochemical processes with an energetic input required to facilitate these systems (Jobling, 1994). However, for increases in growth to be made possible energy must be accumulated over and above that which is required for standard metabolic functions such as respiration.

For fish, the acquisition and utilisation of energy necessary for growth will be affected by many factors. Growth has been found to be influenced by a genetic component (Nilsson, 1990; Gjøen and Bentsen, 1997) with environmental factors also exerting a major role. Primarily, growth will be influenced by the acquisition of food, in terms of the amount of food that a fish gains (i.e. ration) (Storebakken and Austreng, 1987a,b; McCormick *et al.*, 1989; Hillestad *et al.*, 1998) as well as the quality of the food (i.e. chemical composition) (Grisdale-Helland and Helland, 1997; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001). It has also been well documented that light and in particular photoperiod (Stefansson *et al.*, 1989; Stefansson *et al.*, 1991; Hansen *et al.*, 1992; Duncan *et al.*, 1999; Endal *et al.*, 2000) will influence growth by affecting the visual recognition of food items (Higgins and Talbot, 1985; Bolliet *et al.*, 2001) and the efficiency with which food is utilised (Higgins and Talbot, 1985; Jonassen *et al.*, 2000). Finally, the temperature of the water in which fish reside will have a major influence on growth (Elliott, 1975a, b; Clarke *et al.*, 1978) by affecting both the rate and efficiency with which energy is utilised.

However, it is also important to note that the life history strategy of individuals will have an important effect on their yearly profile of growth (Thorpe *et al.*, 1980; Higgins and Talbot, 1985; Skilbrei, 1989; Jobling and Baardvik, 1991) with such effects linked to changes in appetite (Kadri *et al.*, 1995; Simpson *et al.*, 1996) and the utilisation of energy for developmental processes (e.g. Jonsson *et al.*, 1991). Therefore, environmental factors such as photoperiod, temperature and the availability of food items will further influence growth by cueing the daily and seasonal timing of endogenous rhythmic processes that influence developmental strategies (Baggerman, 1972; Erikson and Lundqvist, 1982; Duston and Bromage, 1987; Duncan and Bromage, 1998).

Atlantic salmon are anadromous and the energetic costs of migrating between hyper- and hypo- osmotic environments will have distinct effects on growth (Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988). Furthermore, the variable timing of maturation, with its high energetic requirements (Jonsson *et al.*, 1991), will also influence cycles of growth. Given the plasticity within the life cycle of the Atlantic salmon, it is clear that complex interactions between growth and developmental strategies will occur throughout both the juvenile and adult stages of life.

One important aspect of salmonid growth, that is related to life history strategy, is a divide within the population which results in a bimodal community structure (Thorpe, 1977, 1987a; Bailey *et al.*, 1980; Kristinsson *et al.*, 1985; Stewart *et al.*, 1990). Indeed, the emergence of bimodality can have serious implications for overall productivity within commercial stocks, in particular during the freshwater stage of development. However, in the wild population bimodality is not a problem and it is

usually evident by the autumn of the first growing season (Thorpe, 1977, 1987a; Bailey *et al.* 1980; Kristinsson *et al.*, 1985). Initially, all fish will grow at a similar rate (Kristinsson *et al.*, 1985) although during late summer a clear difference in growth occurs between fish destined to enter the upper mode of the distribution (UMG) and those remaining in the lower mode (LMG) (Higgins and Talbot, 1985; Thorpe, 1987a; Kristinsson *et al.*, 1985; Skilbrei, 1988, 1991; Stewart *et al.*, 1990). UMG fish will typically experience a period of rapid growth (Kristinsson *et al.*, 1985; Skilbrei, 1991) with the LMG fish reducing or ceasing their growth altogether (Higgins and Talbot, 1985; Skilbrei, 1991; Metcalfe and Thorpe, 1992).

Furthermore, it is increasingly evident that the decision to enter the upper mode of the distribution is dependant upon the attainment of a specific size threshold (Elson, 1957; Kristinsson *et al.*, 1985; Skilbrei, 1988; Stewart *et al.*, 1990), with fish that achieve the threshold at a particular time of the year entering the period of extended growth, and those failing to achieve this size reducing their growth. It is therefore likely that the development of bimodality is controlled by environmental factors, with most evidence indicating that photoperiod provides this cue (Thorpe, 1987a; Skilbrei, 1991; Duston and Saunders, 1992; Duncan and Bromage, 1998). However, in the absence of photoperiodic cues it is likely that other environmental factors may become important (Solbakken *et al.*, 1994).

In fresh water growth changes associated with the development of bimodality are clearly linked to life history strategy. Following the emergence of bimodality continued growth in UMG fish leads to the majority of such individuals undergoing the parr-smolt transformation in the following spring (Kristinsson *et al.*, 1985;

Thorpe, 1987a; Skilbrei, 1988; Duston and Saunders, 1992; Saunders *et al.*, 1994). It is likely that the critical threshold sizes that have previously been suggested for smoltification (Elson, 1957; Thorpe *et al.*, 1980) will be linked to those also suggested for the development of bimodality (Kristinsson *et al.*, 1985; Skilbrei, 1988), with the yearly growth profile of smolting individuals the same as that discussed for UMG fish.

However, although mature parr have been found in greater numbers in the lower mode of a population distribution (e.g. Bailey *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1991; Duston and Saunders, 1992; Saunders *et al.*, 1994), population bimodality is not directly dependant on maturity status (Thorpe, 1977; Villarreal and Thorpe, 1985) since mature individuals have been found in both modes of a population distribution (Thorpe, 1977; Bagliniere and Maisse, 1985; Kristinsson *et al.*, 1985; Saunders *et al.*, 1994). It is more likely that the growth profile of maturing fish results in the majority of such individuals failing to reach the size threshold necessary to enter the upper modal group.

For both parr and adult salmon that are destined to mature growth rates are initially greater than for their non-maturing siblings (Saunders *et al.*, 1982; Dalley *et al.*, 1983; Aksnes *et al.*, 1986; Skilbrei, 1989; Rowe and Thorpe, 1990a; Foote *et al.*, 1991; Reimers *et al.*, 1993) which typically results in such individuals being larger during the early stages of the growing season (Aksnes *et al.*, 1986; Rowe and Thorpe, 1990a; Berglund, 1992, 1995; Berglund *et al.*, 1992; Shearer and Swanson, 2000) Subsequently, the somatic growth of maturing individuals decreases (Dalley *et al.*, 1983; Skilbrei, 1989; Rowe and Thorpe, 1990a; Foote *et al.*, 1991) as gonadal

development increases (Hunt *et al.*, 1982; Foote *et al.*, 1991). Furthermore, from this growth profile, as well as the suggestion by Polikansky (1983) that fish will mature as soon as they are able to do so, it is likely that either a size or developmental threshold regulates maturation (Bailey *et al.*, 1980; Thorpe and Morgan, 1980; Saunders *et al.*, 1982; Thorpe, 1986; Berglund, 1995).

It is interesting to note that within the current culture of salmonids some high grilsing stocks are used which might normally be considered detrimental since maturation has been linked to a range of unfavourable traits, in particular a loss in harvest quality (Aksnes *et al.*, 1986) and reduced somatic growth rates (Skilbrei, 1989). However, by utilising the initial fast growth rates of such stocks and then harvesting prior to gonadal development, overall productivity can be greatly enhanced.

1.4. Maturation

1.4.1. Maturation in commercial production

Within the commercial production of salmonids maturation at any stage of development is generally considered as detrimental to productivity. These detrimental effects of maturation can be linked to three main areas: a reduction in flesh quality (Aksnes *et al.*, 1986), a reduction in growth (Skilbrei, 1989; Berglund *et al.*, 1992) and a loss of immunocompetence (Richards and Pickering, 1978; Murphy, 1980). However, it should be noted that parr maturation has also been linked to a loss of seawater tolerance (Clarke and Blackburn, 1994; Saunders *et al.*, 1994) although this subject will be discussed at a later stage.

Maturation involves a loss of body fat content (Aksnes *et al.*, 1986; Rowe *et al.*, 1991; Kadri *et al.*, 1996) as energy reserves are mobilised for the production of gonadal tissue (Hunt *et al.*, 1982; Jonsson *et al.*, 1991; Rowe *et al.*, 1991; Jorgensen *et al.*, 1997). Furthermore, as fat is lost it is replaced by water (Shearer 1994). These combined changes in body composition have the result of reducing flesh quality (Aksnes *et al.*, 1986) and although such a reduction is not necessarily important for juvenile production it has severe implications for adult harvest quality.

Maturation is also linked to a cyclical growth pattern. As mentioned previously, the initial enhanced growth rates of fish that are destined to mature can be utilised in adult production, but if fish are allowed reach maturity their growth rates reduce (Lee and Power, 1976; Dalley *et al.*, 1983; Skilbrei, 1989; Rowe and Thorpe, 1990a; Berglund *et al.*, 1992; Stead *et al.*, 1999) as gonadal growth increases (Hunt *et al.*, 1982; Foote *et al.*, 1991). This reduction in growth has been linked to a decrease in appetite (Rowe and Thorpe, 1990a; Kadri *et al.*, 1996; Tveiten *et al.*, 1996; Stead *et al.*, 1999) although some studies have suggested that maturation does not necessarily result in a reduction in feed intake (Simpson *et al.*, 1996; Arndt, 2000; Shearer and Swanson, 2000). However, even if a reduction in feeding does occur, maturation represents an inefficient utilisation of food with energy received and indeed that accumulated prior to maturation, used for the development of gonadal tissue as opposed to somatic growth. Although there is an increasing interest in the sale of salmon eggs for human consumption (i.e. salmon caviar) this market is currently limited and gonadal tissue must generally be considered as a non-harvestable form. Therefore, the cost of feed that is not utilised for somatic growth represents a significant financial loss to the aquaculture industry.

Another problem linked to maturation is a loss of immunocompetence. Maturing individuals have been found to show an increased rate of infection by pathogens, in particular *Saprolegnia sp.* (Richards and Pickering, 1978; Murphy, 1980) and recently links have been made between maturation and reductions in a range of endogenous immune parameters (Slater and Schreck, 1997; Suzuki *et al.*, 1997). Therefore, not only are mature fish more likely to suffer increased mortality rates but they may also become vectors of disease risking the spread of pathogens to non-maturing individuals within the population. As such the losses of fish through mortality, combined with the increased need for disease treatment measures, mean that the economic costs of maturity rise.

1.4.2. Parr maturation

Typically the maturation of Atlantic salmon is found amongst adult fish that return from a period of seawater residence. However, it is also evident that a proportion of the population can undergo maturation as juveniles in fresh water. This parr maturation is generally restricted to males, almost certainly due to the different energy requirements of male and female maturation (Jonsson *et al.*, 1991; Jorgensen *et al.*, 1997) combined with the limited food resources linked to freshwater residency. Adams and Thorpe (1989) found some gonadal investment in female parr from a range of populations and it is likely that female parr maturation may be possible in environments which allow sufficient nutritional gains. However, such studies are rare (e.g. Ueda *et al.*, 1983; Bagliniere and Maisse, 1985; Hindar and Nordland, 1989) and the frequency of such individuals is extremely low (e.g. 10^{-5}: Hindar and Nordland, 1989). Due to the low recorded incidence of maturing female parr, their reproductive ability and ecological significance is not known.

Shaw (1840) was the first to document the maturation of salmon parr and although studies continued from that early work it was not until the emergence of intensive salmon farming in the late 20th century that interest in the occurrence increased. Typically, in wild populations levels of maturation show great variability (Table 1.1) although it is evident that levels rise with the increasing duration of freshwater residency (c.f. Dalley *et al.*, 1983; Whalen and Parrish, 1999), which is almost certainly due to the increased accumulation of energy reserves.

Although the presence of mature male parr on the adult spawning grounds was not disputed, it was not until Jones and King (1952) documented male parr releasing milt during the spawning act that their role in fertilising adult females' eggs could be confirmed. Mature male parr generally play no role in the courtship act of the adult pair (Jones, 1959), but remain cryptic lying close to the adult nest awaiting the opportunity for "sneak" fertilisation (Jones, 1959; Fleming, 1996). Jones and King (1952) concluded that a significant fraction of eggs could be fertilised by male parr and this suggestion has more recently been confirmed by the genetic assessment of fertilised eggs (Hutchings and Myers, 1988; Jordan and Youngson, 1992; Thomaz *et al.*, 1997). It is therefore evident that in wild populations the maturation of male parr can provide an important ecological life history strategy aiding genetic diversity.

However, parr maturity has serious implications for freshwater production. Previously, it has been suggested that maturation and smoltification are mutually exclusive processes (Thorpe and Morgan, 1980; Thorpe, 1986, 1987a; Herbinger and Friars, 1992), with smoltification a consequence of a fish failing to mature (Thorpe, 1994a, Thorpe and Metcalfe, 1998). However, it does seem that the two

Location	Year class	Incidence (%)	Source
UK	up to 3+	83	Stuart-Kregor *et al*. (1981)
Canada	1+	0 - 100	Dalley *et al*. (1983)
	2+	9.4 - 100	
	3+	72 - 100	
	4+	100	
Canada	up to 2+	80	Myers (1984)
France	0+	4.2 - 6.4	Bagliniere and Maisse (1985)
	1+	0 - 100	
Canada	1+	0 - 100	Myers *et al*. (1986)
	2+	0.7 - 100	
USA	0+	2.8 - 74	Letcher and Terrick (1998)
USA	1+	28 - 52	Whalen and Parrish (1999)
	2+	up to 67	

Table 1.1 The incidence of male parr maturation among wild stocks of Atlantic salmon.

developmental processes do not completely inhibit one another since mature fish have been found to successfully undergo smoltification (Saunders *et al.*, 1982; Bagliniere and Maisse, 1985; Berglund *et al.*, 1991; Saunders *et al.*, 1994; Duston and Saunders, 1997). Indeed, it is likely that the reduced growth rates of maturing individuals and not their maturation *per se* results in the limited numbers of maturing fish that are recruited to the smolting population in the following spring (Bailey *et al.*, 1980; Saunders *et al.*, 1994). Therefore, the smoltification of mature individuals is possible if their growth rates permit the attainment of a particular size threshold (Elson, 1957; Thorpe *et al.*, 1980).

However, it is clear that mature parr do have a reduced seawater adaptability when compared to classical smolts (Foote *et al.*, 1991; Clarke and Blackburn, 1994; Saunders *et al.*, 1994; Staurnes *et al.*, 1994a) with changes in body androgen levels thought to play a role in this inhibitory process (Aida *et al.*, 1984; Ikuta *et al.*, 1985; Miwa and Inui, 1986; Lundqvist *et al.*, 1989). Therefore, although the decisions to mature and smolt appear to be made independently (Bailey *et al.*, 1980) it is likely that the ability of a mature fish to undergo smoltification will be dependant on the degree of recovery from maturation (Skilbrei, 1990). For commercial production though, the smoltification rates of mature parr are not considered to be economically viable. Given this, and to a lesser extent the other detrimental factors linked to parr maturation (i.e. reductions in growth, immunosuppression etc.) such individuals are generally culled from commercial populations as soon as they are identified. This is usually carried out prior to vaccination and subsequent seawater transfer.

1.5. Factors affecting growth, maturation and smoltification

1.5.1. Genetic influences

The genetic manipulation of salmonids is a topic that has received much attention in recent years. Within the trout industry the most notable manipulations to date have been the production of all female and triploid stocks, which enable the industry to produce table-sized fish without the detrimental problems linked to the early maturation of, in particular, male fish (Shepherd and Bromage, 1988; Stickney, 1991). Indeed, the use of such individuals is now widespread with the UK trout industry extensively utilising such techniques. However, the UK Atlantic salmon industry has not moved towards the use of such manipulated stocks primarily due to the possible public perception of techniques such as genetic manipulation. Therefore reductions in the incidence of early maturation, as well as improvements in growth and smoltification, have to be made through manipulating the genetic variation present within domesticated fish stocks.

The majority of genetic enhancement has focused on maturation primarily due to the commercial problems linked to early maturity. Indeed, clear evidence suggests that an underlying genetic component influences maturation (Naevdal, 1983; Thorpe *et al.*, 1983; Gjerde, 1984; Myers and Hutchings, 1986; Herbinger and Newkirk, 1990; Gjøen and Bentsen, 1997). However, although Gjerde (1984) found that rates of maturation during fresh- and sea-water development could not be linked there is a lack of data addressing the relationship between adult and parr maturity status.

As described earlier, stocks with an intrinsically high rate of maturation are often used in order to capitalise on the initial growth rates of maturing fish. However, it has been

suggested that growth is an individually heritable trait (Thorpe *et al.*, 1983: Nilsson, 1990; Silverstein and Hershberger, 1994; Gjøen and Bentsen, 1997) although it is also likely that such increases in growth will subsequently affect maturation, if not through direct genetic manipulation.

Finally smoltification, as with maturation, may be affected by genetic influences on growth. However, it has been suggested that seawater adaptation is an inherited trait (Refstie *et al.*, 1977; Saxton *et al.*, 1984; Nielson *et al.*, 2001) and therefore changes in smolt status of commercial populations may be possible (Saxton *et al.*, 1984).

Growth, maturation and smoltification therefore appear to be influenced by an underlying genetic component with differences in these physiological processes clearly interacting to some degree. However, developmental processes will also be influenced by environmental manipulation.

1.5.2. Environmental influences

Generally, three environmental factors are thought to be of primary importance in the growth, maturation and smoltification of Atlantic salmon: light, diet and temperature. The experiments of the current thesis aim to investigate these influences and they are therefore reviewed in depth within the subsequent chapters. However, a general introduction to each parameter is now provided.

1.5.2.1. Light

Three aspects of light have been identified: spectral composition, intensity and photoperiod (Boeuf and Le Bail, 1999). Of these photoperiod is most commonly manipulated in commercial fish culture.

However, it is first important to categorise some nomenclature that is often used with reference to photoperiod regimes. Frequently daylength regimes are referred to as "long" or "short" day photoperiods. Although this nomenclature can be used as a relative measure, i.e. long day regimes have a longer period of daylight than short day photoperiods, short day regimes are generally less than 12 hours daylight, typically 8 hours of light (i.e. LD8:16) (Fig. 1.2). Long photoperiods will generally be greater than 12 hours daylight, typically 16 hours of light (i.e. LD 16:8) and above. Similarly, "extended" photoperiod regimes refer to daylengths which are greater than the natural daylength at a particular time. An "advanced" or "delayed" yearly photoperiod cycle refers to the shifting of a yearly profile of daylength out of its natural phase. Finally, "accelerated" or "compressed" photoperiod cycles occur when the rate at which a natural photoperiod changes is increased or decreased.

In general increases in daylength result in increased growth with periods of continuous light (LL) or constant long days extensively shown to enhance growth rates (Lundqvist, 1980; Stefansson *et al.*, 1989; Krakenes *et al.*, 1991; Hansen *et al.*, 1992; Solbakken *et al.*, 1994; Sigholt *et al.*, 1995; Handeland and Stefansson, 2001). Although some workers have suggested that it is the rate of the changing photoperiod that is of importance in growth (Clarke *et al.*, 1978), it is generally accepted that the amount of daylight received per day is the primary influence. However, although long

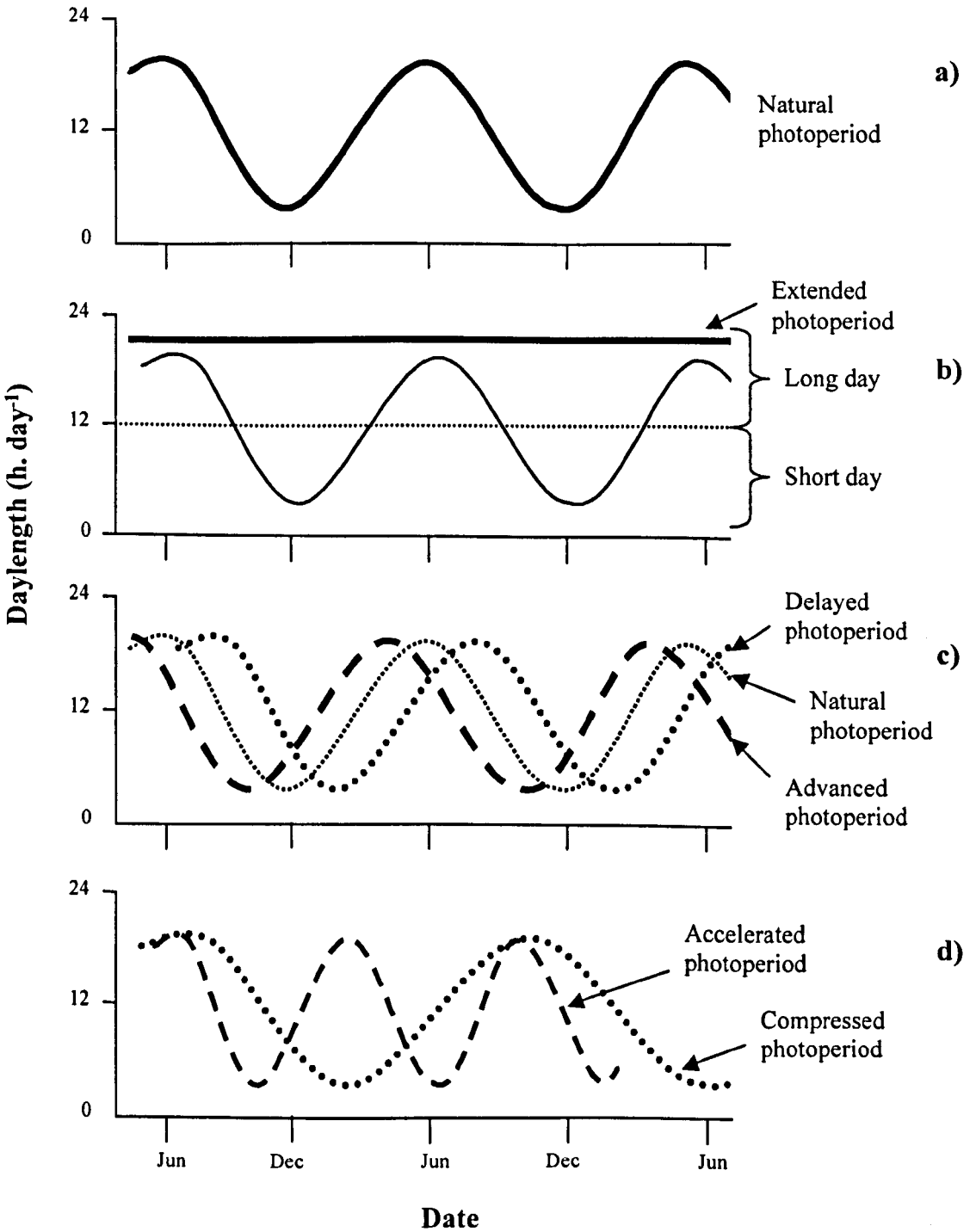


Fig. 1.2 Photoperiod regimes that explain the nomenclature used in experiments that have investigated salmonid development. a) natural photoperiod, b) long/short day photoperiod, c) advanced/delayed natural photoperiod, d) accelerated/compressed natural photoperiod.

day photoperiods are conducive to growth it has been found that when applied over long periods of time such effects may not continue to be advantageous, with shorter day regimes resulting in enhanced growth (Saunders and Henderson, 1988; Stefansson *et al.*, 1989; Saunders and Harmon, 1990; Berg *et al.*, 1994; Solbakken *et al.*, 1994). As a consequence some workers have concluded that there is a seasonal sensitivity to the photoperiodic cues (Saunders and Henderson, 1988; Saunders and Harmon, 1990).

In fresh water, however, further support for the role of either continuous light or constant long days in growth dynamics can be found when population bimodality is considered. Under continuous light or long day regimes bimodality has been shown to be weak (Skilbrei, 1991) or delayed (Duncan and Bromage, 1998) highlighting the photoperiodically-enhanced growth of individuals.

For both maturation and smoltification Duston and Saunders (1992) provided a model explaining the stimulatory effects of the natural photoperiod experienced by Atlantic salmon (Fig. 1.3). Smoltification appears to be initiated during the decreasing phase of the natural photoperiod, with its completion occurring during the increasing phase, whereas the initiation of maturation occurs on the increasing photoperiod, with final gonadal recrudescence completed on the decreasing phase.

However, it has been shown that constant light regimes can result in both maturation (Erikson and Lundqvist, 1980; Bourlier and Billard, 1984a,b; Scott *et al.*, 1984; Duston and Bromage, 1986; Skilbrei, 1991) and smoltification (Eriksson and Lundqvist, 1982; Sigholt *et al.*, 1995) and it is believed that such physiological

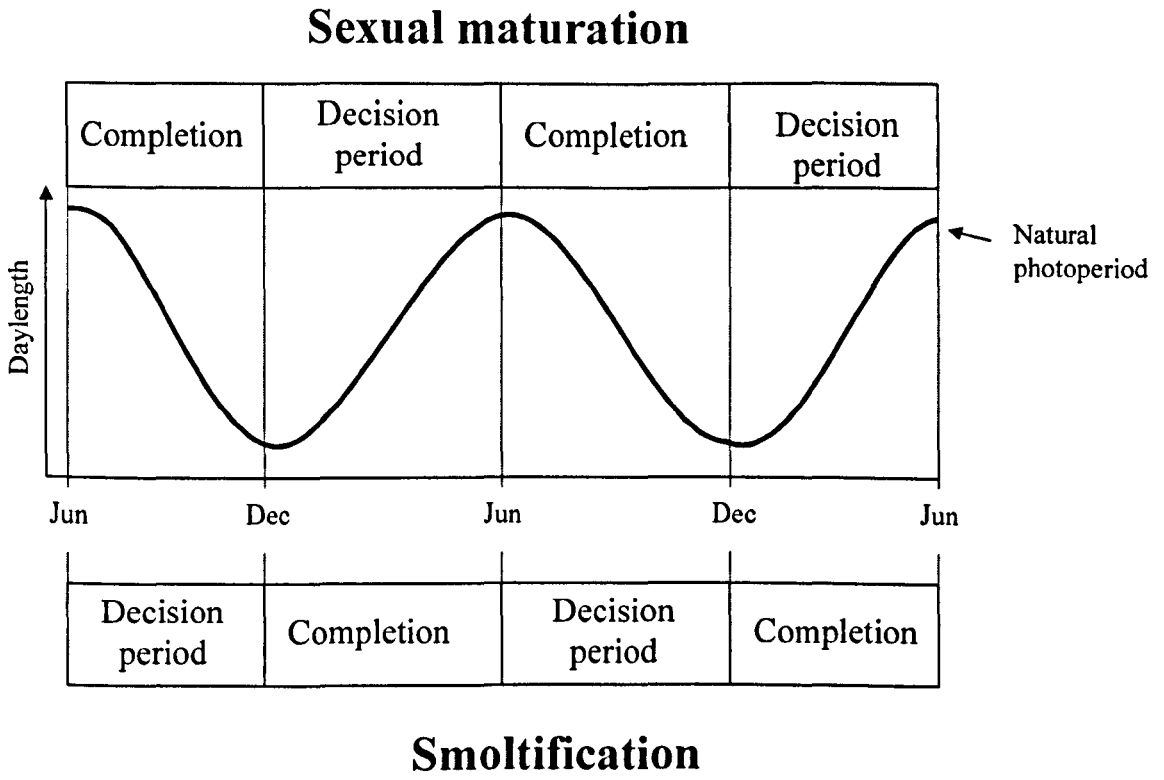


Fig. 1.3 Developmental model detailing the proposed stimulatory effects of photoperiod on maturation and smoltification. Adapted from Duston and Saunders (1992).

processes are under some form of internal cyclic control. In particular, following early work by Whitehead *et al.* (1978), extensive evidence has suggested the presence of an endogenous rhythm of maturation (Lundqvist, 1980; Bourlier and Billard, 1984a; Bromage *et al.*, 1984; Elliott *et al.*, 1984; Duston and Bromage, 1986, 1987, 1991; Hansen *et al.*, 1992). However, although it has been shown that a seasonally-changing daylength is not essential for the cueing and modulation of reproductive development (Bromage *et al.*, 1982) it has been shown that a rhythm of maturation is likely to be entrained by photoperiod, because spawning can occur at any time of the year provided the appropriate photoperiodic cues are received (Elliott *et al.*, 1984).

Indeed, as well a rhythm of maturation substantial evidence exists that other physiological processes are under similar, photoperiodically entrained, endogenous control, in particular growth (Clarke *et al.*, 1978; Saunders and Harmon, 1988; Villarreal *et al.*, 1988; Duncan and Bromage, 1998; Duncan *et al.*, 1999) and smoltification (Clarke *et al.*, 1978; Erikson and Lundqvist, 1982; Stefansson *et al.*, 1989; Thrush *et al.*, 1994; Sigholt *et al.*, 1995).

However, in order to manipulate developmental processes such as maturation and smoltification, within commercial production, it is necessary to understand the photoperiodic cues that entrain such endogenous cycles.

In fresh water the majority of work has focused on photoperiodic effects on smoltification in order to achieve early seawater transfer times and to improve levels of seawater adaptation. Much of the current smolt production is based on S1 regimes (i.e. transfer after 1 year in freshwater) (FRS Annual production survey, 2000), which

generally utilise a natural yearly photoperiod. However, for earlier transfer times, in under one year (i.e. 0+ regimes), altered photoperiods are used. For these regimes, continuous light is generally applied from first-feeding, which results in enhanced growth enabling individuals to reach the critical size for smoltification (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988) at an early time in the year. However, prolonged continuous light regimes can reduce the incidence of smoltification or produce fish with a poor or reduced smolt status (McCormick *et al.*, 1987; Skilbrei, 1991; Solbakken *et al.*, 1994; Duston and Saunders, 1995; Handeland and Stefansson, 2001). Therefore a period of short days (generally of between 8 and 10 hours daylength) of 6 to 10 weeks is required to initiate the parr-smolt transformation (Berg *et al.*, 1994; Duston and Saunders, 1995; Sigholt *et al.*, 1995; Duncan and Bromage, 1998; Duncan *et al.*, 1998). Following the short day treatment a period of continuous light or long days is required during which smoltification is completed (Berg *et al.*, 1994; Duston and Saunders, 1995; Sigholt *et al.*, 1995; Duncan and Bromage, 1998; Handeland and Stefansson, 2001), although it is important to note that the length of these long and short day regimes is clearly affected by temperature (Handeland and Stefansson, 2001). Indeed, photoperiod regimes such as those described above are becoming of considerable importance to the successful out-of-season production of large numbers of competent smolts.

Photoperiod regimes have also been found to advance or delay maturation. Early work by Bromage *et al.* (1984), Elliott *et al.* (1984) and Takashima and Yamada (1984) concluded that maturation could be initiated by a period of long days, with a period of short days necessary in later development during which maturation was completed. Subsequently light regimes have been used to restrict the maturation of

adults in sea water, although photoperiod manipulations have not currently been used to limit maturation in fresh water, due to the complex interactions that occur with the initiation and completion of smoltification. For adults, additional lighting, provided during the early winter months, has been shown to increase growth and reduce grilsing (Hansen *et al.*, 1992; Taranger *et al.*, 1995; Oppedal *et al.*, 1997; Taranger *et al.*, 1998; Porter *et al.*, 1999a; Taranger *et al.*, 1999a) although the results of some continuous light treatments have been contradictory (c.f. Saunders and Harmon, 1988; Krakenes *et al.*, 1991; Endal *et al.*, 2000) with such variations possibly linked to experimental differences, for example the timing of the continuous light treatment or the relative intensity of the lights used.

However, although photoperiod manipulation will influence the timing of both maturation and smoltification it has also been suggested that the initiation of such physiological processes, in particular maturation, will be influenced by certain growth rates during seasonally-critical periods (Thorpe, 1986; 1987b; Duston and Saunders, 1992; Metcalfe, 1998; Thorpe and Metcalfe, 1998; Taranger *et al.*, 1999a).

Thorpe (1986) initially proposed a model including such principles suggesting that if the rate of acquisition of energy was sufficient during early spring, then maturation would be initiated. Subsequently Duston and Saunders (1992) have supported this theory by observing that maturation was initiated during the increasing phase of the photoperiod provided sufficient growth thresholds were achieved. Indeed, there is growing evidence that the decision to mature can be influenced by the potential for growth during the natural spring period (Adams and Thorpe, 1989; Berglund, 1992; Rowe and Thorpe, 1990b; Thorpe *et al.*, 1990; Rowe *et al.*, 1991; Duston and

Saunders, 1997). However, although the attainment of a particular threshold may influence the initiation of maturation the rate at which a particular physiological parameter changes may also be of importance in the initiation of maturation during the seasonally-critical periods (Metcalfe, 1998; Thorpe *et al.*, 1998).

It is also important to note that an adjustment to the original model proposed by Thorpe (1986) has been suggested such that the initiation of maturation occurs in November, one year prior to maturation (Metcalfe, 1998; Thorpe *et al.*, 1998), with a time prior to first-feeding, therefore, possible (Thorpe, 1994b). Subsequently, maturation can be "switched off" during a second sensitive period in spring (Metcalfe, 1998; Thorpe *et al.*, 1998). However, it is remains likely that environmental manipulation during the spring will be of considerable importance in altering the rates of maturation found within commercial populations (c.f. Hansen *et al.*, 1992; Taranger *et al.*, 1999a).

1.5.2.2. Nutrition

The acquisition and utilisation of food has clear effects on the ability of fish to grow in both size and composition (primarily adiposity and protein content). As such the food that a fish receives will ultimately affect its decision to undergo a particular developmental strategy.

In wild populations the ability to find and utilise food is a major limiting factor in growth and development although it is clear that the effects of such seasonal fluctuations can be limited because salmonids, like other fish, are able to undergo periods of recovery growth following periods of nutritional restriction (Weatherley

and Gill, 1981; Dobson and Holmes, 1984; Miglavs and Jobling, 1989a, b; Quinton and Blake, 1990; Metcalfe and Thorpe, 1992).

In commercial salmonid stocks feed is controlled and is rarely a limiting factor in development. However, it is clear that an understanding of how diet affects the growth and the development of fish will be required in order to maximise productivity and investigations have been conducted to consider the effects of feed, in terms of both its quality and its quantity. For the investigations of the current thesis the quality of diets was considered with regard to lipid inclusion, although it is important to note that it may be difficult to separate the effects of ration and diet composition since varying ration ultimately varies the absolute values of constituents a fish receives (Shearer, 1994).

For growth, increases in ration clearly result in elevated growth (Reinitz, 1983; Storebakken and Austreng, 1987a, b; McCormick *et al.*, 1989; Stead *et al.*, 1996) although it is also evident that lipid deposition can increase with ration size (Elliott, 1976; Reinitz, 1983; Storebakken and Austreng, 1987a; Johansson *et al.*, 1995; Hillestad *et al.*, 1998). Increases in dietary lipid inclusion, however, have been shown to result in pronounced increases in body lipid content (Reinitz, 1983; Bjerkeng *et al.*, 1997; Grisdale-Helland and Helland, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001) although a gain in weight has also been recorded (Hemre and Sandnes, 1999; Torstensen *et al.*, 2001). Consequently Shearer *et al.* (1997) have postulated that ration affects growth whereas dietary lipid level affects adiposity.

Limited research presents itself for the effects of diet on the parr-smolt transformation, although it is likely that the attainment of a size threshold of smoltification will be affected by diet through changes in growth. Furthermore, these effects will prove important if they occur during seasonally-critical periods when developmental processes can be influenced by growth (Thorpe, 1986; Duston and Saunders, 1992; Thorpe, 1994b; Metcalfe, 1998; Thorpe *et al.*, 1998). However, during the latter stages of freshwater development feed restriction has been shown to have a negligible effect on smoltification (Dickhoff *et al.*, 1989; Thorpe and Metcalfe, 1998; Larsen *et al.*, 2001) with the long term exposure of parr to diets containing different lipid inclusions also found to have no effect on the parr-smolt transformation (Redell *et al.*, 1988). It is therefore likely that high growth rates will be important for successful smoltification but not necessarily high levels of body lipid (Saunders *et al.*, 1982).

Where maturation is concerned, an initial accumulation of body fat occurs (Aksnes *et al.*, 1986; Rowe *et al.*, 1991; Simpson, 1992; Kadri *et al.*, 1996) with the energetic costs of spawning (c.f. Jonsson *et al.*, 1991; Jorgensen *et al.*, 1997) subsequently resulting in a reduction in lipid reserves. As such there has been the suggestion that maturation is dependent on an individual attaining a certain lipid threshold (Herbinger and Friars, 1992; Simpson, 1992; Shearer, 1994; Silverstein *et al.*, 1997). Consequently, the accumulation of body lipid by feeding diets containing elevated lipid levels has been shown to result in an increase in maturation (Hillestad *et al.*, 1998; Shearer and Swanson, 2000). However, although there is only limited evidence that long-term ration of feed can affect maturation levels (e.g. McCormick and Naiman, 1984) it is evident that attempts to reduce maturation levels in salmon

populations have primarily focused on periods of feed restriction. Indeed it is clear that for both adults (Thorpe *et al.*, 1990; Reimers *et al.*, 1993; Silverstein and Shimma, 1994; Hopkins and Unwin, 1997) and juveniles (Rowe and Thorpe, 1990b; Clarke and Blackburn, 1994; Berglund, 1995; Morgan and Metcalfe, 2001) feed restriction has resulted in a reduction in the incidence of maturation. Although efforts have been made to investigate such restrictions throughout the year it appears that short-term treatments are most effective during spring (Rowe and Thorpe, 1990b). This further highlights the importance of spring as a critical period during which growth rates can influence the decision to undergo maturation (Thorpe, 1986; Duston and Saunders, 1992; Thorpe, 1994b; Metcalfe, 1998; Thorpe *et al.*, 1998).

1.5.2.3. Temperature

Studies into the effects of temperature on salmonid development are particularly problematic primarily due to the costs of either chilling or heating water. However, the manipulation of temperature, in particular heating, is increasingly being used in commercial production.

For the effects of temperature on growth, Allen (1940, 1941) noted that below 7°C Atlantic salmon parr were inactive feeding slowly without growth, although more recent studies suggest that growth will occur at temperatures lower than 7°C (Shelbourne *et al.*, 1973; Elliott, 1975a; Dwyer and Piper, 1987; Koskela *et al.*, 1997). It is also evident that a clear relationship exists between temperature and growth. Typically, as temperature rises growth rates increase (Shelbourne *et al.*, 1973; Elliott, 1975a; Clarke *et al.*, 1981; Dwyer and Piper, 1987; Bjornsson *et al.*, 1989; Siemien and Carline, 1991; Solbakken *et al.*, 1994; Koskela *et al.*, 1997; Edsall *et al.*, 1999)

although such increases will only occur up to a certain temperature, after which growth declines (Shelbourne *et al.*, 1973; Elliott, 1975a; Dwyer and Piper, 1987; Siemien and Carline, 1991; Edsall *et al.*, 1999). Therefore, there is an optimum temperature for growth in Atlantic salmon parr and this appears to occur between 15 and 18°C (Shelbourne *et al.*, 1973; Knutsson and Grav, 1976; Dwyer and Piper, 1987; Siemien and Carline, 1991; Edsall *et al.*, 1999) although it should be noted that other factors such as fish size (Shelbourne *et al.*, 1973; Elliott, 1975a) and ration of feed (Elliott, 1975b) may affect the way in which temperature influences growth.

For smoltification, negligible and even some slight detrimental effects have been found when increased temperatures are applied towards the end of the freshwater period (Dickhoff *et al.*, 1989; Solbakken *et al.*, 1994; Duston and Saunders, 1997; Larsen *et al.*, 2001). However, the majority of evidence suggests that elevated temperatures during winter and spring photoperiod regimes increase the proportion of smolts and their hypo-osmoregulatory ability (Björnsson *et al.*, 1989; Soivio *et al.*, 1989; Berglund *et al.*, 1991; Staurnes *et al.*, 1994b; Duston and Saunders, 1997). Furthermore, it has also been reported that elevated temperatures can aid the further development of hypo-osmoregulatory ability following seawater transfer (Handeland *et al.*, 1998; Handeland *et al.*, 2000).

Evidence relating to the effects of temperature on maturation are limited and contradictory. Herbinger and Friars (1992) found no effect of temperature on maturation in Atlantic salmon parr, whereas Taranger and Hansen (1993) and Taranger *et al.* (1999b) found that elevated temperatures inhibited ovulation in adult female Atlantic salmon. Berglund *et al.* (1991) recorded lower rates of rematuration in

previously mature salmon parr, which had been maintained at elevated winter temperatures. In contrast, Johnston *et al.* (1987) found that a temperature of 12°C stimulated gametogenesis whereas at lower temperatures oocyte maturation did not occur. Furthermore, Nakari *et al.* (1987) found that although an advanced photoperiod regime resulted in early maturation eggs were not ovulated until the temperature was >4°C. Given the contradictory evidence that exists further research is necessary in order to understand the effects of temperature on maturation.

However, in the absence of environmental cues such as photoperiod, it is possible that temperature will act as a seasonal cue for development. It is also possible that temperature will affect the magnitude of the response that fish make to other environmental factors such as photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994) and it may therefore be appropriate to consider temperature as a factor that controls the rate at which physiological processes occur, as opposed to directly entraining development.

1.6 Experimental aims

Currently the freshwater production of Atlantic salmon depends on transferring fish to sea at appropriate times of the year in order to aid the year round production of fish of harvestable size. However, during the freshwater stage it is also important to maximise growth rates as well as achieve a high incidence and quality of smoltification. Environmental manipulation plays a primary role in these physiological processes, but for maturation there is a clear lack of information regarding such effects. The interactions between growth, smoltification and maturation are also poorly understood and these are further investigated in the current thesis where the overall aims were as follows:

1. To investigate the role of photoperiod on growth, maturation and smoltification in Atlantic salmon parr and to understand the interactions determining life history strategy.

    1.a. The effects of winter photoperiod timing on the development of Atlantic salmon parr.

    1.b. The effects of winter photoperiod timing and duration on the development of Atlantic salmon parr.

2. To investigate the role of diet on growth and the accumulation of body lipid content and to elucidate the effects of such gains on development in Atlantic salmon parr.

2.a. The role of dietary lipid level on growth, lipid deposition and development in Atlantic salmon parr.

2.b. The role of ration of feed on growth, lipid deposition and development in Atlantic salmon parr and the subsequent interactions with photoperiod.

In summary, the experiments detailed in this thesis aimed to further our understanding of the environmental influences on freshwater development in a commercially important species and to elucidate the determining factors which result in an individual following a particular life history strategy.

Chapter 2: General Materials and Methods.

2.1. Fish husbandry

2.1.1. Experimental sites.

The fish studied in the current experiments were investigated at seven different sites. Site 1 represented a freshwater hatchery and rearing facility, with water fed by a shallow river. Site 2 was a freshwater rearing site again fed by a shallow river, although the fish used at Site 2 were reared to first-feeding at a separate hatchery where water was again supplied through a shallow river. Sites 3, 4 and 5 were seawater on-growing sites located in sea lochs (salinity = 25.2±0.6 ‰). Site 6 was a freshwater hatchery and rearing facility, with water supplied through a loch fed, shallow river. Finally, Site 7 was a freshwater on-growing facility, with water fed from a shallow reservoir. All sites were located between 56°N and 57°N.

2.1.2. Fish stocks.

The fish used in experiments I and II, detailed in Chapter 3, were from a high grilsing Scottish stock (Marine Harvest Scotland) whereas those studied in experiment III were from a medium grilsing Scottish stock (Marine Harvest Scotland). Those used in the experiments detailed in Chapter 4 were from a low grilsing Scottish stock (Lakeland).

2.1.3. Fish maintenance.

All fish were held under flow through conditions in either square or circular, fibreglass tanks. All tanks were covered with light-proof polythene covers or fibreglass tank lids. Light was artificially supplied (detailed below) and unless otherwise stated, controlled by clockwork timers (±15min.) (Kingshield timer,

Powerbreaker PLC; Harlow, UK). In all cases light intensities were measured using a photosensitive meter (Skye Instruments Ltd.; Powys, UK).

The fish used in experiment I were maintained throughout the experimental period in 2m square, 1.6m^3 tanks, with an external stand-pipe adjusted to maintain a water depth of approximately 0.3m. Light was supplied by one 500 watt halogen light (Ring lighting; Leeds, UK) creating approximately 3800 lux at the water surface and 1200 lux at the tank bottom. In experiment II, fish were initially held under similar conditions before being moved to 4m diameter, 17m^3, circular tanks, with an external standpipe adjusted to maintain a water depth of 0.9m. Light in these tanks was supplied by two 500 watt halogen lights (Ring lighting; Leeds, England) creating 3500 lux at the water surface and 1100 lux at the tank bottom.

The fish described in Chapter 4, experiment IV, were maintained in 0.7m diameter, 0.25m^3, circular tanks, with an internal standpipe adjusted to maintain a water depth of 0.5m. Light was supplied by one 100 watt filament light (RS Components Ltd.; Corby, UK) providing 80 lux at the water surface and 25 lux at the tank bottom. Both of the studies in experiment V were conducted in 1m square, 0.4m^3, tanks, with an external standpipe adjusted to create a water depth of 0.3m. Light was supplied by one 16 watt drum fitting light (RS Components Ltd.; Corby, UK) creating 1550 lux at the water surface and 320 lux at the tank bottom. During the natural photoperiod regime used for experiment Vb, light was controlled using a photosensitive switch (RS components Ltd.; Corby, UK) adapted by Alex Brewsters electrical contractors (Stirling, UK).

2.2. Anaesthesia

For all experiments fish were anaesthetised for sampling purposes in a 1:20,000 bath of 2-phenoxy ethanol (Sigma; Poole, UK) in farm water. Anaesthesia typically took approximately 3 min. Recovery from anaesthesia was achieved using a bath of aerated farm water. No mortalities were recorded following anaesthesia.

2.3. Fish sacrifice

For the removal of blood and tissue samples fish were considered too small for repeated sampling. They were therefore sacrificed prior to the removal of samples. To achieve this fish were anaesthetised in a 1:10,000 solution of 2-phenoxy ethanol and then killed with a strong blow to the dorsal surface of the head such that death was instantaneous.

2.4. P.I.T. tagging

In order to identify individual fish within the respective populations passive integrated transponder (PIT) tags (Avid tags; Norco, USA) were used. Tags (12mm) were placed in the peritoneal cavity, by making a 5mm incision in the posterior, ventral surface of the fish (slightly anterior to the pelvic fins) and injecting the tag into the cavity. The tag reader (Avid tags; Norco, USA) was then used to scan the tag to ensure that it was functioning correctly, after which a 3:1 mixture of Orahesive powder (Squibb and Sons Ltd.; Hounslow, UK) and Cicatrin antibiotic (The Wellcome Foundation Ltd.; Middlesex, UK) was applied to the incision area. To aid the identification of tagged individuals within the much larger non-tagged population the adipose fin of tagged fish was removed during the tagging procedure. Fish were tagged at as small a size as

possible (<1.5g) with mortalities due to the procedure low (<1%) and unrelated to size.

2.5. Growth measurement

Measurements of fish length and weight were made throughout the experiments. In all cases fork length was measured (±1mm) and weights (±0.1g) were recorded using an electronic balance (Model QC7DCE-S, Sartorius AG; Goettingen, Germany).

2.6. Blood sampling

Blood samples were taken via the dorsal caudal aorta of culled fish. Blood was drawn into 1ml syringes (Terumo Europe N.V.; Leuven, Belgium) using either 23 or 25 gauge sterile needles (Terumo Europe N.V.; Leuven, Belgium), for fish smaller and larger than 20g respectively. Where blood was to be used for serum testosterone analysis syringes were first rinsed with a 4mg. ml^{-1} solution of porcine intestinal heparin (Sigma; Poole, UK), whereas for serum osmolarity determination no such procedure was necessary. All blood samples were placed in 1.5ml microcentrifuge tubes and centrifuged at 2500 rpm for 15 min. at 4°C. Sera or plasma was then removed and stored at -70°C, until analysis was performed. Due to fish size blood samples were frequently pooled to achieve the necessary volumes for the respective analytical techniques.

2.7. The identification of maturity.

2.7.1. External identification

During each sampling period experimental populations were examined for signs of maturation. Although mature parr have traditionally been identified based on their

external appearance (i.e. stunted size, dark coloration, increased mucus production) it was possible that such a procedure would not prove sufficiently precise. Therefore, for external identification individuals were only classified as mature if milt could be expressed following slight abdominal pressure.

2.7.2. Internal identification

During the experiments a number of fish were dissected to identify internal signs of maturation. Following dissection such fish were classified as maturing if their testes showed clear signs of development (i.e. thickening and whitening).

2.7.3. Maturation index

During the later experiments a more detailed classification of internal maturity status was made. Previously, Billard (1992) had listed 9 distinct stages of gonadal development including gonadal re-absorption. In the current experiments fish were categorised up to spermiation and a maturity index, adapted from that of Billard (1992), was used as shown below:-

Index 1: Gonadal tissue completely undeveloped, with pink and string-like testes.

Index 2: Slight thickening of gonadal tissue although testes remain pink in coloration.

Index 3: Clear gonadal development with thickened, white testes.

Index 4: Testes developed to full size although the external expression of milt is absent.

Index 5: Full gonadal development and the external expression of milt.

Fish with indices of 3 to 5 were considered to be maturing but not those with indices of 1 to 2.

2.7.4. Plasma testosterone analysis

Changes in the levels of plasma testosterone were used to investigate the development of maturation over time. The determination of plasma testosterone was measured by a direct radioimmunoassay, adapted from that of Duston and Bromage (1987):-

Testosterone standard

A stock standard solution was produced by dissolving 1μg of freeze dried testosterone (Sigma; Poole, UK) in 10ml of Analar grade absolute ethanol (Sigma; Poole, UK) to create a testosterone concentration of 100ng.ml^{-1}. This solution was stored at -20°C. A working standard solution was produced by diluting 100μl of stock standard in 0.9ml of absolute ethanol to create a testosterone concentration of 10ng.ml^{-1}.

Assay buffer

The following constituents were dissolved in 150ml of nanopure water and stirred at 35°C. This solution was then made up to 500ml with nanopure water and chilled to 4°. This buffer could be stored for 7 days at 4°C.

Disodium hydrogen phosphate	8.88g
Sodium dihydrogen phosphate	5.82g
Sodium chloride	4.50g
Gelatin	0.50g

All chemicals were Analar grade, purchased from BDH chemicals Ltd. (Poole, UK).

Antibody

1g freeze dried anti testosterone rabbit antiserum (Biogenesis; Poole, UK) was diluted in 1ml of nanopure water and stored in 100µl aliquots at -20°C until required. A working antibody solution was created by diluting 200µl of antibody in 19.8ml of assay buffer (sufficient for 200 assay tubes).

Extraction

Prior to determination it was necessary to extract the testosterone from the plasma samples according to the following protocol:-

1. Thaw samples thoroughly.
2. Place 50µl plasma into a polypropylene assay tube (LP3P tubes, Thermo Life Sciences; Basingstoke, UK).
3. Add 1ml ethyl acetate (Sigma; Poole, UK), stopper the tube and spin on a rotary mixer for 1 hr.
4. Centrifuge the tubes at 1500rpm for 10min. at 4°C.
5. Store tubes at 4°C until assayed.

Assay protocol

All samples and standards were assayed in duplicate according to the following protocol:-

1. Prepare a series of dilutions of the standard testosterone hormone with absolute ethanol in polypropylene tubes (LP3P, Thermo Life Sciences; Basingstoke, UK) to give a range of concentrations from 0-1000pg/100µl (Fig. 2.1). Include a further tube containing 100µl ethanol which will be used to calculate the non-

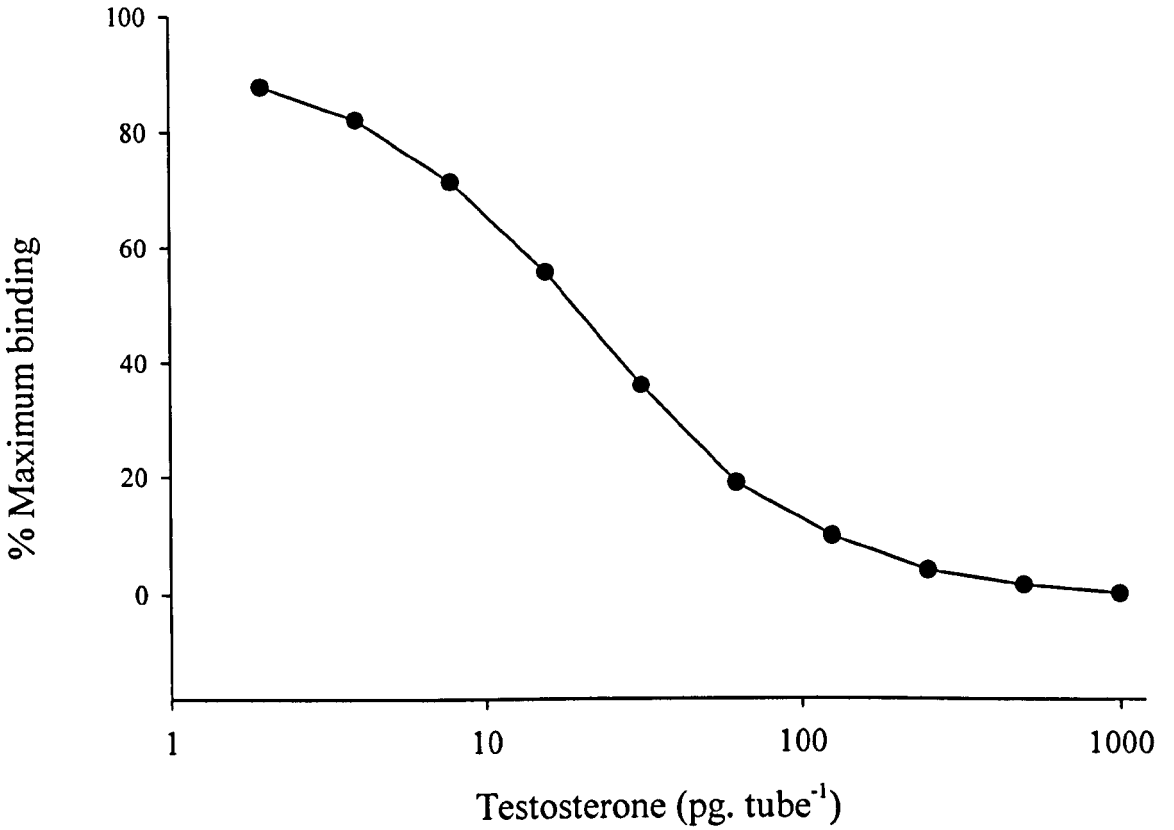


Fig. 2.1 A typical standard curve obtained from the radioimmunoassay of testosterone. Samples of unknown plasma testosterone content were evaluated using the curve, following radioimmunoassay to identify the samples relative percentage binding.

specific binding (NSB).

2. Add 100µl of each sample extract to the sample tubes.
3. Dry down the standards and sample extracts in a vacuum oven at less than 35°C.
4. Cool the dry tubes to 4°C.
5. Add 100µl of antibody to all standard and sample tubes except the NSB tubes.
6. Add 100µl of tritiated testosterone to each tube, vortex and incubate at 4°C for 18 hours.
7. Dissolve 0.3g dextran-coated charcoal (Sigma; Poole, UK) in 125ml of assay buffer and stir on ice for 30min.
8. Add 500µl of charcoal solution to each tube, vortex and incubate for 10 min at 4°C.
9. Centrifuge at 2000rpm for 10 min at 4°C.
10. Transfer 400µl supernatant to 6ml polyethylene scintillation vials (Packard Biosciences; Groningen, The Netherlands) and add 4ml of scintillation fluid (Packard Biosciences; Groningen, The Netherlands). Transfer 4ml of scintillation fluid to 3 empty vials. Add 100µl of tritiated testosterone to two of the vials for the calculation of total radioactivity. Use the remaining vial of scintillation fluid to calculate the background radioactivity.
11. Vortex the vials thoroughly and count the radioactivity for 5 minutes in a scintillation counter (1900TR LSA, Canberra Packard Ltd.; Pangbourne., UK).

Assay disintegration per minute (dpm) values were converted to pg testosterone.tube^{-1} using the "Assayzap" computer program (Elsevier Biosoft) for the Apple Macintosh.

Quality control and validation

The sensitivity of the assay (i.e. the minimum amount of testosterone able to be distinguished from zero) was 1.9pg. $tube^{-1}$. Pooled extractions with a testosterone content of approximately 55pg. $tube^{-1}$ were used to check the reproducibility of measurements, both between and within assays; the intra-assay coefficient of variation was calculated as 4.47% whereas the inter-assay coefficient of variation was 6.60%. Serial dilutions of a pooled sample extract were used to obtain an inhibition curve (Fig. 2.2). No statistical difference ($p>0.05$) was found between the slopes of the inhibition plot and the standard curve regression lines. This confirmed that the testosterone being measured in the samples was immunologically similar to that in the standards.

2.8. Assessment of smoltification

During each experiment it was necessary to assess the development and completion of the parr-smolt transformation. This was achieved using several methods.

2.8.1. Na^+, K^+ -ATPase determination

Gill Na^+, K^+ -ATPase is an enzyme important in the ionic regulation of fish in sea water, with changes in enzyme activity often used as an indicator of the parr-smolt transformation in salmonids (McCormick, 1993).

The determination of Na^+, K^+ -ATPase was performed according to the method detailed by McCormick (1993) which measures the oxidation of NADH by the ouabain sensitive hydrolysis of ATP. This method has been shown to highly sensitive and reproducible (McCormick, 1993).

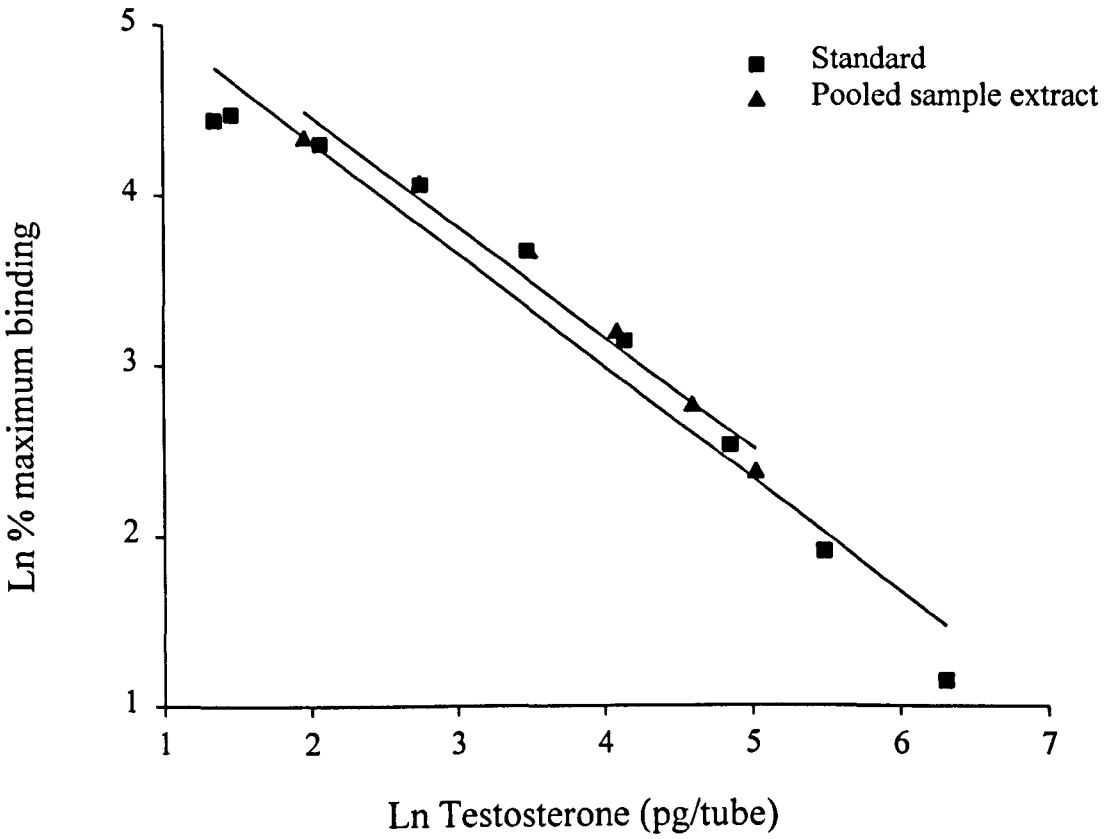


Fig. 2.2 The parallelism of the inhibition curves obtained from the testosterone standard and a serial dilution of salmon plasma extract. The parallel nature of the lines indicates that the testosterone in the standards and samples was immunologically similar, therefore validating the assay for Atlantic salmon parr.

Assay constituents.

All chemicals were Analar grade purchased from Sigma (Poole, UK).

SEI buffer: The following constituents were dissolved in 475ml of nanopure water. The pH was adjusted to 7.3 with 1.0 M hydrochloric acid and a final volume of 500ml achieved by adding nanopure water. At 4 °C this buffer could be stored for three months.

Sucrose	25.67g
Sodium EDTA	1.86g
Imidazole	1.70g

SEID: 0.1g sodium deoxycholic acid dissolved in 20ml SEI buffer. At room temperature this solution could be stored for 1 week.

Imidazole buffer: 1.702g imidazole dissolved in 475ml of nanopure water. The pH was adjusted to 7.5 with 1.0 M hydrochloric acid and a final volume of 500ml achieved by adding nanopure water. At 4 °C this solution could be stored for 3 months.

Salt buffer: The following constituents were dissolved in imidazole buffer to create 500ml of solution. At 4 °C this solution could be stored for 3 months.

Sodium chloride	5.52g
Hydrous magnesium chloride	1.07g
Potassium chloride	1.57g

PEP: 0.491g phosphoenolpyruvate dissolved in 100ml imidazole buffer. Stored in 5ml aliquots at -70°C.

Ouabain solution: 0.382g ouabain dissolved in 50ml imidazole buffer. At room temperature this solution could be stored for three months.

ADP standard: 0.0489g adenosine diphosphate dissolved in a 25ml solution of sodium acetate buffer (0.4627g sodium acetate in 100ml nanopure water with pH adjusted to 6.8). Stored at -70°C in 200µl aliquots.

Gill biopsy.

4-6 filaments of gill tissue were removed from the second gill arch using fine tipped scissors. These were placed into 100µl of ice-cold SEI buffer. Samples were immediately frozen in liquid nitrogen and stored at -70°C until enzyme determination.

Enzyme activity

All assay preparation was carried out on ice.

Assay medium (AM) was prepared immediately prior to analysis with the following constituents:-

Pyrvuate kinase		105µl
Lactate dehydrogenase		16.6µl
NADH		5mg
PEP		5ml
ATP		0.0145g
Imidazole buffer	Make upto:	35ml

Pyruvate kinase (from rabbit muscle: activity 400-600 units per mg protein) and lactate dehydrogenase (from rabbit muscle: activity 800-1200 units per mg protein) were centrifuged at 12000rpm for 8 min. at 5 °C prior to mixing.

NADH was purchased as a disodium salt in pre-weighed vials.

Assay medium was stored at 4 °C until required.

AM preparation:

1. Transfer 17.5ml of AM to a vial and add 1.25ml of imidazole buffer (AM-I).
2. Transfer 17.5ml of AM to a vial and add 1.25ml of ouabain solution (AM-O).
3. Transfer 8.1ml of AM-I to a vial and add 2.7ml of salt buffer.
4. Transfer 8.1ml of AM-O to a vial and add 2.7ml of salt buffer.

These solutions were stored at 4°C until required. Prior to use the AM-I/salt and AM-O/salt were warmed in a water bath at 26°C for 10 minutes.

ADP standard preparation:

ADP standard solution was diluted with imidazole buffer to create ADP standards of 0, 5, 10 and 20 nmoles. $10\mu l^{-1}$ concentration. 10µl of each standard was transferred, in triplicate, to wells on a 96 flat-well multiwell plate (Elkay Laboratories Ltd; Basingstoke, UK).

Sample preparation:

1. Thaw samples thoroughly.
2. Add 25µl SEID to each sample.

3. Homogenise (after homogenisation enzyme activity declines. Therefore subsequent procedures must be completed within 30 min. of homogenisation).
4. Centrifuge at 5000rpm for 30 sec. at 4 °C.
5. Transfer 10µl of sample to each of four wells on a 96 flat well multiwell plate.

Assay completion:

1. Add 200µl of warmed AM-I/salt medium to wells containing all ADP standards as well as 2 replicates of each sample.
2. Add 200µl of warmed AM-O/salt medium to wells containing the remaining 2 replicates of each sample.
3. Measure the oxidation of NADH over 10 min. at 340nm using a multiwell spectrophotometer (Multiskan Ex, Labsystems; Farnborough, UK).
4. Using the plot of ADP standard samples (Fig. 2.3) and the colormetric difference between the AM-I and AM-O wells after 10 min. establish the enzyme activity in terms of ADP hydrolysed per hour.

Protein determination:

Na^+, K^+ -ATPase activity is expressed as µmol ADP hydrolysed. mg $protein^{-1}$. hr^{-1}. Therefore the protein content of each sample was established.

Samples were analysed using a bicinchoninic acid protein assay kit (Sigma; Poole, UK) using the following protocol:

1. Prepare protein standards of 0, 5, 10 and 20µg. $10\mu l^{-1}$ by diluting 2mg. ml^{-1} bovine serum albumin standard with nanopure water.
2. Transfer 10µl of each standard, in triplicate, to a 96 flat-well multiwell plate.

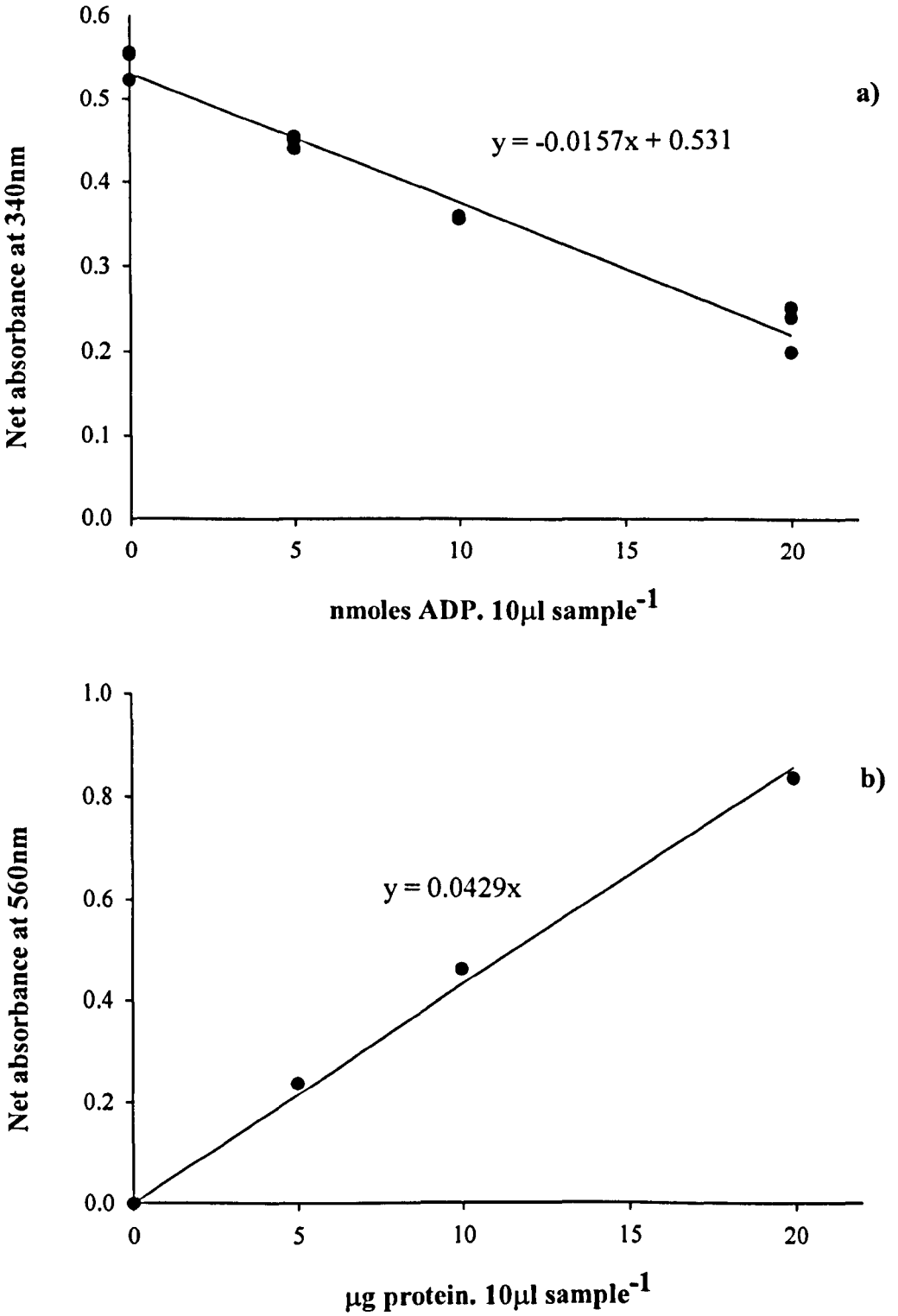


Fig. 2.3 Typical standard plots of enzyme activity (a) and protein content (b) used during the determination of Na^+, K^+ -ATPase activity in salmon parr. Samples of unknown enzyme activity and protein content were evaluated using the regression equations generated from the standard plots.

3. Centrifuge samples at 5000rpm for 30 sec. at 4 °C.
4. Transfer 10µl of each sample, in triplicate, to the 96 flat-well multiwell plate.
5. Add 200µl of a 50:1 solution of bicinchoninic acid and copper II sulphate pentahydrate solution (4%) to each well of the multiwell plate.
6. Incubate at 37 °C for 30 min.
7. Cool to room temperature and measure the absorbance at 560nm using a multiwell spectrophotometer (Multiskan Ex, Labsystems; Farnborough, UK).
8. Using the plot of protein standards (Fig.2.3) the protein content of each gill sample is calculated with ATPase activity then expressed as µmol ADP hydrolysed. mg protein^{-1}. hr^{-1}.

2.8.2. Seawater tolerance

As parr commence the physiological changes associated with the parr-smolt transformation their ability to survive in sea water increases. Seawater (37.5ppt) survival was therefore assessed at regular intervals using a protocol similar to that described by Saunders *et al.* (1985).

1. In large, clear polythene bags, 1.875 kg of Instant Ocean synthetic sea salt (Animal House; Batley, UK) was allowed to dissolve in 50 l of continually aerated (Mistral 3 Air pump, Algarde; Nottingham, UK) water over 24h. Salinity was subsequently checked as being 37.5ppt using an optical refractometer (Amago mill; Japan).
2. From each experimental treatment 15 randomly selected individuals were placed into separate seawater bags. Mortalities were recorded and removed daily for 96h.
3. Total percentage mortality over 96h was then calculated for each treatment group.

2.8.3. Seawater challenge

As parr commence and undergo the parr-smolt transformation they increase their ability to regulate the ion flux associated with seawater residence. This hypo-osmoregulatory ability can be studied using a seawater (35ppt) challenge with the subsequent assessment of the serum osmolality of surviving individuals.

1. In large, clear polythene bags, 1.750 kg of Instant Ocean synthetic sea salt (Animal House; Batley, UK) was allowed to dissolved in 50 l of continually aerated (Mistral 3 Air pump, Algarde; Nottingham, UK) water over 24h. Salinity was subsequently checked as being 35ppt using an optical refractometer (Amago mill; Japan).
2. From each experimental treatment 15 randomly selected individuals were placed into separate seawater bags.
3. After 24h mortalities were recorded. Surviving individuals were culled and blood removed as described in Section 2.6.
4. Blood samples were allowed to clot before being centrifuged at 2500 rpm for 15min. at 4 °C. Serum was removed and stored at -70°C until osmolarity was determined.
5. Samples were allowed to thaw thoroughly after which serum osmolality was determined using a 3MO plus, Advanced Micro-Osmometer (Advanced Instruments Inc.; Massachusetts, USA).

2.8.4. Smolt index

Body silvering and fin coloration has often been used as a general measure of smoltification. During the current experiments the degree of body coloration was determined using a index system described by Sigholt *et al.* (1995) as follows:-

Index 1:	Typical parr, with parr marks clearly visible.
Index 2:	Parr marks visible but some silvering.
Index 3:	Silvered with visible parr marks.
Index 4:	Typical smolt, no parr marks visible.

2.8.5. Cohort analysis

At the conclusion of the experiments detailed in Chapter 3 it was evident that the photoperiod regimes had resulted in fish cohorts that could not easily be identified using either the smolt index system described above or by the division of fish into a particular mode of a bimodal distribution. Therefore a separate analytical system was developed which accounted for both fish size and coloration. The terminology of Birt and Green (1986) and Sigholt *et al.* (1995) was used to aid in the interpretation of the each morphological nomenclature such that:-

Smolts:	Fully silvered fish with no parr marks and blackened fin margins. These fish were typically >30g and <65g.
Large smolts:	Fully silvered fish with no parr marks and blackened fin margins although these fish were significantly larger than the smolts described above (i.e. >100g).

Silvered parr:	Fish that were partially silvered with parr marks that were obscured but still visible. These fish were typically >30g and <65g.
Parr:	Fish showing no signs of silvering with the presence of distinct parr marks. These fish were typically >30g and <65g.
Large parr:	Fish showing some slight silvering although distinct parr marks predominated. However, these fish were significantly larger than the parr described above (i.e. >80g)
Small parr:	Fish showing no signs of silvering with the presence of distinct parr marks although these fish were significantly smaller than the parr described above (i.e. <15g).

Although this nomenclature represents all of the cohorts identified during the experiments detailed in chapter three it is important to note that all cohorts were not necessarily identified within each treatment. Furthermore, the cohorts identified within the non-tagged populations were not necessarily all represented in the PIT tagged populations. Due to this the growth profiles of the PIT tagged fish may display different cohort structures to the graphs documenting total population structure.

2.9. Whole body lipid determination

Whole body samples that were taken for lipid determination were stored at -20°C until analysis. Lipid levels were initially calculated as a percentage of dry weight with samples dried to constant mass in a drying oven (Gallenkamp; Loughborough, UK)

held at 100 °C. Dried samples were ground into a coarse powder using a small domestic electric food blender (Optiblend 2000, Moulinex; Paris, France).

Lipid determination was performed by the soxhlet extraction method using a Soxtec HT 6 extraction unit (Tecator AB; Höganäs, Sweden) as follows:-

1. Approximately 1-3g of sample was weighed into an extraction thimble (Whatman International Ltd.; Maidstone, UK) and the extraction thimbles were fitted to the Soxtec unit.
2. An extraction cup containing 5 glass beads (BDH chemicals Ltd.; Poole, UK) was weighed.
3. 50ml of petroleum ether (Fisher Scientific Ltd.; Loughborough, UK) was added to each extraction cup and the cups were fitted to the Soxtec unit.
4. The extraction thimbles were lowered into the boiling petroleum ether for 20min.
5. The thimbles were then rinsed for 1hr 25min after which the petroleum ether was evaporated from the extraction cup for 15min.
6. The extraction cup was placed into the oven for 1hr at 100°C.
7. The cooled extraction cup was then re-weighed.
8. Percentage dry weight lipid was then calculated as:-

$$\% \text{ lipid} = \text{extracted lipid weight/sample wt.} 100$$

9. These values were then converted to % wet weight lipid content as follows:-

$$\% \text{ wet wt lipid} = (\% \text{ dry wt})/100.\% \text{ dry wt lipid}$$

2.10. Analytical calculations

2.10.1. Condition factor

Condition factor (CF) has previously been used as a measurement of body fat content (Herbinger and Friars, 1991) and as such links have been made to the condition of maturing individuals. Furthermore, changes in condition have been used as a measure of smoltification (with a decline in CF from approximately 1.2 in salmon parr to about 0.9 indicative of the parr-smolt transformation). Therefore in the current experiments condition factor was calculated from the measured length and weight of individual fish as follows:-

$$\text{Condition factor} = [\text{Weight (g)}.100] / \text{length (cm)}^3$$

2.10.2. Specific growth rate (SGR)

Specific growth rate (SGR) was calculated based on changes in weight over a known time as follows:-

$$\text{Specific growth rate (\%. day}^{-1}) = [\text{Ln } Wt_2 - \text{Ln } Wt_1] / (t_2 - t_1)$$

Where: Wt_1 = fish weight (g) at time t_1

Wt_2 = fish weight (g) at time t_2

During the experiments detailed in Chapter 3 PIT tagging allowed the specific growth rate of individual fish to be calculated. In Chapter 4 growth was calculated from mean treatment weights.

2.11. Statistical analysis

The statistical principles used within this thesis are described in Sokal and Rohlf (1995) and Zar (1999). The majority of calculations were performed using the Minitab statistical package (release 13.1). Where non-parametric multi-comparison tests were performed, using Dunn's procedure, "in house" software (courtesy of Dr. Mark Thrush) was executed using Minitab statistical package (release 12.1). Where statistical analyses were calculated by hand Microsoft Excel 97 was used to aid data manipulation. A significance level of 5% was used for all tests.

2.11.1. Estimation of the population mean

The arithmetic mean ($\bar{X}$) was used to provide an estimation of the population mean (μ). In all cases $\bar{X}$ was used along with the standard error of the mean (S.E.M.) to give a representation of the sample distribution.

$$\text{Arithmetic mean } (\bar{X}) = \frac{\sum X}{n}$$

Where: $\sum X$ = the sum of observed samples

n = the number of observations

$$\text{Standard error of the mean (S.E.M.)} = \frac{s}{\sqrt{n}}$$

$$\text{Where } s = \text{sample standard deviation} = \sqrt{\frac{\sum X^2 - \frac{(\sum X)^2}{n}}{n-1}}$$

2.11.2. Parametric assumptions.

All parametric techniques are based on a number of fundamental assumptions. Firstly, all observations must be derived randomly and the variation of these observations should be independently distributed. Furthermore, parametric tests require sample variations to be identically distributed (i.e. homogeneous) with a normal population structure. Therefore all data were investigated to confirm normality and homogeneity of variance prior to detailed statistical analysis.

2.11.3. Testing for normality and homogeneity of variance

Where general linear models were performed (see section 2.11.4.) *n* was typically large enough to allow normality and homogeneity of variance to be confirmed by the examination of the residual plots. However, where *n* was insufficient to allow this or where other statistical techniques were performed the following tests were used:-

Normality

Normality was checked using the Kolmogorov-Smirnov test. This non-parametric test is typically used to compare two cumulative frequency distributions (*F*) but it can be adapted to compare the distribution of a known distribution with an expected distribution. This therefore allows sample populations to be compared to the normal distribution.

Homogeneity of variance

For the comparison of two sample variances the *F*-test was used as follows:-

$$F_s = \frac{s_1^2}{s_2^2}$$

Where s_1^2 and s_2^2 are the greater and lesser variances respectively.

Degrees of freedom $v_1, v_2 = n_1\text{-}1, n_2\text{-}1$

The F value was then compared to tabulated values such that if the calculated value was greater than or equal to the tabulated value, at the 5% level, the variances were considered as heterogeneous.

Bartlett's test (B) was used to compare more than two sample variances. The distribution of B is approximated by the chi-squared distribution although an improved approximation can be obtained as follows:-

$$B_c = \frac{B}{C} \qquad \text{with } k\text{-}1 \text{ degrees of freedom}$$

Where:

$$B = \left(\ln s_p^2\right)\left(\sum_{i=1}^{k} v_i\right) - \sum_{i=1}^{k} v_i \ln s_i^2 \qquad \text{Where } v_i = n_i\text{-}1$$

n_i is the size of sample i

s_p^2 is the pooled variance

$$C = 1 + \frac{1}{3(k-1)}\left(\sum_{i=1}^{k}\frac{1}{v_i} - \frac{1}{\sum_{i=1}^{k} v_i}\right)$$

Again, if the calculated value was greater than or equal to the tabulated value, at the 5% level, the variances were considered as heterogeneous.

2.11.4. Sample comparison

All parametric tests were performed using the analysis of variance technique. However, these calculations were manipulated by the use of General Linear Models

(GLM). Using the Minitab statistical package it was possible to manipulate the ANOVA by constructing model formulae which accounted for a number of factor levels with replication and repeated measures sampling also included. As such it was possible to increase the robustness of each test for the particular parameters that were available. Furthermore, where replicate differences occurred the GLM accounted for the variation between the replicates when presenting statistical significance levels.

For post-hoc multiple comparisons Tukey tests were used. This method involves the pairwise comparison of group means to give the test statistic q such that:-

$$q = \frac{\overline{X}_B - \overline{X}_A}{\sqrt{\frac{s^2}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}}$$

Where: $\overline{X}_a$ and $\overline{X}_B$ are sample means

n_1 and n_2 are the number of observation in each sample

s^2 is the error mean square (calculated by the ANOVA)

If the calculated q value was greater than the tabulated value, at the 5% significance level, the means of the two samples were considered to be significantly different.

2.11.5. Non-parametric techniques

Where the assumptions required for parametric analysis were not met it was necessary to perform a non-parametric equivalent to an ANOVA. In the current experiments a Kruskal-Wallis non-parametric test was performed by ranking the samples and calculating the test statistic H as follows:-

$$H = \frac{12\sum n_i\left[\overline{R}_i - \overline{R}\right]^2}{N(N+1)}$$

Where: n_i is the number of observations in group i

N is the total sample size

$\overline{R}_i$ is the average of the ranks in group i

$\overline{R}$ is the average of all ranks

However where there is tied data it is suggested that H is adjusted such that:-

$$H\ (adj) = \frac{H}{1-\left[\sum\left(d_j^3 - d_j\right)/\left(N^3 - N\right)\right]}$$

Where: J distinct values occur among the N observations and for the Jth value there are d_j tied observations

If the calculated H *(adj)* value was greater than the tabulated value, at the 5% significance level, the samples were considered as significantly different. Differences between samples were then compared using Dunn's multiple range test with the test statistic Q calculated as follows:-

$$Q = \frac{\overline{R}_B - \overline{R}_A}{\sqrt{\left(\frac{N(N+1)}{12} - \frac{\sum\left(t^3 - t\right)}{12(N-1)}\right)\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}}$$

Where: $\overline{R}_A$ and $\overline{R}_B$ are the mean ranks of the samples

N is the total number of observations

t is the number of ties for a given value

n_A and n_B are the number of observations in each sample

2.11.6. Analysis of proportions

During the analysis of the data it was necessary to compare proportions. In order to achieve this 95% confidence limits were calculated for the respective proportions, detailed by Fowler and Cohen (1987) as follows:-

$$95\% \text{ confidence limits} = 1.96\left(\sqrt{\frac{p(1-p)}{n-1}}\right)$$

Where: p is the sample proportion

n is the number of sampling units

If the upper and lower confidence limits of respective proportions were not found to overlap the two proportions were considered as statistically different at the 5% level.

2.11.7. Correlation coefficient

The degree of linear relationship between two variables was considered by calculating the Pearson product moment correlation coefficient (r):-

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{(n-1)s_x s_y}$$

Where: $\bar{x}$ and $\bar{y}$ are the means of the variables

s_x and s_y are the standard deviations of the variables

If the calculated r value was greater than the tabulated r value, at the 5% level, the correlation between variables was considered to be significant.

Chapter 3: Photoperiodic effects on growth, maturation and smoltification.

3.1. Introduction.

3.1.1. Light.

In recent years the manipulation of light has become of increasing significance in salmonid aquaculture due to its influence on physiological processes linked to growth, maturation and smoltification. Accurately understanding how light influences such processes therefore requires investigation into the roles of spectral composition (wavelength), light intensity (energy) and photoperiod (daylength) (Boeuf and Le Bail, 1999). However, there are distinct differences in the amount of literature present for each of these aspects and this has led to some confusion concerning their individual significance (Boeuf and Le Bail, 1999). For the role of light intensity, limited research presents itself (e.g. Wallace *et al.*, 1988; Stefansson, 1990; Hansen and Skilbrei, 1997) with even more sparse data present for the effects of spectral composition (e.g. Stefansson and Hansen, 1989). However, for photoperiod extensive literature can be found and it is generally accepted that it is the most important aspect of light controlling fish development (Stefansson, 1989) although it is also evident that some confusion remains concerning how it influences development, particularly in juvenile salmon.

3.1.2. Light intensity

Interest into the effects of light intensity on fish physiology has only become evident in recent years and is currently both limited and contradictory. Wallace *et al.* (1988)

provided some of the first work into the effects of light intensity by exposing juvenile Arctic charr and Atlantic salmon to intensities of either 700, 200, 50 or 10 lux over a 35 day period. For the charr growth was highest at 50 lux indicating a light intensity optimum, whereas for the salmon ambiguous results were presented. Although 700 lux proved most beneficial for growth this intensity also caused high levels of mortality indicating an increased level of stress (Wallace *et al.*, 1988). Subsequently Oppedal *et al.* (1997) exposed post-smolt salmon to natural daytime light supplemented with additional night-time illumination of low, medium and high intensity, from January until June. Under the high intensity regime increases in live body weight were observed with gutted weight also correlated to light intensity. No mature fish were found amongst the additional light treatments with low levels present in the naturally lit groups and it was concluded that growth and the proportion of fish which mature may be influenced by light intensity thresholds (Oppedal *et al.*, 1997). Although the influence of light intensity on maturation may be disputed in these experiments (primarily due to the influence of continuous light as a function of daylength: see Section 3.1.4) some role of light intensity in fish growth does seem possible (Wallace *et al.*, 1988; Oppedal *et al.*, 1997).

However, Stefansson *et al.* (1993) have provided results indicating that light intensity is not influential in fish development. In their experiments Atlantic salmon parr were exposed to a natural photoperiod regime using either 715, 335 or 27 lux between November and May. No differences in growth were found between groups with all fish completing the parr-smolt transformation successfully. Post-smolt maturation in the Autumn following seawater transfer was also unaffected by the light intensity regimes used in fresh water. Additionally, Oppedal *et al.* (1999) found that continuous

light regimes of either low, medium or high intensity had no effect on growth and the incidence of maturation in post-smolt salmon and it seems that growing evidence provides support that light intensity is not necessarily influential in fish development (Stefansson, 1990; Dahle *et al.*, 1999).

However, studies that investigate light intensity can only provide information regarding changes that occur within the range of intensities used and although in some cases no effects are seen it is likely that certain developmental processes will require a minimum threshold of light. In support of this Hansen and Skilbrei (1997) investigated the growth of salmon parr exposed to continuous light of either 1000, 100, 10, 1 or 0 lux and concluded that growth was lower under the 1 lux intensity than under the other continuous light regimes.

It is also likely that the difference between day- and night-time light intensity will be important in how fish perceive daylength. Thoranensen *et al.* (1988) used a 12 week period of short days, followed by 10 weeks of long days, to stimulate smoltification in coho salmon parr although during the night-time periods illumination of between 0.0001 and 0.5 lux was provided. It was found that growth, body silvering and seawater adaptation were reduced in all groups receiving the night-time light and it was concluded that the threshold level for the inhibition of smoltification may be close to 0.0001 lux (Thoranensen *et al.*, 1988). Similarly, Hansen and Skilbrei (1997) investigated the effects of day- and night-time light intensity on growth using combinations of 1000, 100, 10, 1 or 0 lux during the respective periods of a constant LD 8:16 regime. They found that groups given more than 10 lux during their "dark" period grew at similar rates to fish that were exposed to continuous light of 10 lux or

higher. They also concluded that fish measure daylength by comparing the light intensity of the respective light and dark periods of a photoperiod with 1 lux sufficient to be recognised as night-time illumination if the day-time intensity is sufficiently high. In support, Stefansson *et al.* (1991) found that if fish were exposed to a simulated natural photoperiod regime using 1400 lux during the day-time and 27 lux during the night-time growth was similar to fish exposed to a continuous 1400 lux light regime.

In conclusion, there is now a preliminary understanding of the role that light intensity plays in both fish development and the perception of daylength although our current understanding of these processes is still relatively limited. However, from these principles it is possible that light intensity manipulations may prove important for future commercial gain.

3.1.3. Spectral composition

For the physiological effects of spectral composition Stefansson and Hansen (1989) provide the most notable work to date. In their study different light sources were used to manipulate both the colour temperature (K) and colour reproduction (R_a) of light to which fish were exposed. They concluded that neither fish growth nor the parr-smolt transformation were affected by light sources of different spectral composition. Stefansson and Hansen (1989) did not consider the effects of spectral composition on maturation and to date no such literature presents itself.

3.1.4. Photoperiod

Although some evidence suggests that fish development is affected by, in particular, light intensity, it is generally accepted that photoperiod is the most important aspect of light controlling fish development (Stefansson, 1989). As such the role of photoperiod is now extensively investigated and its manipulation is of increasing importance in commercial aquaculture.

3.1.4.1. Growth

Light periodicity has been shown to exert a primary role on the growth of fish. In particular the effects of photoperiod have been extensively documented in both adult (Krakenes *et al.*, 1991; Hansen *et al.*, 1992; Oppedal *et al.*, 1997; Duncan *et al.*, 1999; Endal *et al.*, 2000) and juvenile salmonids (Saunders *et al.*, 1985; Villarreal *et al.*, 1988; Stefansson *et al.*, 1989; Stefansson *et al.*, 1991; Solbakken *et al.*, 1994; Skilbrei *et al.*, 1997) although similar relationships have been found in a range of other economically important species such as the Atlantic cod (Dahle *et al.*, 1999; Hansen *et al.*, 2001), the sea bass (Rodriguez *et al.*, 2001) and the Atlantic halibut (Jonassen *et al.*, 2000; Simensen *et al.*, 2000).

Daylength primarily influences growth by allowing an increase in food intake (Higgins and Talbot, 1985; Boujard and Leatherland; 1992; Stead, 1997; Bolliet *et al.*, 2001), which is linked to the visual recognition of feed items, as the photoperiod is extended. However, it has also been found that feed conversion efficiency is linked to daylength (Higgins and Talbot, 1985; Jonassen *et al.*, 2000) with changes in activity and anabolic effects of photoperiod thought to contribute to this correlation (Jonassen

et al., 2000). It is therefore, likely that a general correlation will exist between light and growth.

Most evidence suggests that actual daylength is of primary importance to fish growth. For Atlantic salmon in freshwater Komourdjian *et al.* (1976) provided the first early evidence that growth is enhanced by increased photoperiod. By investigating natural and reciprocal photoperiod regimes (Fig. 3.1) it was found that growth was dependant on daylength regardless of the direction of the changing photoperiod (Komourdjian *et al.*, 1976). Subsequently Clarke *et al.* (1978) documented high growth rates in sockeye and coho salmon parr exposed to long day regimes. More recently it has been shown that continuous light results in bimodality being weakened (Stefansson *et al.*, 1989; Skilbrei, 1991) or delayed (Duncan and Bromage, 1998) with periods of extended light increasing the growth rate of small and medium size range fish allowing their entry into the upper modal group (UMG) (Stewart *et al.*, 1990). It therefore seems that a clear link between daylength and growth is evident and indeed supporting evidence for the importance of long day or continuous light is now extensive (Lundqvist, 1980; Saunders and Henderson, 1988; Stefansson *et al.*, 1989; Saunders and Harmon, 1990; Solbakken *et al.*, 1994; Sigholt *et al.*, 1995).

However, it is also apparent that in freshwater, when such photoperiods are applied for long periods of time, conflicting results can occur. Villarreal *et al.* (1988) exposed Atlantic salmon parr to either continuous light or a natural photoperiod shortly after first-feeding and recorded increased growth for continuously illuminated groups throughout the subsequent six month period. More recently Handeland and Stefansson

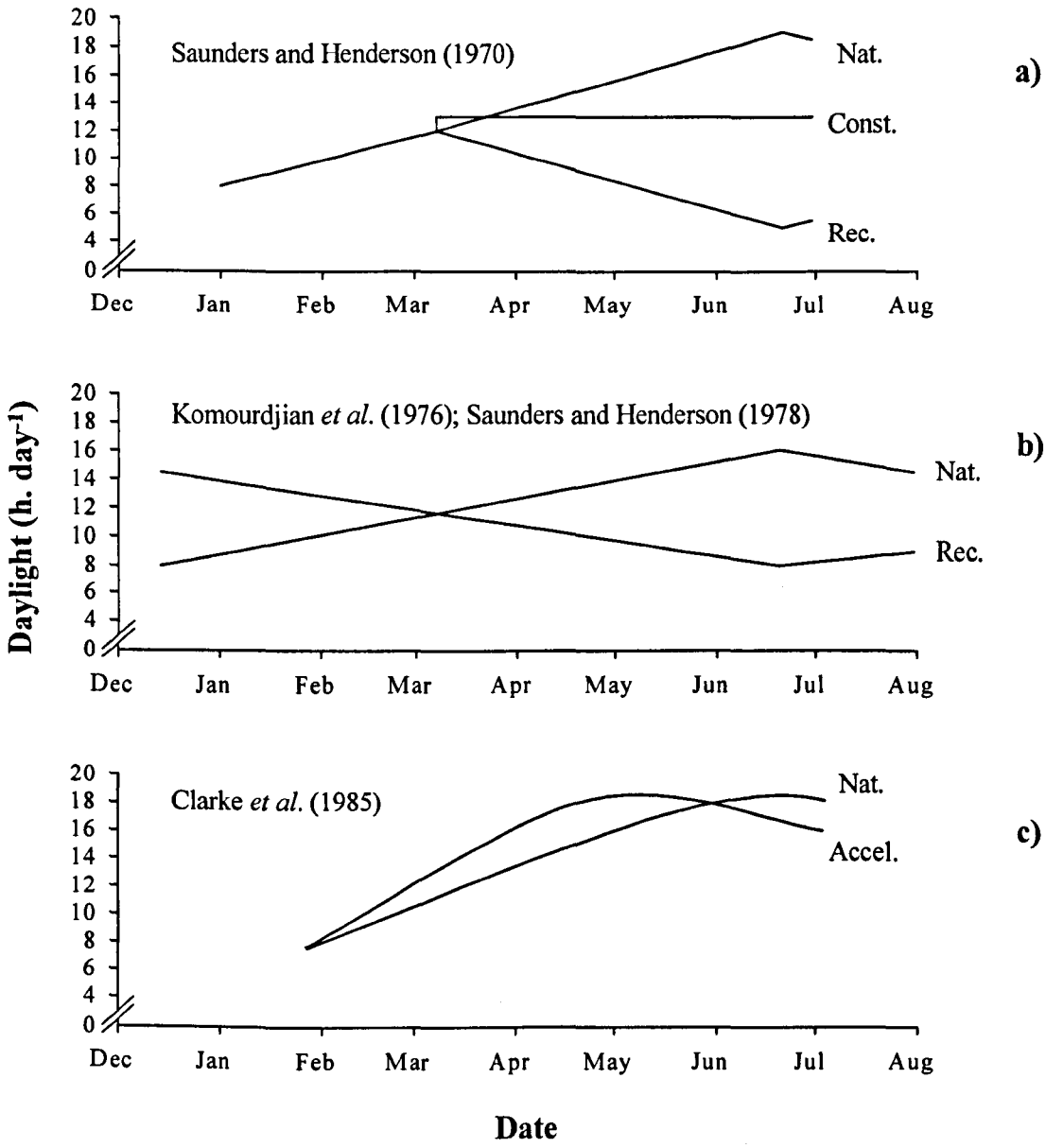


Fig. 3.1 Photoperiod regimes used during experiments conducted by Saunders and Henderson (1970) (a), Komourdjian *et al.* (1976) and Saunders and Henderson (1978) (b), as well as Clarke *et al.* (1985) (c). Nat. denotes a simulated natural photoperiod, Rec. denotes a reciprocal natural photoperiod, Const. denotes a constant photoperiod, Accel. denotes an accelerated natural photoperiod.

(2001) documented the growth of parr exposed to constant LD 24:0 being greater than for those exposed to shorter day regimes during the freshwater phase.

However, over extended periods of time shorter day regimes may prove more beneficial to growth. Saunders and Henderson (1988) exposed first-feeding fry to either constant LD24:0, LD16:8, LD 12:12 or an ambient photoperiod from May until January. Constant LD24:0 resulted in the highest growth rates for the initial three months after which fish exposed to LD16:8 grew faster than all other groups. Similarly, Berg *et al.* (1994) found that when constant LD24:0 was applied to first-feeding fry growth was only elevated for two months. Subsequently Solbakken *et al.* (1994) and Stefansson *et al.* (1989) have recorded similar differences between fish exposed to long days and those held on a range of other shorter day photoperiods. Generally, it seems that extended or continuous illumination for between three (Saunders and Henderson, 1988; Solbakken *et al.*, 1994) and five months (Stefansson *et al.*, 1989; Solbakken *et al.*, 1994) will result in enhanced growth rates compared to individuals exposed to shorter day regimes. After this initial period growth rates tend to decrease (Stefansson *et al.*, 1989; Solbakken *et al.*, 1994) so that shorter day photoperiod regimes may result in higher growth rates with earlier size differentials often reduced (c.f. Stefansson *et al.*, 1989) or even reversed (c.f. Saunders and Henderson, 1988).

In sea water, extended daylengths applied to tank-reared Atlantic salmon post-smolts have also resulted in enhanced growth rates (Saunders and Harmon, 1988; Taranger *et al.*, 1995) with such results observed when extended light has been applied between

August and either September or January (Saunders and Harmon, 1988) and between January and May (Taranger *et al.*, 1995). Comparable results have also been found for Atlantic salmon in sea cages (Krakenes *et al.*, 1991; Hansen *et al.*, 1992; Taranger *et al.*, 1995; Endal *et al.*, 2000). Krakenes *et al.* (1991) recorded that post-smolt growth increased with additional night-time light supplied between late February and June and such growth increases have also been observed when additional lighting is applied from October until June (Hansen *et al.*, 1992), from January until May (Taranger *et al.*, 1995) and from either November, December or January until July (Endal *et al.*, 2000). Oppedal *et al.* (1997) and Oppedal *et al.* (1999) have also recorded increased growth rates for salmon exposed to constant LD24:0 during their experiments into the effects of light intensity on fish growth. Therefore in sea water, unlike fresh water, long-term increases in growth seem to be related to additional lighting although it is important to note that growth differentials will not necessarily be observed during the period when additional light is supplied (c.f. Endal *et al.*, 2000; applied additional lighting from November, December or January until July, with resultant increases in mean body weight only observed from May onwards).

It is also important to note that in sea water increases in growth due to the application of additional lighting may be preceded by an initial 1 to 2 month decrease in growth (Hansen *et al.*, 1992; Taranger *et al.*, 1995; Porter *et al.*, 1999b; Endal *et al.*, 2000). Hansen *et al.* (1992) documented an initial decrease between October and December whereas Endal *et al.* (2000) recorded a six week growth depression before rates rose. A similar initial growth depression has not been recorded in fresh water and it therefore seems to be linked to adult growth. However, there is a limited understanding of the causal factors involved in this growth depression and although

some evidence suggests that additional lighting causes an initial appetite suppression (Taranger *et al.*, 1995) there is currently no detailed explanation for this occurrence.

It is therefore possible to generalise that the growth of both adult and juvenile salmonids is enhanced by either continual illumination or long day photoperiod regimes. As might be expected from these findings periods of illumination shorter than the natural daylength have been shown to cause a reduction in growth. Saunders and Harmon (1990) reported a decrease in the growth rates of Atlantic salmon post-smolts following the reversion to natural light from a two-month period of constant LD24:0. Skilbrei *et al.* (1997) recorded reduced increases in length and a higher proportion of lower modal group (LMG) fish for juvenile Atlantic salmon exposed to short day regimes (i.e. 6 to 12 h daylight) when compared to those held under longer day (i.e. 12+ h daylight) treatments. Similar changes have also been recorded when fish are moved from fresh to sea water. Lower growth rates were recorded for fish that were moved from continuous illumination in fresh water to a natural photoperiod in sea water (Duncan *et al.*, 1998; Duncan *et al.*, 1999), with the observed changes dissociated from any environmental effects linked to the transfer because no growth depression was observed in fish held on LD24:0 throughout both fresh- and sea-water development.

However, although it is clear that actual daylength can be correlated with growth rate a further hypothesis presents itself. It is possible that the rate of change of photoperiod is influential in growth and indeed some works present conflicting results (e.g. Skilbrei, 1991). Komourdjian *et al.* (1976) stated that changes in growth were due to actual daylength regardless of whether the photoperiod was increasing or decreasing,

but Clarke *et al.* (1978) reported the first evidence of a rate of change effect. They found that although growth was greatest for sockeye and coho salmon exposed to constant 20h illumination a decreasing photoperiod was conducive to rapid growth with the lowest rates observed on a rapidly increasing photoperiod. However, in a subsequent experiment conducted earlier in the growth season Clarke *et al.* (1978) recorded that growth was elevated under an increasing photoperiod indicating that as well as direction and rate of change seasonal sensitivity to the changing photoperiod was important.

The results presented by Villarreal *et al.* (1988) and Duston and Saunders (1992) also provide tentative support for a rate of change hypothesis. Villarreal *et al.* (1988) recorded that Atlantic salmon exposed to compressed natural photoperiod regimes grew less well than controls. Similarly, Duston and Saunders (1992) observed that groups exposed to 6-, 12- and 18- month adjusted annual photoperiod cycles had 50, 60 and 100% recruitment into the LMG respectively implying a distinct growth differential. Compressed/extended regimes, such as those observed in these experiments, lead to more rapid changes in photoperiod than would naturally occur and it is possible that changes in growth could occur as a function of the rate of the changing photoperiod.

3.1.4.2. Maturation

Although Villarreal and Thorpe (1985) found photoperiod to exert no role on spermatogenesis in juvenile Atlantic salmon, it is now generally accepted that photoperiod is the major environmental factor influencing maturation (Bromage *et al.*, 1984; Takashima and Yamada, 1984; Duston and Bromage, 1986, 1987, 1991;

Duston and Saunders, 1992; Taranger *et al.*, 1999a). As such the use of photoperiod is now widespread in aquaculture for both the prevention or reduction of maturation as well as its enhancement e.g. for the out-of-season production of eggs and larvae (e.g. Macquarrie *et al.*, 1979: for the pink salmon; Johnson, 1984: for the chinook salmon; Bromage *et al.*, 1984: for the rainbow trout). It is therefore clear that a solid understanding of how photoperiod and maturation interact is required for future aquaculture advancement.

Early work into the role of actual daylength on salmonid maturation provides some of the most useful and important work to date. Bromage *et al.* (1984) investigated the effects of various combinations of long and short day photoperiod on spawning in the rainbow trout. They found that long days early in the year initiated ovarian development, with a long day period of only six weeks sufficient to initiate maturation. Following the initiation with long days, a period of short days resulted in maturation being completed (Bromage *et al.*, 1984). Elliott *et al.* (1984) supported this work by investigating the effects of both constant and seasonally-changing photoperiods on maturation in three strains of rainbow trout. These findings confirmed the importance of a long or increasing daylength cue during the early stages of gonadal development with a short day cue important later in the cycle. Further support for this theory was provided by Takashima and Yamada (1984) working on the masu salmon. In these experiments a range of photoperiod combinations were investigated the conclusions being in agreement with the findings of Bromage *et al.* (1984) and Elliott *et al.* (1984). Subsequently, Duston and Bromage (1987) found that an abrupt reduction in photoperiod successfully advanced maturation in rainbow trout, with Taranger *et al.* (1995) finding that, for adult Atlantic

salmon, an abrupt increase in photoperiod reduced the proportion of fish maturing as grilse. By using 6-, 12- and 18-month annual photoperiod regimes, Duston and Saunders (1992) proposed that under a naturally-changing photoperiod maturation is initiated on the increasing phase (during spring) with its completion on the decreasing phase (autumn).

From these studies, further insight into the role of actual daylength has been made. It has been suggested that the initiation of gonadal investment, by long day photoperiod, requires a certain daylength threshold (Saunders and Henderson, 1988). Elliott *et al.* (1984) have suggested that the amount of daylight received in a daily cycle is important in maturation with a daily sensitivity to photoperiodic cues also indicated (Elliott *et al.*, 1984; Duston and Bromage, 1986). Subsequently, for the completion of maturation it has also been shown that the reduction in photoperiod is more important than its actual magnitude (Duston and Bromage, 1987).

However, it is evident that variations to these perceived models of salmonid maturation are becoming increasingly evident with such deviations most notable when constant photoperiods are applied for long periods of time. During the early work of Bromage *et al.* (1984) and Takashima and Yamada (1984) it was noted that spawning was also advanced by constant long days or continuous light. Similarly, Scott *et al.* (1984) found that constant LD18:6 resulted in the early spawning of rainbow trout and, subsequently, similar advancements have been documented for trout (Duston and Bromage, 1986) as well as both juvenile (Skilbrei, 1991) and adult (Hansen *et al.*, 1992) salmon. Although, it should be noted that for the adults investigated by Hansen *et al.* (1992), maturation was not advanced by constant LD24:0 throughout

development but by continuous additional light applied from October until June. It is also important to note that the constant light regimes used in the experiments detailed above were applied after a natural winter photoperiod and as such the period of short days prior to the experimental light regimes may have influenced the initiation of maturation.

It is also evident that periods of continuous illumination or long days can delay maturation. For salmon parr exposed to constant LD 20:4 regimes a delay in ripening has been observed (Erikson and Lundqvist, 1980; Lundqvist, 1980), with a similar finding documented for rainbow trout exposed to constant LD 24:0 from June onwards (Bourlier and Billard, 1984a, b). For chinook salmon held in fresh water a delay in ripening has also been found when the natural ambient daylength was artificially extended between December and March (Johnson, 1984). However, for adult salmon held in sea water there is no evidence that maturation is delayed by extending the natural winter daylength.

As well as the observed effects on the timing of maturation, the use of continuous light or long days has also been found to result in changes in the incidence of maturation. When daylengths longer than those naturally experienced have been applied between August and January (Saunders and Harmon, 1988) and February and June (Krakenes *et al.*, 1991), the proportion of grilse has been shown to increase when compared to fish exposed to natural light. Duncan *et al.* (1999) also recorded increased levels of maturation when smolts were exposed to continuous light and these findings were further supported by Endal *et al.* (2000) who showed that the

proportion of maturing fish was affected by both early exposure, and the duration of, continuous light applied between November and July.

Decreases in the incidence of maturation have also been found following long day treatment. Saunders and Henderson (1988) exposed Atlantic salmon parr to continuous light from first-feeding and found that levels of maturation decreased from 67% of males in naturally lit groups to 55% in continuously illuminated treatments. Furthermore, decreases in the rate of maturation from 19% to 1% (Berg *et al.*, 1994) and from 26% to 12% (Duston and Saunders, 1995) have also been found when comparing fish exposed to a natural photoperiod to those continuously illuminated from first-feeding. Similar reductions have been noted in Atlantic salmon parr exposed to continuous light from December onwards (Skilbrei, 1991).

In sea water reductions in maturation have also been recorded. However, for adult salmon constant long photoperiods have generally been investigated during the winter months onwards. Hansen *et al.* (1992) recorded a decrease in the level of maturation in sea cage reared Atlantic salmon exposed to continuous illumination from October until June. Similarly, Taranger *et al.* (1995) found reduced levels of maturation in tank-reared salmon exposed to continuous light between January and May. Subsequently, Oppedal *et al.* (1997), Taranger *et al.* (1998), Porter *et al.* (1999a) and Taranger *et al.* (1999) have all documented reductions in the incidence of grilse following exposure to additional lighting from winter through to early summer.

It would therefore, seem that when constant daylength regimes are applied to salmon variations in the levels of maturation will occur. However, as seen with salmonid

growth there is some evidence suggesting that the rate at which a photoperiod changes is of primary importance in cueing reproduction. Eriksson and Lundqvist (1980) investigated the effects of a range of both constant and decreasing photoperiod regimes on maturation in Baltic salmon parr. Their experiments showed that an accelerated decreasing photoperiod brought forward maturation whereas a sudden switch from a long to a short photoperiod was not effective in advancing maturity. As with the effects of photoperiod on growth some support can be found by using compressed and extended annual photoperiod cycles. Duston and Saunders (1992) found negligible levels of parr maturation in groups subjected to an 18 month extended annual photoperiod whereas 6- and 12- month cycles provided high levels of maturity. In such experiments, the rate of change of the photoperiod may be providing the cue for maturation although it is possible that the actual period of time that such fish are exposed to a minimum or maximum daylength is providing the most important cue. The exposure of fish to these actual daylengths may also be occurring at seasonally important times thus creating an effect that is not based on the rate of the changing photoperiod.

Even if experimental designs of this nature can be used to support the rate of change hypothesis there is additional growing evidence that increasing or decreasing photoperiods are not the primary influence in salmonid reproduction. Stuart-Kregor *et al.* (1981) noted that for Atlantic salmon the initiation of maturation occurred around the time of the summer solstice, when little change in photoperiod is evident, although this study did use the relatively insensitive gonadal somatic index (GSI) to measure the timing of the initiation of maturation, with the documented changes in GSI possibly occurring some time after maturation was initiated. Elliott *et al.* (1984) and

Saunders and Henderson (1988) compared constant and seasonally-changing photoperiods concluding that it was the amount of daylight received and not the rate of the changing photoperiod that was the primary factor cueing reproduction. However, it has been found that the advancement of maturation is influenced by the reduction in daylength as opposed to the actual magnitude of the reduction with the importance of the direction of this change also highlighted (Duston, 1987; Duston and Bromage, 1986). It is therefore likely that, although daylength is the primary cue affecting maturation the changing photoperiod will be effective by providing the necessary daylength cue at particular times of the year, along with a required directional change.

It is, therefore, clear that the initiation of maturation will require seasonal environmental signals (Thorpe, 1987b) such as changes in photoperiod. However, Polikansky (1983) has also proposed that fish will mature as soon as they are able to do so although it is likely that this initiation will first require the attainment of specific thresholds of growth or development (Bailey *et al.*, 1980; Thorpe and Morgan, 1980; Saunders *et al.*, 1982; McCormick and Naiman, 1984). Subsequently, it has been suggested that such developmental thresholds may influence maturation during seasonally-critical periods (Thorpe, 1986; 1987b; Duston and Saunders, 1992; Metcalfe, 1998; Thorpe and Metcalfe, 1998; Taranger *et al.*, 1999).

Thorpe (1986) provided one of the first models that explained the initiation of maturation. He proposed that for salmon parr photoperiod had a major role in regulating the decision to either maintain or reduce growth during mid-winter. Furthermore, it was suggested that if the rate of acquisition of energy was sufficient

during early spring, when fish are sensitive to the photoperiodic stimulation of hormone systems, maturation would be initiated (Thorpe, 1986). Subsequently, Duston and Saunders (1992) investigated annual photoperiods that were manipulated to occur in either 6-, 12- or 18-month periods. They concluded that the initiation of maturation would occur on the increasing phase of the photoperiod (i.e. in spring) provided sufficient growth thresholds had been achieved although the length of this decision period remained unknown (Duston and Saunders, 1992).

Indeed, there is growing evidence that the decision to mature will be influenced by the potential for growth during the natural spring period. Rowe and Thorpe (1990b) found that for salmon parr rates of maturation were reduced if feed restriction during spring resulted in sufficient reductions in growth. Berglund (1992) found that size and growth prior to the onset of gonadal growth affected the incidence of maturation, with Adams and Thorpe (1989), Thorpe *et al.* (1990), Rowe *et al.* (1991) and Duston and Saunders (1997) all providing additional evidence that spring is a critical period when the attainment of specific growth or developmental thresholds will influence maturation. It is important to note, though, that it may not necessarily be the attainment of a particular threshold that influences maturation. Metcalfe (1998) and Thorpe *et al.* (1998) have suggested that instead of the attainment of a particular threshold both the current state of a particular developmental parameter as well as its rate of change will be influential during the critical period. As such it may be more appropriate to consider a critical period as a time when maturation is influenced as opposed to it being triggered.

Thorpe (1994b), Metcalfe (1998) and Thorpe *et al.* (1998) have subsequently suggested an adjustment to the model presented by Thorpe (1986). They have suggested that the initiation of maturation occurs in November one year prior to maturation (Metcalfe, 1998; Thorpe *et al.*, 1998), with a time prior to first-feeding therefore possible (Thorpe, 1994b). Subsequently, maturation can be "switched off" during a second sensitive period in spring (Metcalfe, 1998; Thorpe *et al.*, 1998), as previously suggested. However, regardless of whether this new model is accurate it is clear that spring will provide the main period during which environmental manipulations will influence maturation. As such photoperiod regimes can be manipulated in order to adjust the timing of the critical period when maturation is influenced and rates of maturation in commercial populations can, therefore, be altered (c.f. Hansen *et al.*, 1992; Taranger *et al.*, 1999).

Although photoperiod manipulations are clearly influential in the cueing of salmonid reproduction some consideration should be made of the effects that such regimes have on milt and egg quality. There is evidence that photoperiod treatment can result in a decline in gamete quality. Macquarrie *et al.* (1979) noted increased egg mortality in pink salmon exposed to an out-of-phase natural photoperiod, although Macquarrie *et al.* (1979) did consider this species to have a very inflexible life cycle and as such it might not adapt well to photoperiod manipulation. However, photoperiod manipulation will not necessarily affect gamete quality. Bourlier and Billard (1984b) found that although exposure to continuous light reduced the milt yield of rainbow trout egg diameter and fecundity were unaffected. Similarly, Bourlier and Billard (1984a) and Johnson (1984) found that photoperiod did not affect gamete quality, with Bromage *et al.* (1984) recording that although photoperiod affected egg size in

the rainbow trout such changes did not have a detrimental effect on fecundity. It therefore seems that the out-of-season production of gametes may not result in a lack of larval quality.

3.1.4.3. Smoltification

The control of smoltification in commercial salmon production is becoming increasingly important to aid the year round supply of fish of harvestable size. By manipulating the timing of smoltification it is possible for producers to utilise the seawater growth rates of adults, which are greater than those for juveniles in fresh water (c.f. Thrush *et al.*, 1994; Duncan *et al.*, 1998) and as such, by shortening the freshwater phase greater productivity can be achieved. Photoperiod manipulation has proved the most productive method for altering the timing of seawater transfer, although a clear understanding of the photoperiodic control of smoltification is vital for its successful use in production.

Typically in natural populations of Atlantic salmon smoltification occurs during the spring (Jones, 1959; Netboy, 1974; Hoar, 1976; Duston and Saunders, 1992). Saunders and Henderson (1970) conducted one of the first experiments that investigated the actual role of photoperiod on smoltification. In their studies it was found that fish exposed to a constant long days in spring developed as natural smolts and were successfully transferred to sea. However, it was also noted that a reciprocal photoperiod (Fig. 3.1) applied during spring (with a decreasing photoperiod) resulted in fish showing silvering but not developing as true smolts (Saunders and Henderson, 1970). Komourdjian *et al.* (1976) further investigated such regimes by exposing parr from mid-winter to either a natural photoperiod regime or a reciprocal photoperiod

that resulted in a declining daylength during the natural spring. Under the natural photoperiod smoltification occurred in the spring with the reciprocal photoperiod resulting in an earlier parr-smolt transformation in February/March (Komourdjian *et al.*, 1976). Similar findings were documented by Saunders and Henderson (1978) in an identical study suggesting that smoltification could be completed under both an increasing (natural) and a decreasing (reciprocal) photoperiod (Fig. 3.1).

However, Clarke *et al.* (1985) applied natural and accelerated photoperiod regimes between February and June (Fig. 3.1) with the accelerated regime significantly advancing smoltification. From this Clarke *et al.* (1985) concluded that the findings of Komourdjian *et al.* (1976) and Saunders and Henderson (1978) were due to the reciprocal photoperiod being applied in mid-winter, so that although a decreasing photoperiod had been applied initially an abrupt increase from the natural short winter daylength to the long reciprocal photoperiod, had occurred. Therefore, smoltification was advanced when compared to the increase experienced under a natural photoperiod regime.

It is clear that short and then long day regimes are required for the development of smoltification with both the magnitude and duration of these daylengths of importance. Indeed this can be highlighted when constant photoperiods are applied for long periods at different times of the year. Saunders *et al.* (1985) found that salmon parr exposed to continuous light between November and February appeared silvered but did not achieved true smolt status in the spring, although it should be noted that the experimental light regime used would have resulted in an increase in daylength after the natural short November photoperiod and this may have provided a similar

stimulus to that experienced during spring by naturally produced smolts. McCormick *et al.* (1987) exposed fish from first-feeding to continuous light or simulated natural photoperiods. Continuous light inhibited smolting in the spring whereas under the natural photoperiod fish successfully underwent the parr-smolt transformation (McCormick *et al.*, 1987). However, in their experiments it was also found that fish exposed to continuous light until October with a subsequent natural winter and spring photoperiod successfully completed smoltification (McCormick *et al.*, 1987). Subsequently, Okumoto *et al.* (1989), Skilbrei (1991), Solbakken *et al.* (1994), Berge *et al.* (1995), Duston and Saunders (1995), Duncan and Bromage (1998) and Handeland and Stefansson (2001) all found continuous light or constant long days to inhibit smoltification or produce fish with a poor or reduced smolt status in the spring.

For short photoperiod regimes Stefansson *et al.* (1989) found that constant LD 8:16 applied from late winter onwards inhibited smoltification. Furthermore, Okumoto *et al.* (1989) also found a reduction in the smolt status of masu salmon when they were exposed to a constant short day regime from October onwards.

It is therefore becoming increasingly evident that the initiation and completion of smoltification is determined by photoperiod and indeed the model proposed by Duston and Saunders (1992) appears most appropriate: with the initiation of smoltification typically occurring on the decreasing phase of a natural photoperiod (winter) and its completion occurring on the increasing phase (spring).

However, for the commercial manipulation of smoltification to prove productive further insight into these long and short periods has been made. Where the short daylength is concerned Okumoto *et al.* (1989) investigated a range of short day regimes to elucidate the most appropriate "winter " daylength. They observed that only fish exposed to 6 hours of daylight showed signs of smoltification one month after the transfer to long days (LD13:11) with short days of 10 hours or less resulting in smoltification after 2 months. Eleven and twelve hour short day regimes required three months of long days before smolt status was evident. It therefore seems that the initiation of smoltification can occur under a range of photoperiods although shorter daylengths will cause the completion of the parr-smolt transformation to occur more rapidly. However, Björnsson *et al.* (1989) investigated a range of winter photoperiod regimes (i.e. LD2:22, LD8:16 and LD14:10) in Atlantic salmon. In contrast to the work of Okumoto *et al.* (1989) winter photoperiod regime had no effect on the hypo-osmoregulatory ability of fish in the spring (Björnsson *et al.*, 1989).

It is clear that a minimum duration of the winter photoperiod will be necessary to successfully initiate smoltification (Berge *et al.*, 1995). Berg *et al.* (1994) found that 10 weeks of LD14:10 in an otherwise continuous light regime was sufficient to initiate smoltification. However, in their experiments parr were exposed to a very early winter photoperiod during late May after first-feeding in February. Sigholt *et al.* (1995) further investigated the short day initiation of smoltification in Atlantic salmon parr in fish previously grown under a continuous light regime until the development as S1's. In these experiments a short day period of LD 8.45:15.15 for 2 weeks was found to be ineffective at stimulating smoltification with a period of between 5 and 7

weeks considered most beneficial. Subsequently, Duston and Saunders (1995) and Duncan *et al.* (1998) have both suggested that a period of 2 months is sufficient although Duston and Saunders (1995) also discuss unpublished data that indicated three months being more beneficial than two when applied in June. Finally, Duncan and Bromage (1998) have suggested that at least 6 weeks of short days will stimulate the parr-smolt transformation. It would therefore seem that a sensible approximation for short day initiation would be a period of between 8 and 10 hours daylight for about 2 months.

After the short day initiation a period of long days will be necessary during which smoltification can be completed. Typically for the completion of smoltification continuous light regimes are used (e.g. Berg *et al.*, 1994; Sigholt *et al.*, 1995; Duncan and Bromage, 1998; Handeland and Stefansson, 2001) although other long day regimes are also in use (e.g. LD 17:7: Duston and Saunders, 1995; LD 23:1, LD19.5:4.5: Duncan and Bromage, 1998). However, Okumoto *et al.* (1989) investigated such long daylengths in detail after a short day treatment of LD 8:16. Using LD 16:8 smolts were first identified 1 month after the period of short days with LD 13:11 resulting in smoltification after 2 months. Under LD 11:13 three months proved most successful with some pre-smolts also observed on the constant short day regime (LD 8:16) after this time (Okumoto *et al.*, 1989). It therefore seems that the greater the daylength after short day initiation the earlier smoltification occurs.

As far as the optimum duration of long days is concerned Björnsson *et al.* (1989) found that hypo-osmoregulatory ability peaked between 1 and 2 months after short

day treatment had ended. Similarly, Sigholt *et al.* (1995) found that gill Na^+, K^+ - ATPase levels peaked after 6 to 8 weeks of long days in Atlantic salmon parr, although Handeland and Stefansson (2001) found that hypo-osmoregulatory ability peaked after only 2 to 3 weeks. However, it is important to note that temperature will play a significant role in determining the required duration of such long day regimes before smoltification can proceed. Clarke *et al.* (1978), Björnsson *et al.* (1989), Duston and Saunders (1997) and Handeland and Stefansson (2001) have all suggested that increases in temperature will have a positive effect on smoltification. However, Solbakken *et al.* (1994) found that although salmon exposed to elevated temperatures showed normal hypo-osmoregulatory ability they subsequently grew poorly in sea water indicating that the increased temperature had interfered with the parr-smolt transformation. This suggests that an optimum temperature exists for smoltification and that these optima may also influence the role of the stimulatory short day regimes as winter photoperiod temperatures have also been found to affect the parr-smolt transformation (Duston and Saunders, 1997). It would appear, therefore, that temperature will control the rate of response to photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994) and it is likely that this occurs through the temperature sensitive manipulation of enzymatic processes within the fish. Furthermore, from these suggestions Sigholt *et al.* (1998) and Handeland and Stefansson (2001) have proposed that the period of long days, after short day treatment, should continue for at least 400 degree days.

However, there is some confusion as to how long hypo-osmoregulatory ability remains elevated. Erikson and Lundqvist (1982) investigated continuous light regimes and found that silvering remained for up to 4 months, although it has been noted that

coloration is not necessarily a good measure of smoltification (Saunders *et al.*, 1985; Duncan and Bromage, 1998). Björnsson *et al.* (1989) found that hypo-osmoregulatory ability remained for at least one month after peak levels, although Thorpe and Metcalfe (1998) suggested that an interval of only 2 or 3 weeks permits seawater transfer in fish exposed to a natural photoperiod. It therefore seems that although there is a window where seawater transfer is possible the closer to the peak in hypo-osmoregulatory ability the better. Furthermore, it would appear that the determination of this peak will be dependant upon the timing of the return to long days as opposed to the timing of the decrease in photoperiod or the duration of the short day period (Duncan and Bromage, 1998).

It is also possible that as with growth and maturation the rate of the changing photoperiod is important in cueing smoltification. Under a naturally-changing photoperiod smoltification is initiated and completed on the decreasing and increasing phases of the photoperiod respectively (Duston and Saunders, 1992) and manipulating the changing photoperiod has been shown to affect the parr-smolt transformation. Saunders and Henderson (1970) found that following a decreasing photoperiod applied in early spring salmon parr showed signs of silvering in early summer but they had a poor seawater tolerance. Similarly Komourdjian *et al.* (1976) found that a reciprocal photoperiod applied from November onwards resulted in early smoltification during February.

The use of compressed/extended as well as out-of-phase annual photoperiod regimes has also been found to affect the timing of smoltification. Duston and Saunders (1992) found that smoltification was delayed when an 18 month extended photoperiod

regime was applied and Thrush *et al.* (1994) and Duncan *et al.* (1998) have used compressed natural daylight cycles to produce smolts during a range of times of the year. Furthermore, Handeland and Stefansson (2001) used a 10 month out-of-phase natural photoperiod regime to manipulate parr into smolting during November concluding that the use of naturally-changing photoperiod treatments could be used for the successful production of out-of-season smolts. However, variable results have been found when compressed natural photoperiods have been used to produce smolts with aberrations in both the expected timing (Duston and Saunders, 1992) as well as the seawater survival (Thrush *et al.*, 1994) of such individuals, although it is possible that compressed photoperiodic cycles will cause such variations by influencing endogenous rhythms of smoltification (see Section 3.1.4.4). It is clear that the rate of the changing photoperiod is important in cueing smoltification but given the results documented when constant daylength triggers have been investigated it is likely that instead of the rate of changing photoperiod having a direct role its influence is more through manipulating the time to which fish are exposed to a particular stimulatory daylength.

Finally, it is important to consider the long-term quality of smolts produced by photoperiod manipulation and it would seem that seawater growth and mortality are two of the most appropriate determinants. For growth rates Thrush *et al.* (1994), Duncan *et al.* (1998) and Handeland and Stefansson (2001) have all found the growth of out-of-season smolts, produced by photoperiod manipulation, to be comparable to those produced under natural regimes. Indeed, Duston and Saunders (1995) found that fish transferred after a 2 month winter photoperiod treatment showed initial growth rates that were higher than naturally produced smolts. Furthermore, Saunders *et al.*

(1985) and Handeland and Stefansson (2001) have found that continuous light regimes applied in fresh water resulted in poor seawater growth highlighting the poor hypo-osmoregulatory ability of such fish.

Variable levels of post-transfer mortality have been found in out-of-season smolts. Duston and Saunders (1995) and Duncan *et al.* (1998) found that smolts produced by photoperiod manipulation had lower mortality rates than those produced naturally. However, Thrush *et al.* (1994) found that such advanced fish did not necessarily fair as well as natural smolts although it should be noted that the fish investigated were relatively small at seawater transfer (e.g. 33.2 ±0.87g). Sigholt *et al.* (1995) found that for fish exposed to 3 months of continuous light after short day treatment seawater mortality rates of 40% occurred. In groups exposed to 2 months of continuous light 34% mortality occurred with 1 month resulting in 15% mortality. However, given that hypo-osmoregulatory ability peaks after one to two months of long days (Björnsson *et al.*, 1989) and remains elevated for only 2 to 4 weeks (Björnsson *et al.*, 1989; Thorpe and Metcalfe, 1998) it is possible that previously recorded seawater mortality rates may have resulted from transferring individuals that have already passed through the smolt "window" and subsequently started to de-smolt.

Therefore, it is clear that although fish size will be an important determinant in the development of smoltification (Elson, 1957; Thorpe *et al.*, 1980) the quality of out-of-season smolts will also be dependant on the photoperiod used to create them, with the timing of such photoperiodic cues critical in order to transfer individuals when their hypo-osmoregulatory ability is at its greatest.

3.1.4.4. Endogenous rhythms

It is clear from the literature presented that light has a distinct influence on physiological processes linked to growth, maturation and smoltification in salmonids. However, these findings only provide insight into the ultimate responses of fish to light without considering the underlying mechanisms controlling these responses. Although suggestions have been made that such physiological processes are controlled by the direct stimulation of light (i.e. direct photostimulation) (Saunders and Harmon, 1988, 1990; Saunders *et al.*, 1989; Krakenes *et al.*, 1991; Duncan *et al.*, 1999) there is an absence of strong supporting evidence for such a theory. However, from the growing evidence that is now apparent it seems likely that the development of fish is influenced by photoperiodically entrained endogenous rhythm(s).

Baggerman (1972) was one of the first authors to suggest the presence of an endogenous rhythm in fish although such findings had previously been documented in other taxa. In her experiments Baggerman (1972) identified a photoperiodic response to light in the stickleback that followed a circadian rhythm of sensitivity. Daily rhythms of photosensitivity have also been identified in salmonids (Duston and Bromage, 1987) but more interestingly it seems that circannual endogenous rhythms entrained by photoperiod affect growth, maturation and smoltification.

For the presence of an endogenous circannual rhythm to be confirmed, Gwinner (1981, 1986) proposed four criteria that should be adequately satisfied such that: the rhythm should be observed for at least 2 full cycles to establish that it is self-sustaining; it should free-run with a periodicity that approximates to, but is significantly different from, 12 months; it should be entrainable by an environmental

zeitgeber, and it should be temperature compensated. Unfortunately these criteria are seldom achieved in the literature, in particular the monitoring of development over long periods of time has proved problematic, but there are notable works that provide important supporting evidence.

Whitehead *et al.* (1978) presented an early indication of a photoperiodically entrained endogenous circannual rhythm in salmonids by exposing rainbow trout to compressed annual photoperiod cycles as well as constant long and short day regimes. Although these experiments were only conducted over 12 months, changes in both the spawning times and serum components of constant photoperiod groups indicated the presence of an endogenous reproductive rhythm. Similar findings were presented by Bromage *et al.* (1982) when rainbow trout were again exposed to a range of photoperiod regimes including constant photoperiods. This work supported the earlier work of Whitehead in establishing the presence of an endogenous rhythm of reproduction concluding that a seasonally-changing daylength was not essential for the cueing and modulation of reproductive development. In another short-term (14 month) experiment Eriksson and Lundqvist (1982) found a similar rhythm with growth and smoltification showing a cyclic nature in Baltic salmon held under constant LD12:12 conditions.

However, Elliott *et al.* (1984) provided the first long-term experiment using rainbow trout exposed to various photoperiods over a 2.5 year period. The cyclic nature of maturation events observed in these experiments led to the conclusion that an endogenous circannual rhythm was present but that this rhythm was entrained by the ambient photoperiod because spawning could occur at any time of the year provided the appropriate cues were received. Subsequently, long-term experiments have

provided further support for the endogenous rhythm hypothesis (e.g. Duston and Bromage, 1987, 1991; Duston, 1987) but the most thorough work to date has been provided by Duston and Bromage (1986). In their experiments studying spawning in the female rainbow trout groups were exposed to continuous light (LL) and constant long (LD18:6) and short (LD6:18) days, as well as skeleton (6L:4D:2L:12D, 6L:6D:2L:10D and 6L:8D:2L:8D) and resonance (6L:42D, 6L:48D and 6L:54D) procedures. Their observations supported the presence of photoperiodically entrained endogenous rhythms of reproduction and also found that spawning was not influenced by the total length of a light period nor by the accumulation of the number of light-dark cycles.

It is now clear that changes in the profiles of growth (Clarke *et al.*, 1978; Saunders and Harmon, 1988; Villarreal *et al.*, 1988; Duncan and Bromage, 1998; Duncan *et al.*, 1999), maturation (Whitehead *et al.*, 1978; Lundqvist, 1980; Bourlier and Billard, 1984a; Bromage *et al.*, 1984; Elliott *et al.*, 1984; Duston and Bromage, 1986, 1987, 1991; Hansen *et al.*, 1992) and smoltification (Clarke *et al.*, 1978; Erikson and Lundqvist, 1982; Clarke *et al.*, 1985; Stefansson *et al.*, 1989; Thrush *et al.*, 1994; Sigholt *et al.*, 1995) of salmonids can be analysed by considering the actions of photoperiod entrained rhythms. The documented variations of whether maturation will be delayed or advanced when photoperiods are altered can often be explained using a model incorporating photoperiodically entrained endogenous rhythms. Whether maturation is advanced or delayed (to critical periods when maturation can be arrested (Taranger *et al.*, 1999a) or advanced (Hansen *et al.*, 1992)) by photoperiod manipulation will therefore depend on: the natural spawning time of the species (Scott *et al.*, 1984); the relative time of year or position in the phase of the rhythm (Scott *et*

al., 1984; Duston, 1987) and the direction of the change in photoperiod (Duston, 1987).

It is, however, important to note that although endogenous rhythms are found to have a strong influence on both growth (e.g. Clarke *et al.*, 1978; Villarreal *et al.*, 1988; Duncan and Bromage, 1998; Duncan *et al.*, 1999) and maturation (e.g. Elliott *et al.*, 1984; Duston and Bromage, 1986, 1987, 1991; Hansen *et al.*, 1992) such a clear system may not necessarily be the case for smoltification. Erikson and Lundqvist (1982), Clarke *et al.* (1985), Saunders and Harmon (1990), Sigholt *et al.* (1995) and Duncan and Bromage (1998) have all shown smoltification to be strongly influenced by an endogenous cycle but Stefansson *et al.* (1989) found that under a continuous light regime smoltification was incomplete. It was subsequently concluded that an endogenous rhythm was too imprecise to provide complete smolting in the absence of photoperiodic cues.

However, Erikson and Lundqvist (1982) conducted a long-term experiment in which salmon parr were exposed to LD 12:12 for 14 months. In their experiments changes in the growth, condition factor and coloration of individuals all showed cyclical patterns with the conclusion that smolting consists of a number of seasonal processes that will run at their own frequency, being brought into synchrony by the changing photoperiod. Subsequently, Björnsson *et al.* (1989) and Thrush *et al.* (1994) have found increases in hypo-osmoregulatory ability to occur in advance of decreases in condition factor with Duncan and Bromage (1998) also finding the dissociation of a range of smolt parameters under artificial photoperiod regimes. It therefore seems that smoltification is controlled by more than one endogenous rhythm and although these

cycles will be synchronised by a naturally-changing annual photoperiod such synchronisation may not necessarily occur under other photoperiod regimes. As such it is important that when endogenous rhythms of smoltification are considered care is taken to consider the cyclical nature of the various smoltification parameters separately.

3.1.5. Aims

In commercial salmon farming the use of freshwater photoperiod treatments is now widespread producing increasingly early seawater transfer and allowing the year round supply of fish to both seawater on-growing sites and, subsequently, to the market. The influence of these progressively early photoperiod treatments on parr maturation and the interactions with smoltification are currently poorly understood. Therefore the aims of this chapter are:

- To investigate the importance of the timing of winter photoperiod on maturation in Atlantic salmon parr.
- To further investigate this relationship by considering the role of winter photoperiod length on parr maturation.
- To investigate how parr maturation and smoltification interact and to elucidate the role of winter photoperiod in this interaction.

3.2. Experiment I. The effects of winter photoperiod timing on growth, maturation and smoltification.

3.2.1. Objectives.

The experiment detailed in this section aimed to investigate the importance of winter photoperiod timing on growth, maturation and smoltification. Large numbers of PIT tagged fish allowed the growth and development of individuals to be followed, with the retrospective analysis of such fish also possible, and as such investigations were conducted at both the individual and population level.

3.2.2. Materials and Methods.

The experiment was carried out at Site 1 (Section 2.1.1). Ova from a high grilsing Scottish stock (Loch Lochy) were fertilised and held in heated water (8.0°C) under darkness until hatching (20th February 2000). The fry were then held under continuous light (LD24:0) in heated water (13.5±0.5°C) until first-feeding (29th March 2000). At first-feed 6000 fish were transferred into each of two 2m^2 tanks and exposed to a natural temperature regime (Fig. 3.2) under LD24:0. On 18th May 2000 800 fish were transferred to each of eleven 2m^2 tanks and held under LD24:0 until experimental winter photoperiods were applied (see Fig. 3.3 for experiment protocol).

On 18th May three of the 11 tanks of fish were exposed to an 8 week winter photoperiod (LD10:14) after which they were returned to LD24:0 until the conclusion of the experiment. Two further triplicate tanks of fish were exposed to the 8 week winter photoperiod on 9th August and 22nd September respectively.

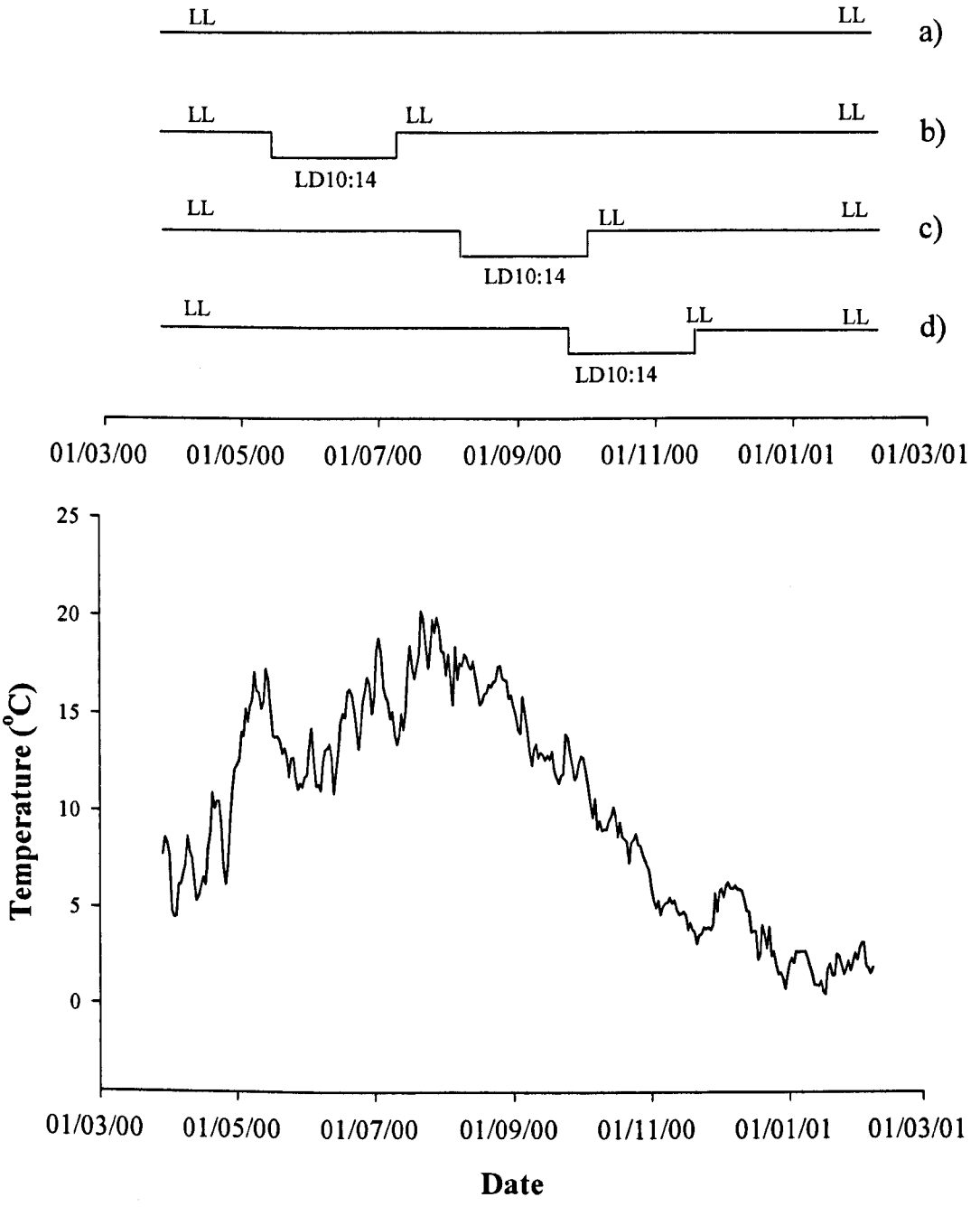


Fig. 3.2 The ambient temperature profile at Site 1 and the experimental photoperiod regimes used during the experiment. a) Continuous light, b) May photoperiod, c) August photoperiod, d) September photoperiod.

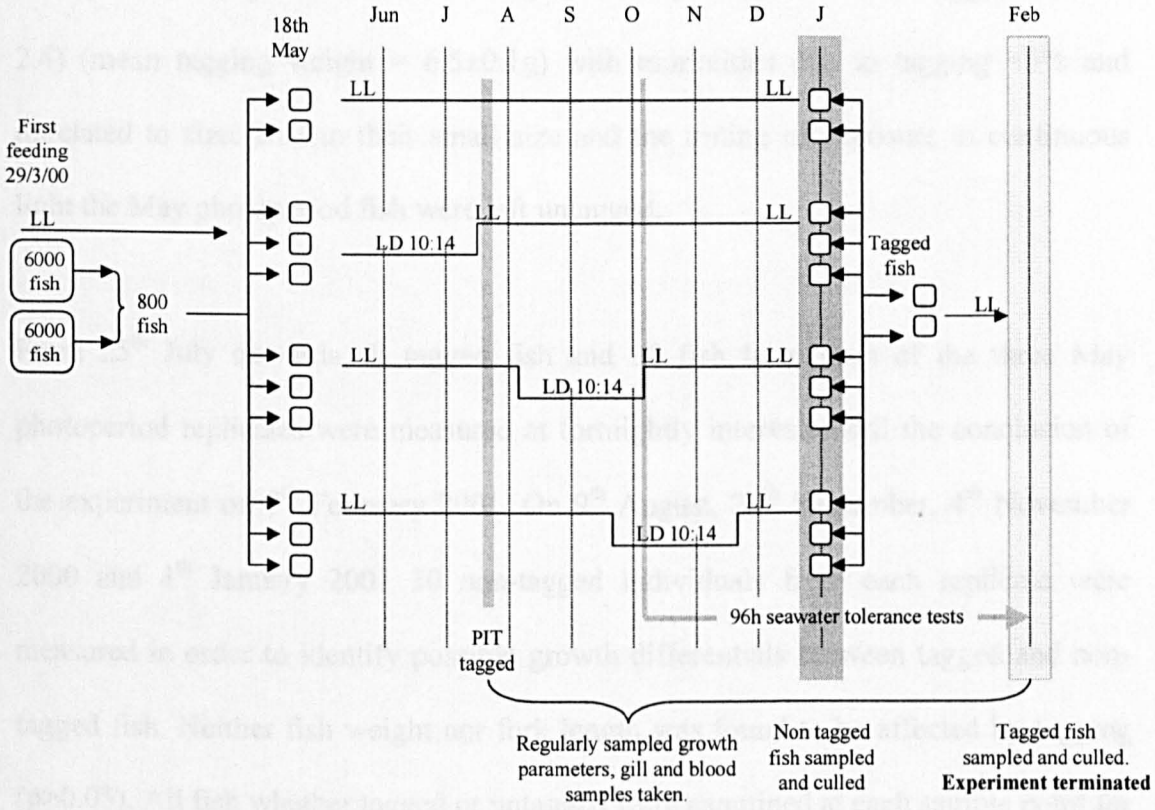


Fig. 3.3 The experimental protocol used during experiment I. For further details of the sampling regime refer to Section 3.2.2.

Treatments were subsequently termed the May, August, and September photoperiod groups (Fig. 3.2). A further group was held in duplicate tanks under LD24:0 throughout the experiment. On 25th July 100 fish per tank were PIT tagged (Section 2.4) (mean tagging weight = 6.5±0.1g) with mortalities due to tagging <5% and unrelated to size. Due to their small size and the timing of exposure to continuous light the May photoperiod fish were left untagged.

From 25th July onwards all tagged fish and 30 fish from each of the three May photoperiod replicates were measured at fortnightly intervals until the conclusion of the experiment on 7th February 2001. On 9th August, 20th September, 4th November 2000 and 4th January 2001 30 non-tagged individuals from each replicate were measured in order to identify possible growth differentials between tagged and non-tagged fish. Neither fish weight nor fork length was found to be affected by tagging ($p>0.05$). All fish whether tagged or untagged were examined at each sample point for signs of maturation (see Section 2.7.1).

At fortnightly intervals from 25th July blood and gill samples were taken from 5-10 culled non-tagged fish per tank to identify changes in serum testosterone (Section 2.7.4) and gill Na^+, K^+ -ATPase (Section 2.8.1). Also from 3rd October at two week intervals 15 individuals per treatment were exposed to a 96h seawater tolerance test (Section 2.8.2.).

On 4th January 2001 all non-tagged fish were culled and the remaining tagged fish randomly divided into two $2m^2$ tanks held under LD24:0. The culled fish were examined for external signs of maturation and their relative cohort recorded (Table

3.1) (see Section 2.8.5). From the culled fish approximately 100 fish per treatment were dissected to establish the sex of the individual and internal signs of maturation (i.e. enlarged gonadal tissue) recorded. On 7th February 2001 all PIT tagged fish were culled. All fish were examined externally and cohort ratios identified, although all of the cohorts that had been identified in the non-tagged fish (see Table 3.1) were not necessarily represented in the PIT tagged populations. All tagged fish were then dissected to remove the PIT tags and internal maturation status recorded.

On 14th October a blocked inlet resulted in the loss of all fish from one of the three August photoperiod replicates. The growth data from this group have therefore been removed from the subsequent analysis although plasma testosterone and gill Na^+, K^+-ATPase measurements taken prior to this incident were included.

Growth data and plasma testosterone levels were compared using a General Linear Model (Section 2.11) although for testosterone levels a natural log transformation was used to improve normality and homogeneity of variance. Gill Na^+, K^+-ATPase levels were analysed using the Kruskal-Wallis non-parametric test with Dunn's multiple range procedure (Sokal and Rohlf, 1995; Zar, 1999). For changes in percentage maturation, population structures and seawater tolerance 95% confidence limits were calculated and compared (Fowler and Cohen, 1987).

Cohort	*Description of fish*
Smolts	Fully silvered fish with no parr marks and blackened fin margins. These fish were typically >30g and <65g.
Large smolts	Fully silvered fish with no parr marks and blackened fin margins although these fish were significantly larger than the smolts described above (i.e. >100g).
Silvered parr	Fish that were partially silvered with parr marks that were obscured but still visible. These fish were typically >30g and <65g.
Parr	Fish showing no signs of silvering with the presence of distinct parr marks. These fish were typically >30g and <65g.
Small parr	Fish showing no signs of silvering with the presence of distinct parr marks, although these fish were significantly smaller than the parr described above (i.e. <15g).

Table 3.1. The nomenclature used to assign individuals to a particular developmental cohort. Cohorts were based on the level of smoltification achieved at the conclusion of the experiment. The cohorts denote groups of fish that were identified within the entire experiment although some treatments as well as the PIT tagged groups did not necessarily contain all of the cohorts that have been described.

3.2.3. Results

3.2.3.1. Growth

Weight

Within treatment differences:

Differences within each cohort over time

All cohorts, except the small parr exposed to LL and mature fish from the May photoperiod group, showed an overall increase in weight ($p<0.01$) over the experimental period (Fig. 3.4).

For the PIT tagged groups parr increased in weight throughout the experiment under all photoperiod regimes whereas mature and small parr groups showed no consistent increases between consecutive sample points. For the smolts some differences occurred between treatments. Smolts exposed to LL showed no consistent increases between consecutive time points whereas under an August photoperiod increases in weight were seen throughout the experiment ($p<0.05$). Under the September photoperiod significant increases in weight were only observed up to November ($p<0.05$).

For the May photoperiod groups immature fish showed increases in weight between consecutive sample points until early October ($p<0.05$) with mature fish exhibiting no significant increases.

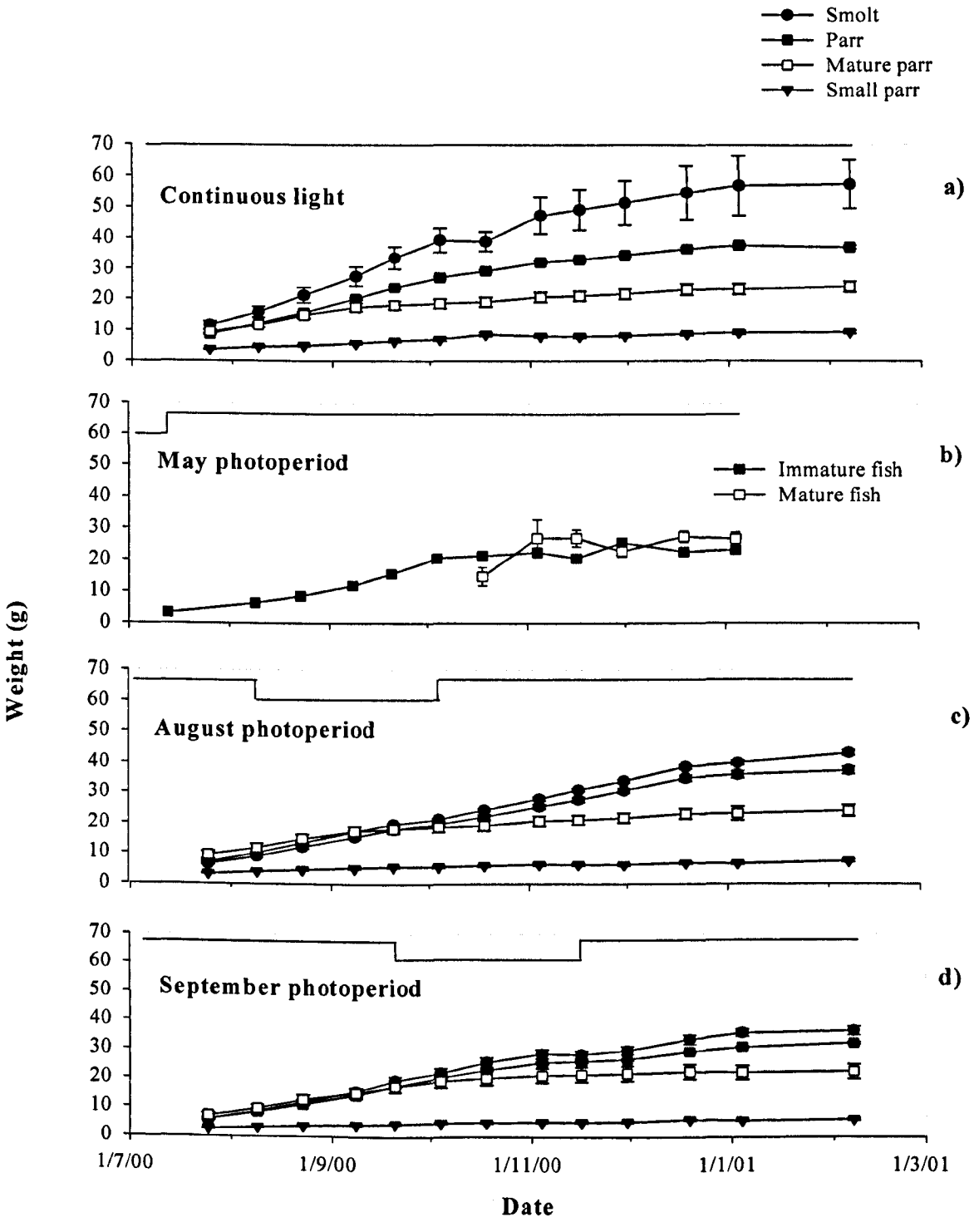


Fig. 3.4 The change in weight (mean ± S.E.M. n=90 to 300) of fish cohorts identified in groups exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c), or September (d), in an otherwise continuous light regime. In some cases error bars may be to small to be depicted. The respective photoperiod regimes are shown to aid interpretation.

Within treatment differences:

Differences between cohorts at each sample point.

Parr and mature parr under LL had similar weights until 4th October after which they were significantly different through to 7th February 2001 ($p<0.01$). All other cohorts differed from one another from 25th July until the conclusion of the experiment ($p<0.01$). Under the August photoperiod mature parr had similar weights to both smolts and parr until 4th October and 4th November respectively although smolts and parr were only similar in July. All other cohorts differed from one another from July until the conclusion of the experiment ($p<0.05$). For fish exposed to the September photoperiod mature parr had similar weights to both smolts and parr until 8th September and 18th October respectively. On 25th July smolts and parr as well as small parr and mature parr were similar but at all other times all cohorts were different ($p<0.05$). For the May photoperiod fish no differences were observed between mature and immature fish.

Between treatment differences:

Differences between cohorts at each sample point

Differences occurred for smolts, parr and mature parr. Smolts exposed to LL were heavier than both August and September photoperiod smolts throughout the experiment ($p<0.01$). September photoperiod smolts were significantly heavier than August smolts from 4th October until 16th November ($p<0.01$) with August smolts heavier than September smolts on 19th December and 7th February ($p<0.01$). Parr exposed to LL were heavier than August and September parr throughout the experiment ($p<0.01$) with the exception that on 7th February parr from the LL and

August groups had similar weights ($p>0.05$). August parr were only heavier than September parr from 16th November onwards. For mature parr those exposed to LL were heavier than August and September fish from 20th September and 9th August respectively ($p<0.01$). Mature parr from the August and September groups were different throughout ($p<0.01$) although the September photoperiod fish were heavier between 4th October and 16th November, with August fish heavier at all other times.

Length

Within treatment differences:

Differences within each cohort over time

All cohort groups, except mature fish from the May photoperiod group, showed an increase in length over the course of the experiment ($p<0.01$) (Fig. 3.5). For PIT tagged groups, parr from all treatments and August photoperiod smolts increased in length significantly throughout the experiment until 4th January ($p<0.05$). September photoperiod smolts increased in length between consecutive time points until early November, although LL smolts showed no consistent increases. Similarly no consistent increases were seen in any treatment for both small parr or mature parr groups. All groups showed increases in length between 23rd August and 8th September. Immature fish from the May photoperiod group increased from July until 23rd August and then from 8th September until 4th October with mature fish showing no significant increases in length.

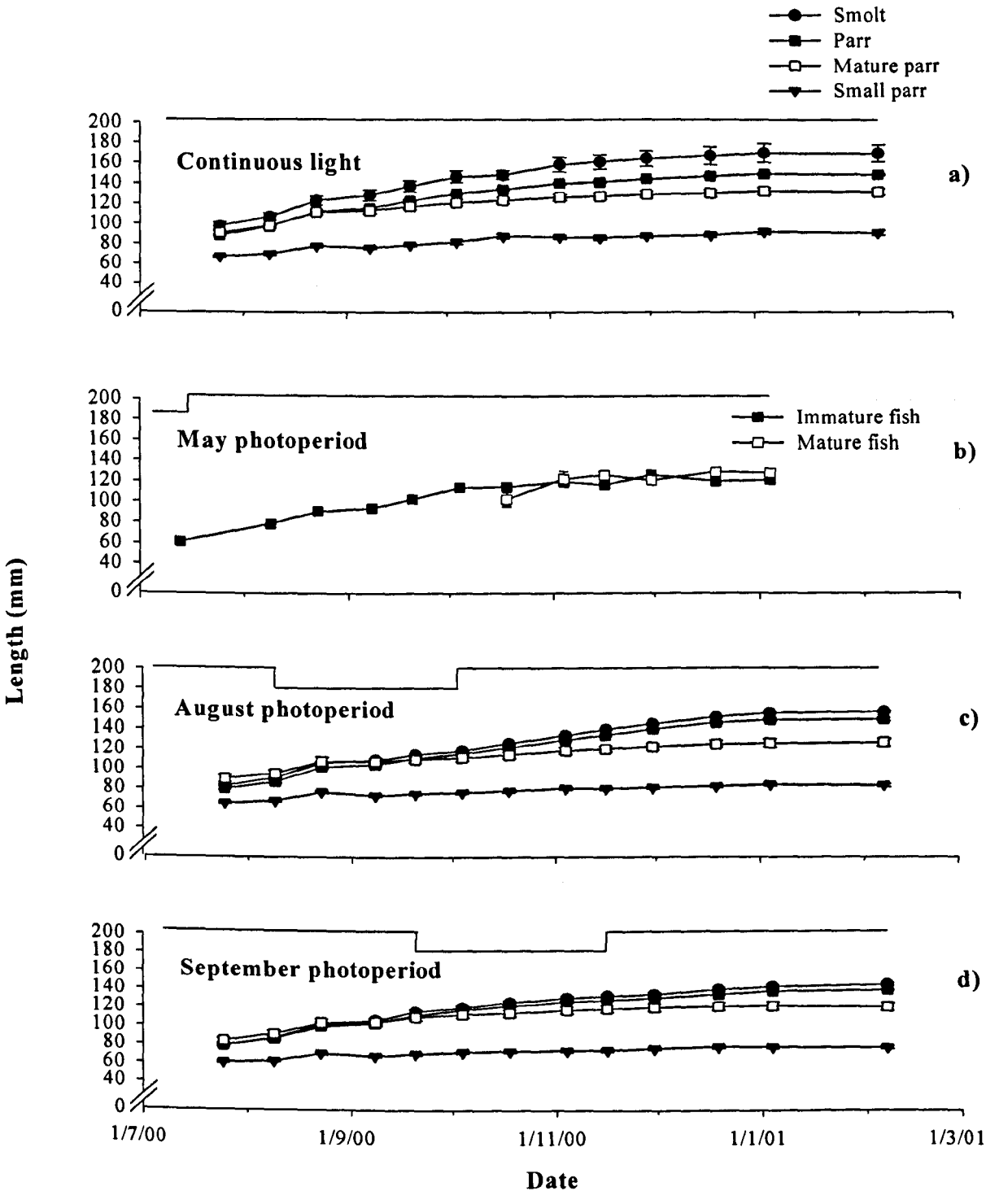


Fig. 3.5 The change in length (mean ± S.E.M. n=90 to 300) of fish cohorts identified in groups exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c), or September (d), in an otherwise continuous light regime. In some cases error bars may be to small to be depicted. The respective light regimes have been shown to aid interpretation.

Within treatment differences:

Differences between cohorts at each sample point.

Under LL, parr and mature parr had similar lengths until 20th September, but at all other time points all cohorts differed from one another ($p<0.01$). For the August photoperiod group parr and smolts were similar in length to mature parr from 23rd August until 18th October and 29th September respectively ($p>0.05$). At all other times all cohorts were significantly different ($p<0.01$). The September photoperiod resulted in all cohorts being different throughout the experiment ($p<0.01$) with the exception of mature parr which were similar to both smolts and parr from July until 8th September and 4th October respectively ($p>0.05$). The immature and mature fish in the May photoperiod group did not differ significantly ($p>0.05$).

Between treatment differences:

Differences between cohorts at each sample point

Smolts exposed to LL were longer than those from the August and September photoperiods throughout the experiment. September photoperiod smolts were longer than those from the August photoperiod from 20th September until 18th October with the August smolts longer from 30th November onwards ($p<0.01$). Parr exposed to LL were longer than those exposed to the August photoperiod until 19th December when the August parr were longer ($p<0.01$) but from then onwards no significant differences occurred ($p>0.05$). The parr exposed to LL were longer than those from the September photoperiod throughout the experiment ($p<0.01$). The parr from the September group were longer than those from the August group from 20th September

until 18th October with August fish longer from 16th November onwards ($p<0.01$). For mature parr those exposed to LL were longer than those from the August group from 23rd August onwards ($p<0.05$) with September parr shorter throughout the experiment ($p<0.01$). Mature parr from the August and September groups had similar lengths throughout the experiment. Small parr exposed to LL and the August photoperiod remained similar throughout the experiment although the September small parr were smaller than those exposed to LL and the August photoperiod from 8th September and 9th August ($p<0.05$) respectively.

Condition factor (CF)

Within treatment differences:

Differences within each cohort over time

Differences in condition factor were observed following photoperiod treatment (Fig. 3.6). Under LL both immature and mature parr showed increases in CF between 25th July and 9th August with an overall decrease by the conclusion of the experiment ($p<0.01$) and with parr showing a decrease in CF between 8th September and 4th October, 18th October and 4th November, and 16th and 30th November. For the smolts and small parr no consistent changes in CF were observed throughout the experiment. Under an August photoperiod all cohorts showed an overall increase in CF from July until early September with a subsequent decline by January ($p<0.01$). Following this smolts, parr and small parr all showed a rise in CF by February ($p<0.01$). Furthermore, smolts showed decreases in CF between consecutive sample points during October ($p<0.01$). Under a September photoperiod all cohorts, except mature parr, showed increases in CF between July and early August ($p<0.01$) with only the

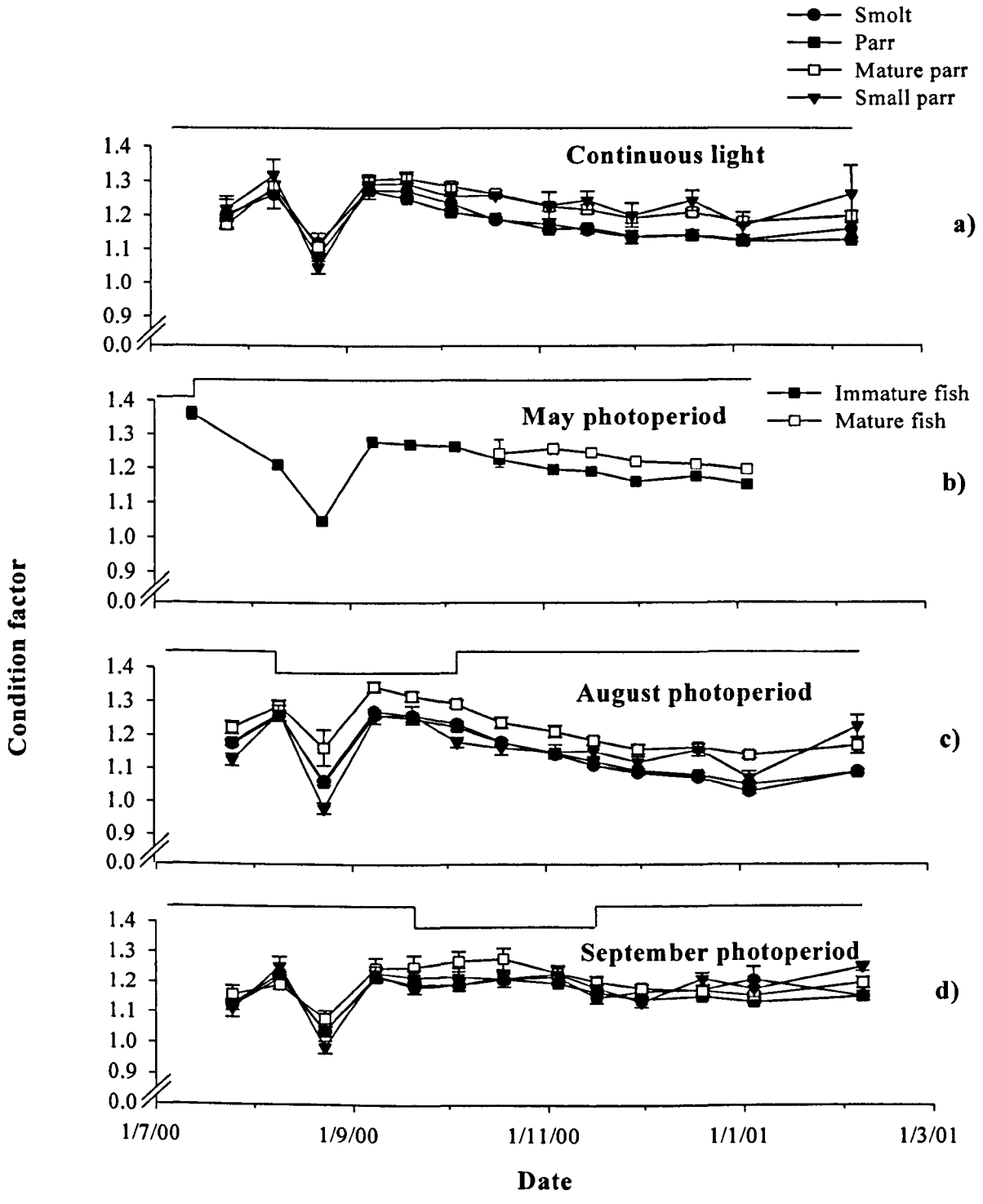


Fig. 3.6 The change in condition factor (mean ± S.E.M. n=90 to 300) of fish cohorts identified in groups exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c), or September (d), in an otherwise continuous light regime. In some cases error bars may be to small to be depicted. The respective photoperiod regimes are shown to aid interpretation.

CF of smolts and parr decreasing by the conclusion of the experiment and with parr showing decreases in CF between consecutive time points during November. For immature fish exposed to a May photoperiod an overall decrease in CF was observed, with mature fish also showing a decline in CF from 18th October onwards ($p<0.01$).

Interestingly all cohorts showed reductions in CF on 23rd August with this decline linked to an increase in length, as opposed to a loss in weight (see Fig. 3.5).

Within treatment differences:

Differences between cohorts at each sample point.

Immature parr exposed to LL had a significantly lower CF from 20th September onwards ($p<0.05$) with all other cohorts similar throughout the experiment. Under an August photoperiod both smolts and parr had a lower CF than the mature and small parr from mid-December onwards ($p<0.01$) with the exception that on 4th January the CF's of parr and small parr were similar ($p>0.05$). Under May and September photoperiods no consistent differences were observed between cohorts.

Specific growth rate (SGR)

Within treatment differences:

Differences within each cohort over time

Due to the absence of tagged data for the May photoperiod it was not possible to statistically analyse the SGR of these fish. However, an overall decrease in SGR was observed in the immature fish from this group (Fig. 3.7). For all tagged cohorts an

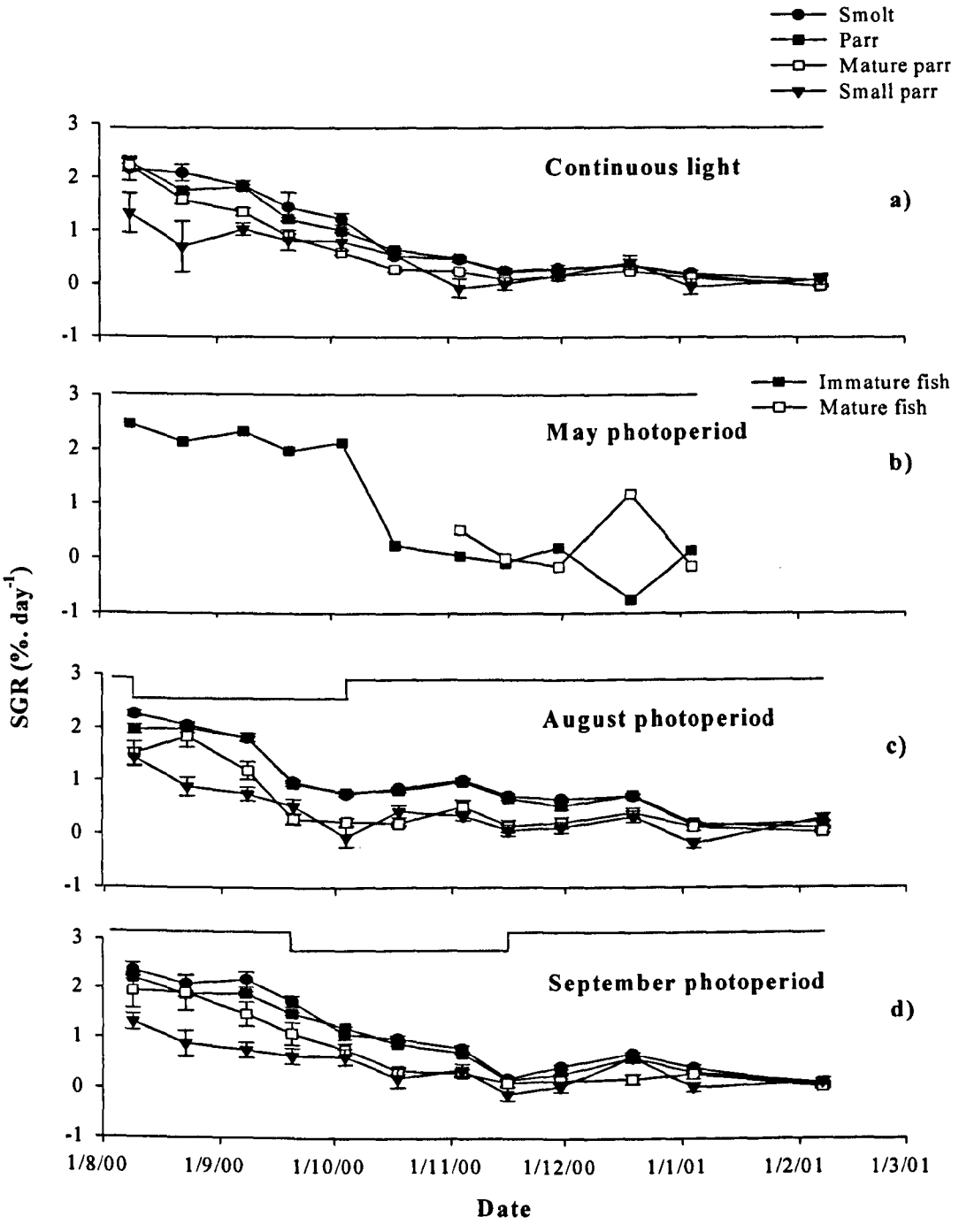


Fig. 3.7 The change in SGR (mean ± S.E.M. n=90 to 300) of fish cohorts identified in groups exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c), or September (d), in an otherwise continuous light regime. In some cases error bars may be to small to be depicted. The respective photoperiod regimes are shown to aid interpretation.

overall decrease in SGR was observed over the experiment ($p<0.01$). Under LL the growth rate of parr decreased between consecutive time points until 18th October ($p<0.05$) with parr from the September photoperiod also showing decreased growth rates until 16th November ($p<0.05$). These September parr then showed an increase in growth rate during December ($p<0.01$) after which SGR declined through to February ($p<0.01$). In all other cohorts and treatments no consistent changes over time were observed.

Within treatment differences:

Differences between cohorts at each sample point.

The SGR of small parr exposed to LL was lower than that of parr and mature parr from July until 8th September and 23rd August respectively ($p<0.05$). The SGR of all other cohorts under LL remained similar throughout the experiment. Under the August photoperiod the SGR of small parr was lower than that of both smolts and parr until 16th and 30th November respectively ($p<0.05$). Until 9th August the SGR of smolts was also higher than for the parr and mature parr ($p<0.05$) although the SGR of all other cohorts remained similar throughout the experiment ($p>0.05$). Under a September photoperiod the SGR of small parr was lower than that of the smolts and parr with this difference remaining until 18th October ($p<0.01$). Apart from small and mature parr being different on 23rd August ($p<0.01$) all other cohorts remained similar throughout the experiment.

Between treatment differences:

Differences between cohorts at each sample point

Differences were only found within in the parr cohort. Parr exposed to LL had a higher SGR than August parr on 20th September and 4th October ($p<0.05$) with the SGR of August parr higher on 9th August, 4th and 16th October and 19th December ($p<0.05$). The SGR of LL parr was higher than that of September parr on 4th October ($p<0.05$) and lower on 20th September, 18th October, 4th November and 19th December ($p<0.01$). September photoperiod parr had a higher SGR than August parr on 20th September and 4th October although it was lower on 16th November.

Weight-frequency

Photoperiod treatment affected the development of weight-frequency structure (Fig. 3.8). However, the timing of the emergence of modality was not greatly affected by photoperiod regime with all groups developing a bimodal divide by either 4th or 18th October. LL resulted in a weak bimodal divide with clearer divisions occurring in all other treatments. The May photoperiod resulted in the greatest percentage of lower modal group fish with low frequencies of very large fish in the LL and May populations.

3.2.3.2. Maturation

Rates of maturation

Photoperiod treatment had clear effects on maturation (Fig. 3.9) with mature fish first identified in early October. Until early November maturation levels in the LL and August photoperiod groups were similar and greater than those of the May and

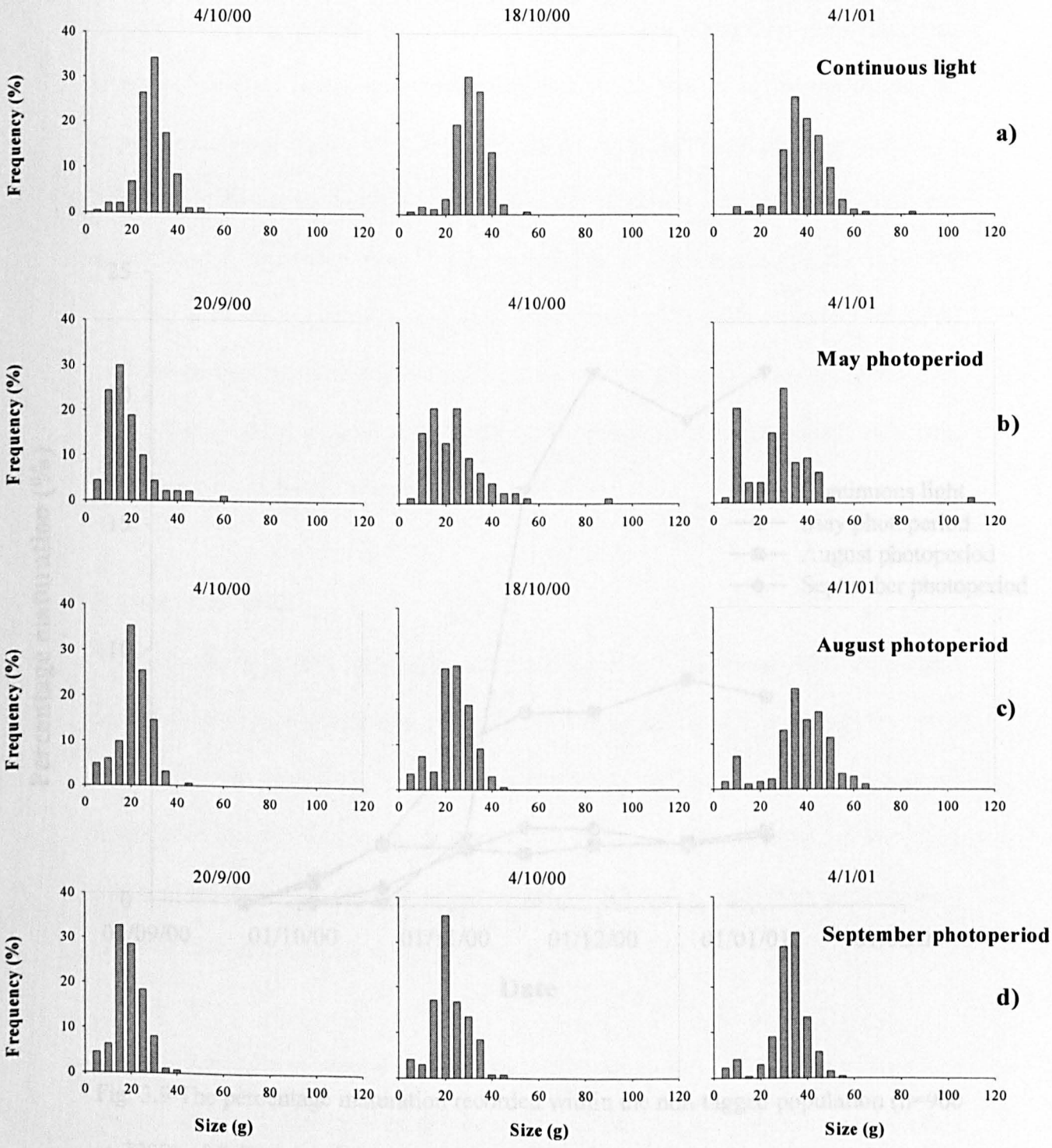


Fig. 3.8 The weight-frequency distribution of populations (n=90-300) exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c), or September (d), in an otherwise continuous light regime. The populations shown represent the weight-frequency distributions just prior to, and at the emergence of modality, as well as at the final sample point.

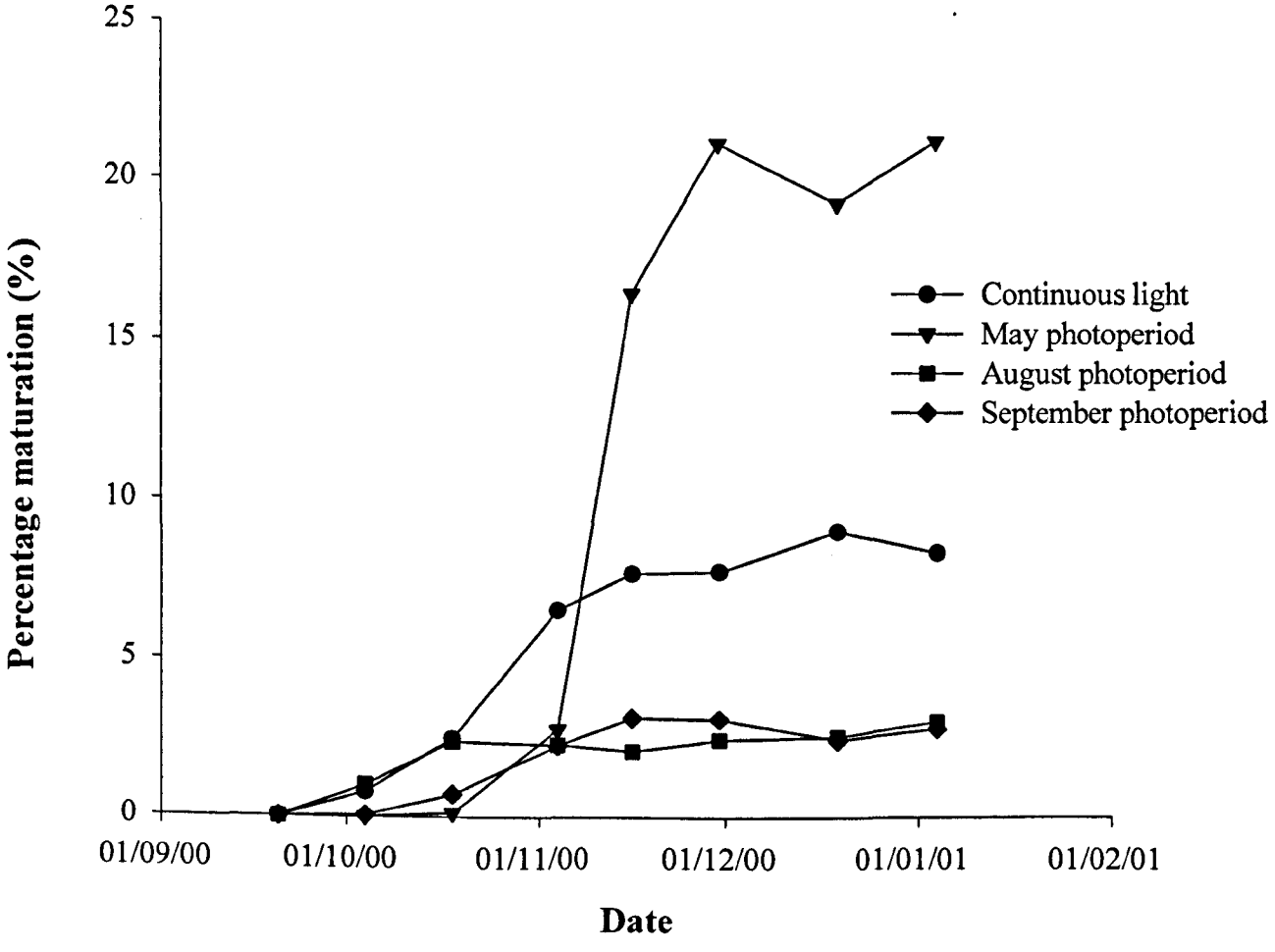


Fig. 3.9 The percentage maturation recorded within the non-tagged population (n=900 to 2200) of fish exposed to either continuous light or to 8 week periods of short days (LD10:14) commencing in May, August or September in an otherwise continuous light regime.

September photoperiod groups ($p<0.05$). By November maturation rates in the LL group had reached levels of approximately 8%, which then remained throughout the experiment, being higher than the levels found in either the August or September photoperiod groups ($p<0.05$). In the August and September photoperiods levels rose during early November with levels remaining at approximately 3% until the conclusion of the experiment. For May photoperiod fish levels remained low until mid-November after which levels rapidly increased to above 20% being significantly higher than all other treatments ($p<0.05$). Levels remained at approximately 20% until the conclusion of the experiment.

Plasma testosterone

When the testosterone profiles of mature and immature individuals were considered separately differences were found (Fig. 3.10). The mean levels of immature fish were similar between treatments, with the exception that on 20th September the levels found in both the LL and August groups were lower than those in the May group ($p<0.01$). Furthermore the testosterone levels of immature fish within each treatment remained unchanged over the course of the experiment.

When the profiles of mature and immature fish within the LL, May and September groups were compared as expected the mature fish had higher testosterone levels than their immature siblings ($p<0.01$). This was also the case for the August group with the exception that on 30th November the testosterone levels of mature and immature fish were similar ($p>0.05$). However, between consecutive time points changes in the testosterone profiles of mature fish only occurred in the August and September groups. For the August treatment a decline in testosterone occurred between 4th and

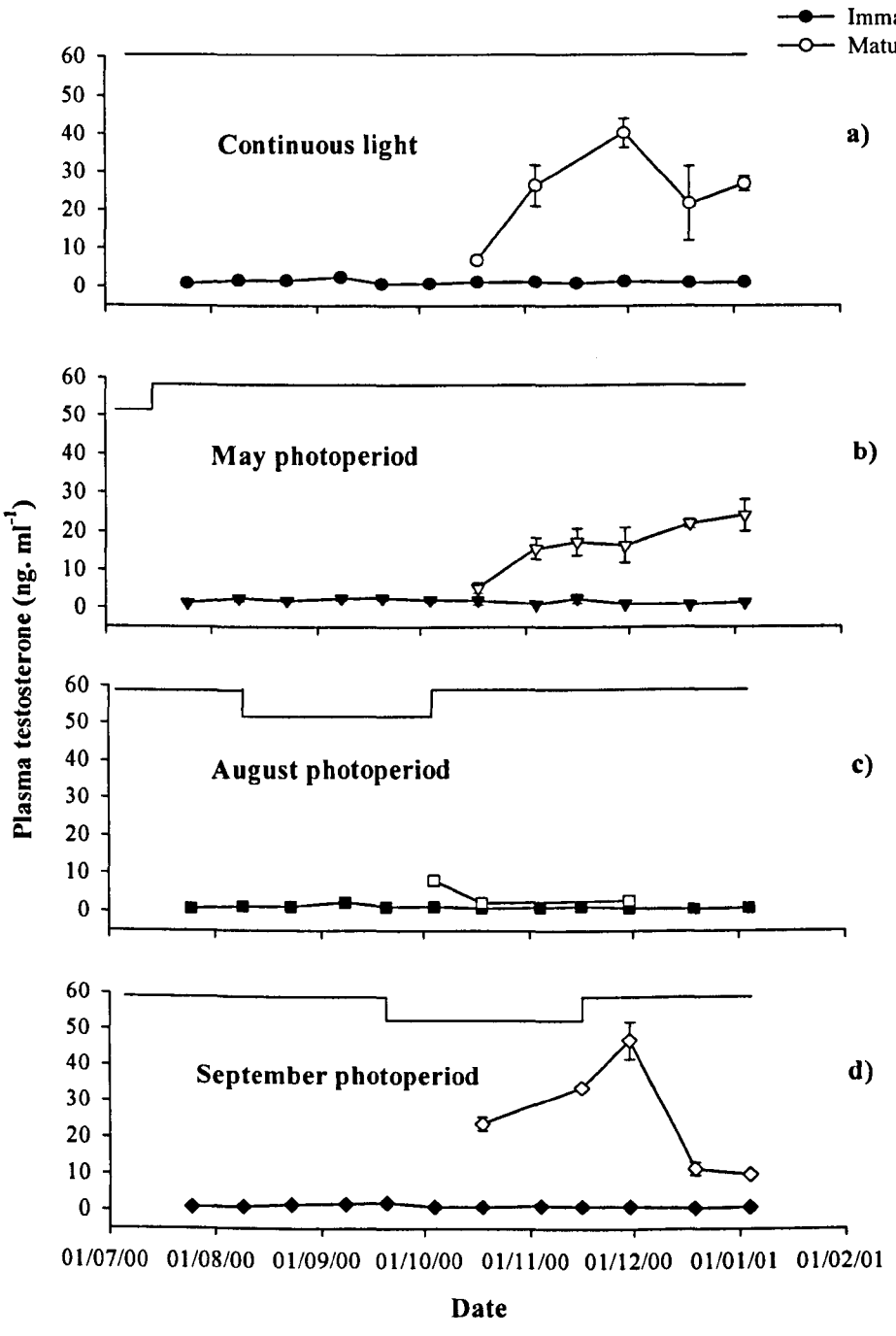


Fig. 3.10 Plasma testosterone levels (mean±S.E.M. n=20-30) of mature and immature fish exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c) or September (d), in an otherwise continuous light regime. Closed symbols denote immature fish, open symbols denote mature fish. The respective photoperiod regimes are shown to aid interpretation.

16th October with a decline also occurring for the September group between 30th November and 19th December ($p<0.01$).

3.2.3.3. Cohort structure

Total population

Total population structure was affected by the timing of winter photoperiod treatment (Fig. 3.11). Under LL 93% of the population developed as parr with only 1% developing as small parr. Furthermore only 5% of the population consisted of silvered parr. For fish exposed to a May photoperiod high numbers of parr were observed (82%) with the lowest incidence of silvered parr (3.0%) ($p<0.05$). However, in the May photoperiod the highest incidence of small parr (12%) was observed ($p<0.05$) and it was the only treatment that resulted in large smolts (2%).

Under an August photoperiod the lowest percentage of parr (7%) was recorded with similar numbers of small parr (8%). However, it was in this treatment that the highest incidence of both silvered parr (60%) and smolts (25%) were observed ($p<0.05$). For the September photoperiod 84% developed as parr with low numbers of both small parr (5%) and silvered parr (11%).

Male: female ratios

Both male and female fish were observed in each of the observed cohorts (Fig. 3.12). Furthermore, within each cohort the incidence of males was the same as that of the females ($p>0.05$).

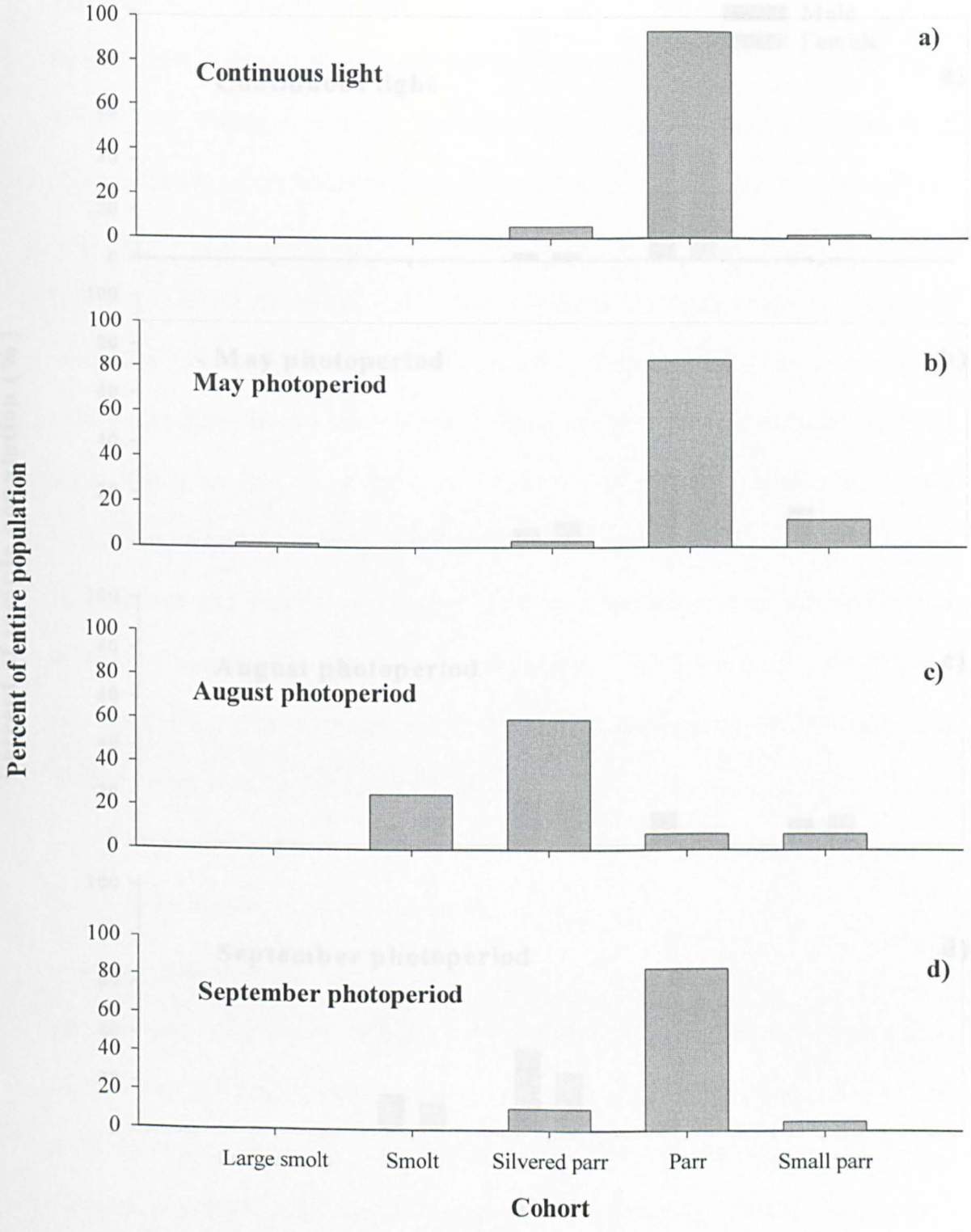


Fig. 3.11 The cohort structure of fish exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c) or September (d), in an otherwise continuous light regime. Data are based on the non-tagged population of each treatment (n=900 to 2000).

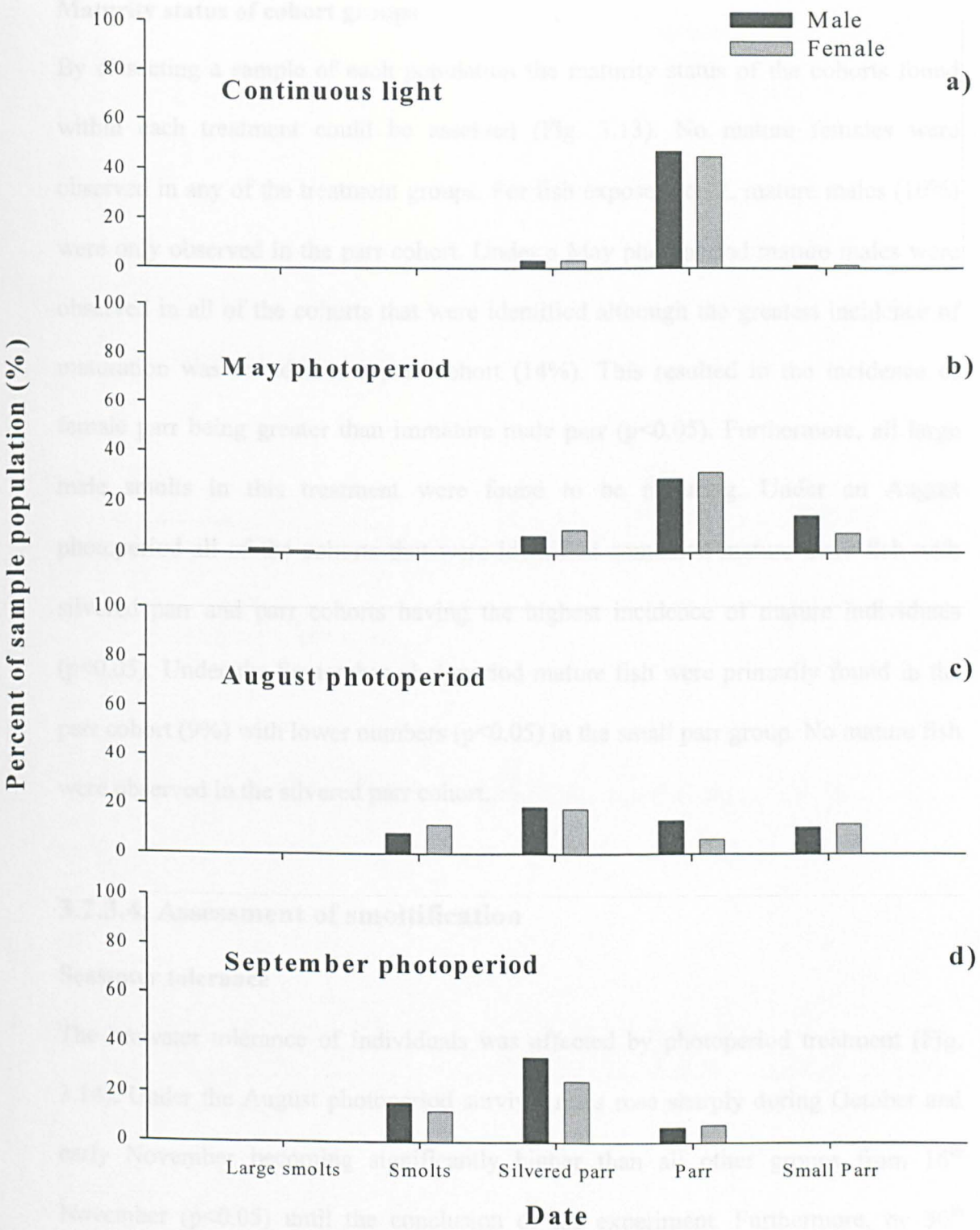


Fig. 3.12 The male: female ratios found in cohorts of fish, that were exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c) or September (d), in an otherwise continuous light regime. Data are based on a sample population of dissected individuals (n=100).

Maturity status of cohort groups

By dissecting a sample of each population the maturity status of the cohorts found within each treatment could be assessed (Fig. 3.13). No mature females were observed in any of the treatment groups. For fish exposed to LL mature males (10%) were only observed in the parr cohort. Under a May photoperiod mature males were observed in all of the cohorts that were identified although the greatest incidence of maturation was found in the parr cohort (14%). This resulted in the incidence of female parr being greater than immature male parr ($p<0.05$). Furthermore, all large male smolts in this treatment were found to be maturing. Under an August photoperiod all of the cohorts that were identified contained mature male fish with silvered parr and parr cohorts having the highest incidence of mature individuals ($p<0.05$). Under the September photoperiod mature fish were primarily found in the parr cohort (9%) with lower numbers ($p<0.05$) in the small parr group. No mature fish were observed in the silvered parr cohort.

3.2.3.4. Assessment of smoltification

Seawater tolerance

The seawater tolerance of individuals was affected by photoperiod treatment (Fig. 3.14). Under the August photoperiod survival rates rose sharply during October and early November becoming significantly higher than all other groups from 16th November ($p<0.05$) until the conclusion of the experiment. Furthermore, by 30th November survival rates reached 100% in the August photoperiod fish remaining so until early January when a slight decline in survival was observed. Under the LL, May and September photoperiods seawater survival showed variable results over time. During early October all groups had low survival rates with the LL and May

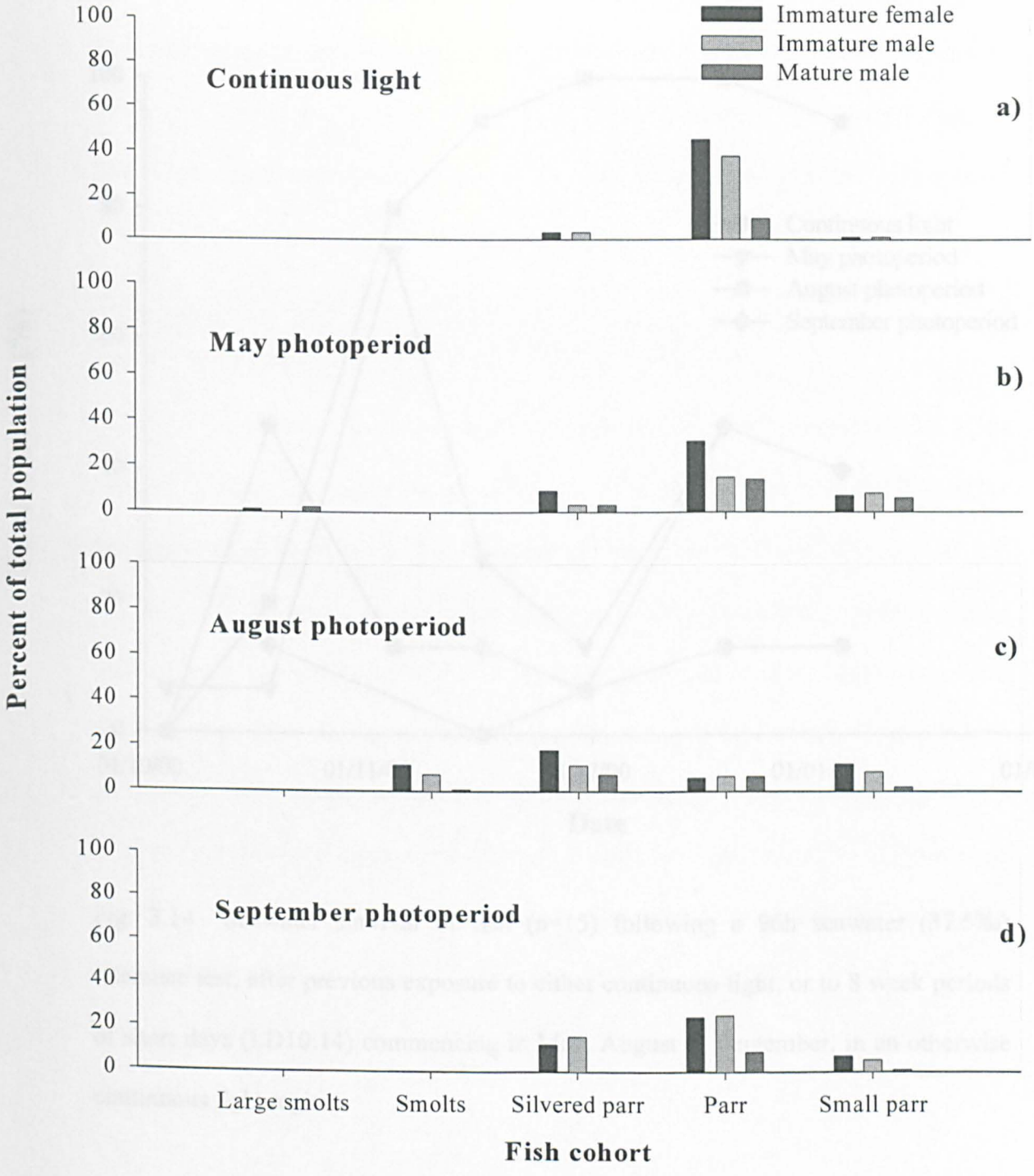


Fig. 3.13 The cohort structure and maturational status found in cohorts of fish, that were exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c) or September (d), in an otherwise continuous light regime. Data are based on a sample population of dissected individuals (n=100).

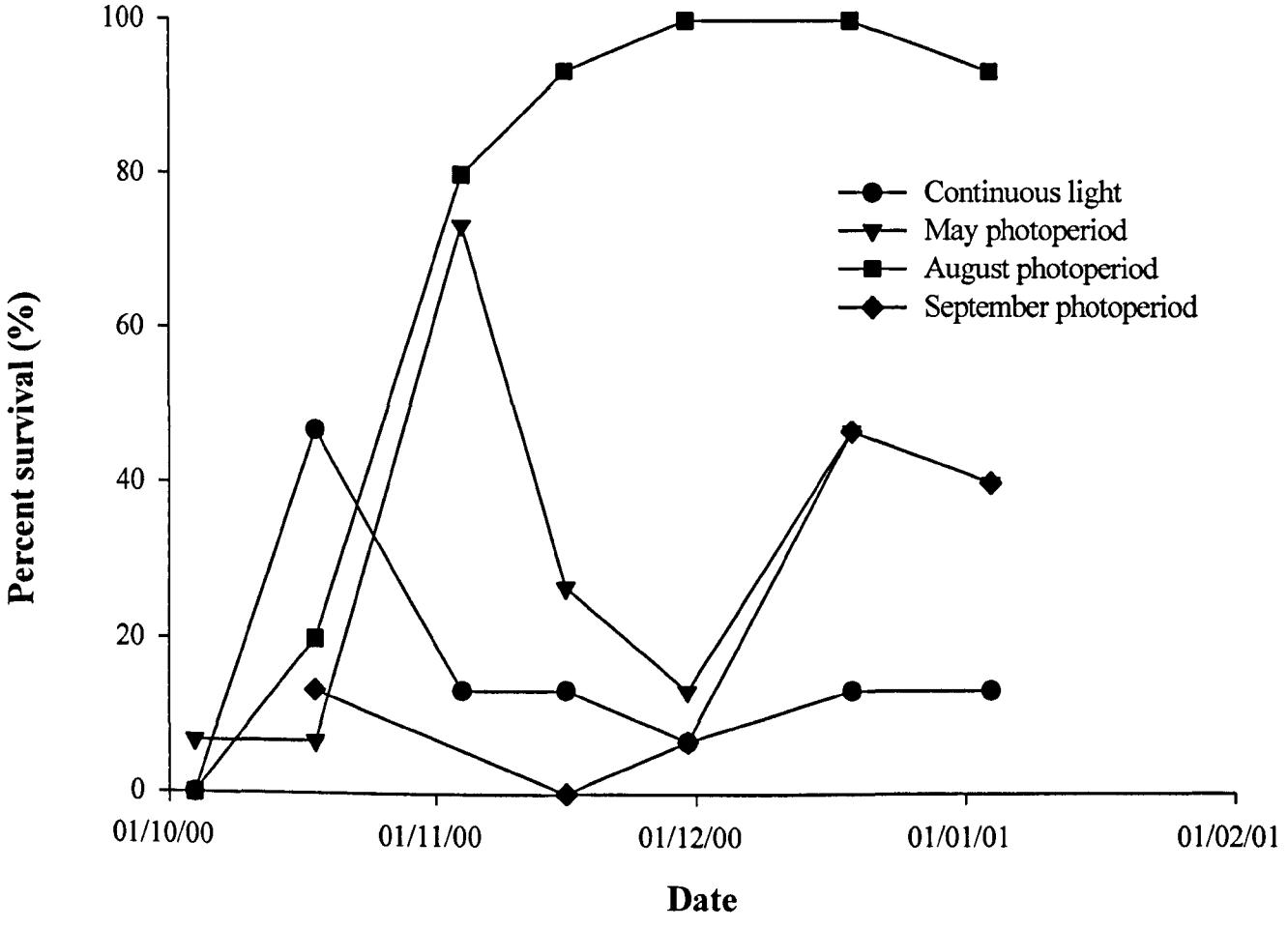


Fig. 3.14 Seawater survival of fish (n=15) following a 96h seawater (37.5‰) tolerance test, after previous exposure to either continuous light, or to 8 week periods of short days (LD10:14) commencing in May, August or September, in an otherwise continuous light regime.

photoperiod groups showing a brief peak in survival on 18th October and 4th November respectively. After this survival dropped, with the May photoperiod fish showing a second peak, along with a peak in the September photoperiod fish, in December. However, survival rates in the LL, May and September photoperiod groups never reached consistently high levels.

Gill Na^+, K^+ -ATPase

Gill Na^+, K^+ -ATPase levels remained at low levels in most treatments (Fig. 3.15) until 4th October; the only exception being on 25th July when levels in the September photoperiod fish were lower than all other groups ($p<0.05$). On 4th October the gill Na^+, K^+ -ATPase levels of the August photoperiod fish became significantly higher than all other groups ($p<0.05$). However, levels observed in the August group then declined to become similar to those of other groups until mid-December after which levels were again higher remaining so until the conclusion of the experiment ($p<0.05$). With the exception of the earlier difference on 25th July, the Na^+, K^+ - ATPase levels found in the LL, May and September groups remained similar throughout the experiment ($p<0.05$).

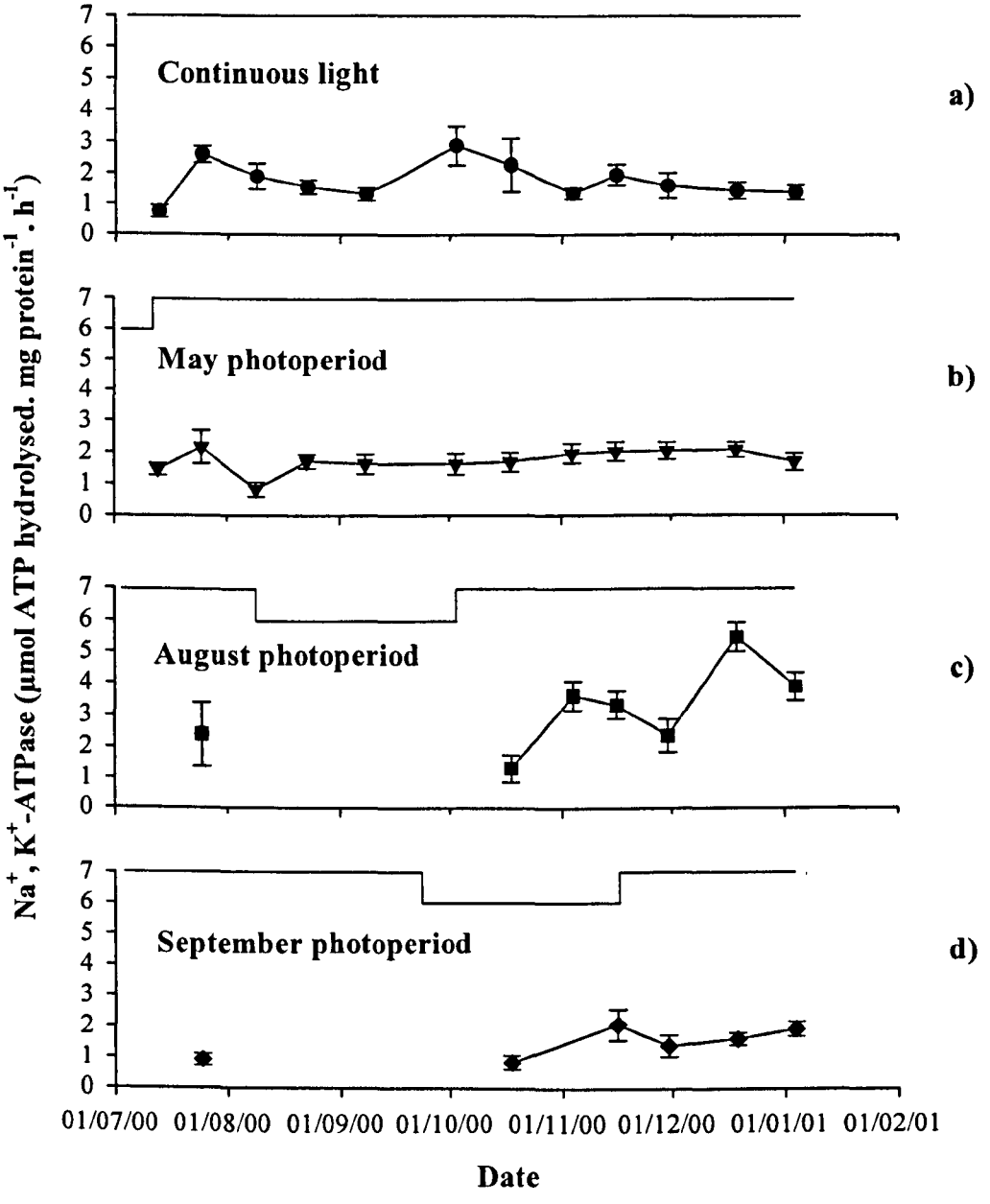


Fig. 3.15 Gill Na^+, K^+ -ATPase levels (Mean ± S.E.M. n=5-10) of fish exposed to either continuous light (a), or to 8 week periods of short days (LD10:14) commencing in May (b), August (c) or September (d), in an otherwise continuous light regime. Regular samples were not taken throughout the August and September photoperiod treatments, therefore not all sample points have been connected to avoid mis-interpretation. The respective photoperiod regimes are shown to aid interpretation.

3.2.4. Summary of the results from Experiment I.

- The weight and length of mature and immature parr were similar throughout the early stages of growth in all treatments.
- The CF of cohorts rose during initial stages of the experiment with most cohorts then showing a decline in CF over the experiment. However, no consistent differences in CF could be identified between cohorts.
- SGR declined for all groups during the experiment with small parr having the lowest SGR.
- All populations developed into bimodal distributions on similar dates regardless of photoperiod treatment. Under LL the division of modal groups was less clear than for the other treatments.
- The May photoperiod resulted in the highest levels of maturation (>20%), with September and August photoperiod groups exhibiting low (<3%), and LL intermediate, levels of maturation (<10%). Photoperiod did not affect the timing of maturation.
- Under LL, May and September photoperiods most fish developed as parr. The August photoperiod resulted in the highest incidence of smolts and silvered parr.
- Large smolts were only observed in the May photoperiod group.
- Mature fish were identified within all cohort groups.
- Only fish exposed to an August photoperiod showed good hypo-osmoregulatory ability (Na^+, K^+ -ATPase and seawater tolerance).
- Data presented in experiment I has been published in a paper entitled: Photoperiodic effects on precocious maturation, growth and smoltification in Atlantic salmon, *Salmo salar* (see Appendix 1).

3.3. Experiment II. The effects of early winter photoperiod timing and duration on growth, maturation and smoltification.

3.3.1. Objectives.

The experiment detailed in this section was developed from the findings presented in Section 3.2. The timing of early winter photoperiod was further investigated with the addition of winter photoperiods of different duration. Again large numbers of PIT tagged fish allowed the growth and development of individuals to be followed with the retrospective analysis of such fish also possible. As such investigations were possible at both the individual and population level.

3.3.2. Materials and Methods.

The experiment was carried out at Site 2 (Section 2.1.1). Ova from a high grilsing Scottish stock (Loch Lochy) were fertilised at a separate hatchery (Section 2.1.1) and held in heated water (6.4±2.2°C) in darkness until first-feeding (18th April 2001). Hatching occurred on 4th March 2001. On 19th April the fry were transferred to Site 2 and held under LD24:0 and ambient temperature conditions (Fig. 3.16) in two 2m^2 tanks. On 21st May 2001 1000 fish were transferred into each of eight 2m^2 tanks and held under LD24:0 until experimental winter photoperiods were applied (see Fig. 3.17 for experiment protocol).

On 21st May two groups, each in duplicate tanks, were exposed to either an 8 or 12 week winter photoperiod (LD10:14) after which they were returned to LD24:0 until

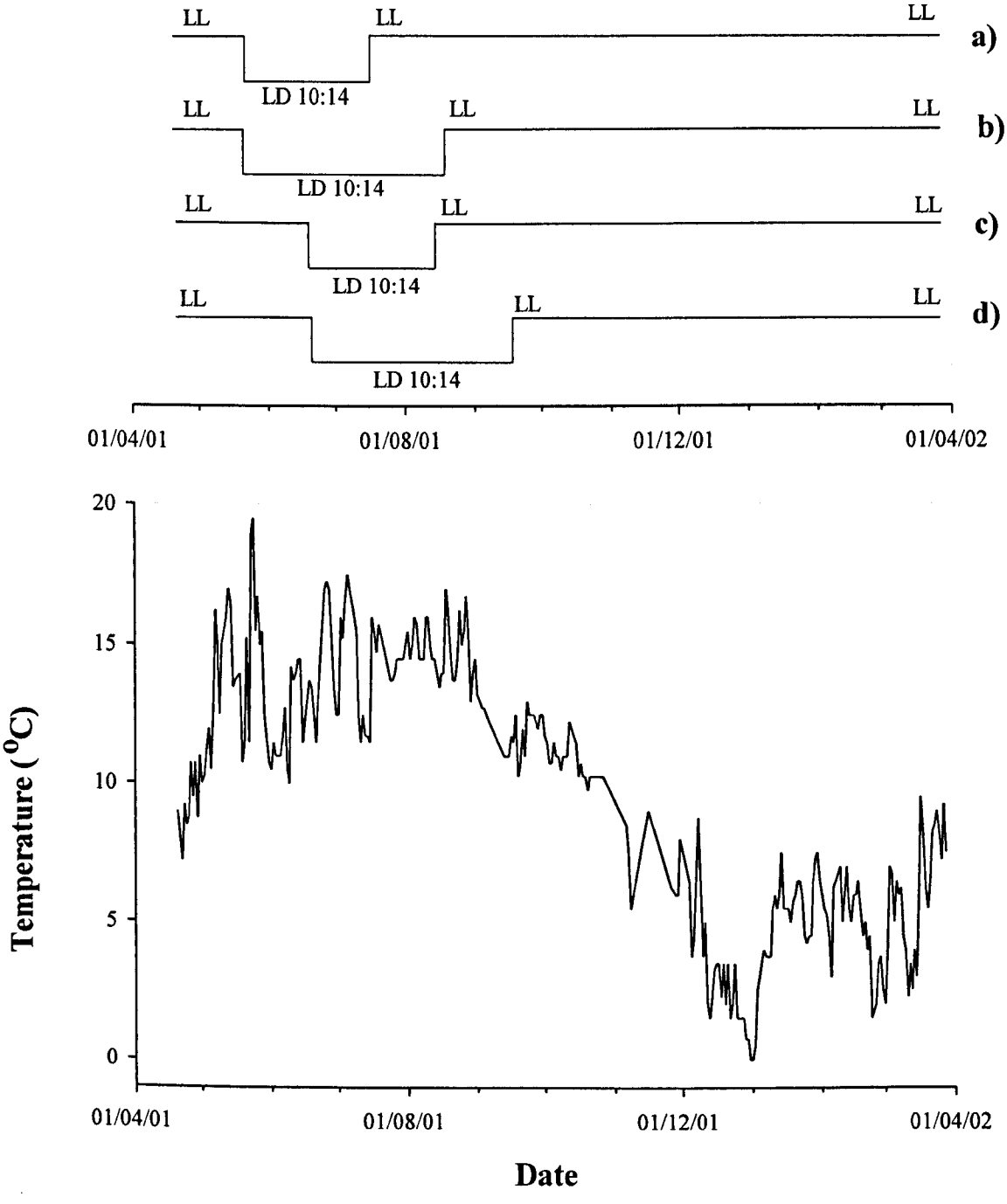


Fig. 3.16 The ambient temperature profile at Site 2 and the experimental photoperiod regimes used during the experiment. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod.

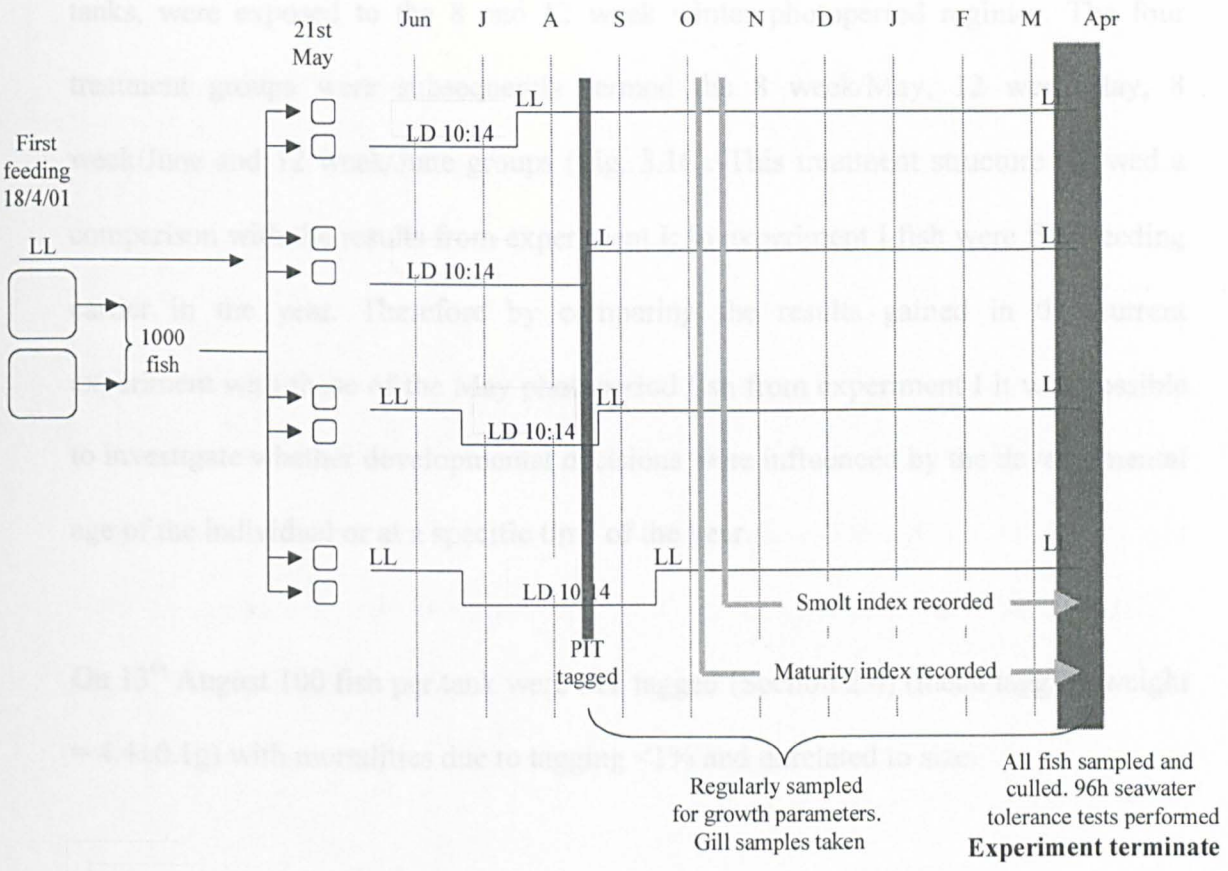


Fig. 3.17 The experimental protocol used during experiment II. For further details of the sampling regime refer to section 3.3.2.

the conclusion of the experiment. On 18th June two further groups, each in duplicate tanks, were exposed to the 8 and 12 week winter photoperiod regimes. The four treatment groups were subsequently termed the 8 week/May, 12 week/May, 8 week/June and 12 week/June groups (Fig. 3.16). This treatment structure allowed a comparison with the results from experiment I: In experiment I fish were first-feeding earlier in the year. Therefore by comparing the results gained in the current experiment with those of the May photoperiod fish from experiment I it was possible to investigate whether developmental decisions were influenced by the developmental age of the individual or at a specific time of the year.

On 13th August 100 fish per tank were PIT tagged (Section 2.4) (mean tagging weight = 4.4±0.1g) with mortalities due to tagging <1% and unrelated to size.

From 14th August onwards all tagged fish were measured at fortnightly intervals until 29th January 2002 and then monthly until the conclusion of the experiment on 23rd March 2002. On 3rd October, 14th November, 8th January and 23rd March 40-60 non-tagged individuals from each tank were measured in order to identify possible growth differentials between tagged and non-tagged fish. Neither fork length nor weight was found to be affected by tagging ($p>0.05$). All fish, whether tagged or untagged, were examined at each sample point for signs of maturation (see Section 2.7.1).

At each sample point from 13th August gill samples were taken from 20 culled non-tagged fish per treatment to identify changes in gill Na^+, K^+-ATPase (Section 2.8.1). At fortnightly intervals from 4th October, 40 fish per treatment were dissected and maturity index scores recorded (Section 2.7.3). At each sample point, from 18th

October, the smolt index score (see Section 2.8.4) of these fish was also recorded.

On 23rd March 75 individuals from each treatment (except the 12 week/May group where n=20) were exposed to a 96h seawater tolerance test (Section 2.8.2) with the fork length of both mortalities and surviving individuals recorded.

On 23rd March 2002 all fish (both tagged and non-tagged) were culled and examined for external signs of maturation (Section 2.7.1) and their relative cohort recorded (Table 3.2) (see Section 2.8.5), although all of the cohorts identified in the experiment were not necessarily represented within each treatment. Furthermore all tagged and approximately 150 non-tagged fish per treatment were dissected with the sex of the individual and internal signs of maturation (i.e. enlarged gonadal tissue) recorded.

Between the 14th September and 4th October sample points a blocked inlet resulted in the loss of one replicate of the 12 week/May group. The second replicate was subsequently divided to create a replicated group, although no further fish were tagged and sacrificial sampling in these groups was reduced to maintain population numbers. A broken stand-pipe resulted in further losses on 29th January, therefore the treatment was terminated at that time.

Growth data as well as changes in gill Na^+, K^+ -ATPase were compared using a General Linear Model (Section 2.11) although for some weight comparisons natural log transformations were used to improve normality and homogeneity of variance. For changes in percentage maturation and population structures 95% confidence limits were calculated and compared (Fowler and Cohen, 1987).

Cohort	*Description of fish*
Large smolts	Fully silvered fish with no parr marks and blackened fin margins. These fish were typically >100g
Parr	Fish showing no signs of silvering with the presence of distinct parr marks. These fish were typically >30g and <65g.
Large parr	Fish showing some slight silvering, although distinct parr marks predominated. However, these fish were significantly larger than the parr described above (i.e. >100g).
Small parr	Fish showing no signs of silvering, with the presence of distinct parr marks, although these fish were significantly smaller than the parr described above (i.e. <15g).

Table 3.2. The nomenclature used to assign individuals to a particular developmental cohort. Cohorts were based on the level of smoltification achieved at the conclusion of the experiment. The cohorts denote groups of fish that were identified within the entire experiment although some treatments, as well as the PIT tagged groups, did not necessarily contain all of the cohorts that have been described.

3.3.3. Results

3.3.3.1. Growth

Weight

Within treatment differences:

Differences within each cohort over time

All cohorts showed an overall increase in weight ($p<0.001$) over the experimental period (Fig. 3.18).

Parr increased throughout the experiment ($p<0.01$), except in the 12 week/May group where increases occurred until 16th October and then from 14th November until 17th December and finally from 8th until 29th January.

In the 8 week/May and 8 week/June groups mature parr increased until 4th October and then from 8th January onwards. However, for the 12 week treatments no increases were observed between consecutive time points ($p>0.05$).

For large parr the 8 week/May and 8 week/June groups increased until 16th October ($p<0.01$) and then from 8th until 29th January for the June group and between 30th November and 17th December ($p<0.05$) and from 8th January ($p<0.01$) onwards in the May group. For large parr under the 12 week/May treatment no consistent increases occurred whereas those in the 12 week/June group increased throughout the experiment ($p<0.01$).

For small parr no consistent increases occurred in either of the May groups with those

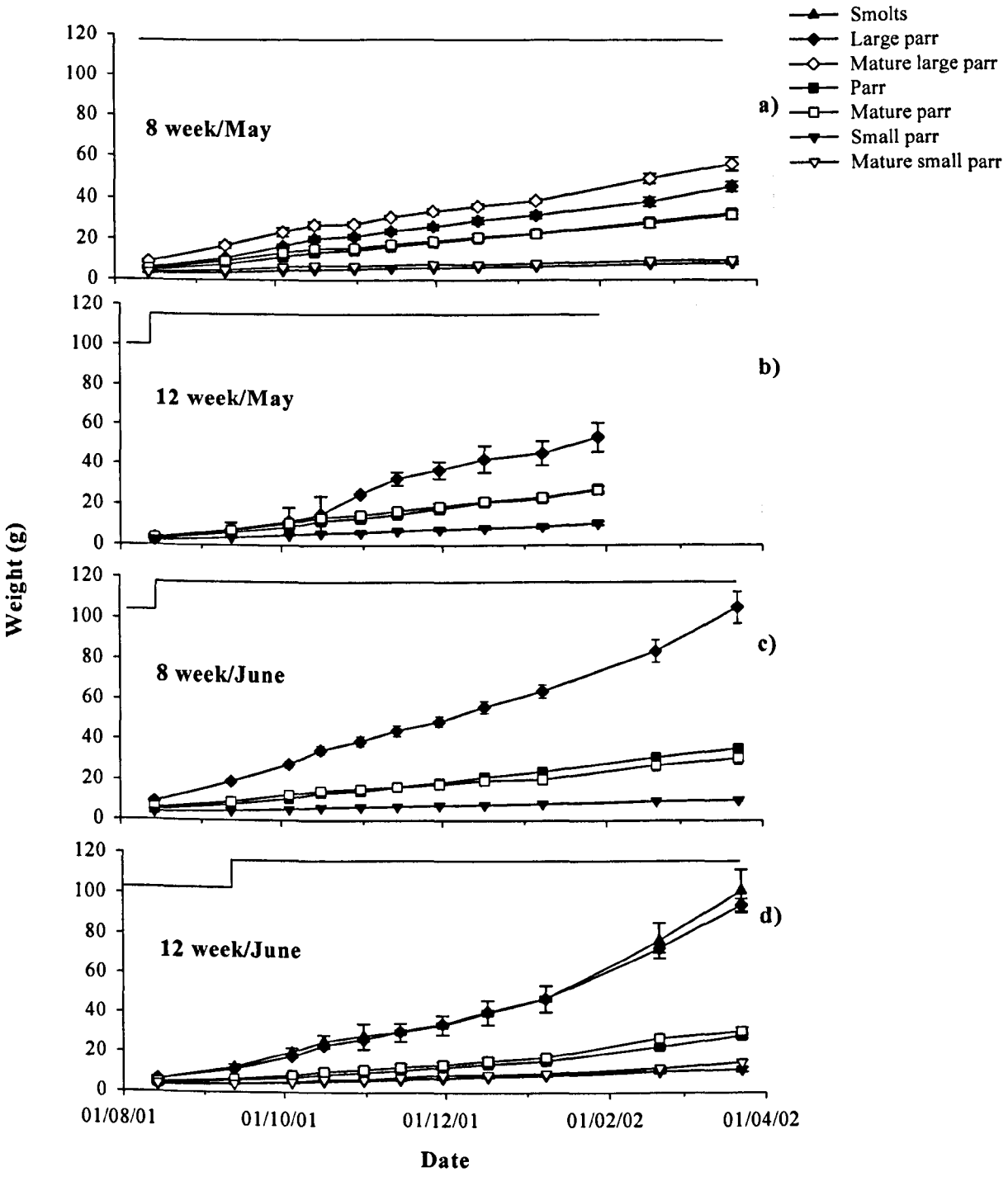


Fig. 3.18 The change in weight (mean ± S.E.M., n=100-200) of fish cohorts identified in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. In some cases error bars may be too small to be depicted. The relative photoperiod regimes are shown to the time to aid interpretation.

from the 8 week/June group only increasing until 16th October. The 12 week/June small parr increased until February ($p<0.01$).

The 8 week/May and 12 week/June treatments were the only groups where further cohorts were observed. In the 8 week/May group mature large parr increased until 12th September ($p<0.01$) and from 8th January onwards ($p<0.05$). In this group only one mature small parr was observed. In the 12 week/June group smolts increased until early October ($p<0.01$) and then between 8th January and 20th February ($p<0.01$) with mature small parr showing no consistent increases over time ($p>0.05$).

Within treatment differences:

Differences between cohorts at each sample point.

In the 8 week/May treatment large parr, mature large parr, parr and mature parr were all similar until 12th September with mature parr also similar to small parr until this time. Parr and mature parr remained similar throughout the experiment ($p<0.01$) with all other groups being significantly different ($p<0.05$). In the 8 week/June group parr and mature parr were similar from 13th August until early January with all other groups different throughout the experiment ($p<0.01$). Under the 12 week/May photoperiod all groups were similar until 4th October with large parr remaining of similar weight to both mature and immature parr until 31st October. Parr and mature parr had similar weights throughout the experiment. In the 12 week/June group, smolts were similar to large parr throughout the experiment with this also the case for parr and mature parr, and small parr and mature small parr. Parr were similar to mature small parr until 17th December with smolts and large parr also similar to

mature parr until 12th September. At all other times cohorts were significantly different ($p<0.05$).

Length

Within treatment differences:

Differences within each cohort over time

All cohorts showed an overall increase in length (Fig. 3.19) over the experimental period ($p<0.001$). Parr grew consistently throughout the experiment in all photoperiod groups ($p<0.05$).

In all but the 12 week/June group, where no increases occurred between consecutive time points, mature parr increased consistently until 4th October ($p<0.05$) with those from the 8 week/May group continuing to increase until 16th October ($p<0.01$). The mature parr from this 8 week/May group also increased from 8th January onwards ($p<0.01$), with those from the 8 week/June group increasing between 8th January and 20th February ($p<0.01$).

Under both 8 week regimes large parr grew consistently until 31st October and then from 8th January onwards ($p<0.01$) with those from the 12 week/June group increasing throughout the experiment ($p<0.01$). However, for the large parr from the 12 week/May increases only occurred until 12th September and then between 16th and 31st October ($p<0.01$).

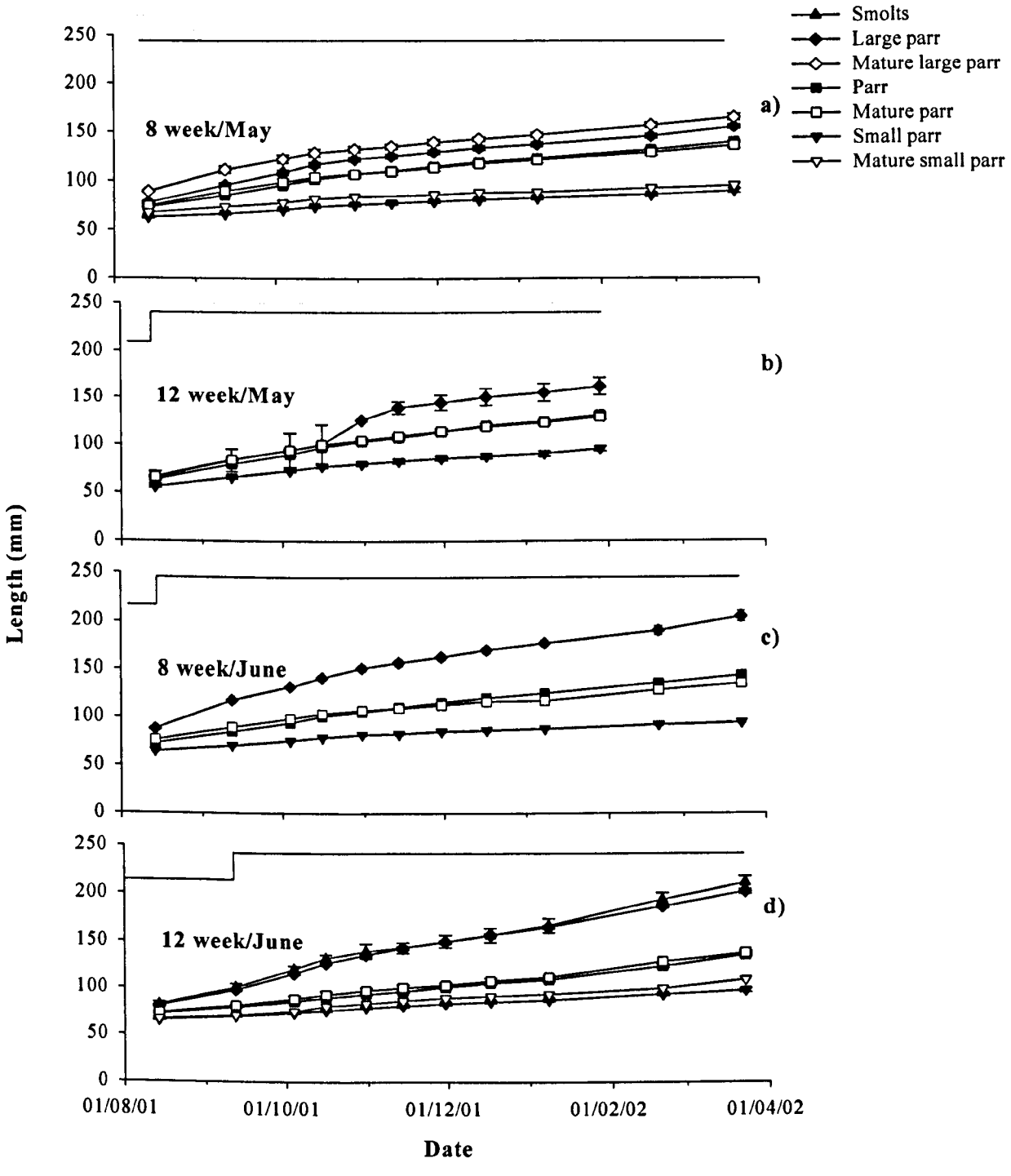


Fig. 3.19 The change in length (mean ± S.E.M., n=100-200) of fish cohorts identified in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. In some cases error bars may be too small to be depicted. The relative photoperiod regimes are shown to the time to aid interpretation.

In all groups small parr increased in length until early October ($p<0.05$) with those from the 12 week/June group continuing to increase until 31st October ($p<0.01$). Finally, the mature large parr in the 8 week/May group only increased until 12th September ($p<0.01$) with mature small parr in the 12 week/June group showing no increases. Smolts from this group, however, increased until 16th October ($p<0.05$) and then from early January onwards ($p<0.01$).

Within treatment differences:

Differences between cohorts at each sample point.

In the 8 week/May group immature and mature parr had similar lengths throughout the experiment with all other cohorts different at all sample points ($p<0.05$). For the 8 week/June group the lengths of immature and mature parr remained similar until 17th December with all cohorts differing at all other times ($p<0.05$). Under the 12 week/May photoperiod large parr had similar lengths to both immature and mature parr until 31st October, with immature and mature parr having similar lengths throughout the experiment. All other cohorts differed from one another throughout the experiment ($p<0.01$). For the 12 week/June group, smolts and large parr were similar in length throughout the experiment with this also the case for immature and mature parr, and immature and mature small parr. Both immature and mature parr were also similar in length to mature small parr until 17th December and 8th January respectively, with smolts, large parr and small parr all statistically similar on 14th August ($p>0.05$). At all other times cohorts differed in length ($p<0.05$).

Condition factor (CF)

Within treatment differences:

Differences within each cohort over time

Differences in condition factor were observed following photoperiod treatment (Fig. 3.20). Under the 8 week/May photoperiod only large parr, parr and small parr showed an overall decline in CF over the course of the experiment ($p<0.05$) with mature parr and mature large parr showing no overall decline. Between consecutive time points both large parr and parr showed an initial decline in CF to 12th September with a subsequent rise by 4th October ($p<0.01$) that was also observed in the mature parr cohort. Following this all three cohorts showed a decline between 16th and 31st October with the CF of parr rising again by 14th November ($p<0.01$). No further changes were seen in any cohort until February when both parr and small parr showed a decline in CF ($p<0.05$).

In the 8 week/June group all cohorts showed an overall decline in CF ($p<0.01$). Until 12th September large parr, parr and small parr all showed a decline in CF with these groups showing a further decline between 16th and 31st October ($p<0.01$) although parr did show an increase immediately prior to this decrease. All groups then remained unchanged until 20th February when parr showed a further decrease in CF ($p<0.01$).

For the 12 week/May group only parr and small parr showed overall declines in CF ($p<0.05$). However, a decline in CF between consecutive time points only occurred between 14th August and 12th September for the parr group and between 16th and 31st

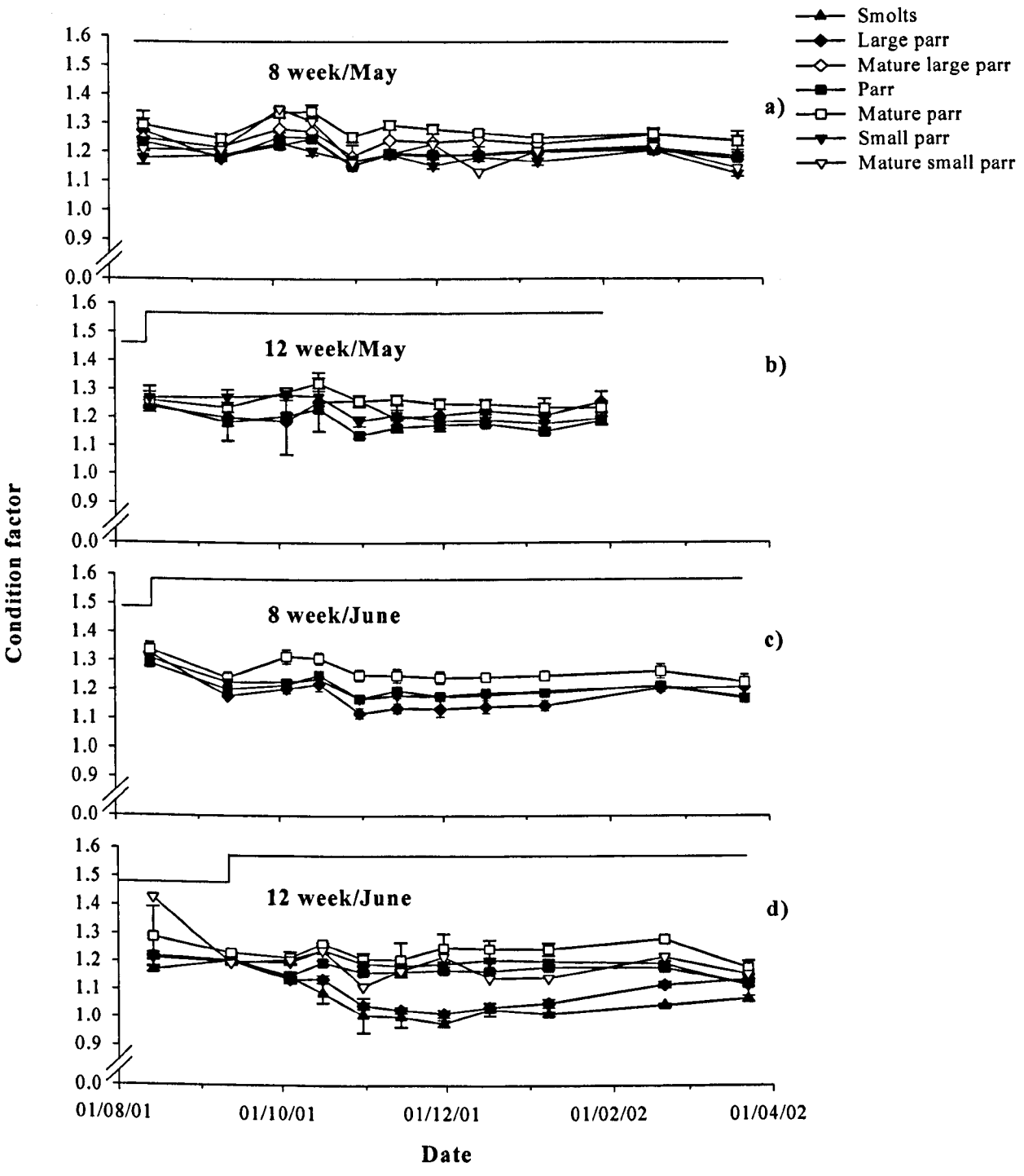


Fig. 3.20 The change in CF (mean ± S.E.M., n=100-200) of fish cohorts identified in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. In some cases error bars may be too small to be depicted. The relative photoperiod regimes are shown to the time to aid interpretation.

October for both parr and small parr cohorts ($p<0.05$).

For the 12 week/June group large parr, parr and small parr showed overall decreases in CF over the experimental period ($p<0.01$). However, between consecutive time points decreases in CF were only observed between 12th September and 4th October for large parr and parr cohorts and then between 16th and 31st October for the large and small parr ($p<0.01$). No further changes were observed until 20th February when both parr and small parr showed a decline ($p<0.01$). However, both smolts and large parr showed decreases in CF between 12th September and 30th November ($p<0.05$) with large parr then showing an increase by the conclusion of the experiment ($p<0.01$).

Within treatment differences:

Differences between cohorts at each sample point.

In the 8 week/May group the only consistent differences were that the CF of mature parr was higher than that of large parr and parr from early October until 17th December, with the CF of small parr lower than that of large parr and mature parr between 4th October and 17th December ($p<0.05$). For the 8 week/June group mature parr had a higher CF than large and small parr between 31st October and 8th January, and 4th and 31st October respectively ($p<0.05$). On 4th and 31st October as well as 30th November mature parr had a higher CF than parr ($p<0.05$). For the 12 week/May group no consistent differences in CF could be observed between groups ($p>0.05$). Under the 12 week/June photoperiod differences only occurred when the smolt and large parr cohorts were compared with the other groups. The smolt cohort had a lower

CF than parr, mature parr and small parr from 14th November, 30th November and 16th October respectively until 8th January with small parr different until 20th February ($p<0.05$). For large parr a similar situation occurred with the CF of parr, mature parr and small parr lower from 16th October, 14th November and 4th October respectively until 8th January ($p<0.01$), with small parr again different until 20th February ($p<0.01$).

Specific growth rate (SGR)

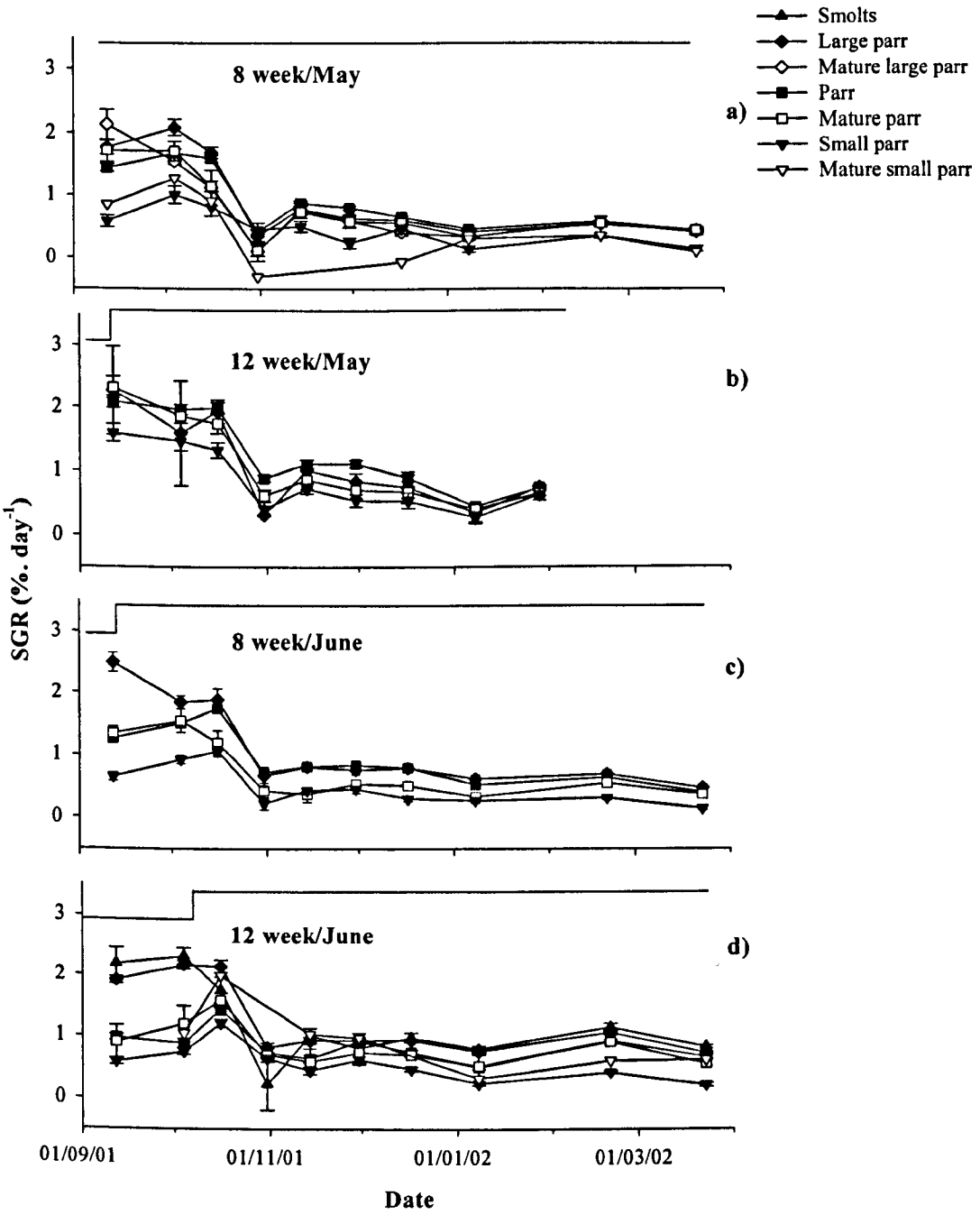
Within treatment differences:

Differences within each cohort over time

All groups under the 8 week/May photoperiod regime, except the small parr, showed an overall decline in SGR ($p<0.01$) (Fig. 3.21). However, both small parr and parr showed an initial increase in SGR until 4th October after which the small parr group displayed an overall decline in SGR until the conclusion of the experiment ($p<0.01$). For the parr and mature parr decreases in SGR were only observed between consecutive time points from 16th October until 14th November, with decreases occurring from 16th until 31st October for the large parr ($p<0.01$).

For the 12 week/May photoperiod group all cohorts showed an overall decline in SGR over the experiment ($p<0.05$). All cohorts showed a significant decrease in SGR between 16th and 31st October ($p<0.05$) with parr also showing a decrease in SGR from 17th December until early January ($p<0.05$).

For the 8 week/June group all cohorts showed an overall decrease in SGR over the experiment ($p<0.01$). Parr showed initial increases in growth until 16th October with



3.21 The change in SGR (mean ± S.E.M., n=100-200) of fish cohorts identified in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. In some cases error bars may be too small to be depicted. The relative photoperiod regimes are shown to the time to aid interpretation.

large parr having a decrease in SGR until 4th October ($p<0.05$). Then between 16th and 31st October all groups showed a decrease in growth rate ($p<0.05$).

Under the 12 week/June photoperiod smolts, large parr and small parr all showed a decline in SGR over the experiment ($p<0.05$). However, both small parr and parr showed an increase in SGR from 4th until 16th October with the parr group subsequently having an overall decline in growth until the conclusion of the experiment ($p<0.01$). Between 16th and 31st October smolts, large parr, parr and small parr all showed significant decreases in growth rate ($p<0.05$).

Within treatment differences:

Differences between cohorts at each sample point.

Small parr from the 8 week/May group initially had a lower SGR than all other cohorts. For mature large parr this difference only occurred on 12th September but for mature parr the differential remained until 16th October ($p<0.01$), with large parr and parr growth higher until 31st October ($p<0.01$). In the 12 week/May treatment all groups had similar SGR's except the parr and small parr, which differed from each other until 31st October ($p<0.05$) and then on 30th November ($p<0.01$). For those exposed to the 8 week/June photoperiod the most consistent difference was observed between the parr and small parr groups, with the growth of small parr lower than that of parr throughout the experiment ($p<0.05$). Large parr had a higher growth rate than all other cohorts on 12th September, with the growth of small parr remaining lower until 31st October ($p<0.01$). The SGR of small parr was also lower than that of both the parr and mature parr fish on 12th September ($p<0.05$). Finally, for the 12

week/June treatment smolts had a higher SGR than both parr and small parr until 16th October, with mature small parr growth also lower on 12th September ($p<0.01$). The SGR of large parr was higher than that of parr until 31st October, with mature small parr growth lower on 12th September ($p<0.01$). However, the growth of small parr was lower than that of large parr throughout most of the experiment ($p<0.01$).

Weight-frequency distribution.

Photoperiod treatment affected both the structure and timing of modality (Fig. 3.22). The emergence of modality was first observed on the 18th October in both June photoperiod groups. Subsequently, the 12 week/May group displayed a bimodal distribution on 30th November, with the 8 week/May group the last population to divide on 19th December. In both May photoperiod groups two clear modes were evident by the conclusion of the experiment although in both June photoperiod treatments the division of the population into two distinct modes was less clear with a more complex population structure present.

3.3.3.2. Maturation

Rates of maturation

Mature fish were first identified in late October (Fig. 3.23) in both 8 week photoperiod treatments although the numbers found in the June treatment were extremely low. From late October levels of maturity steadily increased in the 8 week groups with the numbers of mature fish in the 12 week/May group increasing during early December reaching peak levels by 8th January (>11%). The 8 week/May and 8 week/June groups had similar levels of maturity ($p<0.05$) from 16th November until 23rd March, peaking on 17th December and 8th January respectively, with levels at

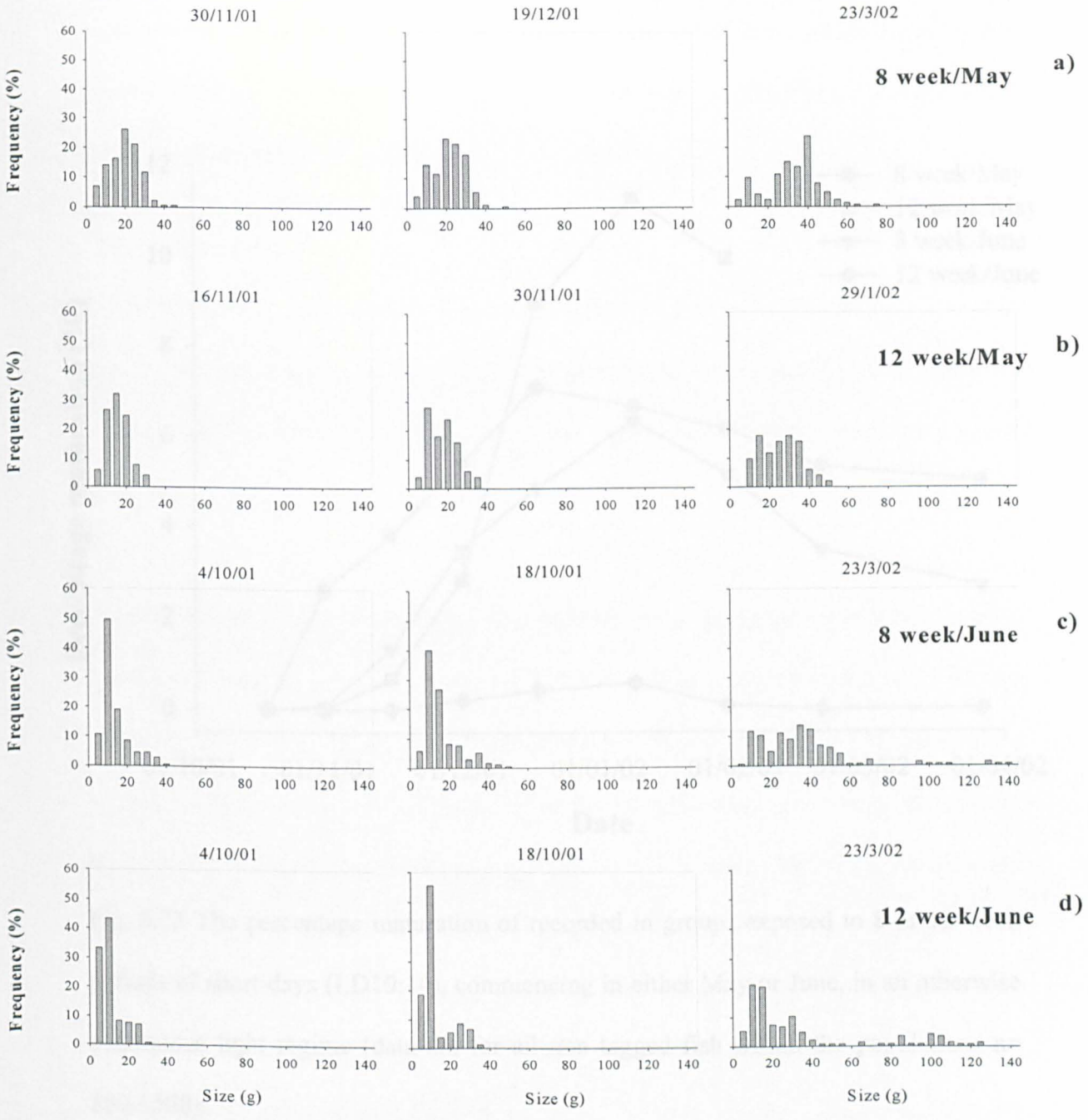


Fig. 3.22 The weight-frequency distribution of populations exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. (n=50-200). The populations shown represent the weight frequency distributions just prior to, and at the emergence of modality, as well as at the final sample point (except for the 12 week/May group where, due to population numbers, the 29th January sample point was displayed).

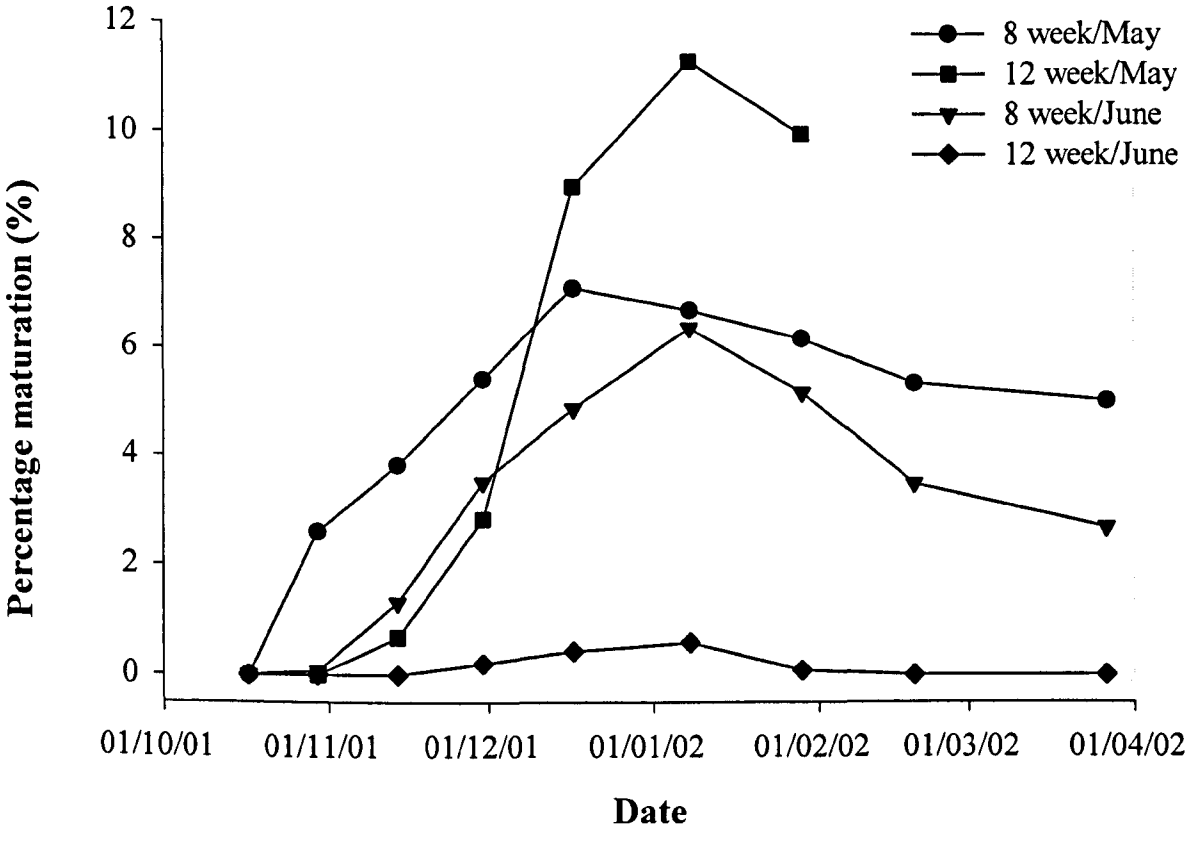


Fig. 3.23 The percentage maturation of recorded in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime (data are for all non tagged fish within the populations n= 350-1500).

approximately 6%. Maturity in the 12 week/June group remained lower than all other groups from 16th November onwards (<0.5%) although a minor peak in maturation could be identified on 8th January.

Maturation index

No differences in the mean maturity index scores of treatment groups were found at specific sample points ($p>0.05$) (Fig. 3.24) with the exception that on 31st November the maturity index of 12 week/May fish was higher than for the 8 week/May group ($p<0.05$).

For fish exposed to an 8 week/May photoperiod the mean index score rose initially, but then decreased reaching a low on 31st November. Following this a sharp rise occurred with levels peaking on 8th January before the score started to decline through to the end of the experiment. The score for the 12 week/May group was consistently high from 4th October onwards reaching a peak on 29th January before the mean index decreased. Under the 8 week/June photoperiod levels remained fairly stable at approximately 1.5, falling slightly to 1.4 by 8th January with a subsequent rapid increase in mean score peaking on 29th January. Levels then declined to the end of the experiment. Finally, maturity scores of the 12 week/June group increased initially reaching a low peak on 17th December before declining over the latter stages of the experiment.

When the structure of the index scores for each treatment were analysed in more detail (Fig. 3.25), all photoperiods resulted in a high incidence of index scores 1 and 2. Both of these scores are inconclusive in confirming maturation (Section 2.7.3). However, in the 8 week/May group index 3 fish were identified from 4th October,

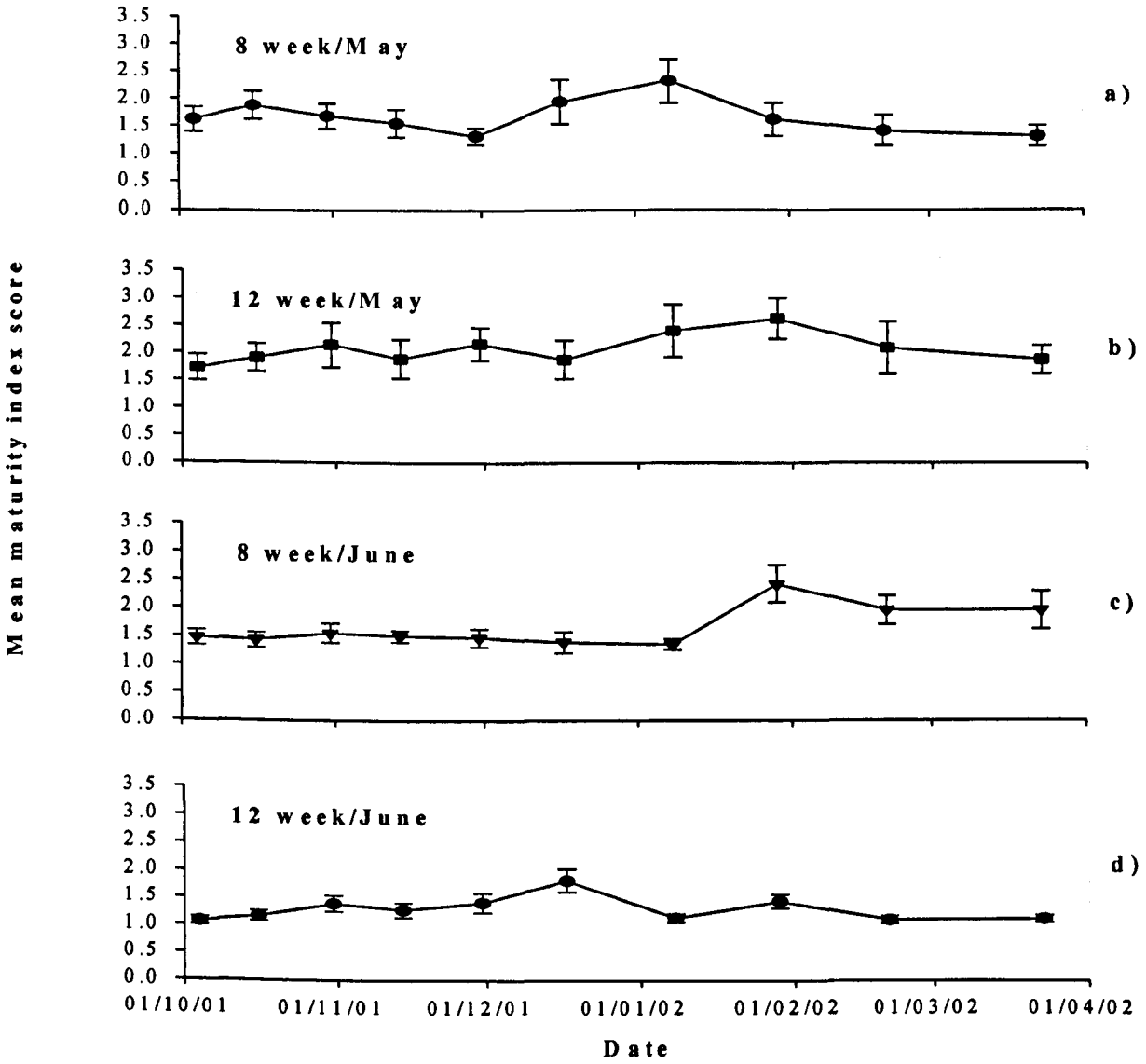


Fig. 3.24 Mean maturity index scores (mean±S.E.M., n=7-27) of male fish, in populations previously exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime (points represent all male fish identified from a dissected population sample of 40 fish). a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. Maturity scores: 1 = gonadal tissue pink and undeveloped, 2 = gonadal tissue thickened, but remaining pink, 3 = gonadal tissue clearly thickened and white, 4 = testes fully developed without external expression of milt, 5 = testes fully development, with external expression of milt (see Section 2.7.3). Photoperiod regimes are omitted due to all groups being held on LD24:0 during the sample points.

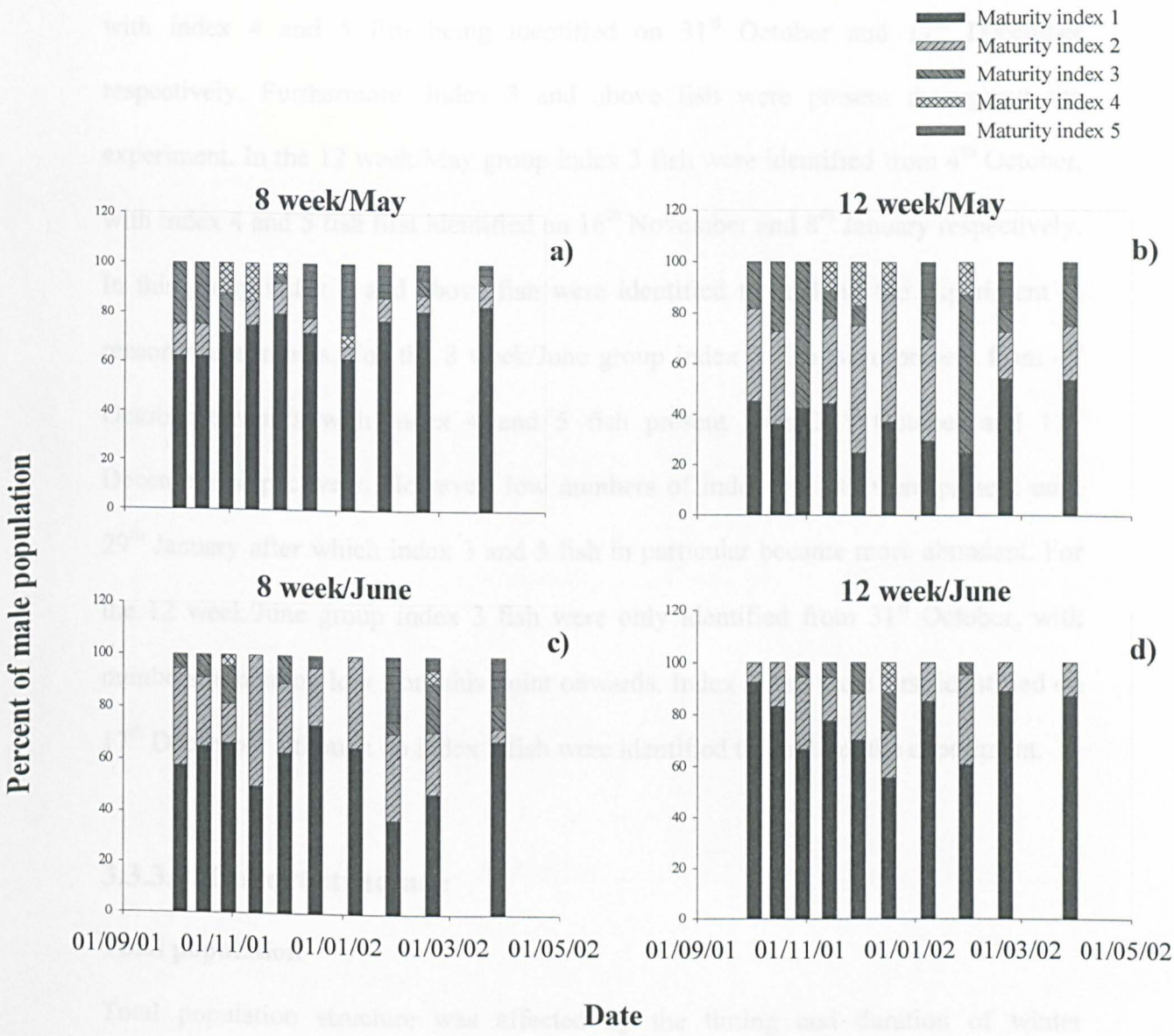


Fig. 3.25 Maturity index scores of male fish, in populations previously exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime (points represent all male fish identified from a dissected population sample of 40 fish, n=7 to 27). a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. Maturity scores: 1 = gonadal tissue pink and undeveloped, 2 = gonadal tissue thickened, but remaining pink, 3 = gonadal tissue clearly thickened and white, 4 = testes fully developed without external expression of milt, 5 = testes fully development, with external expression of milt (see Section 2.7.3 for details).

with index 4 and 5 fish being identified on 31st October and 17th December respectively. Furthermore, index 3 and above fish were present throughout the experiment. In the 12 week/May group index 3 fish were identified from 4th October, with index 4 and 5 fish first identified on 16th November and 8th January respectively. In this group index 3 and above fish were identified throughout the experiment in reasonable numbers. For the 8 week/June group index 3 fish were present from 4th October onwards with index 4 and 5 fish present from 31st October and 17th December respectively. However, low numbers of index 3+ fish were present until 29th January after which index 3 and 5 fish in particular became more abundant. For the 12 week/June group index 3 fish were only identified from 31st October, with numbers remaining low from this point onwards. Index 4 fish were first identified on 17th December although no index 5 fish were identified throughout the experiment.

3.3.3.3. Cohort structure

Total population

Total population structure was affected by the timing and duration of winter photoperiod treatment (Fig. 3.26). The 8 week/May, 12 week/May and 8 week/ June groups exhibited similar population structures with large parr, parr and small parr present. The 8 and 12 week/May photoperiods resulted in similar numbers of parr (87% and 82% respectively) ($p>0.05$), with the 8 week/June photoperiod resulting in a lower ($p<0.05$), but still high, incidence (77%). Likewise, the 8 and 12 week/May populations had similar ($p>0.05$) numbers of small parr (10% and 13% respectively) although a higher incidence ($p<0.05$) was found in the 8 week/June population (15%). The lowest incidence of large parr (3%) was identified in the 8 week/May group ($p<0.05$) with the 12 week/May and 8 week/June groups having similar, low numbers

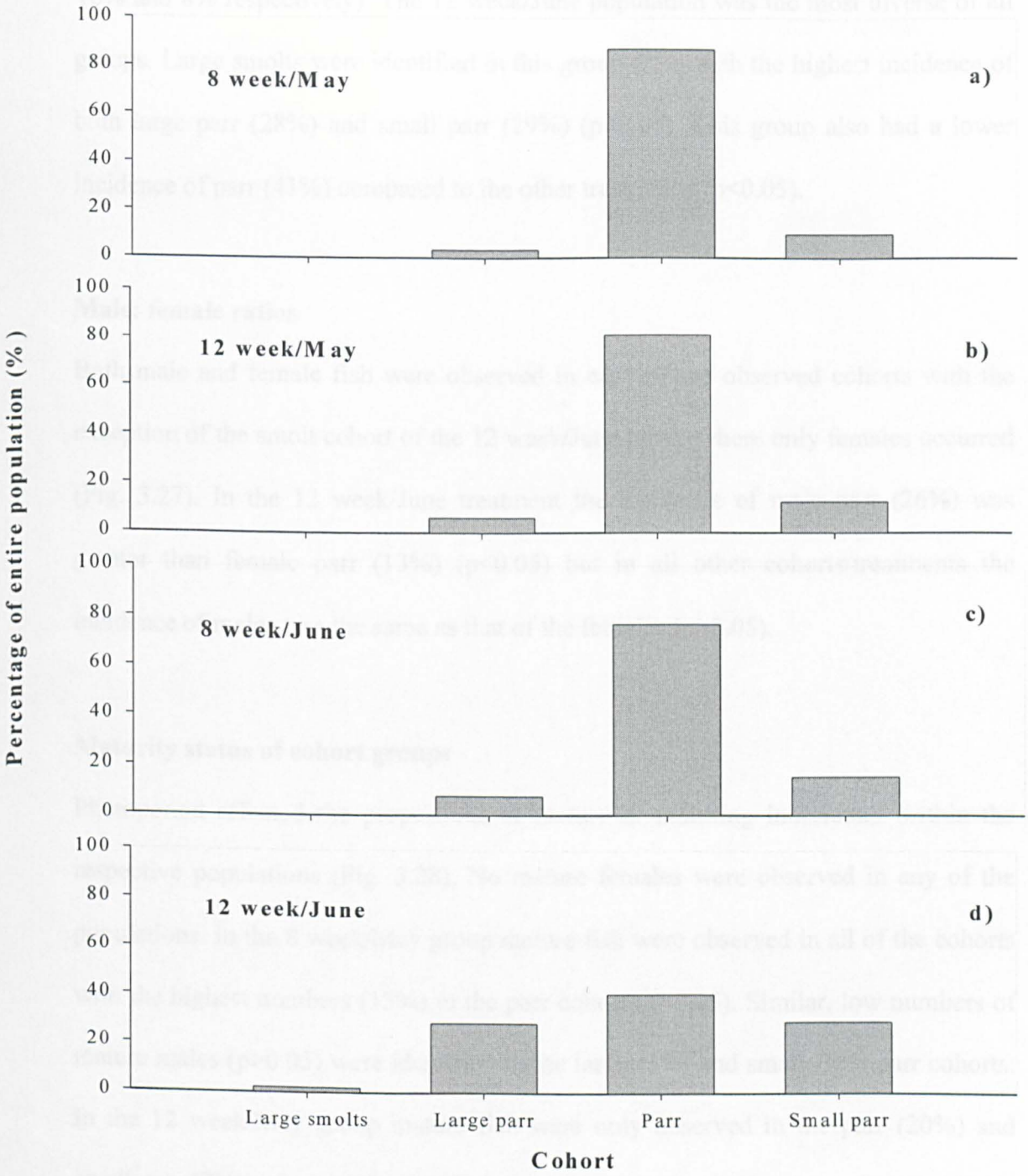


Fig. 3.26 The cohort structure of groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. Data are based on the entire non-tagged population of each treatment (n=350 to 1250).

(6% and 8% respectively). The 12 week/June population was the most diverse of all groups. Large smolts were identified in this group (2%) with the highest incidence of both large parr (28%) and small parr (29%) ($p<0.05$). This group also had a lower incidence of parr (41%) compared to the other treatments ($p<0.05$).

Male: female ratios

Both male and female fish were observed in each of the observed cohorts with the exception of the smolt cohort of the 12 week/June group where only females occurred (Fig. 3.27). In the 12 week/June treatment the incidence of male parr (26%) was greater than female parr (13%) ($p<0.05$) but in all other cohorts/treatments the incidence of males was the same as that of the females ($p>0.05$).

Maturity status of cohort groups

Photoperiod affected the proportions of mature or maturing individuals within the respective populations (Fig. 3.28). No mature females were observed in any of the populations. In the 8 week/May group mature fish were observed in all of the cohorts with the highest numbers (13%) in the parr cohort ($p<0.05$). Similar, low numbers of mature males ($p>0.05$) were identified in the large (1%) and small (1%) parr cohorts. In the 12 week/May group mature fish were only observed in the parr (20%) and small parr (2%) groups with significantly more occurring in the parr cohort ($p<0.05$). This resulted in a difference in the incidence of immature males and females ($p<0.05$). In the 8 week/June group mature fish were observed in all of the cohorts. However, the greatest incidence was found in the parr cohort (13%) ($p<0.05$) with similar low numbers in the large (1%) and small parr (1%) groups ($p>0.05$). Under the 12 week/June photoperiod mature fish were only found in the parr and small parr

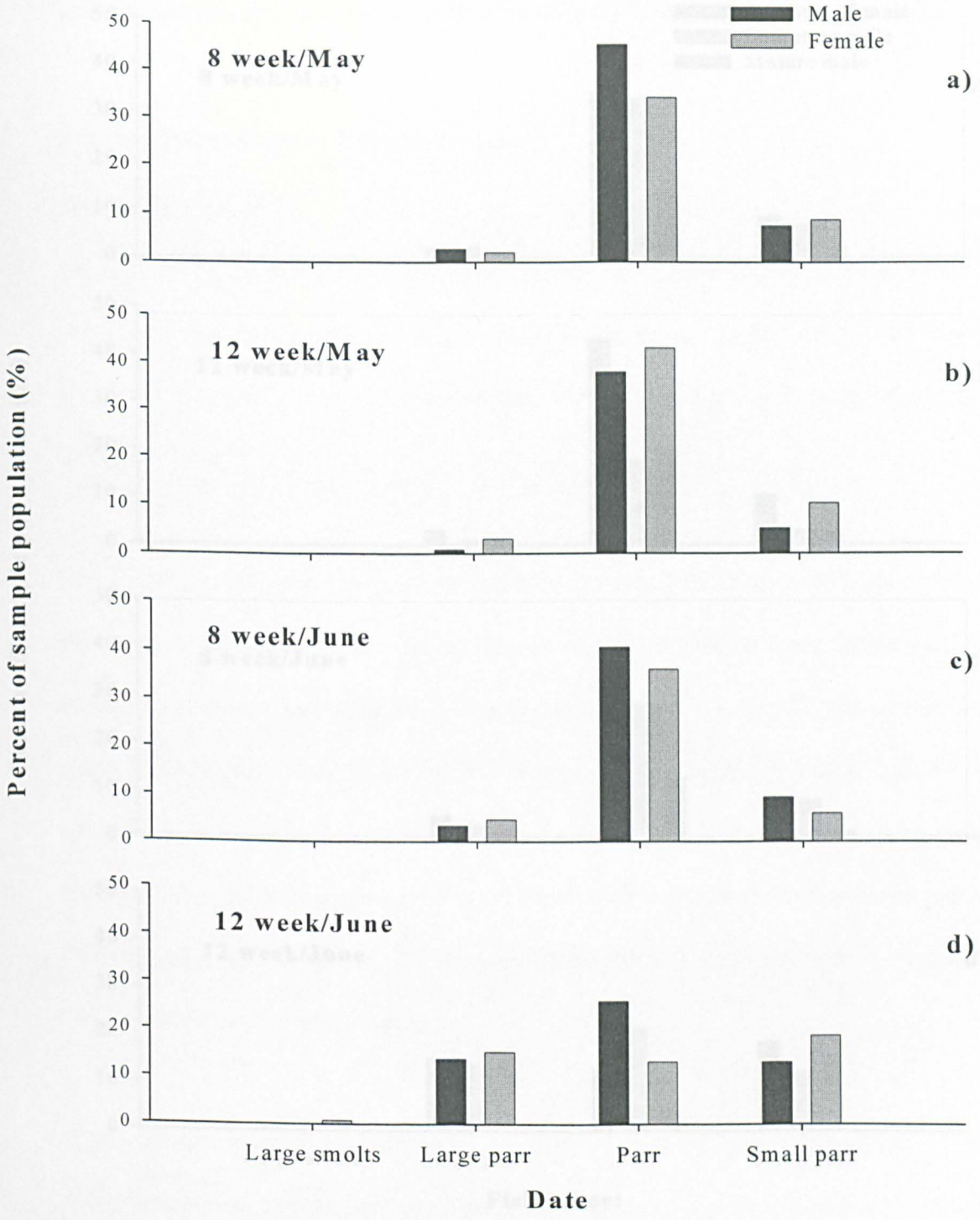


Fig. 3.27 The male: female ratio of fish cohorts, identified in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. Data are based on a dissected population sample (n=150). a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod.

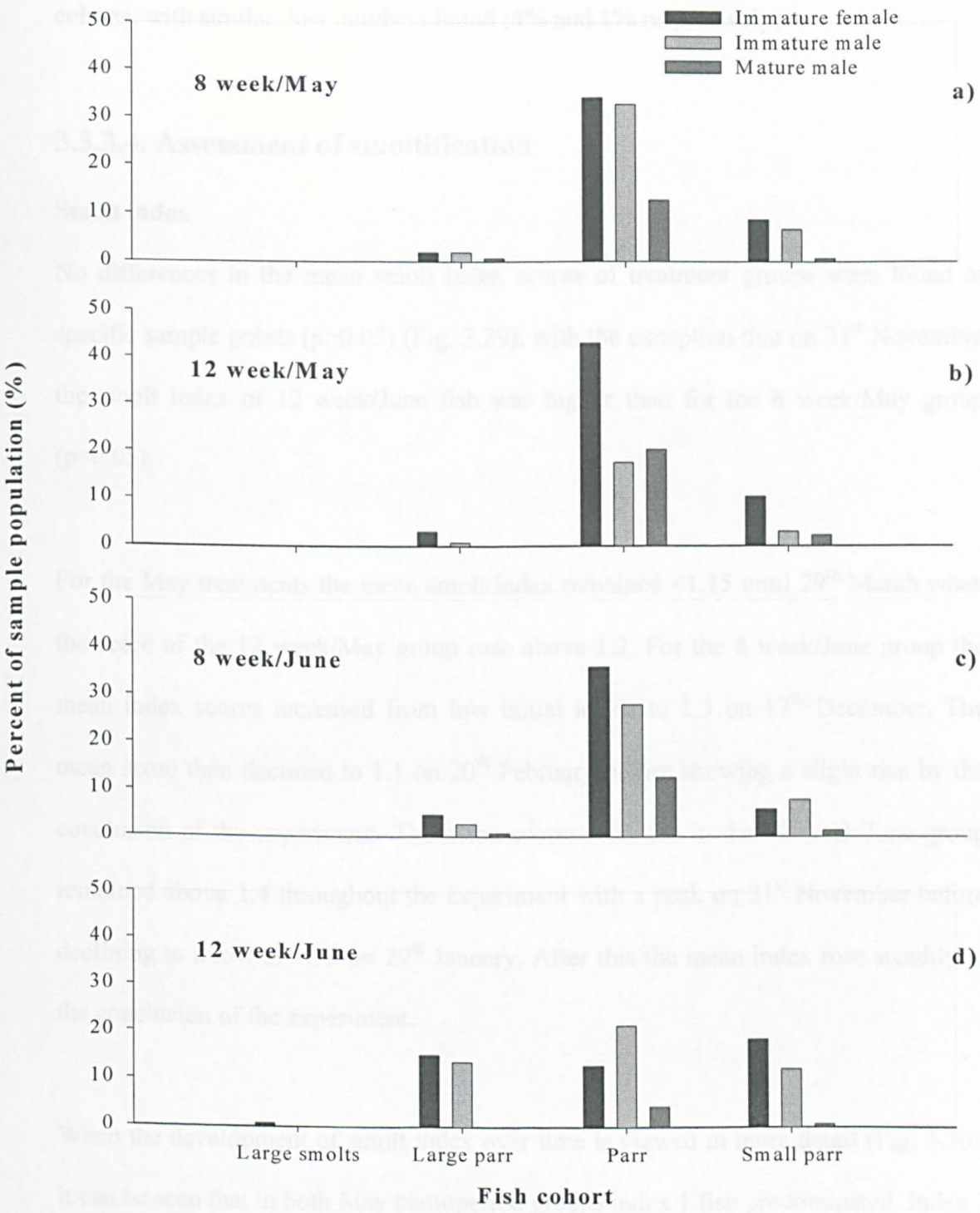


Fig. 3.28 The maturity status of fish cohorts, identified in groups exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. Data are based on a dissected population sample (n=150). a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod.

cohorts, with similar, low numbers found (4% and 1% respectively).

3.3.3.4. Assessment of smoltification

Smolt index

No differences in the mean smolt index scores of treatment groups were found at specific sample points ($p>0.05$) (Fig. 3.29), with the exception that on 31st November the smolt index of 12 week/June fish was higher than for the 8 week/May group ($p<0.05$).

For the May treatments the mean smolt index remained <1.15 until 29th March when the score of the 12 week/May group rose above 1.2. For the 8 week/June group the mean index scores increased from low initial levels to 1.3 on 17th December. The mean score then declined to 1.1 on 20th February before showing a slight rise by the conclusion of the experiment. The mean scores recorded in the 12 week/June group remained above 1.4 throughout the experiment with a peak on 31st November before declining to a low of 1.35 on 29th January. After this the mean index rose steadily to the conclusion of the experiment.

When the development of smolt index over time is viewed in more detail (Fig. 3.30), it can be seen that in both May photoperiod groups index 1 fish predominated. Index 2 fish were present in low numbers throughout the experiment for both May treatments, with index 3 fish only observed on 23rd March for the 12 week/May group. No index 4 fish were identified within either May photoperiod group. In the 8 week/June group a slightly higher incidence of index 2 fish were observed with index 3 fish also noted from 16th November onwards. However, as with the May groups no index 4 fish were

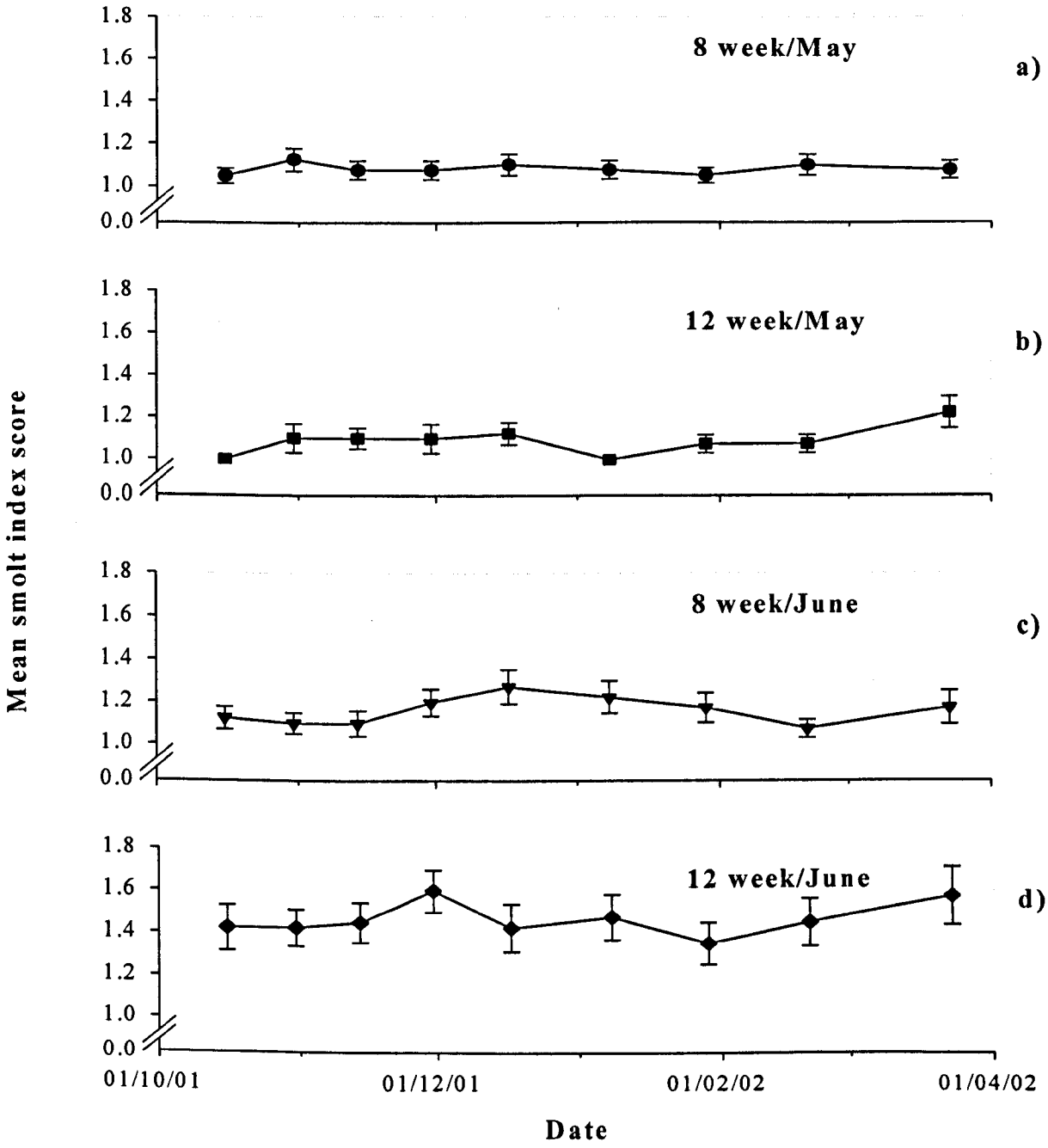


Fig. 3.29 Mean smolt index scores (mean±S.E.M., n=40) of fish exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. Smolt index scores: 1 = parr, 2 = parr with some silvering, 3 = silvered fish, with parr marks still visible, 4 = smolt (see Section 2.8.4 for details). Photoperiod regimes are omitted due to all groups being held on LD24:0 during the sample points.

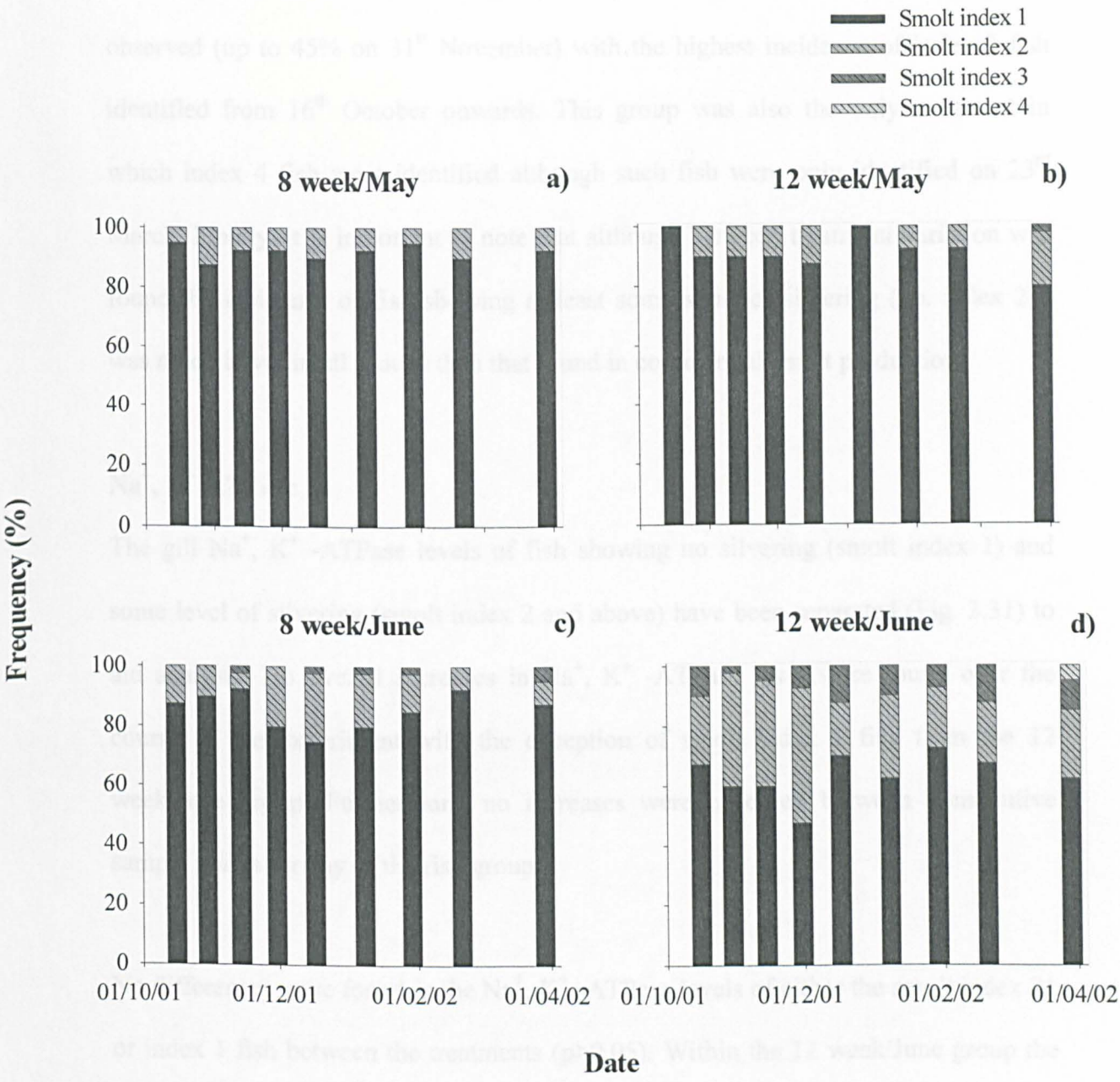


Fig. 3.30 The smolt index of fish exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. Smolt index scores: 1 = parr, 2 = parr with some silvering, 3 = silvered fish, with parr marks still visible, 4 = smolt (see Section 2.8.4 for details).

identified. For the 12 week/June group the greatest incidence of index 2 fish was observed (up to 45% on 31st November) with the highest incidence of index 3 fish identified from 16th October onwards. This group was also the only treatment in which index 4 fish were identified although such fish were only identified on 23rd March. Finally, it is important to note that although between treatment variation was found the incidence of fish showing at least some signs of silvering (i.e. index 2+) was much lower in all groups than that found in commercial smolt production.

Na^+, K^+-ATPase

The gill Na^+, K^+ -ATPase levels of fish showing no silvering (smolt index 1) and some level of silvering (smolt index 2 and above) have been separated (Fig. 3.31) to aid analysis. No overall increases in Na^+, K^+ -ATPase level were found over the course of the experiment with the exception of smolt index 1 fish from the 12 week/June group. Furthermore, no increases were observed between consecutive sample points for any of the fish groups.

No differences were found in the Na^+, K^+ -ATPase levels of either the smolt index 2+ or index 1 fish between the treatments ($p>0.05$). Within the 12 week/June group the Na^+, K^+ -ATPase levels of the smolt index 2+ were higher than those found in the index 1 fish until 17th December ($p<0.05$). However, within the 8 week/May, 12 week/May and 8 week/June groups no consistent differences could be found between the Na^+, K^+ -ATPase levels of the index 2+ and index 1 fish.

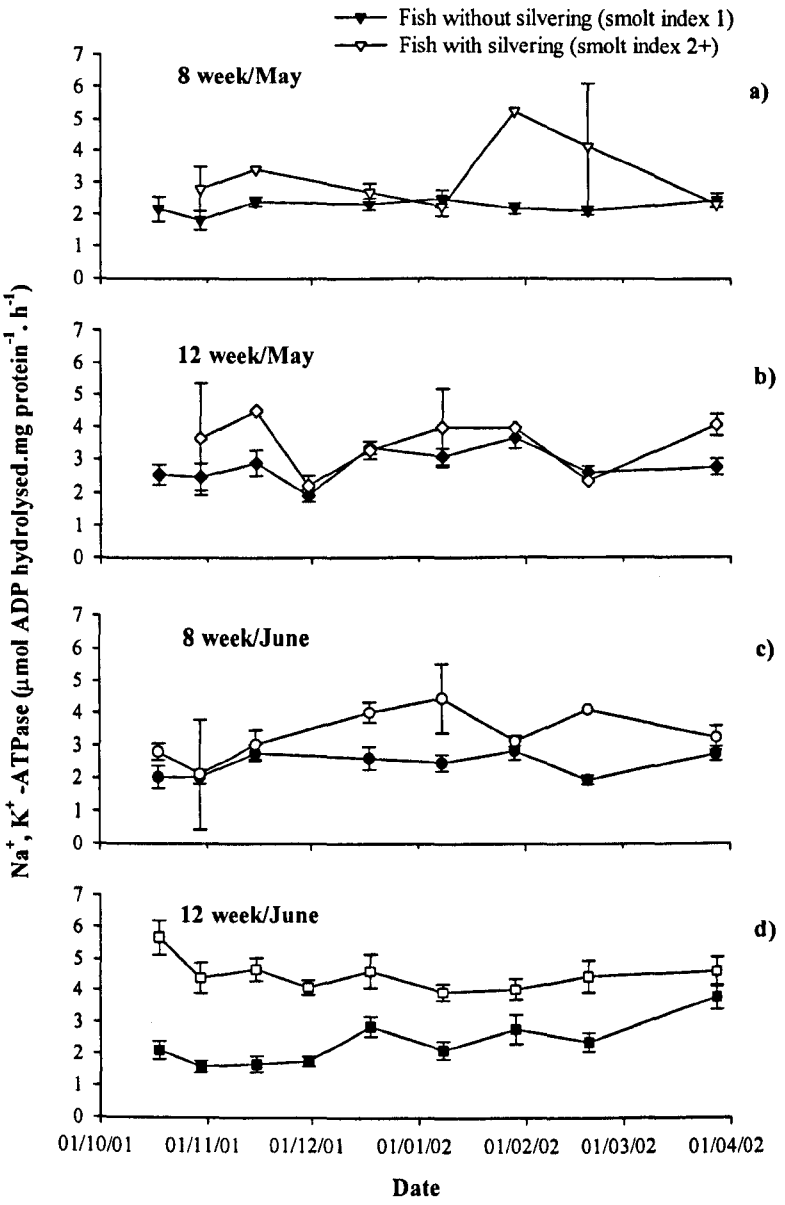


Fig. 3.31 The Na^+, K^+ -ATPase levels (mean±S.E.M., n=20) of fish exposed to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod. The Na^+, K^+ -ATPase levels of fish showing no signs of silvering, i.e. smolt index 1 (closed symbols), and those showing silvering, i.e. smolt index 2+ (open symbols), are separated to aid analysis. Photoperiod regimes are omitted due to all groups being held on LD24:0 during the sample points.

Seawater tolerance

When fish were exposed to a 96h seawater tolerance test those in the 8 week/May treatment were the first to suffer mortalities after 7 h (Fig. 3.32). Subsequently, fish from the 8 and 12 week/June groups suffered their first mortalities after 21 h, with the cumulative mortalities recorded at this time significantly greater than for the 8 week/May group ($p<0.05$). The first mortalities from the 12 week/May group occurred after 30 h. However, from 30 hours onwards the cumulative mortality of the 8 week/May, 8 week/June and 12 week/June groups remained similar and higher than for the 12 week/May treatment ($p<0.05$). The overall increases in mortality, therefore, resulted in the 12 week/May group having 55% survival after the 96 h with all other groups only achieving 12% survival.

When the lengths of mortalities were considered (Fig.3.33), for all groups the length of surviving individuals was significantly greater than for the mortalities ($p<0.05$). However, all groups showed poor linear correlations between fish length and duration in sea water. The 8 week/June treatment resulted in the highest r^2 value (0.475) with the 12 week/May group showing the least confident linear regression ($r^2=0.051$).

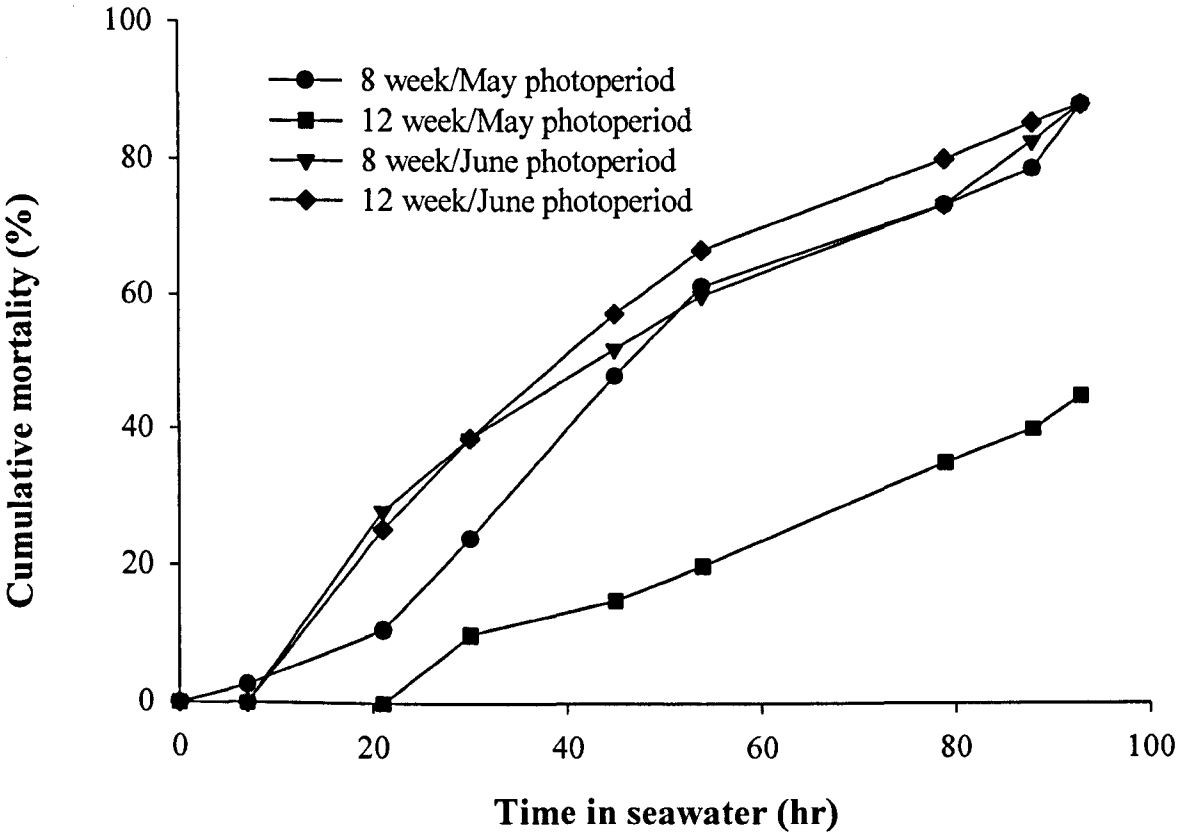


Fig.3.32 The percentage cumulative mortality of fish exposed to a 96 hour sea water (37.5‰) tolerance test after previous exposure to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. (for the 8 week/May, 8 week/June and 12 week/June photoperiods n=75, for the 12 week/May photoperiod n=20).

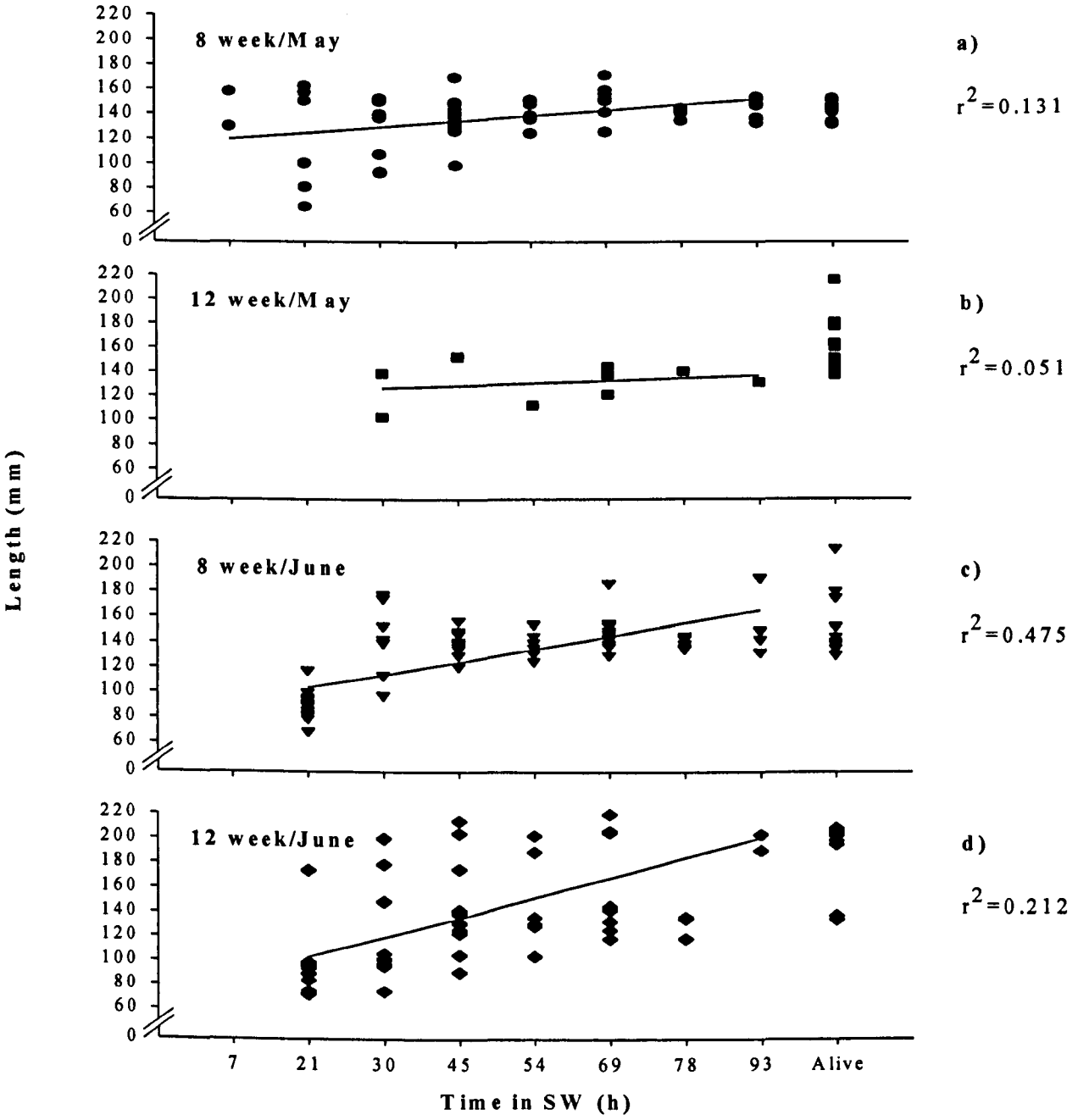


Fig. 3.33 The fork length of mortalities during a 96 hour seawater (37.5‰) tolerance test, after previous exposure to 8 or 12 week periods of short days (LD10:14), commencing in either May or June, in an otherwise continuous light regime. (for the 8 week/May, 8 week/June and 12 week/June photoperiods n=75, for the 12 week/May photoperiod n=20). Linear regression plots have been included with r^2 values quoted. a) 8 week/May photoperiod, b) 12 week/May photoperiod, c) 8 week/June photoperiod, d) 12 week/June photoperiod.

3.3.4. Summary of the results from Experiment II.

- The weight and length of mature and immature parr was similar throughout the experiment in all treatments. In the 12 week/June group this was also the case for large smolts and large parr as well as small parr and mature small parr.
- The CF of all cohorts, except mature parr, declined over the experimental period. In the 8 week groups mature parr had high CF's during the initial stages of the experiment. In the 12 week/June group the CF of large smolts and large parr was lower than for other cohorts.
- SGR declined for all groups during the experiment, with decreases occurring during the initial stages of the experiment. Small parr consistently had the lowest SGR within groups.
- The 12 week/May photoperiod resulted in the highest levels of maturation (>11%), with the 12 week/June group having the lowest levels (<1%). The 8 week photoperiods resulted in similar intermediate levels (>6%).
- The 12 week/June photoperiod resulted in the most diverse population structure with the only incidence of large smolts and the highest numbers of both large and small parr. All other treatments had similar population structures with a high incidence of parr and low numbers of both large and small parr.
- Mature fish were identified within the large parr, parr and small parr cohorts. In the 8 week photoperiod groups mature fish occurred in all cohort groups but under the 12 week photoperiods mature fish only occurred in the parr and small parr cohorts.
- Smolt index scores were slightly higher in the 12 week/June group compared to the other treatments, although the scores were relatively low for all groups.

- The gill Na^+, K^+ -ATPase levels of silvered fish were slightly elevated in only the 12 week/June photoperiod group.
- Fish exposed to the 12 week/May photoperiod exhibited moderate survival in sea water (55%). All other groups had high levels of mortality after 96h in sea water (i.e. 88%).
- Some evidence suggested that larger fish were less susceptible to seawater mortality.

3.4. Experiment III. The effects of an individuals' sex and maturation status on post transfer mortality in smolts.

3.4.1 Objectives

Based on the findings of experiments I and II, where maturation had occurred in some fish which were also undergoing smoltification, it was suggested that post seawater transfer mortality rates may be linked to maturational status or an individuals' sex. Therefore, the current survey of commercial data aimed to investigate whether smolts suffering from post transfer mortality displayed any incidence of maturation or whether mortality was linked to an individuals' sex.

3.4.2. Materials and Methods.

A survey of the rates of maturation and sex, of individuals suffering post transfer mortality was conducted at three sites: Site 3, Site 4 and Site 5 (Section 2.1.1). For each site surveys of both 0+ and 1+ production were conducted. From the transfer date of each group (Table 3.3) each site was visited at weekly or two weekly intervals until approximately 6 weeks after transfer when mortalities due to transfer were found to become reduced (M. Thomson *pers. comm.*). At each sample point all mortalities identified in the respective sea cages were removed and dissected with the individuals sex and maturation status recorded.

To analyse differences between the proportions of male and female mortalities 95% confidence intervals were calculated and compared (Fowler and Cohen, 1987) (Section 2.11).

Site	Transfer date	
	0+	*1+*
Site 3	09/02/02	02/04/02
Site 4	21/02/02	04/04/02
Site 5	16/02/02	03/04/02

Table 3.3. The dates on which both 0+ and 1+ smolts were transferred from freshwater hatcheries to three commercial sea cage rearing sites. Subsequently the mortality rates of these fish were recorded.

3.4.3. Results.

0+ production

No signs of maturity were observed at any of the sites investigated. When the total numbers of fish were considered for each site (Fig. 3.34) more male mortalities were found than females at sites 3 (64% and 36% respectively) and 4 (60% and 40% respectively) ($p<0.05$). However, when the number of mortalities are considered at the respective sample points only on 17th May at Site 3 were there differences with more male mortalities (8% of the total mortality at Site 3) recorded than females (0% of the total mortality at Site 3).

1+ production

No signs of maturity were observed at any of the sites investigated. When the total fish numbers (Fig. 3.35) were considered no differences were found between male and female mortalities at any site ($p>0.05$). Furthermore, no differences were found when mortality rates at individual sample points were considered ($p>0.05$).

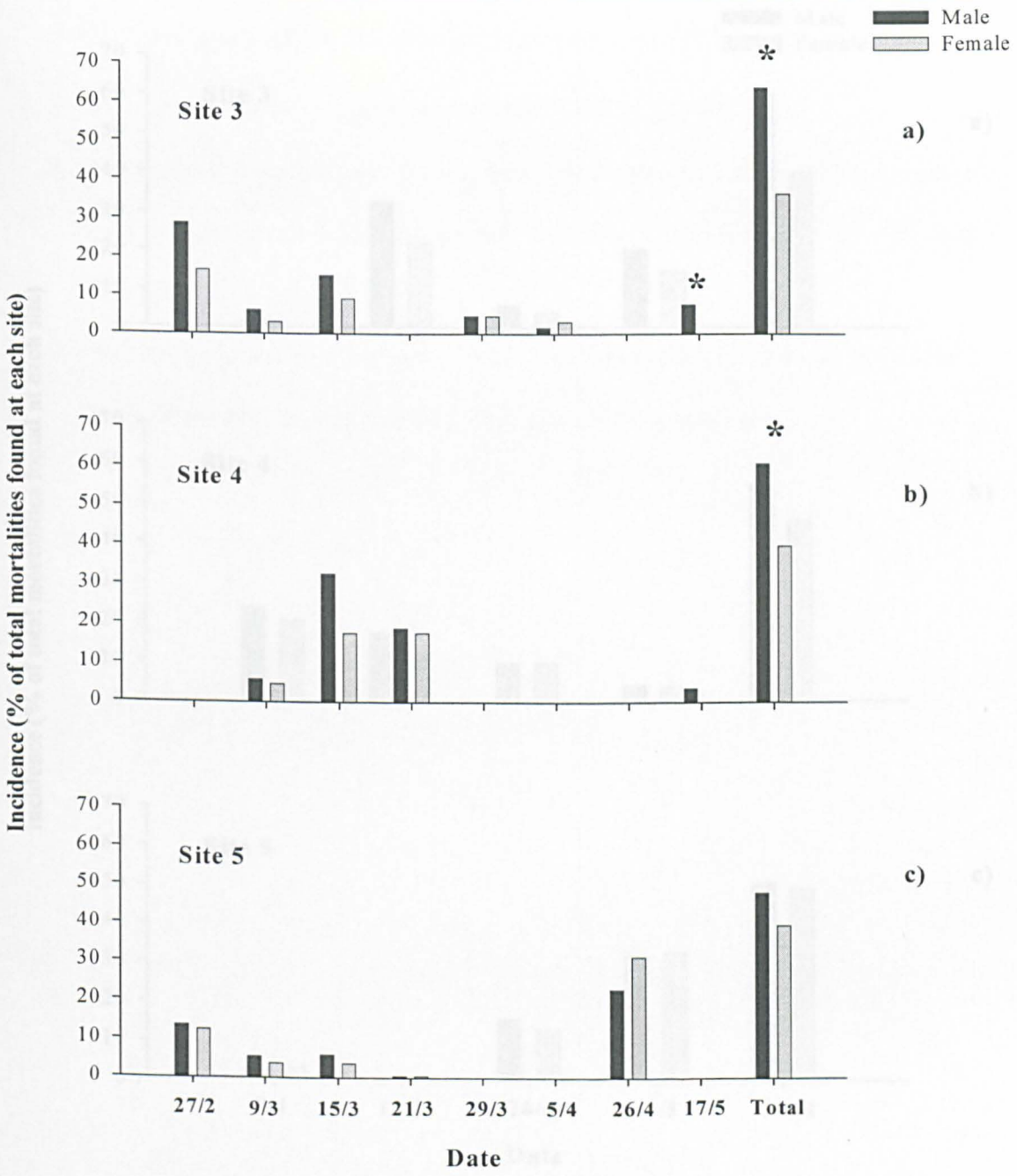


Fig. 3.34 The male: female ratio recorded in mortalities that occurred following transfer to sea water (n=66 to 185). Mortality rates were recorded, for up to six weeks after sea water transfer, at three commercial on-growing sites: a) Site 3, b) Site 4, c) Site 5 (see Section 2.1.1 for details). Smolts were produced under an 0+ photoperiod production regime. * = a significant difference ($p<0.05$) between the incidence of males and females.

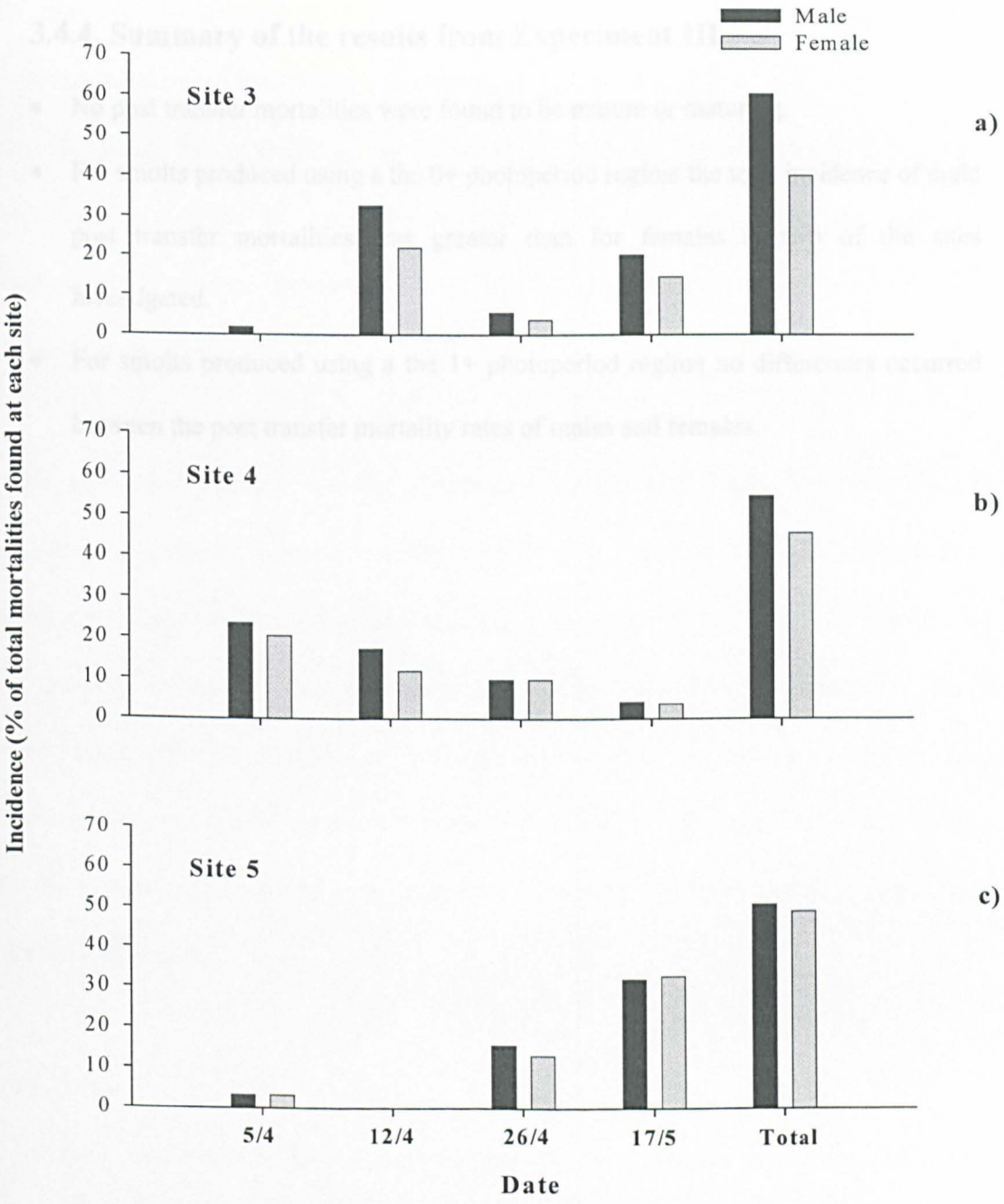


Fig. 3.35 The male: female ratio recorded in mortalities that occurred following transfer to sea water (n=55 to 220). Mortality rates were recorded, for up to six weeks after sea water transfer, at three commercial on-growing sites: a) Site 3, b) Site 4, c) Site 5 (see Section 2.1.1 for details). Smolts were produced under an 1+ photoperiod production regime.

3.4.4. Summary of the results from Experiment III.

- No post transfer mortalities were found to be mature or maturing.
- For smolts produced using a the 0+ photoperiod regime the total incidence of male post transfer mortalities was greater than for females at two of the sites investigated.
- For smolts produced using a the 1+ photoperiod regime no differences occurred between the post transfer mortality rates of males and females.

3.5. Discussion

The experiments detailed in this chapter have shown that winter photoperiod has important effects on the growth, maturation and smoltification of Atlantic salmon parr. By using large numbers of individually tagged fish the development and retrospective analysis of such physiological processes was analysed with the interactions between maturation and smoltification investigated at both the individual and population level.

Before a detailed discussion of the results presented in this chapter it is important to mention the different rearing conditions that were used in the two experiments. Because the fish were reared to first feeding with commercial production fish it was not possible to control the photoperiod regimes used during incubation. As a consequence different incubation regimes were used in the two experiments. It is therefore important to be aware that some of the findings documented in this chapter may have been influenced to some unknown degree by the photoperiod regimes used prior to first-feeding.

3.5.1. Growth

It is well documented that salmonid growth is enhanced by either long day (Komourdjian *et al.*, 1976; Clarke *et al.*, 1978; Lundqvist, 1980; Saunders and Harmon, 1990) or continuous light regimes (Saunders and Henderson, 1988; Villareal *et al.*, 1988; Handeland and Stefansson, 2001) applied in both fresh- (Saunders and Harmon, 1990; Sigholt *et al.*, 1995) and sea water (Saunders and Harmon, 1988; Hansen *et al.*, 1992; Taranger *et al.*, 1995). In experiment I similar findings were observed with continuous light resulting in smolts, parr and mature parr being longer

and heavier than those from either the August or September winter photoperiod regimes. Furthermore, the growth of these cohorts appeared to display a seasonal sensitivity to photoperiod as previously noted by Stefansson *et al.* (1989), Saunders and Henderson (1988), Saunders and Harmon (1990) and Berg *et al.*, (1994) and it would appear that although long day regimes are conducive to growth (Komourdjian *et al.*, 1976; Clarke *et al.*, 1978; Lundqvist, 1980; Saunders and Harmon, 1990; the present study), with short days regimes found to reduce growth (Higgins and Talbot, 1985; Skilbrei *et al.*, 1997), a seasonal sensitivity to such photoperiodic cues may result in daylength-related growth being affected.

An overall increase in both the weight and length of individual cohorts occurred regardless of photoperiod regime. This would indeed be expected because although population bimodality has been shown to result in growth differentials between fish destined to enter different modes (Thorpe, 1977; Kristinsson *et al.*, 1985; Stewart *et al.*, 1990) there are no examples of fish failing to significantly increase their somatic growth over a yearly profile.

There were differences in the profiles of growth of particular cohorts between the photoperiod groups. In both experiments parr grew consistently throughout the year. Smolts from the August photoperiod of experiment I also grew throughout the experiment although smolts from the continuous light and September groups did not show consistent increases and it is likely that this was due to the low observed incidence of such fish, which would have resulted in a loss of statistical robustness. None of the mature parr or small parr within experiment I showed increases during the experiment. In experiment II growth differentials between treatments were found.

Mature parr from the 8 week as well as the 12 week May photoperiod grew until October and then from January onwards with a similar growth arrest observed for the large parr from the 8 week photoperiods and the large smolts from the 12 week June photoperiod.

These differences lend support to the theory that changes in winter growth are under photoperiod control. Although mature fish have previously been linked in greater numbers to the lower modal group (LMG) of a bimodal population (Kristinsson *et al.*, 1985; Duston and Saunders, 1992, 1995) maturation is not necessarily the primary cause of modality (Thorpe, 1977; Villareal and Thorpe, 1985; Thorpe, 1987a) with mature fish often identified within the different modes of a distribution (Bailey *et al.*, 1980; Baglinere and Maisse, 1985; Kristinsson *et al.*, 1985; Saunders *et al.*, 1994). Lower modal group fish generally show reduced growth rates during winter months with upper modal group (UMG) fish (such as the large parr and large smolts of experiment II) continuing to grow during the winter (Kristinsson *et al.*, 1985; Duston and Saunders, 1992). However, in the current experiment a two month growth arrest was observed in large parr, large smolts and mature parr during the natural winter. Continuous light was applied during this time and it is possible that in the absence of any changing photoperiod cue the natural growth profiles of the respective modal group fish were influenced by the seasonal changes in temperature.

Additionally, the winter growth arrest observed for the large parr and large smolts occurred when a continuation of growth, linked to photoperiod, might have been expected. This indicates support for the role for a photoperiodically entrained endogenous rhythm of growth (Clarke *et al.*, 1978; Villareal *et al.*, 1988; Duncan *et*

al., 1998). It therefore seems unlikely that growth is under a direct photostimulation as previously suggested by Saunders and Harmon (1988), Krakenes *et al.* (1991) and Duncan *et al.* (1999).

In experiment I mature parr appeared to be initially amongst the largest fish in the August and September photoperiod groups (although such differences were not significant). Indeed it is well documented that mature fish are initially amongst the fastest growing individuals within a population (Saunders *et al.*, 1982; Dalley *et al.*, 1983; Foote *et al.*, 1991; Heath *et al.*, 1996), which results in those fish that are destined to mature being heavier than their immature siblings during the early stages of a growing season (Lundqvist, 1980; Rowe and Thorpe, 1990a; Prevost *et al.*, 1992; Berglund, 1995). In experiment I the mature fish remained of similar sizes to their immature siblings for a short period of time with the observed decreases in somatic growth and the divergence in size between mature and immature fish possibly linked to the energetic costs of gonadal recrudescence (Lee and Power, 1976; Dalley *et al.*, 1983; Foote *et al.*, 1991) as well as a reduction in the feed intake of maturing individuals (Rowe and Thorpe, 1990a; Kadri *et al.*, 1996; Stead *et al.*, 1999).

However, in experiment I there was a difference in the timing of the divide in growth between maturing and immature parr and it is likely that photoperiod was important in influencing this division. The earliest division between mature and immature parr occurred under continuous light (4th October) with the divide in growth of the August and September photoperiod fish occurring in early November and mid-October respectively. Although distinct differences in specific growth rate between the photoperiod groups could not provide support for these differences in timing it is

possible that subtle, photoperiod induced, growth differences could have occurred. Under continuous light growth potential is enhanced (Villareal *et al.*, 1988; Berg *et al.*, 1994; Sigholt *et al.*, 1995) allowing maturing fish to reach the point where energy is diverted from somatic growth into gonadal development (Foote *et al.*, 1991) at an early stage. Growth potential when fish are exposed to short days is reduced (Higgins and Talbot, 1985; Berge *et al.*, 1995; Skilbrei *et al.*, 1997) so the mature and immature parr exposed to a September photoperiod diverged at a slightly later date than those in the continuous light treatment due to experiencing a long period of continuous light and then only four weeks of short days prior to the divergence. Finally, the August photoperiod group experienced 8 weeks of short days prior to the divide in growth, which resulted in the mature fish diverging at a later date than both the continuously illuminated and September photoperiod fish.

However, it is evident that temperature would also have played an important role in the timing of these divergences in growth. The action of temperature is generally accepted to be one that controls the rate of response to photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994). Therefore, due to the increased natural summer temperatures the relative growth potential of the August photoperiod fish during their winter photoperiod would have been greater than for the September fish. Subsequently, the magnitude of the response of growth to photoperiod would have been different for the August and September fish. This would have resulted in the timing of the divergence between mature and immature fish being different to that which might have occurred under standardised temperatures.

In experiment II it was evident that mature and immature parr remained of similar length and weight throughout the experiment. Previously this finding has been observed by Naevdal (1983) although the majority of literature documents a size divergence between mature and immature fish during early development (e.g. Dalley *et al.*, 1983; Rowe and Thorpe, 1990a; Berglund, 1995; experiment I). Naevdal (1983) assigned no reasoning to the finding but it is possible that the early timing of the experimental photoperiods can explain the observed profiles of growth. It has been hypothesised that the decision to mature is made at an early stage in development (Saunders and Henderson, 1988; Thorpe, 1994b; Metcalfe, 1998) with a time prior even to first-feeding suggested (Saunders and Henderson, 1988; Thorpe, 1994b). If such an early decision were made then a winter photoperiod applied during warmer months of the year, when the temperature sensitive growth response to photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994) is enhanced, could result in fish that have chosen to mature maintaining similar sizes to immature fish. In the current experiment fish were fed to satiation and as such food availability would not have been a limiting factor in growth.

If this is the case similar growth patterns would be expected in the fish exposed to continuous light in experiment I. However, if the decision to mature is not made prior to first-feeding (Saunders and Henderson, 1988; Thorpe, 1994b) and individuals are affected by a stimulus in early development (as will be suggested and discussed later), fish exposed to continuous light would not necessarily show the growth profiles observed in experiment II. Unfortunately, the lack of individual growth data for the May photoperiod fish of experiment I means that such a theory cannot be confirmed

but the high levels of maturation observed in this group certainly do not contradict the hypothesis.

Changes in condition factor have been linked to both maturation (Rowe and Thorpe, 1990a; Jobling and Baardvik, 1991; Duston and Saunders, 1997; Tveiten *et al.*, 1998) and smoltification (Thrush *et al.*, 1994; Berge *et al.*, 1995; Duncan *et al.*, 1998; Handeland and Stefansson, 2001). Generally, for fish destined to mature condition factor becomes elevated during spring and summer (Rowe and Thorpe, 1990a; Jobling and Baardvik, 1991; Duston and Saunders, 1997) although levels subsequently fall (Aksnes *et al.*, 1986; Tveiten *et al.*, 1998). Saunders *et al.* (1982) suggested that the high condition factor of mature fish was due to increased gonadal mass although it has also been suggested that such changes in condition occur during times when gonadal increases are not significant (Tveiten *et al.*, 1998). However, although distinct differences in condition factor have been found between mature and immature individuals Jobling and Baardvik (1991) and Duston and Saunders (1997) have both found it an unreliable parameter for predicting which fish will undergo maturation.

In the present experiments differences in condition factor were found between fish destined to mature and those remaining immature. In experiment I, differences were only observed under continuous light and in the August photoperiod groups from late September and mid-December onwards. In experiment II mature fish from the 8 week photoperiods had higher condition factors than both the large parr and parr from October until December in the May group and only during the early stages of the experiment in the June treatment. These findings are in agreement with the those of Jobling and Baardvik (1991) and Duston and Saunders (1997) that such

measurements are unreliable for predictive purposes. However, in the present study mature fish had elevated conditions during periods when gonadal increases were present indicating that the condition factor of mature fish is due to increased gonadal mass as previously suggested by Saunders *et al.* (1982).

A significant decline in condition is documented as fish undergo the parr-smolt transformation (Thrush *et al.*, 1994; Berge *et al.*, 1995; Duncan *et al.*, 1998; Handeland and Stefansson, 2001). In the present experiments smolts from the August photoperiod group in experiment I showed a lower condition factor than mature and small parr and in experiment II large smolts and large parr from the 12 week June group had a lower condition factor than other fish from October until February. However, in both of these experiments the reduction in condition was not of the magnitude normally seen in photoperiodically manipulated commercial smolts (c.f. Thrush *et al.*, 1994; Duncan *et al.*, 1998) suggesting that although some level of smoltification had been achieved the fish had not achieved full smolt status.

In experiment I, a sudden decline in condition occurred during the early stages of the experiment. The reasoning for this is difficult to determine. By viewing the temperature profile it can be seen that during this period a brief decline in temperature was observed. However, such deviations occurred throughout the experiment without any effect on condition and it is probable that temperature was not the sole cause of this decline. It is notable, though, that from the growth profiles it appears that the decrease in condition was due to an increase in length as opposed to a loss of weight. Changes in length will affect condition to a greater extent than weight and it is possible that the actual length change resulting in this decrease in condition was quite

small. As such any number of factors could have been influential such as water quality or feeding regime. Unfortunately, due to the nature of the experimental site detailed records of such parameters are not maintained and therefore is not possible to establish if one of these factors was influential in the decline. However, it is also important to note the decline in condition may have been due to sampling error. Given that the length of individuals decreased on the sampling point following the decline in condition it is possible that some form of experimental error had occurred.

Over the experimental period an overall decrease in SGR was observed in all groups with the most significant decreases occurring during the early stages of the experiment. This finding is in agreement with the documented observations of fish growth (Jobling, 1994) with small fish growing at a greatly increased rate during very early development after which growth falls fairly rapidly to be maintained at lower levels. However, the recorded changes in SGR between groups proved insensitive in supporting the overall changes in weight and length that were observed in both experiments.

However, one notable exception was that the small parr (that could be considered as LMG fish) had lower growth rates than all other cohorts during the early stages of the experiments. Therefore, the current experiments indicate that a short-term growth differential occurs between upper and lower mode fish. Previously, it has been suggested that during September fish destined to enter the upper mode of a population will undergo a short period of rapid growth (Kristinsson *et al.*, 1985; Stewart *et al.*, 1990). However, from the current findings it would appear that instead of a period of rapid growth by UMG fish (Kristinsson *et al.*, 1985; Stewart *et al.*, 1990) it is more

accurate to suggest a period when upper modal group fish maintain a higher SGR than LMG fish. Kristinsson *et al.* (1985) and Stewart *et al.* (1990) suggested that differential growth between the UM and LM fish occurred from September under natural photoperiod regimes. Skilbrei (1990) suggested that the changes in winter growth were under photoperiodic control with Thorpe (1987a) also indicating a role for photoperiod in the development of population bimodality. In the current experiments such differentials were observed at an earlier time of year than September although fish in many of the treatments were experiencing no change in photoperiod at that time. It therefore seems that in the absence of changing photoperiods other environmental cues, such as temperature, may become important in cueing growth (Solbakken *et al.*, 1994) or that growth is controlled by an endogenous rhythm (Clarke *et al.*, 1978; Villareal *et al.*, 1988; Duncan and Bromage, 1998).

In the current experiments similar profiles of growth were observed in all cohorts during the time of year when winter would normally occur, under a naturally changing photoperiod (i.e. November to January). This provides further support that growth and bimodality are under photoperiodic control (Thorpe, 1987a; Skilbrei, 1990). Thorpe *et al.* (1980) and Skilbrei (1991) found that during winter lower modal group fish cease or reduce their growth compared to those of the upper mode. However, in the current experiments instead of a changing photoperiod continuous light was applied throughout November to January resulting in fish from different modes maintaining similar profiles of growth. This suggests that photoperiod affects the growth dynamics of fish from different modal groups during winter in a naturally changing photoperiod regime.

Population bimodality has been well documented in salmonids (Thorpe, 1977, 1987a; Higgins and Talbot, 1985; Stewart *et al.*, 1990; Skilbrei, 1991; Duston and Saunders, 1992; Saunders *et al.*, 1994) and in both experiments of the current study population modality was observed. However, it was evident that under continuous light the timing of the population divide was less clear compared to the other treatment groups and by the conclusion of the experiment modality was fairly weak. Similar findings have been documented for fish exposed to continuous light or constant long days (Skilbrei, 1991) with a delay in the emergence of modality also noted (Duncan and Bromage, 1998). Duston and Saunders (1995) suggested that because modality occurs under continuous light regimes it is not dependant on a decrease in photoperiod and as such it was suggested that bimodality was endogenously controlled. However, from the literature that presents itself it does seem likely that photoperiod has some influence on the development of population modality (Thorpe, 1987a; Stefansson *et al.*, 1989; Skilbrei, 1991).

In the current experiments some conflicting data are provided. In experiment I the timing of the population divide was unaffected by photoperiod. However, photoperiod did result in differences in the percentages of UM and LM fish. Under continuous light low numbers of LMG fish were observed with slightly higher percentages in the August and September photoperiod groups. For the May photoperiod group the highest percentages of LMG fish were found. Although it may be sensible to correlate this high incidence with the high percentage of mature fish within this group such links should be made cautiously because it has previously been found that maturation is not the primary cause of population modality (Thorpe, 1977, 1987a; Villarreal and Thorpe, 1985). It seems more likely that the division of modes is determined by a size

threshold and that such a size must be attained prior to winter before individuals can develop into UM fish (Kristinsson *et al.*, 1985; Skilbrei, 1991). If this is the case it is probable that in experiment I the May photoperiod fish were unable to attain the necessary size threshold prior to the application of the winter regime and as such a high incidence of LMG fish was recorded. In the August and September groups long periods of continuous light as well as elevated summer rearing temperatures enhanced growth so that a greater proportion of the fish could enter the upper mode.

It is also possible that for the May group the high levels of maturation actually restricted the numbers of LMG fish. Mature fish are often found to have elevated growth rates and sizes during early development (Saunders *et al.*, 1982; Dalley *et al.*, 1983; Berglund, 1995). In a situation where only a short period of continuous light is applied after first-feeding, prior to the application of short days (e.g. in the May population of experiment I), individuals destined to mature may be amongst the largest fish and contribute to those reaching the UM threshold.

In experiment II there is evidence that both photoperiod and temperature affected the structure and timing of modality. Under the June photoperiod regimes the emergence of modality occurred in mid-October with this division developing later in the year for the May photoperiod treatments. For the May groups during the early stages of their winter photoperiods a brief reduction in temperature was observed but by the beginning of the June photoperiods the natural temperature had risen. Clarke *et al.* (1978) and Solbakken *et al.* (1994) have found that the growth response to photoperiod is affected by temperature and as such the growth response to the winter photoperiods of the June treatments would have been greater than for the May groups.

If a growth differential subsequently occurred between the UM and LM group fish during the winter photoperiod, as has been previously suggested (Kristinsson *et al.*, 1985; Skilbrei, 1991; this study), then where growth potential is enhanced during the short day regime (such as in the June photoperiod groups) the differential in size between modes may emerge more rapidly.

Previously, it has been suggested that the size threshold necessary to enter a particular mode is influential prior to winter photoperiod treatment (Kristinsson *et al.*, 1985; Stewart *et al.*, 1990; Skilbrei, 1991). As such it should follow that the duration of a winter photoperiod will not affect the resultant percentage of fish within each mode. However, the results presented in the current study indicate that such a rigid determination time is unlikely with the final percentage of LMG fish, recorded in experiment II, higher in the 12 week photoperiods than in the 8 week groups. It therefore seems that if a size threshold influences the development of modality the recruitment of LMG fish into the upper mode will be possible during the winter photoperiod (Duston and Saunders, 1997). It would also follow that a growth reduction, due to the longer period of short days (Higgins and Talbot, 1985; Skilbrei *et al.*, 1997), would affect the proportion of fish developing into each mode.

In the present study the current views regarding the development of modality in salmonid populations (e.g. Thorpe, 1977; Kristinsson *et al.*, 1985; Stewart *et al.*, 1990; Skilbrei, 1991; Duston and Saunders, 1992, 1997) do not fully explain the results gained. In the June photoperiods of experiment II there appeared to be more than two modes within each population. It is likely that this was due to both the timing of the early winter photoperiod and the subsequent long period of continuous

light. As mentioned previously winter photoperiod may influence the development of modality with fish recruited into the UM throughout the winter period (Duston and Saunders, 1997; this study). With extended periods of continuous light after a winter regime it is probable that growth divergences become further complicated, especially if the UMG fish that should have constituted the smolting population (Kristinsson *et al.*, 1985; Thorpe, 1987a), remain in fresh water.

It is also possible that endogenous rhythms of growth (Clarke *et al.*, 1978; Villareal *et al.*, 1988; Duncan *et al.*, 1998) may have played a role in the development of such population structures. In the current study the early winter photoperiod regimes experienced by the June treatments of experiment II may have acted as a zeitgeber entraining an endogenous rhythm of growth, although the winter photoperiod would also have resulted in the development of modality. Following this winter photoperiod the UM and LM fish were held in fresh water and during the subsequent long period of continuous light an endogenous rhythm of growth may have "free run", which could have resulted in a further population divide of the respective modes later in the year. However, it is also possible that in the May groups of experiment I and II, the duration of continuous light, prior to winter photoperiod treatment, was too brief to allow the entrainment of a rhythm of growth, therefore resulting in a distribution similar to those of a population in its first year of development.

Clearly photoperiod has a complex role in population modality and although in natural populations bimodality may be the norm, where increasingly early photoperiods are used in commercial production the emergence of further modes may become of significance.

3.5.2. Maturation

Photoperiod treatment had distinct effects on maturation in both experiments. Currently, it is believed that maturation is under endogenous control with the rhythm of maturation entrained by photoperiod (Lundqvist, 1980; Bromage *et al.*, 1984; Elliott *et al.*, 1984; Duston and Bromage, 1986, 1987, 1991). As such the timing of maturation can be advanced using a period of long days or continuous light followed by a period of short days in later development during which maturation can be completed (Bromage *et al.*, 1984; Elliott *et al.*, 1984; Takashima and Yamada, 1984).

In experiment I further evidence was provided that maturation is controlled by an endogenous rhythm. Mature fish were identified in the continuous light group indicating that in the absence of any photoperiodic change an internal rhythm had influenced maturation. However, it seems that a period of short days will not necessarily advance a rhythm of maturation. In experiment I although maturation occurred in the continuous light treatment no phase shift in the timing of maturation was observed amongst the respective winter photoperiod treatments as first maturation was observed on a similar date in all groups. Furthermore, from the testosterone profiles of the mature fish it seems that there was no phase shift in the endocrinological control of maturation. Similarly, Eriksson and Lundqvist (1980) found that a sudden switch from a long day photoperiod to short days was ineffective in advancing maturation in salmon parr although it is important to note that in their experiments the change in daylength was applied in August and the natural decline in photoperiod prior to the experimental regimes may have influenced the results to some degree. However, given the findings of the current experiments it may be that

juvenile Atlantic salmon are not as sensitive to the photoperiodic initiation of maturation as may be the case for adults.

However, the results documented in experiment II, indicate that a phase shift in the endogenous rhythm of maturation may have occurred. Although, a phase shift was not observed in the 12 week photoperiod regimes it is important to note that due to the low levels of maturation recorded in the June group it would not be possible to accurately compare the levels of maturation recorded with these groups. However, when the 8 week photoperiods were compared the timing of the emergence of maturity as well as the peak of maturation occurred at least two weeks earlier in the May photoperiod group. From the findings of experiment II, it also seems that the timing of the increase in photoperiod may be most influential in the advancement of endogenous rhythms. Although some variation in timing was observed the timing of peak maturation was most accurately correlated with the end date of each winter regime.

It is possible that the role of such internal rhythms influenced the low maturational levels found in the August and September photoperiod groups of experiment I. Up until their respective winter treatments the August and September groups experienced the same light regime as the continuous light group although lower maturational levels were observed. It is therefore possible that the late winter photoperiod treatment phase shifted such a rhythm to a point where maturation could not be completed before the conclusion of the experiment.

Interestingly, it is also possible that an endogenous rhythm of maturation influenced the high levels of maturity observed in the early photoperiod treatments of both experiments (i.e. the May and June treatments). Prior to photoperiod treatment continuous light was used indicating a spring/summer period to the fish. Subsequently, by applying the early winter photoperiod the individuals may have had their rhythm of maturation entrained such that they believed that they were in their second year of life. Dalley *et al.* (1983), Baglinere and Maisse (1985) and Whalen and Parish (1999) have documented the incidence of maturation rising during successive years in fresh water and it is possible that a photoperiodically entrained endogenous rhythm may result in maturation increasing over successive years. If so it is likely that such a rhythm acts on the assumption that with prolonged freshwater residency the size and energetic status of an individual increases and as such its maturational success will be enhanced. However, such an endogenous mechanism would not necessarily have to be directly linked to the size or nutritional status of the fish, although it is likely that the ability of an individual to respond to the rhythm would be dependant on their size or nutritional status at a specific period of the photoperiodic cycle. Therefore, if in the current experiments the photoperiod regimes resulted in individuals believing that they were in their second year of life they may have been more likely to mature than if they believed they were only in their first year of life.

In experiment I it was found that the May photoperiod resulted in the highest levels of maturation. Similar findings have been recorded by Berg *et al.* (1994) who found that a 7 week period of LD14:10 applied in May resulted in high levels of parr maturation compared to natural and continuous light regimes.

Previously, Thorpe (1986) and Duston and Saunders (1992) have proposed a model suggesting that the initiation of maturation will occur if particular growth or developmental thresholds are achieved during a critical period in spring. Subsequently, Metcalfe (1998) and Thorpe *et al.* (1998) have postulated that the initiation of maturation occurs during November, one year prior to final maturation with spring important as a period when maturation can be suppressed. This indicates that in some cases the initiation of maturation may occur during early development (Saunders *et al.*, 1982; Saunders and Henderson, 1988; Thorpe, 1994b; Metcalfe, 1998) or even prior to first-feeding (Saunders and Henderson, 1988; Thorpe, 1994b).

The high levels of maturation in the May photoperiod group provide support that the initiation of maturation may be influenced during early development although they indicate that a period prior to first-feeding may be unlikely. However, it is possible that instead of initiating maturation the May photoperiod occurred during a developmentally critical stage when maturation could be enhanced or suppressed (Thorpe, 1986; Duston and Saunders, 1992; Metcalfe, 1998; Thorpe *et al.*, 1998). Therefore if the initiation of maturation does take place prior to first-feeding then a short day regime during a critical time in early development may provide the stimulus for a greater incidence of maturation. As such it is likely that instead of a critical period occurring during spring *per se* it occurs during a particular period in development that follows a yearly cycle.

Duston and Saunders (1992) found that maturation was initiated during the spring photoperiod regardless of whether the naturally-changing annual photoperiod regime had been extended or compressed. It is therefore possible that although such a critical

period will be influenced by a particular developmental stage individuals will utilise the seasonally-changing photoperiod to measure that developmental age or their ability to reproduce.

However, the current findings do not support the theory that the initiation of maturation is dependant on the attainment of growth or developmental thresholds during such critical periods (Thorpe, 1986; Duston and Saunders, 1992; Metcalfe, 1998; Thorpe *et al.*, 1998). In the May photoperiod group maturation was enhanced using periods of short days, which have previously been shown to reduce growth (Skilbrei *et al.*, 1997). Therefore some other environmental factor may be influential during developmentally critical periods.

As well as the early period during which maturation is influenced the results of experiment I indicate that a further period is of importance. Under both the August and September photoperiod groups significantly lower levels of maturation were recorded when compared to the May photoperiod and continuous light groups. It therefore seems that a period of short days some time after the early initiation period will suppress maturation, possibly through subtle reductions in growth (Thorpe, 1994b) influenced by the short day regime. Although the results of the current experiment indicate that this period occurs during late summer it is possible that it is influential throughout a much longer period following the period when maturation can be enhanced.

In experiment II photoperiod treatment resulted in variation in the incidence of maturation. The greatest incidence of maturation was observed in the 12 week/May

photoperiod group, with similar intermediate levels found in both of the 8 week regimes. It therefore seems that maturation is not inversely related to winter duration as previously suggested by Prevost *et al.* (1992) although it should be noted that low levels of maturation were observed in the long, 12 week, June photoperiod group. It is possible to suggest that the differences between the two studies were influenced by temperature given that the natural winter experienced in the study by Prevost's group occurred at colder regimes than those experienced in experiment II. However, temperature has been shown to control the rate of response to photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994) with photoperiod being the main environmental factor influencing maturation (Whitehead *et al.*, 1978). Furthermore, although Duston and Saunders (1997) found that elevated winter temperatures increased the incidence of maturation Herbinger and Friars (1992) found no effect of temperature on maturation. Therefore, it seems that although in the absence of changes in photoperiod temperature will be an important seasonal cue (Solbakken *et al.*, 1994) it is likely that when manipulated (c.f. Duston and Saunders (1997) photoperiod will become the primary stimulus affecting maturation.

The results of experiment II indicate that the suggested period in early development when maturation can be enhanced may be affected by the duration of stimulatory winter photoperiod used. Metcalfe (1998) suggested that small changes in growth rate may influence the proportion of maturing fish within a population and it is possible that such subtle changes, over extended periods, may have important effects on maturation. In experiment II, increasing the duration of the early winter photoperiod regime resulted in an increase in maturation with the highest incidence observed in the 12 week/May treatment and similar intermediate levels recorded in the 8 week

photoperiod regimes. However, the 12 week/June group resulted in low levels of maturation and it is possible that the timing of a period in early development when maturation can be enhanced may be inflexible. As such if the extended short day treatments continue outside of the period (e.g. in the 12 week/June group) maturation levels may start to reduce.

If such an inflexible period exists is it important to elucidate whether it is influential at the developmental/chronological age of the fish or at a specific time of year? In experiment II first-feeding occurred approximately 3 weeks later in the year than in experiment I. Therefore, for developmental comparisons to be made the May photoperiod of experiment I and the June photoperiods of experiment II can be considered. Lower levels of maturation were found in the 8 week/June photoperiod of experiment II when compared to the May group from experiment I. Combined with the negligible levels of maturation in the 12/week June group this infers that during the June photoperiod of experiment II the period when maturation can be enhanced was nearing its end. Given the high levels of maturation in the May photoperiod in experiment II it may seem that the actual time of the year is most influential in determining the timing of an early enhancement period (c.f. Berg *et al.*, 1994). However, if the May photoperiod of experiment I were applied during the latter stages of the enhancement period then an exposure to winter photoperiod earlier in development would have increased the levels of maturation even further. It is therefore difficult to elucidate whether developmental or yearly timing is of importance in influencing maturation. Indeed it may be that both yearly timing and developmental age interact to some degree with a seasonal adjustment in the timing of the period when maturation can be enhanced.

3.5.3. Smoltification

During the experiments conducted in this chapter low levels of smoltification were observed. In experiment I, large smolts, smolts and silvered parr were identified although it is important to note that the smolts and silvered parr observed in this experiment were smaller than those grown in commercial freshwater rearing sites, possibly due to the experimental rearing conditions being different to those of production fish (e.g. lower stocking densities in the experimental groups and as such different social hierarchies and competition, different tank sizes/shapes/volumes, different water flow rates). As such some caution is required when comparisons are made to the findings of production fish. In experiment II large smolts and large parr (with some level of silvering) could be considered as those showing external signs of smoltification.

It is well documented that the initiation of smoltification requires a period of short days with a subsequent period of long days during which the parr-smolt transformation is completed (Berg *et al.*, 1994; Duston and Saunders, 1995; Sigholt *et al.*, 1995; Duncan and Bromage, 1998; Duncan *et al.*, 1998). The results of experiment I provide further support for this theory with fish exposed to continuous light having a low hypo-osmoregulatory ability throughout the experiment. The August photoperiod group exhibited the greatest numbers of silvered parr and smolts and although external appearance is not always a good measure of smoltification (Saunders *et al.*, 1985; Duncan and Bromage, 1998) these fish also showed the greatest hypo-osmoregulatory capacity (in terms of both Na^+, K^+ -ATPase and seawater survival).

However, it was noted that fish exposed to the May and September photoperiod regimes had a low hypo-osmoregulatory ability, similar to that of the continuous light fish. Although some disagreement can be found (e.g. Økland *et al.*, 1993; McKinnell and Lundqvist, 1998) it is generally accepted that fish must first reach a particular size threshold before smoltification can be attempted (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988). Therefore, it is probable that for the May photoperiod group it was not possible for high numbers of fish to achieve such a size prior to the winter photoperiod and as such a low incidence of smoltification was recorded. In the August group a longer period of continuous light prior to the winter photoperiod allowed more fish to reach the necessary size threshold for smoltification and therefore a high number of smolts were recorded. However, in the September group although the size threshold necessary for smoltification must have been achieved by a large number of fish (as the fish were of a similar size or larger than those from the August group on entry to their photoperiod) the numbers of smolts remained low. Previously, Johnston and Eales (1970), Björnsson *et al.* (1989), Duston and Saunders (1997), Sigholt *et al.* (1998) and Handeland and Stefansson (2001) have found temperature to play an important role in smoltification with Sigholt *et al.* (1998) and Handeland and Stefansson (2001) suggesting that at least 400 degree days of long days, following short day treatment, are be required before for smoltification can be completed. It is therefore probable that the low temperatures experienced by the September fish during and after their winter photoperiod regime caused the lower numbers of smolting fish. In the September group although an extended period of long days was applied after the short day treatment (80+ days), the low winter temperatures resulted in only 260 degree days being achieved by the conclusion of the

experiment. Therefore, it is unlikely that the fish were able to develop into competent smolts.

The findings of the current study provide further support that the development of hypo-osmoregulatory ability is reduced or inhibited (Saunders *et al.*, 1985; McCormick *et al.*, 1987; Solbakken *et al.*, 1994; Berge *et al.*, 1995; Duston and Saunders, 1995) by continuous light regimes. However, some fish that were exposed to continuous light did show signs of smoltification and as such there is support for the theory that an endogenous rhythm helps to control smoltification (Erikson and Lundqvist, 1982; Thrush *et al.*, 1994; Duston and Saunders, 1995; Sigholt *et al.*, 1995; Duncan and Bromage, 1998). However, it is important to note that in the current experiment low numbers of smolting fish were observed in the continuous light treatment. Previously, Stefansson *et al.* (1989) has stated that endogenous cycles are too imprecise to provide complete smolting in the absence of a naturally changing photoperiod. However, the fish reared in the current experiment were small compared to those produced on commercial freshwater rearing sites (G. Beaton, M. Porter, N. Bromage *pers. comm.)* and given that they were of a similar size to those studied by Stefansson *et al.* (1989) it is possible that the role of an endogenous rhythm of smoltification will only become of importance if a particular size threshold for smoltification (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988) is also reached.

In experiment II only fish from the 12 week/June photoperiod appeared as large smolts with this group also providing the highest incidence of large parr. Furthermore, this was the only group where gill Na^+, K^+ -ATPase levels in the silvered fish

increased compared to the other groups although the levels of ATPase never reached those previously recorded in competent smolts (c.f. Berge *et al.*, 1995; Handeland and Stefansson, 2001). In contradiction to these findings, following exposure to the 96 hour seawater tolerance test, the 12 week/June group suffered high levels of mortality with the 12 week/May group displaying the greatest seawater survival (55%). However, in the 12 week/June group the gill Na^+, K^+ -ATPase levels of silvered fish were only elevated until 17th December with the external appearance of these fish showing levels of silvering for over 5 months. Previously, Björnsson *et al.* (1989) observed that hypo-osmoregulatory ability rose 1 to 2 months after short day treatment remaining high for at least one month, with Sigholt *et al.* (1995) suggesting that it is better to transfer fish to sea when hypo-osmoregulatory parameters are increasing as opposed to decreasing regardless of actual level. As such it is possible that the silvered fish from the 12 week/June group were ready for seawater transfer some time before the conclusion of the experiment and as such by the final sample point they had passed through the window when smoltification and seawater transfer was possible. In support of this Sigholt *et al.* (1998) and Handeland and Stefansson (2001) have suggested that following the conclusion of a stimulatory winter photoperiod approximately 400 degree days will be required before smoltification can be completed. Given the high summer temperatures experienced by the 12 week/June fish following their winter photoperiod regime it is likely that 400 degree days would have been achieved by mid to late October. As such it is possible that by the conclusion of the experiment the window during which smoltification can occur had passed and individuals were experiencing some level of de-smoltification.

However, it is important to note that given the gill Na^+, K^+ -ATPase levels and the smolt index values recorded in the 12 week/June fish it is more likely that those individuals that made an attempt at smoltification never truly achieved a good level of hypo-osmoregulatory ability (c.f. McCormick *et al.*, 1987; Duncan and Bromage, 1998; Handeland and Stefansson, 2001).

During experiment II it was evident that individuals that attempted smoltification never achieved a good smolt status. Certain individuals within each population had clearly made the decision to smolt and they subsequently received the necessary photoperiodic cues and thermal requirements that would allow them to successfully complete the parr-smolt transformation. The reasoning for the clear lack of hypo-osmoregulatory ability is confusing and it is only possible to postulate why such results were observed. It seems most likely that environmental factors and rearing conditions were influential. For example, light contamination may have occurred during the dark phase of the winter photoperiod regimes. Similarly, water quality may have been influential in some way as could the size and shape of the rearing tanks, the water flow rates or the stocking densities used.

The 12 week/May fish displayed the highest levels of seawater survival in the absence of high increases in body silvering or gill Na^+, K^+ -ATPase. However, it has been shown that body coloration is a poor indicator of smolt status (Saunders *et al.*, 1985; Duncan and Bromage, 1998). It has also been found that gill Na^+, K^+ -ATPase levels do not necessarily correspond with the peak in hypo-osmoregulatory ability (Langdon and Thorpe, 1985; Saunders and Harmon, 1990; Solbakken *et al.*, 1994; Handeland and Stefansson, 2001) with gill Na^+, K^+ -ATPase levels increasing once fish are

transferred to sea water (Saunders and Henderson, 1978; Solbakken *et al.*, 1994). Although gill Na^+, K^+ -ATPase levels were not measured during the seawater tolerance test it is probable that such a mechanism aided the survival of the 12 week/May fish and that these fish were in fact the most pre-adapted to seawater survival.

The results of experiment II indicate that the duration of the winter photoperiod was important in the development of smolt status with only groups exposed to the 12 week photoperiods showing signs of seawater adaptation (silvering, gill Na^+,K^+ -ATPase, seawater survival). Previously, Sigholt *et al.* (1995), Duncan and Bromage (1998) and Duncan *et al.* (1998) have suggested that approximately 2 months of short days are sufficient to initiate smoltification, with Berg *et al.* (1994) observing that 10 weeks of short days were required. Although such differences in the time required to initiate smoltification may be explained by between experiment variations (e.g. different fish stocks or rearing temperatures), Duston and Saunders (1995) quote unpublished data indicating that 3 months of short days in June were better at stimulating smoltification than 2 months. It therefore seems that although 8 weeks of short days may be sufficient to initiate smoltification under previously good conditions of growth, where smaller fish or those experiencing poorer pre-photoperiod growth are present (such as in experiment II), the application of a longer period of short days will result in a greater incidence of smoltification. Therefore, it may be that smoltification is dependant on attaining a specific number of thermal days during the winter photoperiod (c.f. Sigholt *et al.*, 1998; Handeland and Stefansson, 2001) and that the duration of these degree days will be influenced by the individuals size either prior to or during the winter photoperiod.

From the results of the seawater tolerance tests conducted in experiment II it seems that size is not necessarily important in the survival of individuals following seawater transfer. As mentioned earlier it has been suggested that a size threshold exists for smoltification (Elson, 1957; Thorpe *et al.*, 1980; Skilbrei, 1988) and from this it has been found that larger fish show better signs of seawater adaptation when compared to smaller siblings (Thrush *et al.*, 1994; Duston and Saunders, 1995) although Økland *et al.* (1993) and McKinnell and Lundqvist (1998) have presented data contradicting such a theory. In experiment II although individuals surviving the 96 hour seawater tolerance test were larger than those which died this takes no account of fish that made no attempt at smoltification (i.e. small parr). Furthermore it was clear that the length of individuals which died and the time in sea water necessary to cause death were poorly correlated. Indeed the poorest correlations were found in the groups which exhibited the greatest seawater survival (12 week/May photoperiod) and the highest levels of silvering and gill Na^+,K^+ -ATPase (12 week/June photoperiod). However, in the current experiment the incomplete smoltification of groups, as well as the diverse population structures, mean that is not possible to compare such information to commercial populations where a high smolt status and larger size of fish is achieved.

3.5.4. Maturation and smoltification interactions.

Previously it has been suggested that maturation and smoltification are mutually exclusive processes (Thorpe and Morgan, 1980; Thorpe, 1986, 1987b; Herbinger and Friars, 1992) and that smolting occurs as a consequence of a fish failing to mature (Thorpe, 1994a; Thorpe and Metcalfe, 1998). Indeed there is much evidence that mature fish are not well adapted for seawater survival (Foote *et al.*, 1991; Clarke and

Blackburn, 1994; Staurnes *et al.*, 1994a). However, it is clear that mature fish are able to migrate to sea (Baglinere and Maisse, 1985; Saunders *et al.*, 1994; Duston and Saunders, 1997) and in the current experiments fish showing signs of smoltification were found to be mature with such fish identified within all of the cohorts studied. It was also evident that in the fish showing signs of both maturation and smoltification milt was freely running for some weeks.

However, it should be emphasised that in the current experiments mature fish that showed signs of smolting did not necessarily display the secondary sexual characteristics often linked to parr maturation (i.e. reduced length/size, high condition factor, darkened coloration and distinct parr marks). A similar lack of secondary sexual characteristics has also been found in mature adult salmon (Duston and Saunders, 1995; Thrush *et al.*, 1994) and it seems that in commercial production care must be exercised in identifying mature parr, which are often culled from a population prior to seawater transfer.

It is clear that there is some interaction between maturation and smoltification and it is possible that a better understanding of these interactions can be gained by considering the size/developmental thresholds that influence freshwater life history strategy. It has been suggested that a size threshold influences both maturation (Bailey *et al.*, 1980; Thorpe and Morgan, 1980; Saunders *et al.*, 1982; Thorpe, 1986) and smoltification (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988) although it is probable that considering such a threshold related to size alone may be inaccurate (Saunders *et al.*, 1982; Økland *et al.*, 1993) and that it would be more precise to consider a developmental threshold (Saunders *et al.*, 1982). Polikansky (1983) has

stated that fish will mature as soon as they are ready to do so indicating that maturation is the primary physiological route for fish. Additionally several authors have made attempts to determine the actual length of such physiological thresholds (e.g. for maturation 70-72mm: Myers *et al.*, 1986; 70mm: Berglund, 1992, for smoltification 100mm: Elson, 1957; 100-120mm Kristinsson *et al.*, 1985; 70-80mm Skilbrei, 1988).

It therefore seems likely that the threshold for maturation will be lower than that for smoltification (Saunders *et al.*, 1982). Bailey *et al.* (1980) and Saunders *et al.* (1982) suggested that due to the reduced growth rate linked to gonadal recrudescence mature parr will rarely achieve the larger threshold required for smolting but that if such growth was possible smoltification could occur (Saunders *et al.*, 1994). However, given the use of improved feeds, additional lighting regimes and elevated temperatures it is likely that the commercial freshwater production of salmon could result in the attainment of both thresholds within one year of life.

The findings of the current study provide support for the two threshold hypothesis. High levels of maturation were observed in the May photoperiod group of experiment I and it is likely that this was due to the long periods of continuous light that were applied during the warm summer months. This would have allowed both mature and immature fish to grow and develop into either silvered parr or large smolts. However, it is interesting that in the continuous light group although mature silvered parr were observed the high growth potential of these fish (Saunders and Henderson, 1988; Villarreal *et al.*, 1988; Handeland and Stefansson, 2001) did not result in the development of either mature or immature large smolts as might have been expected

from the results of the May photoperiod group in experiment I. Therefore, although the presence of silvered parr under the continuous light regime indicates the action of an endogenous rhythm(s) controlling smoltification (Erikson and Lundqvist, 1982; Thrush *et al.*, 1994; Duston and Saunders, 1995; Sigholt *et al.*, 1995; Duncan and Bromage, 1998) the finding of large smolts in only the May photoperiod group indicates that even in early development a winter photoperiod had been influential in advancing smoltification and that the subsequent long period of continuous light had allowed the smolting fish to develop as large smolts.

In the August photoperiod group mature fish were seen in the smolt and silvered parr groups. It would therefore seem that although the winter photoperiod had advanced smoltification shorter periods of continuous light after the winter photoperiod were not sufficient for the fish to develop as large smolts. In the September group only immature fish were able to achieve any level of smolt status and it is probable that this was due to the short period of continuous light after the winter photoperiod combined with the low winter temperatures.

However, it is important to note that in these current experiments it is not possible to state unequivocally that the mature fish that were identified in the cohorts with signs of smoltification were initially mature fish that developed into smolts. It is possible that such fish were initially smolts and that an internal decision was subsequently made to mature. If this is the case, however, it provides further evidence that maturation is the primary physiological process because the fish that had prepared themselves for seawater migration had then chosen to mature.

The results observed in experiment II provide further understanding of the interactions that occur between maturation and smoltification. Although all treatments resulted in large parr only those from the 8 week photoperiods underwent maturation. Under the 12 week photoperiods mature fish were only found within the parr and small parr cohorts. Therefore, the duration of the winter photoperiod affected the way in which the early stimulus and subsequent growth potential, under continuous light (suggested for the May photoperiod group in experiment I), influenced the initiation of both maturation and smoltification. The extended period of short days (12 week group) resulted in those individuals that had made the decision to mature having a longer photoperiod induced reduction in growth (Higgins and Talbot, 1985; Skilbrei *et al.*, 1997) and as such they were only able to mature as parr or small parr. Under the 8 week photoperiod the early winter stimulated maturation in certain individuals but the short winter photoperiod allowed a longer, subsequent exposure to continuous light and as such they were able to grow sufficiently to attempt some level of smoltification as large parr (Saunders *et al.*, 1982).

Therefore, the current study is at variance with the suggestions of Thorpe (1994a), Thorpe and Metcalfe (1998) and Metcalfe (1998) that maturing fish are those that have failed to smolt. Maturing fish can make the decision to smolt after maturation but it is also possible that fish choosing to smolt may, under good conditions, undergo the physiologically dominant process of maturation. It therefore seems that where conditions for growth are good (such as in commercial freshwater rearing sites) and developmental thresholds can be exceeded fish can undergo a developmental decision that maximises their reproductive success.

Previously, developmental models have been proposed that aim to explain the factors involved in the initiation and completion of maturation and smoltification. Thorpe (1986) provided one of the first models that explained the initiation of maturation suggesting that if the rate of acquisition of energy was sufficient during early spring maturation would be initiated. Subsequently, Duston and Saunders (1992) proposed that the initiation of maturation and smoltification occurs on the increasing (i.e. in spring) and decreasing (i.e. autumn) phases of the photoperiod respectively provided sufficient growth thresholds had been achieved. However, the length of these decision periods was unknown (Duston and Saunders, 1992). More recently, Thorpe (1994b), Metcalfe (1998) and Thorpe *et al.* (1998) have suggested that the initiation of maturation occurs in November one year prior to maturation (Metcalfe, 1998; Thorpe *et al.*, 1998) and that maturation can be "switched off" during a second sensitive period in spring (Metcalfe, 1998; Thorpe *et al.*, 1998). Indeed growing evidence suggests that spring provides a sensitive period when growth rates influence the decision to mature (Adams and Thorpe, 1989; Rowe and Thorpe, 1990b; Thorpe *et al.*, 1990; Rowe *et al.*, 1991; Duston and Saunders, 1997). Furthermore, given that both maturation (Elliott *et al.*, 1984; Duston and Bromage, 1986, 1987, 1991; Hansen *et al.*, 1992) and smoltification (Erikson and Lundqvist, 1982; Clarke *et al.*, 1985; Saunders and Harmon, 1990: Sigholt *et al.*, 1995; Duncan and Bromage, 1998) are influenced by photoperiodically entrained rhythm(s) it is likely that following critical initiation periods the cueing of final maturation and smoltification will be assisted by internal processes.

From the findings and discussions detailed in the current study adjustments to these models can be suggested. It is likely that maturation will be influenced during a

period in early development although it is not clear whether this period initiates maturation or whether it acts as a period when maturation can be enhanced/suppressed (Fig. 3.36). This period will be influential at a particular chronological or developmental age the timing of which will be aided by endogenous rhythm(s) that are adjusted by photoperiod. An endogenous rhythm of maturation will proceed, although this rhythm can be arrested during a second "sensitive" period later in development.

Smoltification is primarily initiated by photoperiod (a period of short days) providing an individual has achieved certain size/developmental thresholds. The timing of this stimulatory photoperiod is relatively flexible although endogenous rhythm(s) of smoltification are also likely. If an individual has previously made the decision to mature it may subsequently undergo smoltification providing the necessary thresholds have been achieved. Typically in wild populations individuals that mature in autumn will not smolt in the following spring due to size constraints, although during the commercial culture of Atlantic salmon heightened production regimes may allow individuals to mature and undergo smoltification in one year. Finally it is possible that the period when maturation can be arrested could coincide with the photoperiod that is used to initiate smoltification (c.f. the August and September treatments of experiment I). As such a particular photoperiod may result in an individual smolting as opposed to undergoing maturation. In wild populations it is possible that these two developmental periods naturally coincide, which has led to suggestion that maturation and smoltification are mutually exclusive (Thorpe and Morgan, 1980; Thorpe, 1986, 1987b; Herbinger and Friars, 1992).

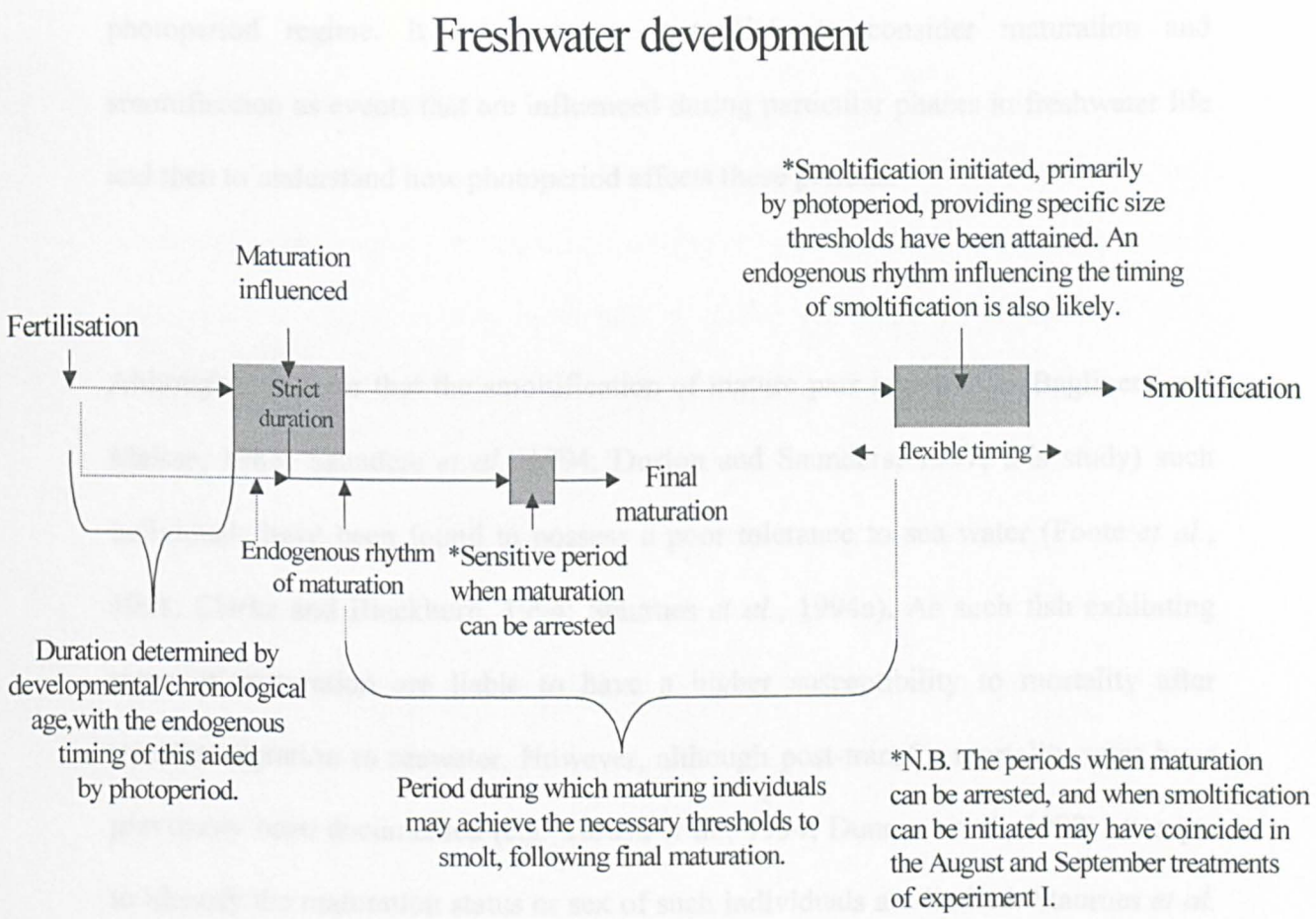


Fig. 3.36 A proposed model detailing the freshwater development of Atlantic salmon parr.

In conclusion it seems appropriate that models which aim to explain freshwater development avoid linking life-history strategies directly to phases of the natural photoperiod regime. It seems more appropriate to consider maturation and smoltification as events that are influenced during particular phases in freshwater life and then to understand how photoperiod affects these periods.

Although it is clear that the smoltification of mature parr is possible (Baglinere and Maisse, 1985; Saunders *et al.*, 1994; Duston and Saunders, 1997; this study) such individuals have been found to possess a poor tolerance to sea water (Foote *et al.*, 1991; Clarke and Blackburn, 1994; Staurnes *et al.*, 1994a). As such fish exhibiting signs of maturation are liable to have a higher susceptibility to mortality after transfer/migration to seawater. However, although post-transfer mortality rates have previously been documented (e.g. Thrush *et al.*, 1994; Duncan *et al.*, 1998) attempts to identify the maturation status or sex of such individuals are limited. Staurnes *et al.* (1994a) found mature males to be more susceptible to mortality than immature individuals after 10 days in sea water, with Saunders *et al.* (1994) also finding a higher mortality rate in mature fish following seawater transfer. In experiment III, no mature mortalities were found at any sites for both 0+ and 1+ production cycles. However, it was evident that at two sites 0+ males were more susceptible to transfer mortality than females. Skilbrei (1990) suggested that smolting in mature parr may be due to the degree of recovery from maturation and it is possible that the higher incidence of male mortalities was due to either a previous maturation episode or an attempted maturation, which was ceased due to a physiological decision made before final maturation could be completed. If this were the case then the physiological and

nutritional status of such individuals may result in a reduced smolt status without necessarily showing signs of gonadal development.

However, it is important to note that the sites investigated during experiment III were located in regions where strong tidal currents are present and this physical stressor may have also been influential in the high mortality rates of males in particular when the fish were moved from calm freshwater rearing sites. Fish that exhibit a poor smolt status may be able to tolerate the salinity changes experienced as they are transferred to sea but the inclusion of a further stressor may have resulted in higher mortality rates than normal. As such males that have undergone some level of maturation previously may have a reduced nutritional or physiological status and as such they are likely to suffer from these combined stressors more than may be the case if only a change in salinity has to be overcome.

3.5.5. Conclusions

- Photoperiod is an important environmental parameter influencing the growth of salmon parr.
- Maturation can be greatly enhanced during a short, inflexible period during early development. Later in the year a second period may influence maturation acting in a suppressive role.
- The timing of winter photoperiod affects smoltification mainly due to an individuals ability to achieve a critical size threshold prior to photoperiod treatment. Where conditions for the attainment of such a threshold are not favourable extending the winter photoperiod may improve the incidence of smoltification.
- Growth, maturation and smoltification are all influenced by photoperiodically entrained endogenous rhythms.
- Maturation and smoltification are not completely mutually exclusive processes.

Chapter 4: Nutritional effects on growth, maturation and smoltification.

4.1. Introduction

Growth involves the complex formation and interaction of many physiological and biochemical processes. As such increases in growth require an energetic input with the calorific intake of feed ultimately affecting the rate at which internal processes occur (Jobling, 1994). Physiologically demanding processes such as maturation and migration require inputs over and above those required for growth. Therefore, a clear understanding of how diet influences fish growth, maturation and smoltification is required before its manipulation can be utilised in current culture conditions. However, due to the commercial importance of harvest size and flesh quality it is evident that literature citing such dietary effects often focus on adult salmonids with studies concerning effects on juvenile development less frequent.

4.1.1. Growth

Since calorific intake ultimately influences fish growth (Jobling, 1994), diet regime will affect increases in size through changes in either diet composition or ration. Shearer (1994) stated that it is difficult to separate the effects of ration from those of dietary composition, since variations in feed amount ultimately affect the absolute values of constituents a fish receives. However, it is important to attempt such a separation of information in order to accurately understand their relative effects.

4.1.1.1. Diet composition

Reinitz (1983) was one of the first authors to investigate the role of dietary lipid level in juvenile salmonids. Rainbow trout fry (initial weight of 2.1g) were fed diets containing either 13.6% or 7.2% lipid over a 308 day period. Three feed rates were investigated utilising arbitrary hatchery constants (HC) of either 4, 8 or 12 in order to differentially adjust the ration to fish size. Reinitz (1983) concluded that under the low (HC 4) and medium (HC 8) feed rates growth was enhanced by increased dietary lipid level whereas at the high feed rate (HC 12) no differential was found. Shearer *et al.* (1997) investigated the effects of diets containing either 3% or 23% lipid on growth in chinook salmon parr in a 247 day experiment. Initially a slight increase in weight occurred with the higher dietary lipid level but it was noted that inaccuracies in the amount of feed used as well as the feed efficiency were influential in this gain. Consequently, when these interactions were included into the analysis the effects of dietary lipid on growth were negated (Shearer *et al.*, 1997).

Grisdale-Helland and Helland (1997) found that although low dietary lipid resulted in some detriment to growth this only occurred when the dietary protein content fell below 55% irrespective of energy level. Shearer and Swanson (2000) recently investigated the effects of a range of dietary lipid levels (from 4% to 22% inclusion) on growth with effects only observed at the end of a 13 month treatment period. However, it should be noted that the feed rates used in this experiment were calculated from only one treatment group and as such some ration related effect may have been influential in this difference. Therefore, it is probable that changes in dietary lipid will only be responsible for minor effects on the growth of juvenile salmonids and where feed is unlimited it is unlikely that such differences will arise.

For adults dietary lipid manipulation has resulted in more significant variations. Hemre and Sandnes (1999) investigated the effects of 310, 380 or 470 g kg^{-1} dry weight dietary lipid inclusion on muscle composition and growth in Atlantic salmon (start weight: 1.2kg) between April and November. Dietary lipid level significantly affected growth with groups fed the 310 g kg^{-1} diet having the lowest increases in weight and SGR and significantly greater weight gains and growth rates recorded in the 380 and 470 g kg^{-1} treatments (Hemre and Sandnes, 1997). Similarly, Torstensen *et al.* (2001) have recorded increased growth rates in Atlantic salmon post-smolts following long-term exposure to different dietary lipid inclusions (from 15±2 to 32±2 g. kg^{-1} dry weight lipid inclusion).

By contrast, Refstie *et al.* (2001) recorded only a slight increase in the body weight of adults fed diets containing either 32% or 39% lipid for 235 days with 91g of the 122g average weight gain attributed to increases in the accumulation of body fat. In a similar experiment Hillestad *et al.* (1998) found that long-term dietary lipid variation had no clear effects on adult growth. Therefore, it is possible that previously reported differences in the growth of adult salmon may have been influenced by lipid deposition, although without detailed information on lipid accumulation such suggestions cannot be confirmed. However, it is important to note that aside from specific nutritional requirements the source of dietary lipid appears to have little influence on the principles of growth documented above (Refstie *et al.*, 2001; Rosenlund *et al.*, 2001).

4.1.1.2. Ration

Ration of feed has clear effects on growth. Although Quinton and Blake (1990) found no difference in the growth of fish fed at a ration of 3, 5 or 7% body weight per day for three weeks following a three week period of starvation, a distinct relationship between increased ration and growth is likely. Reinitz (1983) found a clear gain in weight with increasing ration of feed for rainbow trout fed at low, medium or high rates for 308 days. Similarly, McCormick and Naiman (1984) showed that brook trout fed at either high or low rates from shortly after first feeding grew at differential rates. More recently Shearer *et al.* (1997) found that the growth of chinook salmon parr was enhanced by increased feed rate and it is evident that such a correlation is well documented in a range of juvenile salmonids (Storebakken and Austreng, 1987b; Stead *et al.*, 1996; Nicieza and Metcalfe, 1997; Silverstein *et al.*, 1998). Therefore, it would seem that in juvenile salmonids the ration of feed and not dietary lipid level will be of primary importance in fish growth (Shearer *et al.*, 1997; Silverstein *et al.*, 1998).

For adult salmonids a similar relationship between ration and growth is documented. McCormick *et al.* (1989) fed Atlantic salmon post-smolts at rates of 0, 0.2, 0.8 or 1.6% wet body weight per day and concluded that growth increased with increasing ration. More recently further support for the ration related growth of fish immediately following seawater transfer was provided by Stead *et al.* (1996). Storebakken and Austreng (1987a) investigated the growth of large (0.5-1.0kg) rainbow trout fed a range of rations with growth increasing with ration up to a point where feed rate provided maximum growth. Indeed similar ration/growth correlations are well

documented in other, similarly sized, adult salmonids (Kreiberg, 1991; Johansson *et al.*, 1995; Hillestad *et al.*, 1998).

The relationship between ration and growth can be further supported by the finding that population bimodality, commonly observed in juvenile salmonids (Thorpe, 1977, 1987a; Kristinsson *et al.*, 1985; Stewart *et al.*, 1990; Skilbrei, 1991), is also affected by ration. Bimodality results from a size and growth differential between prospective upper and lower mode fish (Kristinsson *et al.*, 1985; Stewart *et al.*, 1990) and as such ration-related differences in growth would be expected to affect the structure of salmonid populations. Storebakken and Austreng (1987a, b) and Nicieza and Metcalfe (1997) reported that population structure was skewed towards the lower modal group when salmonids were fed low rations highlighting the importance of ration to growth.

However, although ration clearly influences growth it is important to consider the scope for growth that increasing rations provide. From previous investigations into ration it has been possible to predict the maintenance ration required by a particular salmonid species and/or stock. Storebakken and Austreng (1987a) found that feeding 0.5-1.0kg rainbow trout 25% of the ration necessary for maximum growth resulted in neither an increase nor a decrease in weight. Similarly, by manipulating ration McCormick *et al.* (1989) suggested that 1.4% of dry body weight per day would provide maintenance rations for Atlantic salmon held in fresh water immediately prior to seawater transfer. Unfortunately such measurements may not be an accurate indication of maintenance ration (Storebakken and Austreng, 1987a). Elliott (1975b) has found that optimum ration size for growth decreased with decreasing temperature and O'Connor *et al.* (2001) noted that the standard metabolic rate of salmon declined

as ration was reduced. It is therefore likely that a range of environmental, physiological and genetic influences will hinder the accurate determination of maintenance ration by such methods although these experiments will allow a generalised understanding of the required basal feed rate.

It is also evident that variations will occur in the response to high feed rates and accurately determining the scope for growth is further hindered. Storebakken and Austreng (1987a, b respectively) fed rainbow trout and Atlantic salmon parr at various ration levels above and below that which was calculated to provide maximum growth. As might be expected rations above the rate necessary for maximum growth did not result in further increases in weight highlighting the fact that ration increases are only influential up to a certain level. Subsequently, McCormick *et al.* (1989) showed that although food conversion efficiency and growth rate increased with ration, feed conversion efficiency increased at a diminishing rate. Elliott (1975b) found that the optimum ration for efficient growth was close to that which was required for 80% of maximum growth. Interestingly, Kreiberg (1991) reported that adult chinook salmon fed rations equivalent to 80% of the maximum ration grew at a similar rate to those on the 100% ration. Therefore it seems that the efficiency of feeding and growth are not necessarily maximised when feed rates are near to those which provide maximum growth (Storebakken and Austreng, 1987b; Nicieza and Metcalfe, 1997).

4.1.1.3. Compensatory growth

Following periods of starvation or feed restriction salmonids, as well as other species, have been shown to adjust their growth and correct for the lost growth incurred during

the period of reduced feeding. Weatherley and Gill (1981) highlighted this by investigating periods of restricted feeding (i.e. 3% dry body weight per day for 16 weeks) or starvation (for either 3 or 13 weeks) in fingerling rainbow trout. Following these periods full rations were applied for 14 and 12 weeks for the restricted and starved fish respectively. Full compensation of size was recorded for both the restricted fish and those starved for 3 weeks with the fish starved for 13 weeks subsequently exceeding the size of controls, which were maintained on full rations throughout (Weatherley and Gill, 1981). Similarly, Dobson and Holmes (1984) investigated 3 week periods of starvation followed by 3 weeks of *ad libitum* feeding in rainbow trout at five different times of the year. In all but one of these periods compensatory responses equalled or exceeded those of controls (Dobson and Holmes, 1984). Compensatory growth responses following periods of feed restriction or starvation have also been reported in other salmonids (Miglavs and Jobling, 1989b; Thorpe *et al.*, 1990; Reimers *et al.*, 1993; Hopkins and Unwin, 1997). Therefore, following periods of nutritional stress (i.e. starvation or feed restriction) feed efficiency can be maximised so that growth differentials are eliminated.

Although increases in growth are required to facilitate the recuperation of size Quinton and Blake (1990) found that growth responses do not necessarily follow a linear relationship. During an experiment in which rainbow trout were starved for three weeks and subsequently fed full rations for three weeks a distinct cyclical growth response was observed. During the starvation period weight loss in the first week was large although in the subsequent two weeks weight loss was significantly reduced. Following the return to feeding there was a moderate gain in weight in the first week with a slight decline during the second week. However, in the third week of

feeding the gain in weight and growth rate increased rapidly (Quinton and Blake, 1990). It is also important to note that during the early stages of recovery growth feed conversion efficiency may be enhanced (Miglavs and Jobling, 1989b) although it is unlikely that such a short lived period of enhanced feed conversion efficiency will greatly affect compensatory responses (Miglavs and Jobling, 1989a).

Although compensatory growth can result in fish fully regaining their lost size (Weatherley and Gill, 1981; Thorpe *et al.*, 1990; Reimers *et al.*, 1993; Hopkins and Unwin, 1997) or indeed exceeding the size of continuously fed controls (Weatherley and Gill, 1981; Dobson and Holmes, 1984) some evidence indicates that individuals do not necessarily regain the size lost through nutritional stress. Dobson and Holmes (1984) starved rainbow trout for three week periods at five different times of the year with growth measured over a subsequent three week period of feeding. Although full compensation of the growth lost was recorded in four of the five experiments, in one group individuals did not regain the size of continuously fed controls. Similarly, Miglavs and Jobling (1989a) found that Arctic char fed between 10% and 20% of the satiation ration (fed to controls) for 8 weeks did not regain the size of controls during the subsequent full ration feeding period. It would therefore seem that although the utilisation of sub-maximal growth performances near to the full ration rate can provide sufficient increases in growth to counteract nutritional deficiencies, such increases are not necessarily guaranteed.

As a closing point it is also important to mention that the ability of an individual to regain its size will be affected by further biological and environmental influences. As mentioned previously Dobson and Holmes (1984) found that although at four times of

the year growth responses resulted in fish regaining the size of controls, during one of the periods an incomplete recovery of size occurred suggesting a possible seasonality to the compensatory response. It has also been shown that following periods of restricted feeding the compensatory response of pre-migratory Atlantic salmon parr is significantly greater than fish destined to remain in fresh water for a further year (Nicieza and Metcalfe, 1997) indicating a seasonal, developmental or size related effect on compensatory growth. Hence, comparisons of compensatory responses during different times of the year, or with different sized individuals, may not be appropriate.

4.1.2. Lipid accumulation and feeding behaviour

Salmonids exhibit a yearly cycle of lipid deposition with the feeding behaviour required to accumulate lipid stores also showing seasonal variation. However, the accumulation of fat reserves will also be affected by physiologically demanding processes such as migration and maturation. Therefore the decision of whether an individual will undergo a particular developmental route will affect its feeding behaviour.

Juvenile salmonids show an accumulation of lipid during the summer (Vanstone and Markert, 1968; Saunders and Henderson, 1978; Gardiner and Geddes, 1980; Simpson, 1992) with a decline noted during winter (Vanstone and Markert, 1968; Saunders and Henderson, 1978; Woo *et al.*, 1978; Gardiner and Geddes, 1980; Rowe *et al.*, 1991). However, the magnitude and timing of these changes is clearly affected by both smoltification and maturation.

Although Larsen *et al.* (2001) and Nordgarden *et al.* (2002) found that smoltification did not result in a loss of body fat during winter the majority of evidence suggests that whole body lipid levels decrease during the parr-smolt transformation. Komourdjian *et al.* (1976) and Saunders and Henderson (1978) both provided evidence that smoltification results in a decrease in lipid content by investigating natural (increasing) and reciprocal (decreasing) photoperiod regimes during spring (refer to Fig. 3.1) in Atlantic salmon parr. Smolting fish had a lower muscle fat content than non-smolting individuals generated from the non-stimulatory photoperiod regime (Komourdjian *et al.*, 1976; Saunders and Henderson, 1978). Woo *et al.* (1978) provided support by investigating the whole body lipid changes occurring in parr, smolts or fish undergoing de-smoltification. Furthermore, these fish were reared under the same photoperiod regime so unlike the studies of Komourdjian *et al.* (1976) and Saunders and Henderson (1978) individuals undergoing different developmental routes could be compared at the same time of the year. It was found that individuals that were undergoing smoltification had lower serum, total liver and muscle fat levels than parr. Furthermore, if fish underwent de-smoltification in fresh water they regained the biochemical characteristics of parr (Woo *et al.* 1978).

Subsequently, Higgins and Talbot (1985) found that upper mode fish had higher whole body lipid levels than lower mode fish at the emergence of bimodality in September with levels remaining higher throughout the winter. However, during winter the lipid levels of both the upper and lower mode fish showed a decline of similar magnitude (Higgins and Talbot, 1985). Therefore, although growing support can be provided that smoltification results in a loss of body lipid (Birt and Green,

1986; Helland and Grisdale-Helland, 1998) it is likely that individuals remaining in fresh water will also loose some fat during the winter.

Clearly the physiological constraints of smoltification play an important role in the changes that occur in lipid content but for a full understanding of these variations the feeding behaviour of both parr and smolts must be considered during the winter period.

It is evident that a division of feeding behaviour occurs between upper and lower mode fish. Higgins and Talbot (1985) found that upper mode fish consistently took larger meals than lower mode individuals with Metcalfe *et al.* (1986) and Metcalfe and Thorpe (1992) both observing a decrease in the feeding of lower mode fish during autumn. Metcalfe *et al.* (1988) found that the appetite of upper and lower mode fish remained similar until August after which the upper mode fish increased their appetite until October with a decreased appetite in the lower mode fish. Additionally, it has been noted that upper mode fish have a far greater feed efficiency than their lower mode siblings (Valdimarsson and Metcalfe, 1999). Therefore, a difference in the feed intake of upper and lower mode fish will occur during autumn and winter with this occurring despite decreases in the whole body lipid level of fish from both modal groups (c.f. Higgins and Talbot, 1985).

In support it has been shown that following a period of feed restriction during winter lower mode fish show only a brief period of re-feeding (Metcalfe and Thorpe, 1992), with the compensatory response of prospective smolts stronger and more persistent (Nicieza and Metcalfe, 1997). Therefore, during over-wintering the lipid losses

incurred by lower mode fish appear to be due to a reduction in feeding motivation (anorexia) whereas in the upper mode fish losses are primarily due to the energetic demands of smoltification. Consequently, under some circumstances, such as yearly fluctuations in temperature or feed availability, smolting individuals may exhibit a lower body lipid level than non-migratory individuals (c.f. Woo *et al.*, 1978). It would also seem that if the lipid reserves of both upper and lower mode fish fall below a certain internally determined level a compensatory feeding response will occur, although it is likely that this response will be different for the two developmental groups (Metcalfe and Thorpe, 1992; Nicieza and Metcalfe, 1997).

When the changes in lipid content of fish destined to mature are considered care must be taken not to make direct links to the findings documented above. Previously more mature male parr have been found in the lower mode of bimodal populations (Kristinsson *et al.*, 1985; Saunders and Henderson, 1988; Herbinger and Friars, 1992) and it may be suggested that changes in the lipid content of maturing fish are linked to those of lower modal group fish. However, bimodality is not thought to be caused by differences in maturational status (Thorpe, 1977; Villarreal and Thorpe, 1985) and such inferences should not be made.

Where maturation is concerned recorded changes in the lipid content of juveniles are similar to those found in adults and consequently data from maturational episodes in both adults and parr can be viewed together in order to understand the changes that are observed.

Although Herbinger and Friars (1992) found that maturation was not very dependent on spring lipid storage growing evidence highlights a cyclical nature of lipid deposition in fish destined to mature. Rowe *et al.* (1991) investigated fat accumulation in juvenile Atlantic salmon. For individuals that were destined to mature both total and mesenteric fat levels began to accumulate during April and May respectively, although a similar lipid deposition did not occur for a further month in fish destined to remain immature. Therefore, by June the mesenteric fat levels of maturing fish were significantly greater than those of immature fish, although by September these levels had declined with this reduction occurring as GSI (gonadal somatic index) increased (Rowe *et al.*, 1991). Similarly, Simpson (1992) noted that the fat content of Atlantic salmon parr, which were destined to mature, was higher than their immature counterparts by February. This differential remained until October by which time the fat levels of maturing individuals had declined to levels similar to those found in immature fish. However, Simpson (1992) also noted that although the fat content of mature individuals was greater than that of immature fish during the summer, from February onwards the difference was not increasing suggesting that the mature fish had experienced an early accumulation of fat.

For adults Aksnes *et al.* (1986) found that the fat level of fillets from maturing fish peaked between June and July with levels higher than those in the immature fish. Subsequently, a decline occurred through to November with the lipid levels of mature fish clearly lower than immature individuals during September, November and December. Kadri *et al.* (1996) also found that the lipid content of mature adult Atlantic salmon peaked in June with a subsequent decline. However, it was also noted that although the lipid content of all fish was similar in the September one month later

those destined to mature had a higher fat content. The lipid levels of both fish destined to mature and those remaining immature then rose during early winter. It therefore seems that maturing salmonids show a brief period of enhanced lipid deposition with peaks during spring and early summer. Subsequently levels decline as gonadal development progresses.

The feeding behaviour of maturing individuals also shows a clear cyclic behaviour. Early work by Scott (1962) showed that the feed intake of rainbow trout was lower during the 3 to 4 months of final maturation with Rowe and Thorpe (1990a) observing that between August and October maturing Atlantic salmon parr had a higher proportion of non-feeding individuals than their immature siblings. For the Arctic charr Tveiten *et al.* (1996) found that low feed intake occurred for all fish from December until April with appetite increasing in late spring/summer. However, increases in appetite were observed between 1 and 2 months earlier in maturing fish although such fish subsequently ceased or reduced feeding during late summer (Tveiten *et al.*, 1996). Kadri *et al.* (1996) found that mature adult salmon had a high feeding rate from April with a cessation of feed intake during spring/summer indicating a non-temperature related 2 month surge in feeding before the onset of anorexia and indeed further support for this two-phase feeding response linked to maturation is present (Stead *et al.*, 1999).

However, Simpson *et al.* (1996) found that the appetite of both maturing and non-maturing individuals declined after a peak in feed intake during May with the appetite of maturing individuals never greater than immature fish and it was concluded that maturation had no direct role on appetite. Similarly, it has been found that maturing

individuals may show a considerable feed response during the time of maturation (Arndt, 2000; Shearer and Swanson, 2000). Where maturation-induced anorexia has been found there has often been considerable variation in the response of maturing individuals within a particular population (Kadri *et al.*, 1995; Stead *et al.*, 1999). Kadri *et al.* (1995) linked the cessation of feeding to fat reserves, with Tveiten *et al.* (1996) observing that the anorexic response of maturing individuals was possibly correlated to condition factor. It has been suggested that if maturation progresses without sufficient lipid stores then the rate of post-spawning mortality is likely to increase (Thorpe, 1994b). Therefore, where the lipid reserves of previously anorexic, maturing individuals fall below a certain level the fish may recommence feeding in order to prevent post-maturation mortality in a similar manner to the re-feeding response of over-wintering immature parr.

Following periods of starvation or restricted feeding a recovery in the size of individuals is well documented (Weatherley and Gill, 1981; Dobson and Holmes, 1984; Quinton and Blake, 1990; Thorpe *et al.*, 1990; Reimers *et al.*, 1993; Hopkins and Unwin, 1997). However, it is also important to consider the behavioural responses to such periods of nutritional stress. After an 8 week period of restricted feeding (i.e. feeding individuals between 10 and 20% of the food consumed by fish that were fed to satiation) Miglavs and Jobling (1989b) found that Arctic charr increased their food intake (hyperphagia) in the weeks immediately following the transfer back to a satiation diet with this response peaking after three to four weeks. Similarly, Metcalfe and Thorpe (1992) found that following a period of induced starvation during winter Atlantic salmon parr showed a subsequent hyperphagic response for four weeks. More recently, Nicieza and Metcalfe (1997), Johansen *et al.*

(2001) and Morgan and Metcalfe (2001) have all documented a hyperphagic response following periods of nutritional stress.

Traditionally, recovery growth has been viewed in terms of the ability of a fish to replenish a certain size following a period of starvation or restricted feeding. However, some evidence shows that fish fed restricted rations can either exceed the size of fully-fed controls (Weatherley and Gill, 1981; Dobson and Holmes, 1984; Nicieza and Metcalfe, 1997) or that they may not achieve such sizes (Miglavs and Jobling, 1989a). More recently the focus of these compensatory responses has moved towards investigating the changes that occur in body lipid content. Metcalfe and Thorpe (1992) found that following a period of starvation feeding increased until fat stores were restored to the levels of constantly fed controls. Similarly, Simpson *et al.* (1996) found that reductions in body fat were counteracted by increases in appetite. Johansen *et al.* (2001) investigated two restricted feeding regimes observing that in one instance hyperphagia continued where body size had been fully compensated, whereas in the second experiment fish were smaller following the decrease in hyperphagic response although their body lipid contents were similar to those of controls. Furthermore, Jobling and Miglavs (1993); Shearer *et al.* (1997); Silverstein *et al.* (1999) and Morgan and Metcalfe (2001) have all provided evidence that supports a role for lipid content in regulating feeding and recovery growth during and following periods when feed availability is limited. It is therefore becoming increasingly evident that the appetite and growth of salmonids is not set to control a particular size status but that a mechanism using lipostatic regulation is more likely (Jobling and Miglavs, 1993; Silverstein *et al.*, 1999; Johansen *et al.*, 2001).

4.1.3. Proximate composition correlations

Although it is clear that both seasonal and developmental variations occur in the lipid content of salmonids it is also evident that further correlations concerning proximate composition can occur with such relationships possibly affecting the way in which experimental data are treated.

In their comprehensive reviews of salmonid proximate composition Shearer (1994) and Rasmussen (2001) highlighted the importance of fish size on proximate composition and in particular its effect on lipid content. Shearer (1994) considered such a relationship significant enough that in experimental analyses it was suggested that size should be included as a covariate in lipid determinations. It seems that there is significant evidence to suggest that such a procedure is necessary in order to avoid inaccuracies. Early evidence of such a relationship was provided by Reinitz (1983) when the feeding of juvenile (2.1g) rainbow trout was investigated. Although nutritional history was found to have a primary role in proximate composition it was also found that fat content increased with increasing fish size (Reinitz, 1983). Similarly, Storebakken and Austreng (1987b) reported an increase in the fat content of Atlantic salmon parr with increasing size and suggested that such effects may have masked the effects of the different rations used in their experiments. More recently, further support can be found particularly when considering adult salmonid composition (Bjerkeng *et al.*, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001).

However, Mørkøre and Rørvik (2001) stated that the relationship between fish size and adiposity is ambiguous since the fat content of post-smolts in their experiments

remained stable or declined slightly during winter and spring whereas fish weight increased. Shearer (1994) concluded that lipid content increases with fish size although it is also affected by life cycle stage. Interestingly much of the evidence for such a relationship presents itself for adult salmonids and it is evident that where such a correlation has been found in juvenile salmon it has only been recorded during early development (e.g. Storebakken and Austreng, 1987b) or in fish which do not exhibit natural smoltification (e.g. Reinitz, 1983). It therefore seems likely that although whole body lipid levels may be correlated to body size during the early development of salmonids, with the onset of physiologically demanding processes such as maturation or smoltification the relationship becomes less apparent. Consequently, including fish size as a covariate in analyses of juvenile fish may result in a loss of statistical robustness.

Interestingly, a further correlation can be found when considering the whole body fat and moisture content of individuals. A negative correlation has been found between the moisture content and whole body lipid level of both adult and juvenile salmonids (Elliott, 1976; Reinitz, 1983; Saunders and Henderson, 1978; Bjerkeng *et al.*, 1997; Reviews by Shearer, 1994; Rasmussen, 2001). Indeed this negative correlation is strong enough that it may be possible to predict the level of whole body fat from the moisture content of fish (Elliott, 1976).

4.1.4. Dietary influences on development

Previously, it has been suggested that the developmental decision of whether an individual juvenile salmonid will undergo maturation and/or smoltification is determined by the attainment of a certain critical size threshold (for smoltification:

Elson, 1957; Thorpe *et al.*, 1980; Skilbrei, 1988; Kristinsson *et al.*, 1985; for maturation: Bailey *et al.*, 1980; Saunders *et al.*, 1982; Berglund, 1995; Silverstein *et al.*, 1997). However, there is evidence that size is not correlated to either maturation (Prevost *et al.*, 1992) or smoltification (McKinnell and Lundqvist, 1998) with Saunders *et al.* (1982) and Økland *et al.* (1993) suggesting that a size threshold alone may not account for such physiological decisions. McCormick and Naiman (1984) proposed that other factors that correlate well with size may be more influential. It has therefore been suggested that an energetic or nutritional threshold may have to be surpassed before either maturation (Herbinger and Friars, 1992; Simpson, 1992; Shearer, 1994; Silverstein *et al.*, 1997) or smoltification (Thorpe, 1986; Shearer, 1994) can be successfully completed.

It has also been suggested that such thresholds may influence development, in particular maturation, during seasonally-critical periods (Thorpe, 1986; 1987b; Duston and Saunders, 1992; Metcalfe, 1998; Thorpe and Metcalfe, 1998; Taranger *et al.*, 1999a). Thorpe (1986) proposed that if the rate of acquisition of energy was sufficient during early spring maturation would be initiated. Further support was provided by Duston and Saunders (1992) who investigated annual photoperiods that were manipulated to occur in either 6-, 12- or 18-month periods, concluding that the initiation of maturation would occur on the increasing phase of the photoperiod (i.e. in spring) provided sufficient growth thresholds had been achieved. Indeed growing support for such a theory is present in the literature (Adams and Thorpe, 1989; Rowe and Thorpe, 1990b; Thorpe *et al.*, 1990; Rowe *et al.*, 1991; Berglund, 1992; Duston and Saunders, 1997) although it is likely that both the current state of a physiological

parameter as well as its rate of change will be influential during the suggested critical period (Metcalfe, 1998; Thorpe *et al.*, 1998).

Thorpe (1994b), Metcalfe (1998) and Thorpe *et al.* (1998) have suggested that the initiation of maturation occurs in November one year prior to maturation (Metcalfe, 1998; Thorpe *et al.*, 1998) with a time prior to first-feeding therefore possible (Thorpe, 1994b). Subsequently, maturation will be influenced during a second sensitive period in spring (Metcalfe, 1998; Thorpe *et al.*, 1998) as previously suggested. As such it remains clear that spring will provide the main period during which environmental manipulations can influence maturation. It is also likely that if such a model influences maturation other developmental processes such as smoltification may be influenced during critical periods of the year.

Although lipid levels may influence development during seasonally-critical periods, Herbinger and Friars (1992) have suggested that a lipid threshold for parr maturation may be very low, with Saunders *et al.* (1982) also suggesting that high fat levels are not necessary for smoltification. Given that only low levels of fat may be required to exceed developmental lipid thresholds it is unlikely that the non-sacrificial determination of fat content using electronic instruments (e.g. the Torrymeter) will prove accurate enough to identify individuals that have reached a particular lipid threshold. Furthermore, the estimation of fat content using morphometric measurements (e.g. Herbinger and Friars, 1991; Simpson *et al.*, 1992; Sutton *et al.*, 2000) has provided variable results. Consequently, it can be concluded that the use of energetic thresholds for predicting which fish will undergo a particular developmental route may not be practicable on a commercial scale.

Although it may not be possible to use lipid thresholds to accurately predict which individuals will undergo either smoltification or maturation in commercial populations it is possible that the attainment of such thresholds can be affected through diet manipulation. In this way dietary manipulations can be used to adjust either the timing or the incidence of fish choosing to undergo a particular developmental route.

During commercial production an understanding of the dietary influences affecting maturation is important primarily to limit the numbers of mature fish during the ongrowing stages of production, but also to enhance the productivity of broodstock programmes. Although ration level has been shown to influence growth, with dietary lipid level affecting adiposity (Shearer *et al.*, 1997), it is dietary restrictions that are generally used to reduce maturation.

Shearer and Swanson (2000) investigated the role of dietary lipid on maturation in chinook salmon parr. Following a 7 month period where fish were maintained on a commercial diet groups were fed diets containing either 4, 9, 14, 18 or 22% lipid for 13 months. Maturation levels were 34% in the group fed the 4% lipid diet increasing to 45% in the 22% lipid group with the growth of all groups similar throughout the experiment. Therefore, whole body lipid levels derived from the dietary lipid regime and not growth had influenced maturation (Shearer and Swanson, 2000). Hillestad *et al.* (1998) found a higher incidence of maturation in adult Atlantic salmon in groups fed a diet containing 300g kg^{-1} fat, rather than 220 g kg^{-1} fat. Unfortunately, the experiment of Hillestad *et al.* (1998) focused primarily on growth and carcass quality and it is evident that due to the effects of dietary fat on adiposity such experiments

tend to focus on ultimate harvest quality as opposed to maturation. However, the works of Hillestad *et al.* (1998) and Shearer and Swanson (2000) provide evidence that increases in dietary fat will result in an increased incidence of maturation almost certainly through elevated lipid deposition and the attainment of maturational thresholds by a greater number of individuals within the population.

Silverstein *et al.* (1998), working with male chinook salmon parr, found that dietary lipid level only affected maturation through an interaction with ration rate. When dietary lipid was considered alone no effect was observed although ration did singularly influence maturation. It is therefore clear that further investigation is required to help identify the true role of dietary lipid on maturation.

For investigations regarding the effects of feed restriction on maturation Scott (1962) provided some preliminary work by studying the effects of periods of starvation on maturation and gamete quality in female rainbow trout. During the summer months prior to spawning, at both the second and third year of age, female rainbow trout were exposed to reduced feeding (i.e. fed for three days each week) for differential periods. It was concluded that semi-starvation in the second year clearly reduced the percentage of mature fish in the third year. Furthermore, in mature fish an increase in atresia and a reduction in fecundity was noted, although feed restriction did not affect the size of eggs (Scott, 1962). Subsequently, Bagenal (1969) found that feeding brown trout either half or one-third rations for much of the year resulted in a lower number of maturing fish than under full ration regimes. However, it was also noted that unlike the work of Scott (1962) fish fed restricted rations had larger eggs than full

ration controls, although feed restriction had resulted in a reduction in the number of eggs (Bagenal, 1969).

Although, it would seem that long-term feed restriction will be detrimental maturation (Scott, 1962; Bagenal, 1969) it is unlikely that short-term starvation immediately prior to spawning will have an effect on gamete quality (Ridelman *et al.*, 1984). Consequently, the manipulation of maturation in commercial populations, for example to improve broodstock programmes, will require long-term dietary manipulation.

Given these early works it is clear that feed restriction can be used to reduce the incidence of maturation and indeed investigations aimed at limiting maturation in commercial salmon production have focused on such manipulations. Rowe and Thorpe (1990b) exposed 2+ Atlantic salmon parr to 2 month periods of either enhanced or restricted feeding opportunity between November and September. It was found that feed enhancement during April to July increased the incidence of maturation, with the two month period between April and May most influential. Furthermore, only restricted feeding during April, May and June resulted in a reduction in the numbers of mature individuals (Rowe and Thorpe, 1990b) although it is important to note that the sample populations of 2+ parr used in these experiments were fairly small. Further evidence has documented that restricted feeding regimes applied between November and June (Clarke and Blackburn, 1994), March and September (Silverstein *et al.*, 1998) and September and October (Morgan and Metcalfe, 2001) can reduce the incidence of male parr maturation.

Similar findings have been presented for adults. Thorpe *et al.* (1990) found a decrease in the incidence of maturation in adult female Atlantic salmon that were supplied feed every second week between December and April, with February and March the most effective times for limiting maturation in both sexes. By starving adult Atlantic salmon between February and April, Reimers *et al.* (1993) have reduced female and male maturation by 48% and 32% respectively and similar reductions in adult maturity have been observed following feed restriction in other salmonid species (Silverstein and Shimma, 1994; Hopkins and Unwin, 1997).

However, dietary manipulations have been found to result in extremely varied effects on maturation in particular for juvenile salmon. Although decreases in the incidence of maturation have been documented following periods of feed restriction (e.g. Clarke and Blackburn, 1994; Morgan and Metcalfe, 2001) some studies have recorded only slight or negligible effects on maturation (Herbinger and Friars, 1992; Berglund, 1995). Interestingly, although feeding regimes have been found to affect maturation at a range of times during the year (c.f. Thorpe *et al.*, 1990; Clarke and Blackburn, 1994; Morgan and Metcalfe, 2001) an increase in the response to feed restriction occurs during spring. Rowe and Thorpe (1990b) and Thorpe *et al.* (1990) showed that maturation could be influenced to the greatest extent during a short period in spring and subsequently many authors have focused their experimental restrictions on this period (Reimers *et al.*, 1993; Silverstein and Shimma, 1994; Berglund, 1995; Hopkins and Unwin, 1997). Indeed, this early spring period is becoming increasingly viewed as a developmental period during which maturation can be influenced (Thorpe, 1986; Duston and Saunders, 1992; Thorpe, 1994b; Metcalfe, 1998; Thorpe *et al.*, 1998) and

dietary manipulations in particular will influence the decision as to whether a fish will mature later in the year.

There is limited literature on the effects of dietary lipid level on the parr-smolt transformation. Redell *et al.* (1988) provided the most comprehensive experiment to date. In their experiments no variation was found in the quality of smolting individuals that were fed diets containing different levels of dietary lipid (between 11 and 18% inclusion) (Redell *et al.*, 1988).

Limited literature is also available on the effects of ration on smoltification. However, given the changes in fat accumulation and feeding behaviour that occur during the parr-smolt transformation, as well as the documented effects of feed restriction on maturation, a greater level of investigation might be expected. Dickhoff *et al.* (1989) reported that fully-fed Atlantic salmon parr showed similar increases in gill Na^+, K^+ - ATPase and seawater tolerance in spring as individuals which were starved during November and December. More recently Thorpe and Metcalfe (1998) found that restricted feeding (i.e. full ration for only one week in four) between February and June did not directly affect smolt status. However, an indirect role of ration was suggested because growth was limited under the restricted feed regime and these smaller fish faired less well in sea water (Thorpe and Metcalfe, 1998). It has also been shown that fasting between January and February does not impair smoltification in coho salmon parr (Larsen *et al.*, 2001). However, in this study fish that were fed during January and February were larger than those that were starved and the indirect role of feed regime on growth suggested by Thorpe and Metcalfe (1998) may therefore have been important in smoltification given that a distinct size relationship

in seawater survival has been well documented (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988; Økland *et al.*, 1993).

Therefore, although clear changes in body lipid occur during smoltification diet regime during the latter stages of freshwater development has negligible effects on smoltification. The suggestion by Saunders *et al.* (1982) that high fat levels might not be necessary for smoltification may therefore be true. As such differences between the effects of feed regime on maturation and smoltification may be linked to differences in the feeding opportunity that occurs in fresh and sea water, as well as the subsequent survival that occurs after maturation or seawater transfer. However, for smoltification in particular investigation into the effects of long-term diet manipulation is necessary in order to enhance the understanding of such physiological processes.

4.1.5. Experimental aims

Within commercial salmon culture complex diet formulations are increasingly used primarily to aid growth and harvest quality. However, the effects of diet manipulation on juvenile life history strategy are not well understood, in particular with reference to the incidence of maturation and the developmental decision of whether an individual undergoes maturation and/or smoltification. Therefore, the aims of this chapter are:-

- To investigate the effects of long-term differences in dietary lipid regime on growth, maturation and smoltification in Atlantic salmon parr.
- To consider the effects of differing rations of feed on the "decisions" to mature and/or to smolt.

- To investigate the interaction between the rations of feed and photoperiod in Atlantic salmon parr reared under both natural and photoperiodically-manipulated regimes.

4.2. Experiment IV. The role of dietary lipid level on growth, maturation and smoltification.

4.2.1. Objectives.

The experiment detailed in this section aimed to investigate the role of differing levels of dietary lipid on growth, maturation and smoltification.

4.2.2. Materials and Methods.

The experiment started at Site 6 (Section 2.1.1). Ova from a low grilsing Scottish stock were fertilised and held in heated water (6.0±1.2°C) under darkness until hatching (24th January 2000). The fry were then held under a natural photoperiod in heated water (6.2±1.4°C) until first-feeding (16th March 2000). At first-feed 2500 fish were transferred into each of two, 1m square, 0.4m^3 tanks and exposed to LD24:0 with the gravity fed water heated slightly above the natural temperature regime (Fig. 4.1). From first-feed each tank was supplied with one of two experimental diets (EWOS; Scotland, UK) containing either 12.5% or 25% lipid (Table 4.1) fed at the manufacturers' recommended rate throughout the 24h illuminated period (see Fig. 4.2 for experiment protocol). Although the lipid levels of the diets deviated slightly from those formulated similar variations would normally be expected during the manufacture of diets and as such the diets were considered appropriate for the experiment.

On 16th May 2000 the fish were moved to Site 7 (Section 2.1.1) with 400 fish from each diet group placed into each of six, 0.7m diameter, 0.25m^3 tanks and exposed to

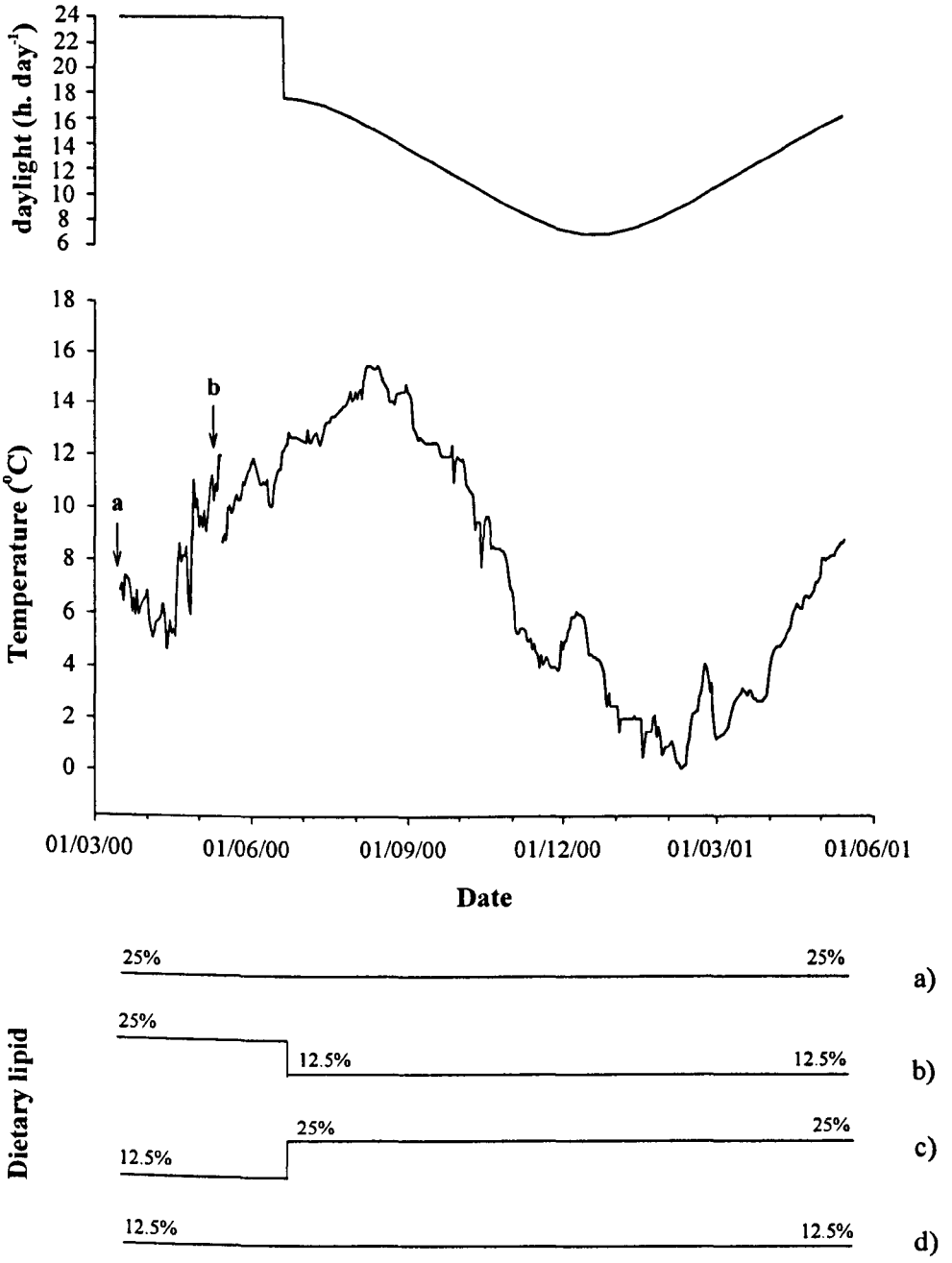


Fig. 4.1 The photoperiod, temperature and feed regimes at Sites 6 and 7 during the 2000-2001 dietary lipid experiment, where groups were fed diets containing either 25% or 12.5% lipid. First-feeding is denoted by 'a', 'b' denotes the date that fish were moved from Site 6 to Site 7. Between a and b the water was artificially heated. a) 25% lipid throughout the experiment, b) 25% lipid until 21st June, 12.5% thereafter, c) 12.5% lipid until 21st June, 25% thereafter, d) 12.5% lipid throughout the experiment.

Feed size	Diet			
	12.5% lipid		25% lipid	
	Mean	*S.E.M*	*Mean*	*S.E.M.*
Crumble 1.0	12.6	0.04	25.5	0.2
Crumble 2.0	12.5	0.08	25.7	0.06
Crumble 3.0	12.4	0.06	24.8	0.2
1.5mm Pellet	10.4	0.1	22.7	0.01
2.0mm Pellet	13.3	0.03	24.7	0.08

Table 4.1 Lipid levels (mean±S.E.M., n=3) in the different sizes of diet containing either 12.5% or 25% lipid.

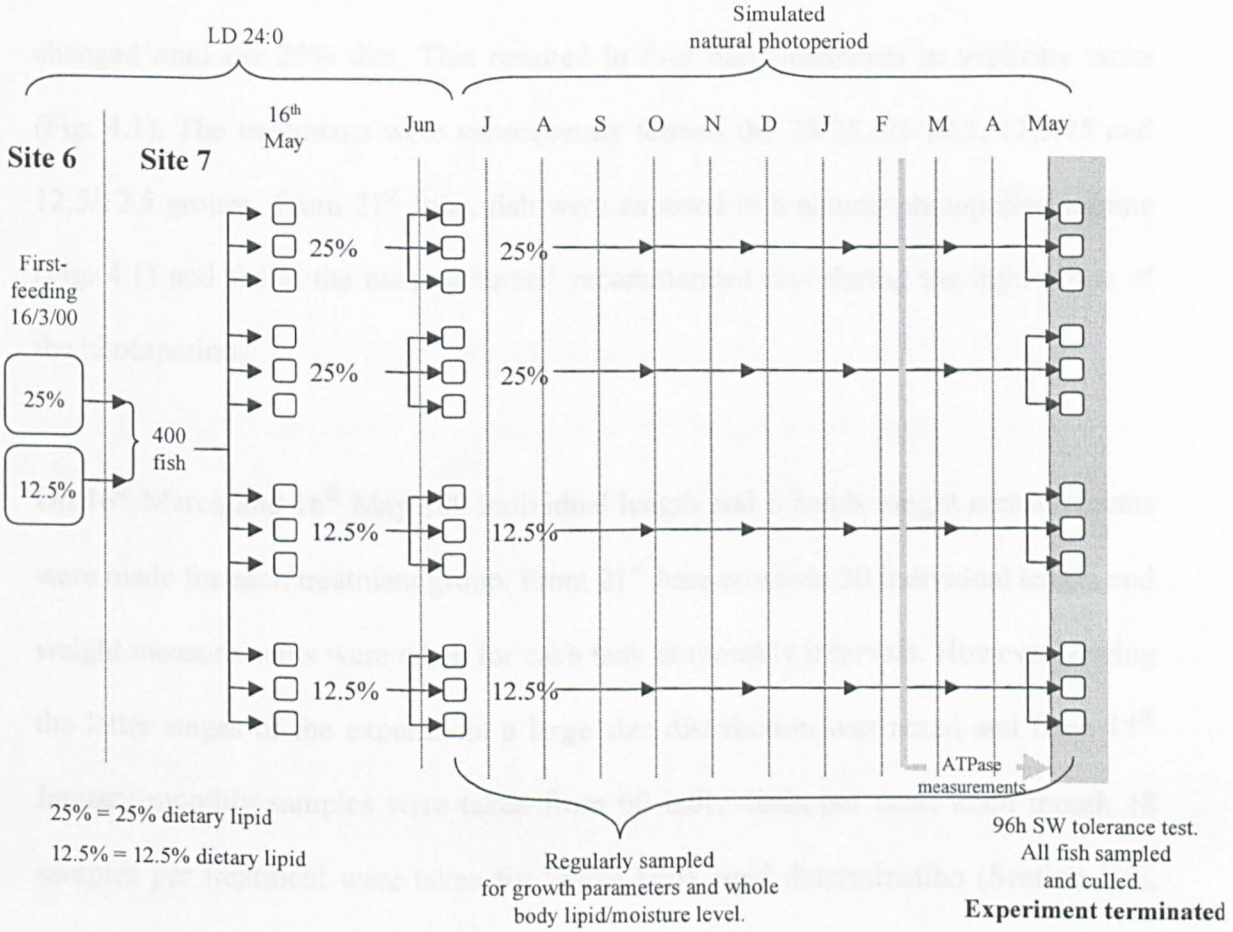


Fig. 4.2 The experimental protocol used during experiment IV. For further details of the sampling regime refer to section 4.2.2.

ambient temperature regimes under LD24:0 (Fig. 4.1). Fish were then maintained on their respective diets until 21st June 2000 after which the fish in three tanks from the 25% diet group received the 12.5% diet and those in three of the 12.5% diet tanks changed onto the 25% diet. This resulted in four diet treatments in triplicate tanks (Fig. 4.1). The treatments were subsequently termed the 25/25, 25/12.5, 12.5/25 and 12.5/12.5 groups. From 21st June, fish were exposed to a natural photoperiod regime (Fig. 4.1) and fed at the manufacturers' recommended rate during the light phase of the photoperiod.

On 16th March and 16th May 100 individual length and 6 batch weight measurements were made for each treatment group. From 21st June onwards 30 individual length and weight measurements were taken for each tank at monthly intervals. However, during the latter stages of the experiment a large size distribution was noted and from 15th January monthly samples were taken from 60 individuals per tank. Each month 18 samples per treatment were taken for whole body lipid determination (Section 2.9). Up until 19th September samples were pooled in order to achieve the necessary dry weight to accurately perform lipid analysis.

At each monthly sample all measured fish were examined for external signs of maturation (Section 2.7.1). From 13th November due to the low incidence of mature fish an additional 70 individuals per tank were checked for maturity.

On 16th February 2001 and twice monthly from 15th March gill samples were taken from 5 individuals from the upper modal group of the population per tank for the determination of gill Na^+, K^+ -ATPase.

On 14th May 2001 20 individuals selected at random per treatment were exposed to a 96h seawater tolerance test (Section 2.8.2). The remaining fish were culled with the numbers of 1+ smolts and parr in each group recorded based on both size and the presence of external silvering.

Growth data, whole body lipid level, moisture content and gill Na^+, K^+ -ATPase level were compared using a General Linear Model (Section 2.11) although for changes in weight, length and condition factor a natural log transformation was used to improve normality and homogeneity of variance. Correlations between whole body lipid and moisture level were analysed using the Pearson's product moment method. For the analysis of population structure, 95% confidence limits were calculated and compared.

4.2.3. Results.

4.2.3.1. Growth

Weight

All treatments resulted in an overall increase in weight ($p<0.001$) over the experimental period (Fig. 4.3) with fish from all groups increasing between consecutive time points until 19th September ($p<0.05$) and fish in the 12.5/12.5 group increasing until 15th October ($p<0.01$). However, no differences were observed between the weight of fish from each treatment at individual time points ($p>0.05$).

Length

All treatments resulted in an overall increase in length ($p<0.001$) over the experimental period (Fig. 4.4) with all groups increasing between consecutive time

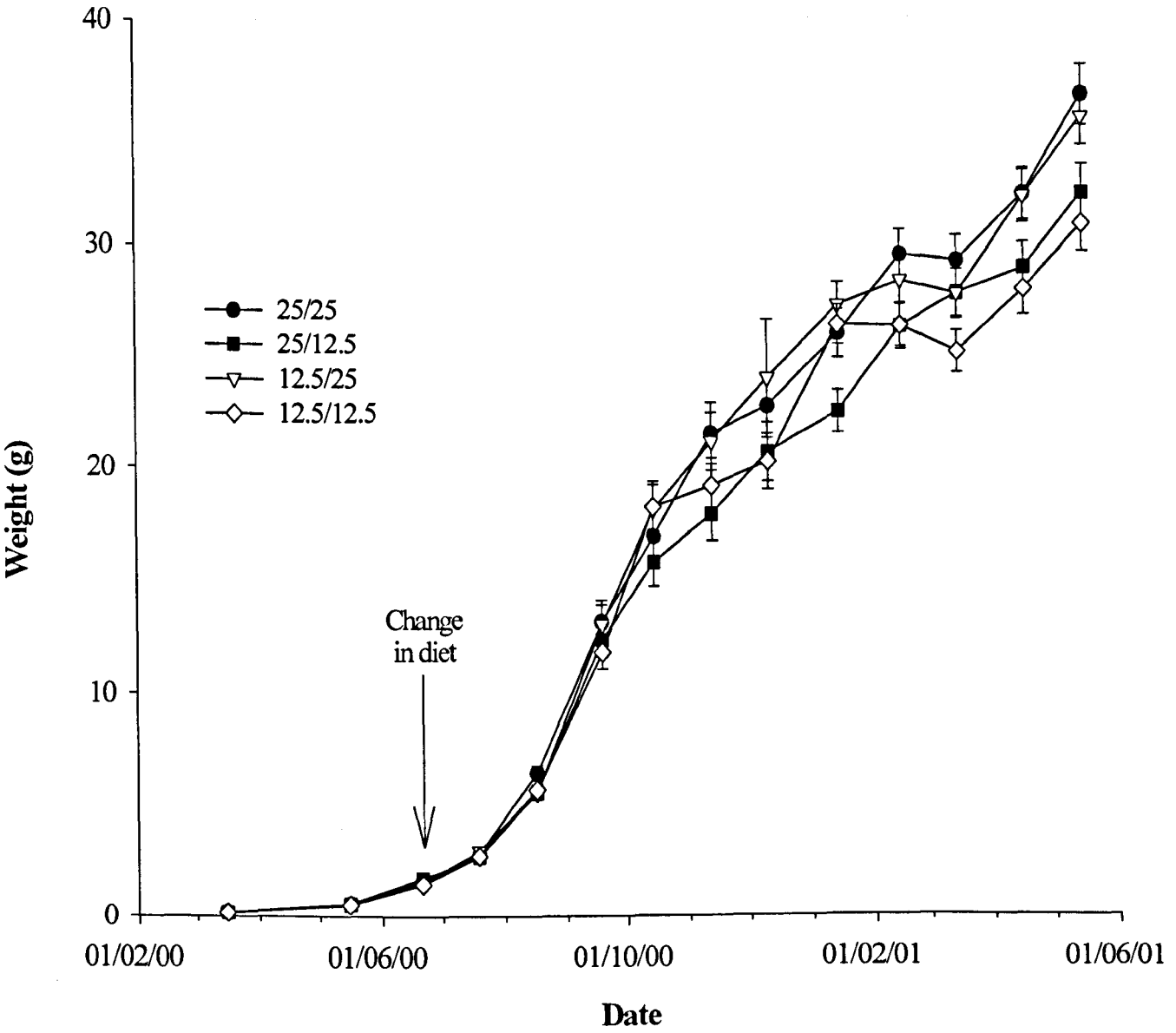


Fig. 4.3 Changes in weight (mean ±S.E.M., n=90-180) of parr fed diets containing different levels of lipid, for different periods of development.

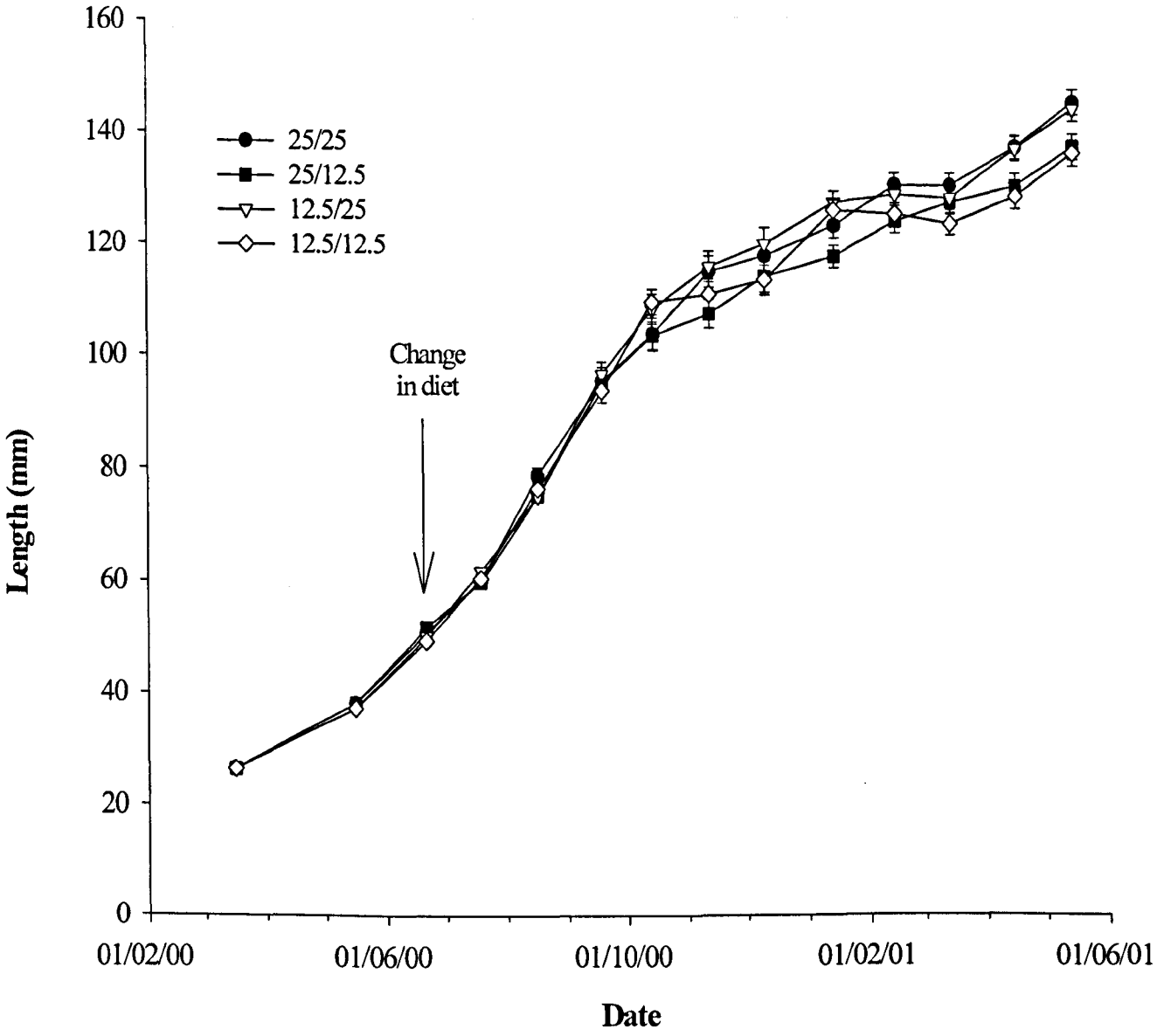


Fig. 4.4 Changes in length (mean ±S.E.M., n=90-180) of parr fed diets containing different levels of lipid, for different periods of development.

points all groups until 19th September ($p<0.05$) and fish from the 12.5/12.5 group increasing until 15th October ($p<0.01$). However, no differences were observed between the length of fish from each treatment at individual time points ($p>0.05$).

Condition factor

All treatments except the 25/25 group showed an increase in CF between 21st June and 19th September ($p<0.05$) with all groups then displaying an overall decline to the end of the experiment ($p<0.001$) (Fig. 4.5). However, between consecutive time points the CF of the 25/25 fish only increased between 17th August and 19th September with a decrease only observed between 15th March and 17th April ($p<0.01$). For the 25/12.5 fish, a decrease in CF was found between 19th September and 15th October ($p<0.05$). The 12.5/25 fish exhibited an increase in CF between 21st June and 19th August with a decline occurring between 13th November and 15th January ($p<0.01$) and between 15th March and 17th April ($p<0.01$). However, for the 12.5/12.5 fish changes only occurred between 17th April and 14th May when a decline in CF was observed ($p<0.01$).

For differences between treatments at individual time points no consistent trends could be identified. However, on 21st June 25/25 and 25/12.5 fish both had higher CF values than the 12.5/25 and 12.5/12.5 fish ($p<0.05$).

SGR

Because changes in SGR were calculated from the mean weights of fish from each treatment no statistical analysis could be performed. However, a generalised trend in SGR could be found (Fig. 4.6). Between 16th May and 21st June SGR increased in

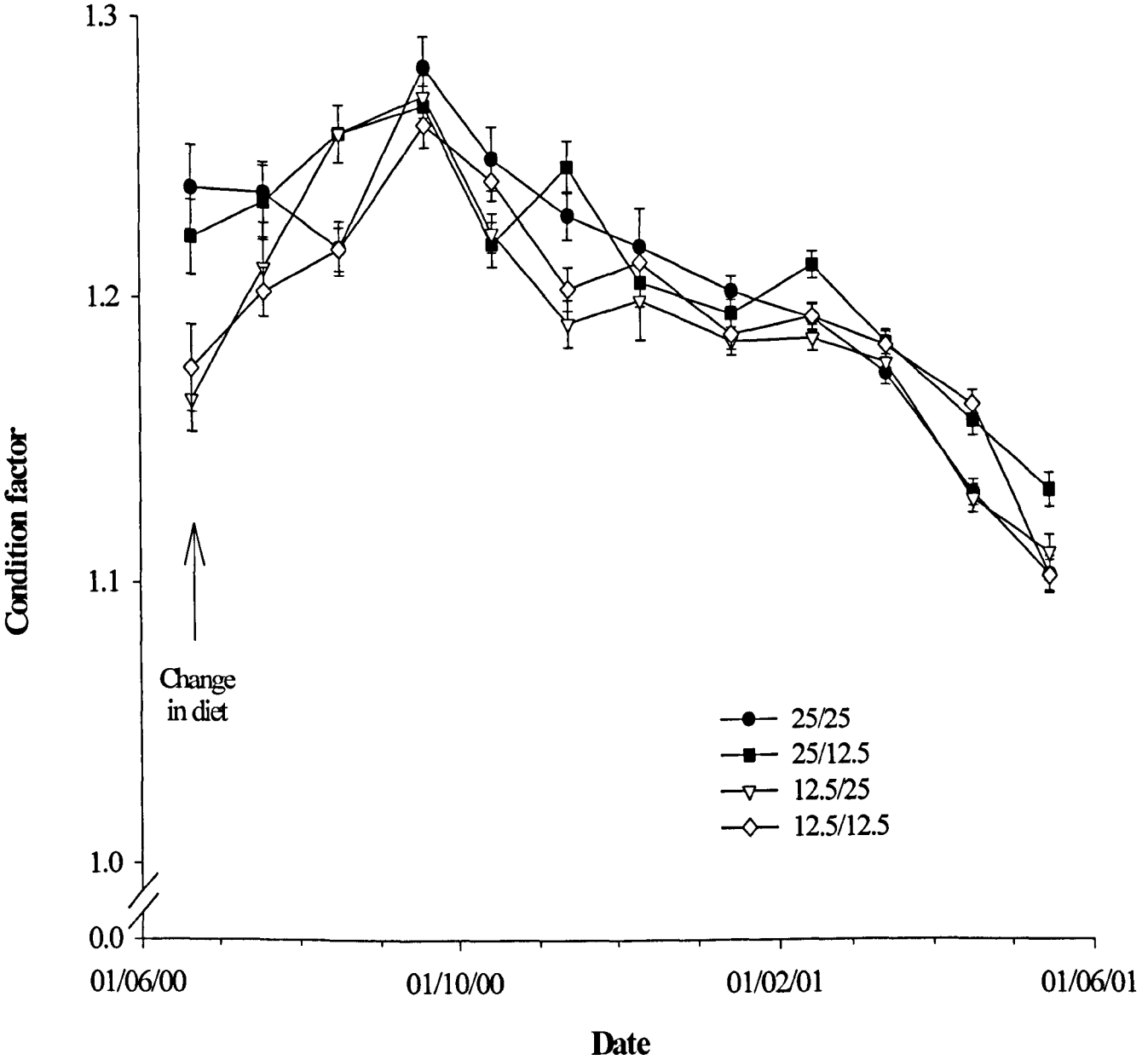


Fig. 4.5 Changes in condition factor (mean ±S.E.M., n=90-180) of parr fed diets containing different levels of lipid, for different periods of development.

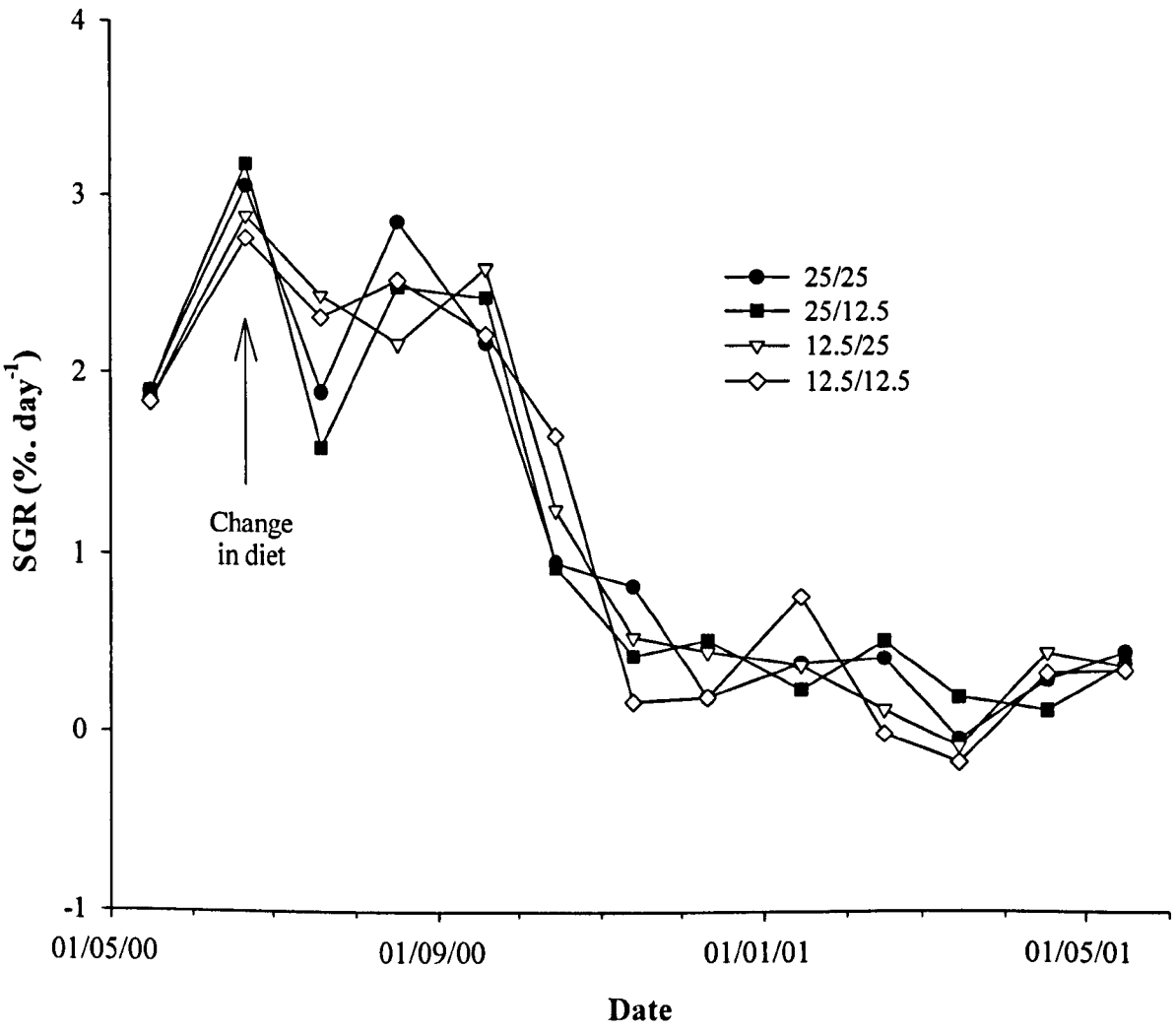


Fig. 4.6 Changes in specific growth rate (SGR) of parr fed diets containing different levels of lipid, for different periods of development.

all groups to give a peak of between 2.8 and 3.2. Growth then declined in all groups by 19th July with the 25/25, 25/12.5 and 12.5/12.5 treatments exhibiting increased growth by 17th August. The SGR of the 12.5/25 fish showed a later increase on 19th September. Subsequently, the SGR of all groups decreased up to the end of the experiment.

Weight-frequency distribution

All treatments resulted in the development of bimodality (Fig. 4.7). For the 25/25, 25/12.5 and 12.5/25 treatments the bimodal divide was first evident on 15th October. However, for the 12.5/12.5 group the emergence of bimodality seemed to occur at an earlier date on 19th September. Furthermore, by the conclusion of the experiment the 25/25, 25/12.5 and 12.5/25 groups had similar population structures although the 25/25 group did contain the largest individuals of all the groups. For the 12.5/12.5 group a similar UM as the other treatment groups occurred but the LM fish were smaller than those from the other treatments.

4.2.3.2. Body composition

Lipid content

All treatments resulted in an overall increase in whole body lipid level until 15th October with a subsequent decline by the end of the experiment ($p<0.001$) (Fig. 4.8). For between treatment differences at individual time points the lipid content of individuals remained at levels that were relative to the dietary lipid inclusion they were being fed. As such on 16th May the 25/25 and 25/12.5 fish had higher lipid levels than the 12.5/25 and 12.5/12.5 fish. Then following the change in diet on 21st June the lipid level of the 12.5/25 group increased significantly ($p<0.01$) until 19th

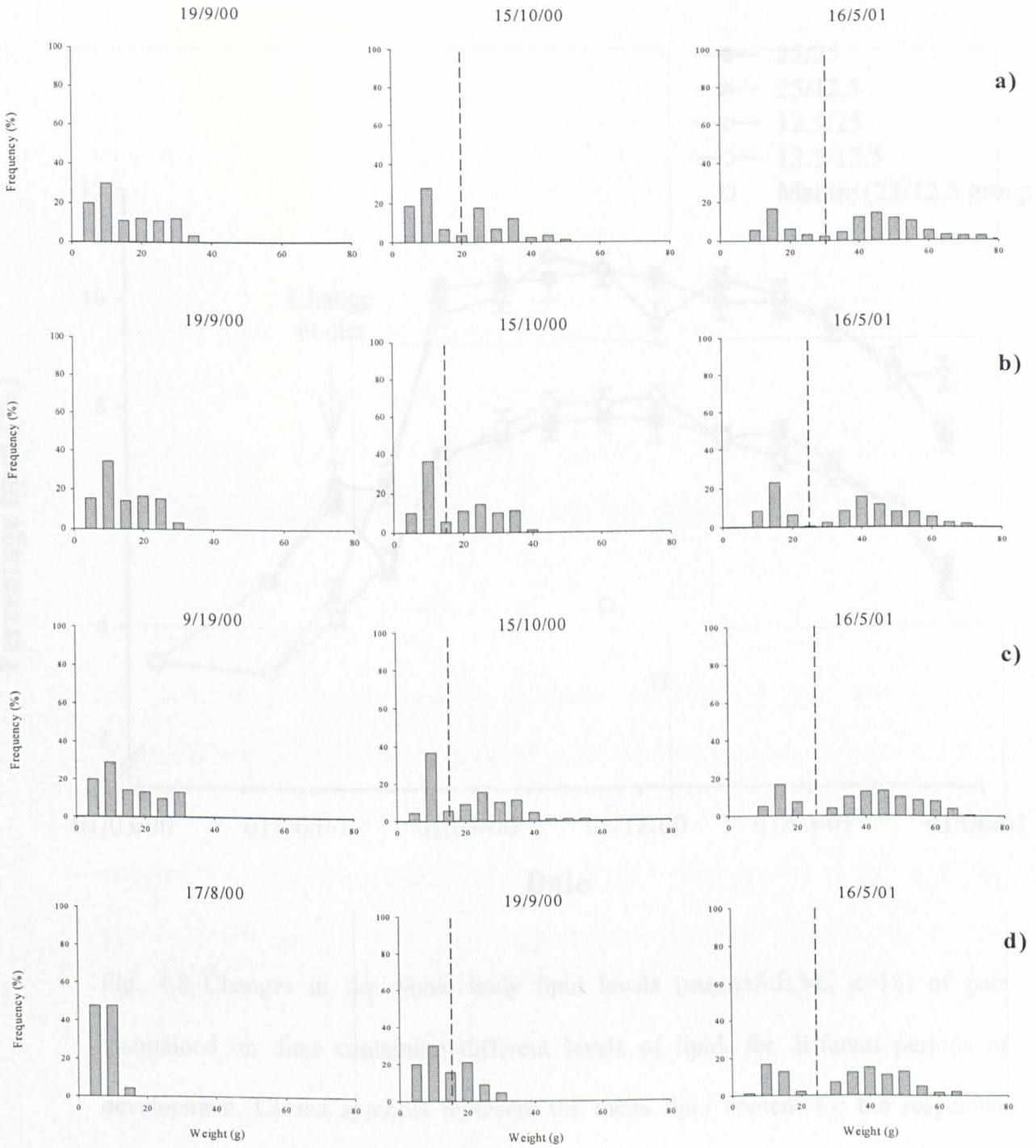


Fig. 4.7 The weight-frequency distributions of parr maintained on diets containing different levels of lipid, for different periods of development (n=90-180). Plots represent the sample points just prior to, and at the emergence of bimodality, as well as at the final sample point. Dotted lines depict the division of modes. a) 25% lipid throughout the experiment, b) 25% lipid until 21st June, 12.5% thereafter, c) 12.5% lipid until 21st June, 25% thereafter, d) 12.5% lipid throughout the experiment.

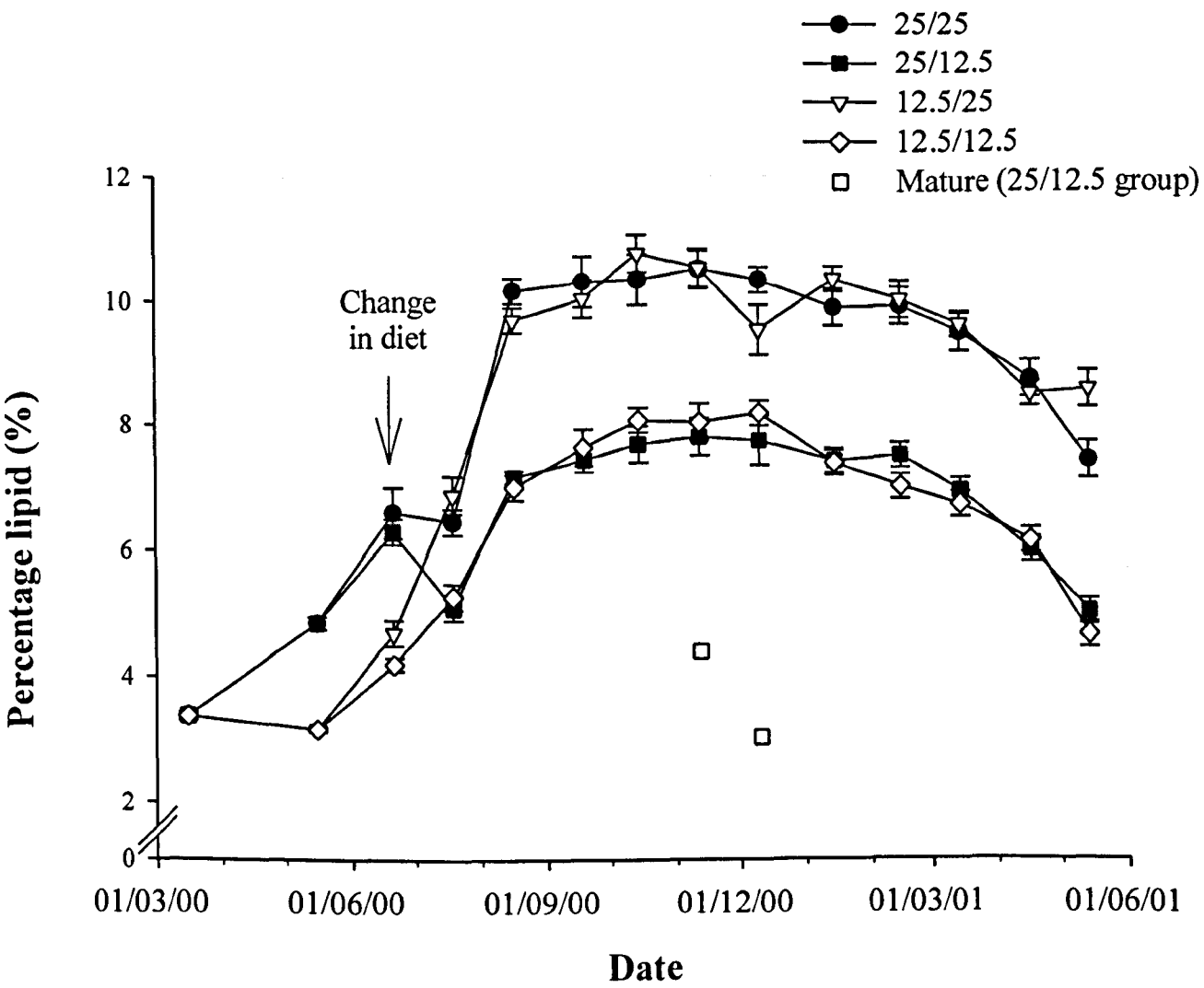


Fig. 4.8 Changes in the whole body lipid levels (mean±S.E.M., n=18) of parr maintained on diets containing different levels of lipid, for different periods of development. Closed symbols represent the mean lipid content for the respective treatment, open symbols represent the lipid content of mature fish identified within a population.

July to give similar lipid levels as those in the 25/25 fish. Then from 17th August until the conclusion of the experiment the lipid levels of the 25/25 and 12.5/25 fish remained similar but different from those of the 25/12.5 and 12.5/12.5 groups ($p<0.05$).

During the random sampling of fish for lipid determination two mature individuals were identified and analysed. Both fish were found in the 25/12.5 group and although statistical analysis could not be performed on the data from these fish it was clear that maturation had resulted in a reduction of whole body lipid.

Moisture content

All treatments resulted in an overall decrease in whole body moisture level until 19th December with a subsequent increase by the end of the experiment ($p<0.001$) (Fig. 4.9). For between treatment differences at individual time points the moisture content of individuals remained at levels that were relative to the dietary lipid inclusion they were being fed. As such on 16th May the moisture levels of the 25/25 and 25/12.5 fish were lower than those of the 12.5/25 and 12.5/12.5 fish. Then from 17th August onwards, following the change in diet, the 25/25 and 12.5/25 fish maintained lower moisture levels than those of the 25/12.5 and 12.5/12.5 fish ($p<0.05$).

Although statistical analysis could not be performed on the moisture content data of the two mature fish that were identified from the 25/12.5 group it was clear that maturation had resulted in an increase in moisture content.

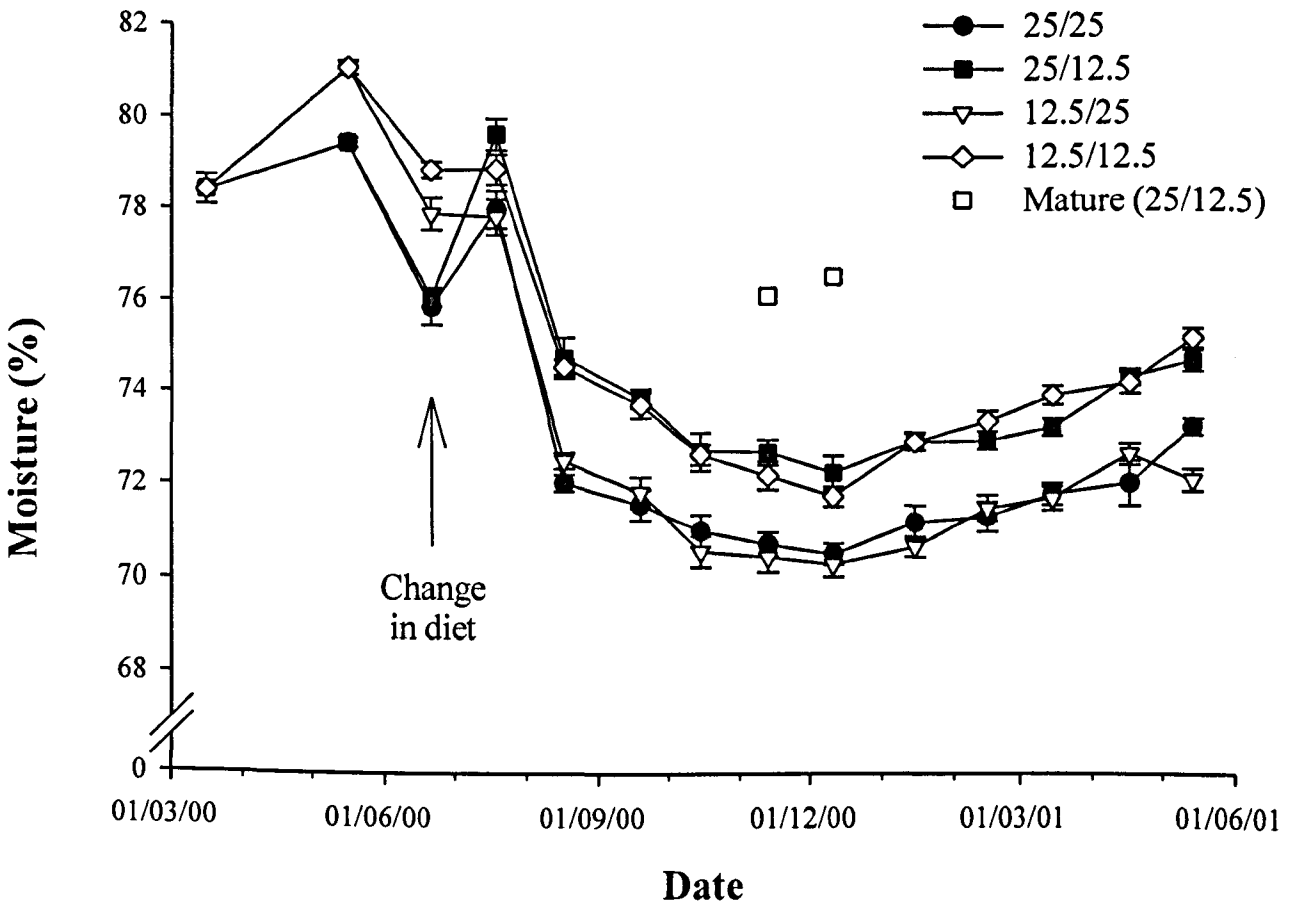


Fig. 4.9 Changes in the whole body moisture levels (mean±S.E.M., n=18) of parr maintained on diets containing different levels of lipid, for different periods of development. Closed symbols represent the mean moisture content for the respective treatment, open symbols represent the moisture content of mature fish identified within a population.

Lipid/Moisture correlation

When the lipid contents of individual fish were plotted against their respective moisture content for each treatment but regardless of sample time good linear regressions were achieved (Fig. 4.10). Furthermore, for each treatment the correlation between lipid and moisture level was found to be highly statistically significant ($p<0.001$).

Lipid/Size correlation

In order to investigate whether the changing lipid level of fish was affected by fish size scatter plots of individual fish weight and their respective lipid level were made. Due to the changes in lipid and size during smoltification it was necessary to consider such a relationship at each time point. However, this resulted in low numbers of fish used for each regression. Therefore, to give a general representation of the accuracy of any size/lipid level relationship only the changing r^2 values of such regressions have been presented for the experimental period (Table 4.2).

A poor relationship between size and lipid level was found during the initial stages of the experiment. Between 21st June and 13th November linear regressions were higher with the r^2 values of all treatments subsequently declining through to the conclusion of the experiment.

4.2.3.3. Maturation

Incidence

Unfortunately the incidence of maturity was extremely low (Table 4.3) and as such statistical analyses were not possible. Mature fish were only present in the 25/12.5

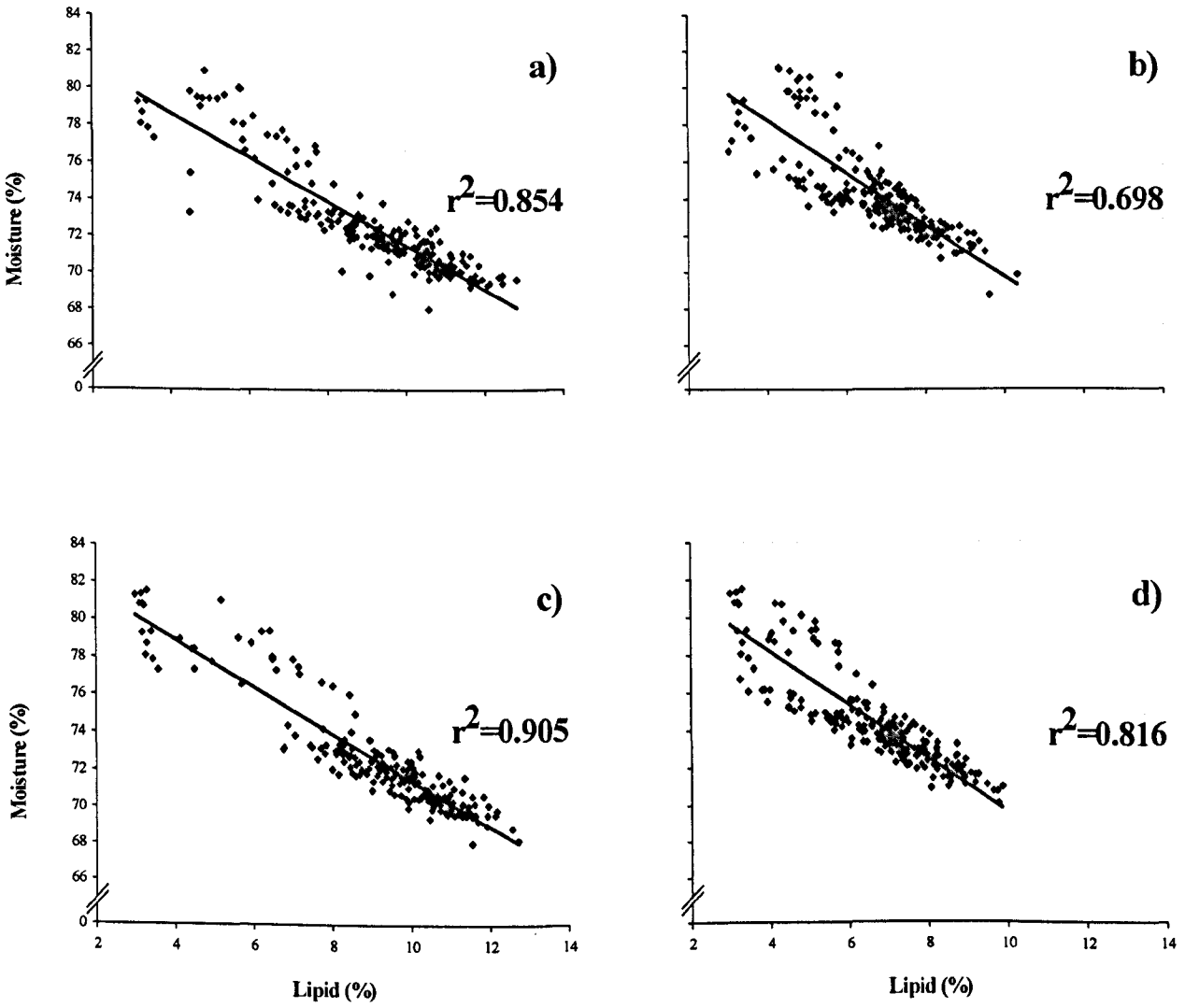


Fig. 4.10 The correlation between whole body lipid level and moisture content in parr fed diets containing different levels of lipid during different periods of development (n=230). a) 25% lipid throughout the experiment, b) 25% lipid until 21st June, 12.5% thereafter, c) 12.5% lipid until 21st June, 25% thereafter, d) 12.5% lipid throughout the experiment.

Date	Diet regime			
	25/25	*25/12.5*	*12.5/25*	*12.5/12.5*
16/3/00	0.285	0.285	0.285	0.285
16/5/00	0.078	0.078	0.177	0.177
21/6/00	0.731	0.701	0.446	0.574
19/7/00	0.540	0.368	0.771	0.536
17/8/00	0.411	0.132	0.171	0.816
19/9/00	0.746	0.715	0.862	0.836
15/10/00	0.738	0.676	0.561	0.332
13/11/00	0.781	0.423	0.627	0.440
11/12/00	0.155	0.364	0.300	0.014
15/1/00	0.178	0.156	0.254	0.125
15/2/00	0.397	0.001	0.159	0.374
15/3/00	0.066	0.008	0.269	0.058
17/4/00	0.126	0.000	0.313	0.082
14/5/00	0.038	0.021	0.049	0.059

Table 4.2 The changing r^2 values of linear regressions between the weight and whole body lipid level of parr fed diets containing different levels of lipid during different periods of development (n=18).

Date	Incidence of maturity (%) *Diet regime*			
	25/25	*25/12.5*	*12.5/25*	*12.5/12.5*
13/11/00	0	1.1	0	0
19/12/00	0	0	0.7	0
15/01/01	0	0	0.3	0
15/02/01	0	0	0.2	0
15/03/01	0	0	0.5	0
17/04/01	0	0	0	0
16/05/01	0	0	0	0

Table 4.3 The incidence of maturity observed in groups of parr that were fed diets containing different levels of lipid during different periods of development (n=90-300).

and 12.5/25 treatments. In the 25/12.5 group mature fish were only observed on 13th November whereas in the 12.5/25 group low levels were found from 19th December until 15th March.

4.2.3.4. Smoltification

Gill Na^+, K^+ -ATPase

An overall increase in gill Na^+, K^+ -ATPase level occurred in all treatment groups between 16th February and 14th May ($p<0.001$) (Fig. 4.11). Between consecutive time points an increase in the ATPase levels of the 12.5/12.5 treatment group was observed from 29th March until 17th April ($p<0.05$). The levels recorded in the 25/25, 25/12.5 and 12.5/25 groups then increased between 17th April and 3rd May ($p<0.05$) with those of the 25/25 fish continuing to increase until 14th May ($p<0.01$). However, when between treatment variations were considered differences only occurred on 14th May when the gill Na^+, K^+ -ATPase levels of the 25/25 fish were greater than those of the 12.5/12.5 fish ($p<0.01$).

Seawater survival

All groups showed good levels of survival following a 96h seawater tolerance test (Fig. 4.12). The 25/25 fish exhibited the highest level of mortality after 96h (25%) although throughout the test no statistical differences were found between the mortality rates of the treatment groups ($p>0.05$). Furthermore, although not quantified, it was noted that all mortalities were small fish with distinct parr markings (i.e. no silvering).

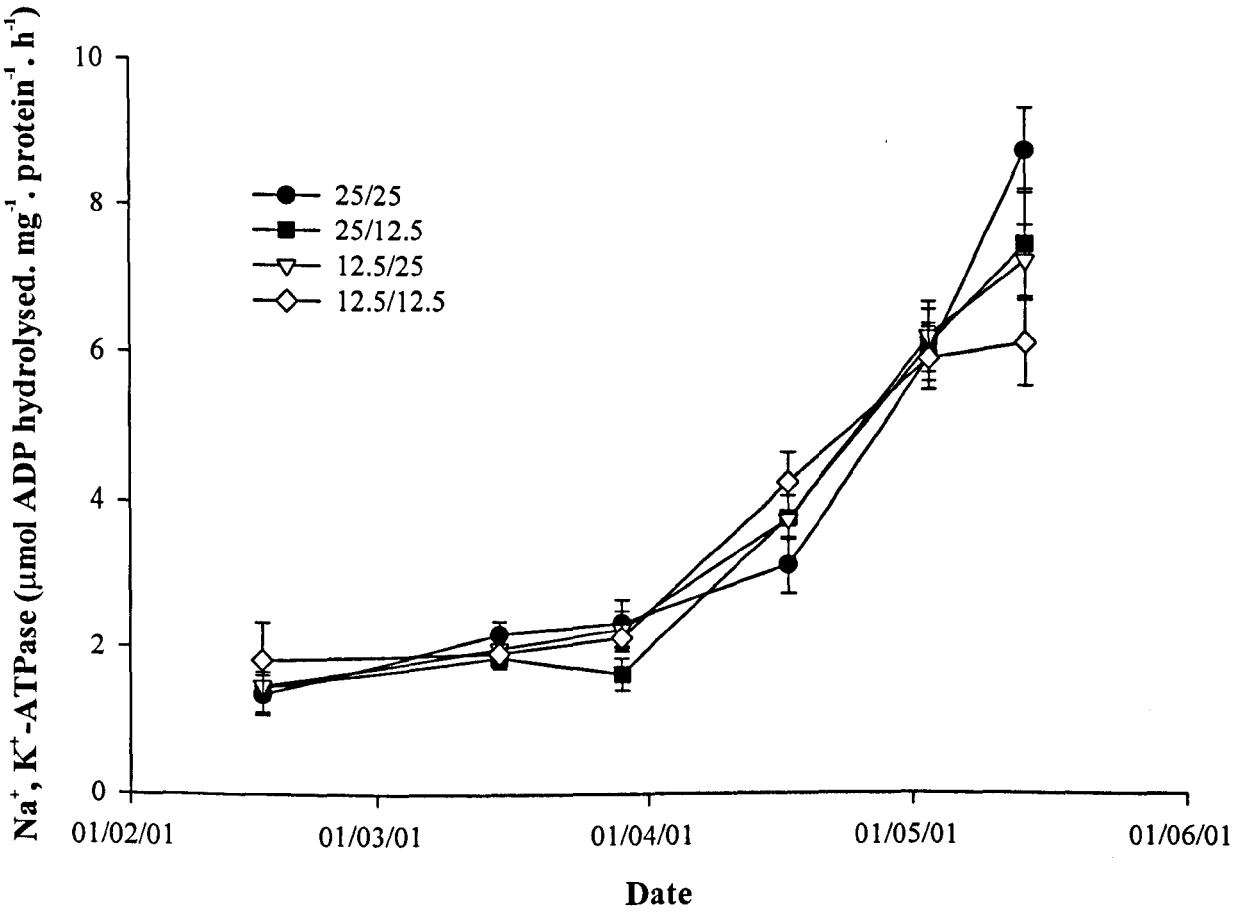


Fig. 4.11 Changes in the gill Na^+, K^+ -ATPase level (mean±S.E.M., n=15), during spring, of parr fed diets containing different levels of lipid, during different periods of development.

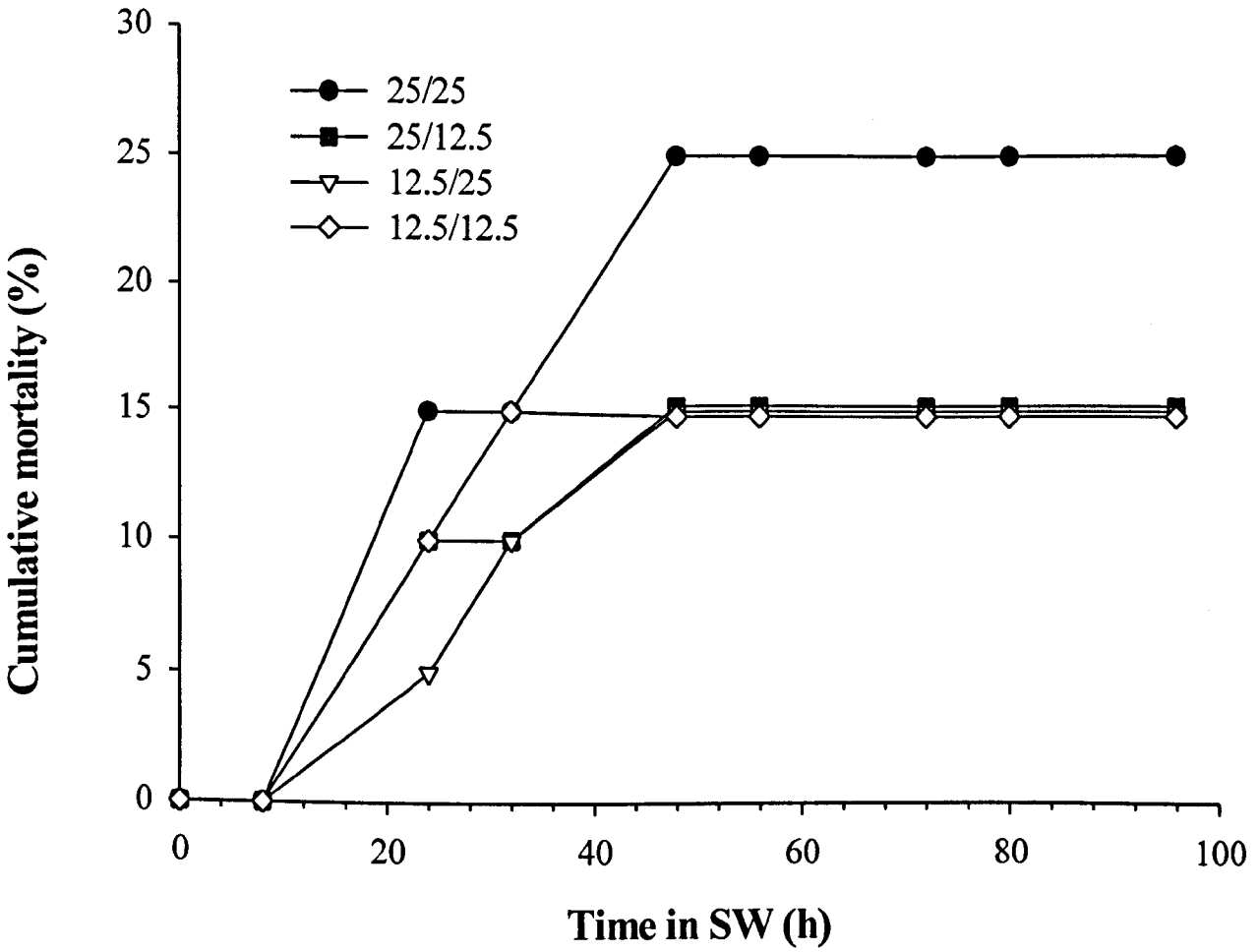


Fig. 4.12 The cumulative mortality rates of fish exposed to a 96h seawater tolerance test, after being fed diets containing different levels of lipid during different periods of development. Seawater tolerance tests were performed on 14th May 2001 (n=20).

Population structure

Dietary lipid level had only a slight effect on the incidence of 1+ smolts and parr at the conclusion of the experiment (Fig. 4.13). In all treatment groups the incidence of 1+ smolts was greater than that of parr ($p<0.05$) although there were differences between the numbers of 1+ smolts and parr within each treatment group. The number of 1+ smolts in the 25/12.5 group (69%) exceeded those within the 12.5/12.5 group (60%) whereas the incidence of parr in both the 25/25 (31%) and the 25/12.5 groups (69%) was lower than that in the 12.5/25 group (76%) ($p<0.05$).

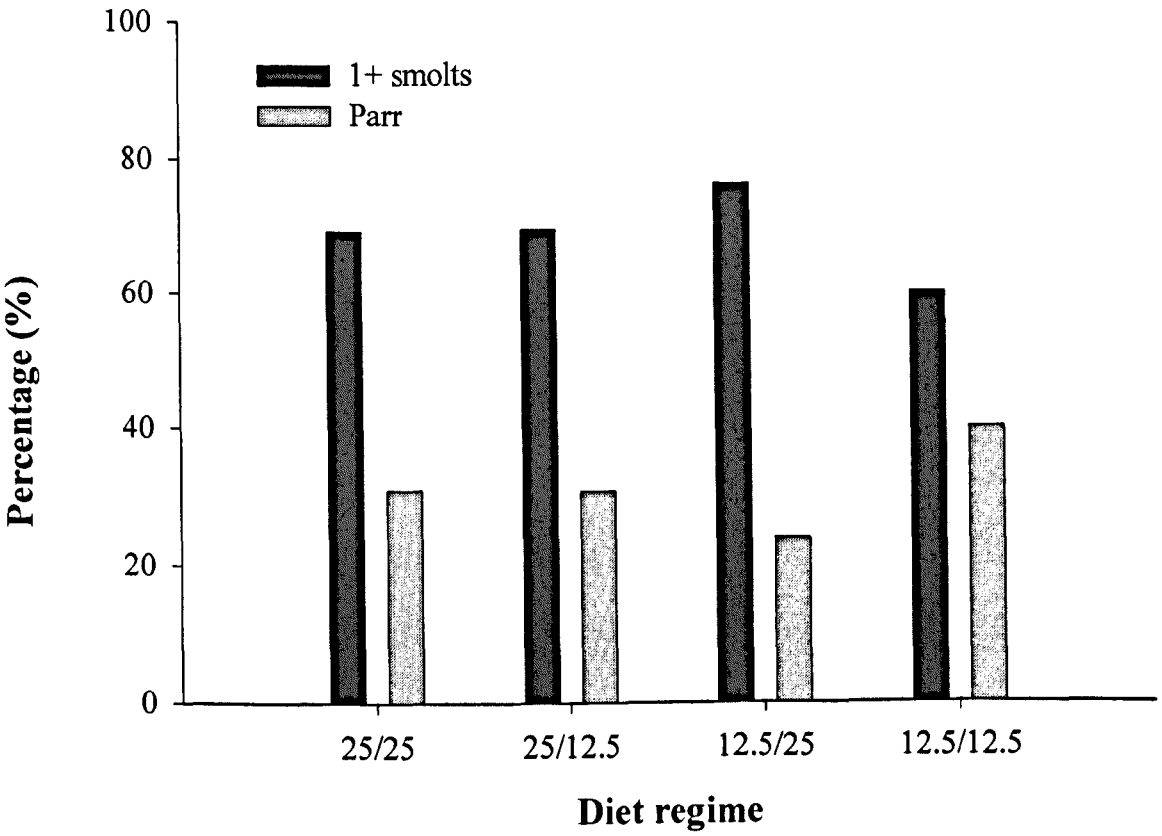


Fig. 4.13 The population structure recorded at the conclusion of the experiment where groups were fed diets containing different levels of lipid during different periods of development (n=320-440).

4.2.4. Summary of the results from Experiment IV.

- The weight and length of fish within all treatments increased during the experiment. There were no differences in the weight and length of individuals between treatments at individual time points.
- CF rose initially then declined to the end of the experiment. Fish fed the 25% lipid diet initially had the highest CF values but no consistent differences were subsequently found.
- SGR increased initially then declined up to the end of the experiment. Following the change in diet/photoperiod regime a decline in SGR occurred.
- All treatment groups developed a bimodal population structure. The 25/25 group contained some of the largest individuals whereas in the 12.5/12.5 group the LM fish were smaller than in all other groups. The emergence of bimodality occurred at an earlier date in the 12.5/12.5 group than in the other three treatments.
- Whole body lipid levels increased initially then declined up to the end of the experiment. Parr fed the high lipid diet had higher whole body lipid levels than parr fed the low dietary lipid.
- Moisture levels declined throughout the experiment. Parr fed high lipid diet had lower whole body moisture levels than the parr fed the low dietary lipid. There was a good negative correlation between whole body lipid and moisture level.
- Initially a poor correlation occurred between whole body lipid level and fish size. Subsequently the correlations improved and then decreased from 13th November up to the end of the experiment.
- Low levels of maturity were found in all treatment groups.
- Gill Na^+, K^+ -ATPase levels increased in all groups during spring. Differences between the ATPase levels of the groups was only found at the final sample point.

- Following a 96h seawater tolerance test high survival rates were recorded in all treatment groups.
- At the final sample point the incidence of 1+ smolts was greater than that of parr, in all treatment groups.

4.3. Experiment V. The effects of different rations of feed on growth, maturation and smoltification.

4.3.1. Objectives.

The experiment detailed in this section aimed to investigate the role of ration of feed on growth, maturation and smoltification. However, the interaction between photoperiod and ration was also investigated by rearing different groups under two photoperiod production regimes.

4.3.2. Experiment Va. The effects of ration of feed on growth, maturation and smoltification in 0+ production fish.

4.3.2.1. Materials and Methods.

The experiment was started at Site 6 (Section 2.1.1). Ova from a low grilsing Scottish stock were fertilised and held in heated water (6.0±1.2°C) under darkness until hatching (11th February 2001). The fry were then held under a natural photoperiod in heated water (6.1±1.9°C) until first-feeding (10th March 2001). From first-feed, fish were maintained in hatchery stock tanks (2m square, 4m^3 tanks) and exposed to LD24:0 with water heated slightly above the natural temperature regime (Fig. 4.14). From first-feed fish were supplied a commercial diet (EWOS; Scotland, UK) fed at the manufacturers' recommended rate throughout the 24h illuminated period. On 29th May 2001 3000 fish were moved to Site 7 (Section 2.1.1) with 500 individuals placed

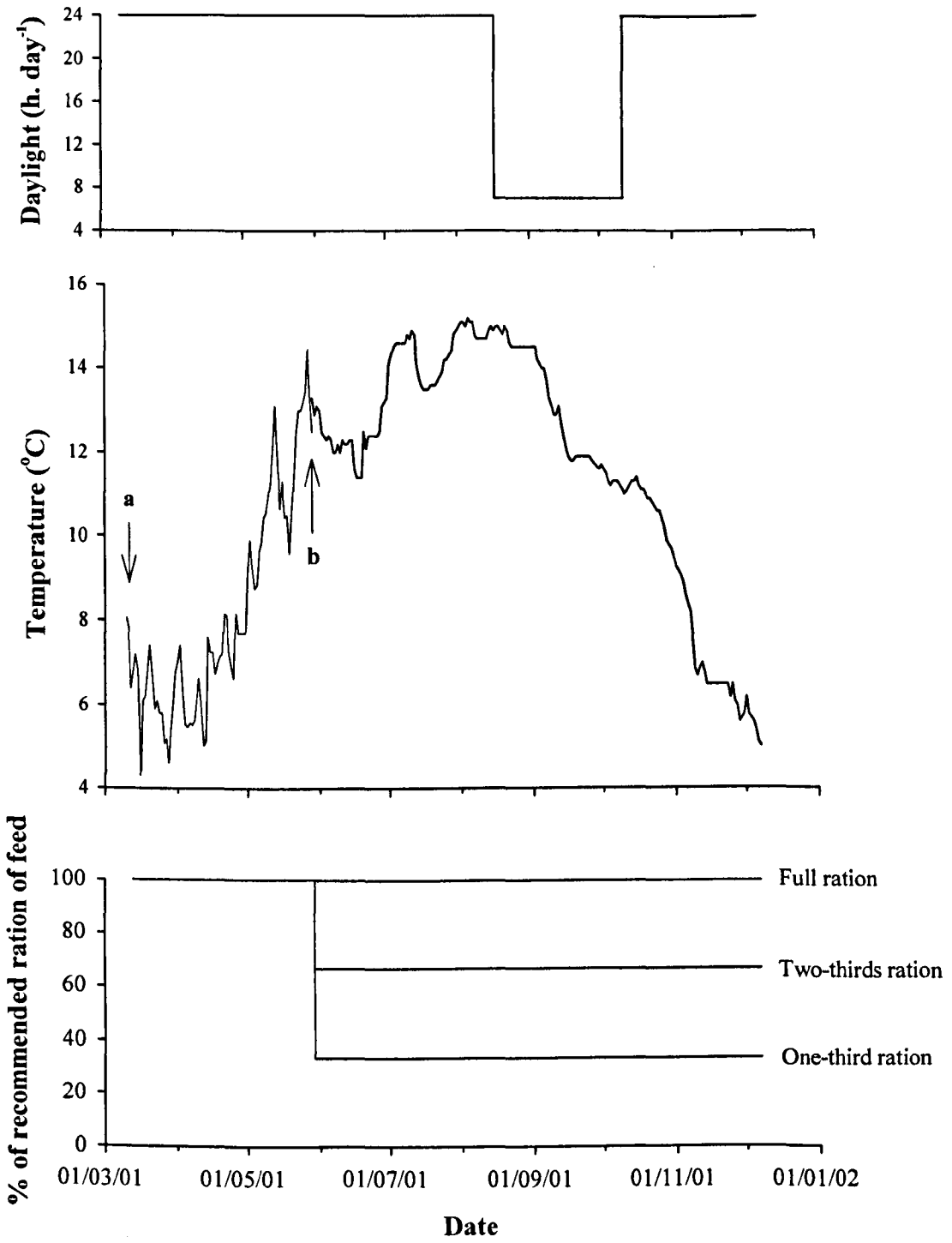


Fig. 4.14 The photoperiod, temperature and feed regime of parr during experiment Va. The time of first-feed is denoted by 'a', 'b' denotes the time when fish were moved from Site 6 to Site 7. Between a and b the ambient temperature was artificially raised.

into each of six, 1m square, 0.4m^3 tanks that were exposed to ambient temperature regimes under LD24:0 (Fig. 4.14). Duplicate groups were then fed a commercial diet (EWOS; Scotland, UK) (Table 4.4) at either full, 2/3 or 1/3 of the manufacturers' recommended daily ration throughout the 24h light period (see Fig. 4.15 for experiment protocol) with the actual weight of feed given to each tank of fish re-calculated at weekly intervals. On the 16th August 2001 all groups were exposed to an 8 week period of short days (LD7:17) during which rations were only applied during the light phase of the photoperiod. On 11th October all groups were returned to the LD24:0 regime and held for a further 8 weeks after which the experiment was terminated.

On 23rd March and 20th April 100 individual length and 6 batch weight measurements were made and then on 16th and 29th May 100 individual length and weight measurements were made prior to the fish being moved to Site 7. At twice monthly intervals from 11th June 50 individual length and weight measurements were taken per tank.

Until 29th May 6 samples were taken at each sample point for whole body lipid determination (Section 2.9). Then at twice monthly intervals from 11th June 12 samples were taken per treatment for lipid determination. Until 23rd July samples from individual fish were pooled in order to achieve the necessary dry weight to accurately perform lipid analysis; this was necessary until 27th September for the 1/3 ration group. Whilst drying the samples taken on 29th May an oven fault resulted in the tissues burning and these data were lost.

Diet size	Diet lipid levels (%)		
	Quoted level	*Actual level*	
		Mean	*S.E.M*
Crumble 1.0	16.0	17.2	0.04
Crumble 2.0	20.0	21.1	0.09
Crumble 3.0	20.0	18.2	0.18
Crumble 4.0	22.0	21.7	0.31
1mm Pellet	20.0	19.7	0.10
2mm Pellet	22.0	20.7	0.14

Table 4.4 The levels of lipid quoted as being in different sizes of the commercial diet used in the ration of feed experiment, as well as the actual levels of lipid identified following lipid determination (n=3).

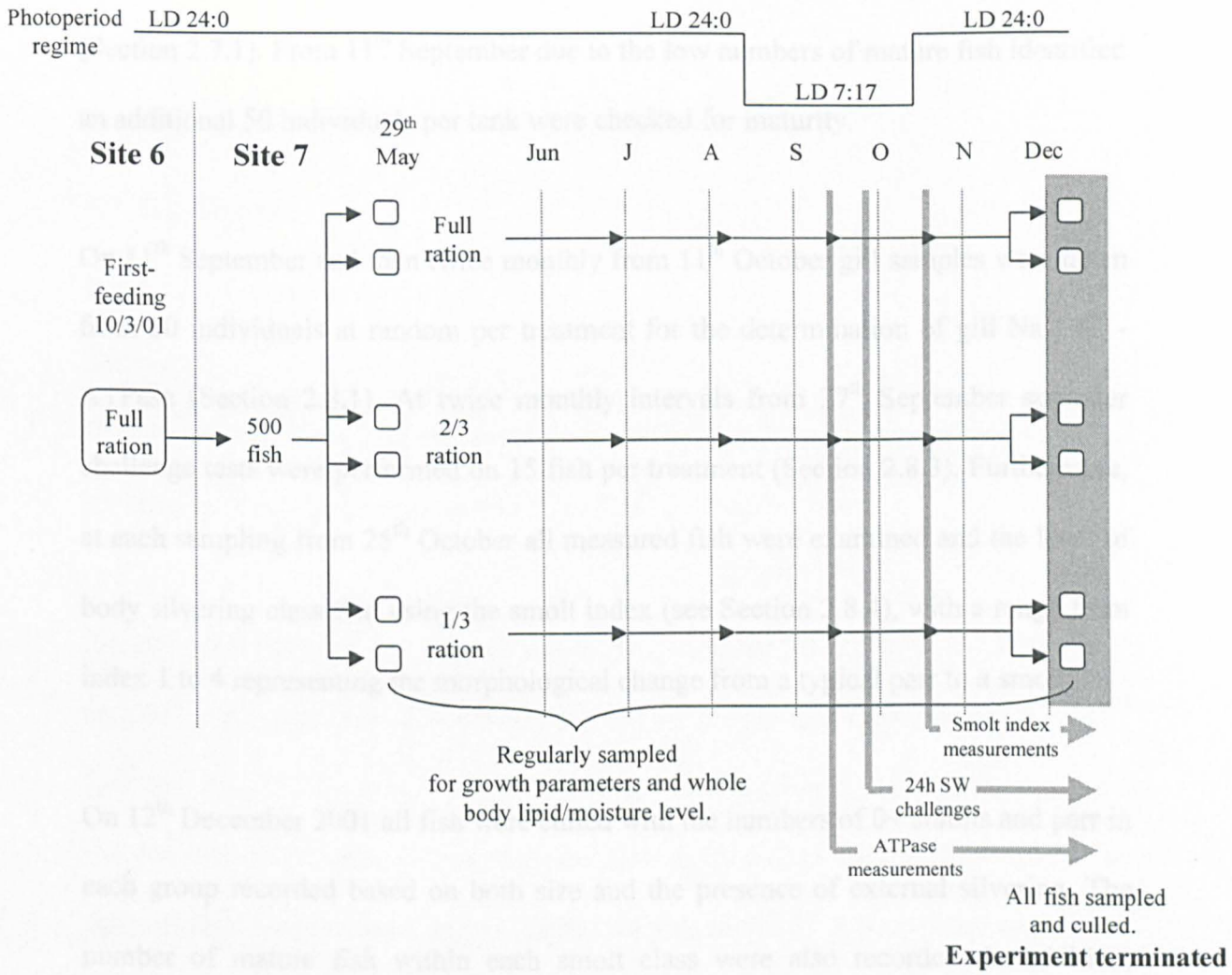


Fig 4.15 The experimental protocol used during experiment Va. For further details of the sampling regime refer to section 4.3.2.1.

At each sampling all measured fish were examined for external signs of maturation (Section 2.7.1). From 11th September due to the low numbers of mature fish identified an additional 50 individuals per tank were checked for maturity.

On 11th September and then twice monthly from 11th October gill samples were taken from 20 individuals at random per treatment for the determination of gill Na^+, K^+ - ATPase (Section 2.8.1). At twice monthly intervals from 27th September seawater challenge tests were performed on 15 fish per treatment (Section 2.8.3). Furthermore, at each sampling from 25th October all measured fish were examined and the level of body silvering classified using the smolt index (see Section 2.8.4), with a range from index 1 to 4 representing the morphological change from a typical parr to a smolt.

On 12th December 2001 all fish were culled with the numbers of 0+ smolts and parr in each group recorded based on both size and the presence of external silvering. The number of mature fish within each smolt class were also recorded. In addition, approximately 100 individuals per treatment were dissected with both the individuals' sex and internal signs of maturation recorded.

Growth data, whole body lipid level, moisture content and gill Na^+, K^+ -ATPase level were compared using a General Linear Model (Section 2.11). Natural log transformations were used to improve the normality and homogeneity of variance of the weight, length and condition factor data. Changes in serum osmolality were compared using the non-parametric Kruskal-Wallis test, with Dunn's multiple range procedure. Pearson's product moment correlation was used to compare scatter plots of whole body lipid and moisture level. For the analysis of population structure, sex

ratios, mortality and seawater survival, 95% confidence limits were calculated and compared.

4.3.2.2. Results.

4.3.2.2.1. Growth

Weight

Feeding with different rations resulted in an overall increase in the weight of all groups over the experimental period (Fig. 4.16) ($p<0.001$).

Prior to the application of different rations fish increased in weight between 20th April and 29th May. After the rations were applied all groups had similar weights on 11th June but from 25th June until the conclusion of the experiment the weight of both the full and two-thirds ration fish were greater than the one-third ration fish ($p<0.05$). The full and two-thirds ration fish had similar weights until 16th August from which time the full ration fish became heavier remaining so until the conclusion of the experiment ($p<0.01$).

Between consecutive sampling points the weight of the full and two-thirds ration fish increased until 11th and 27th September respectively ($p<0.05$) although the one-third ration group showed no consistent increases.

Length

All groups showed an overall increase in length over the experimental period (Fig 4.17) ($p<0.001$).

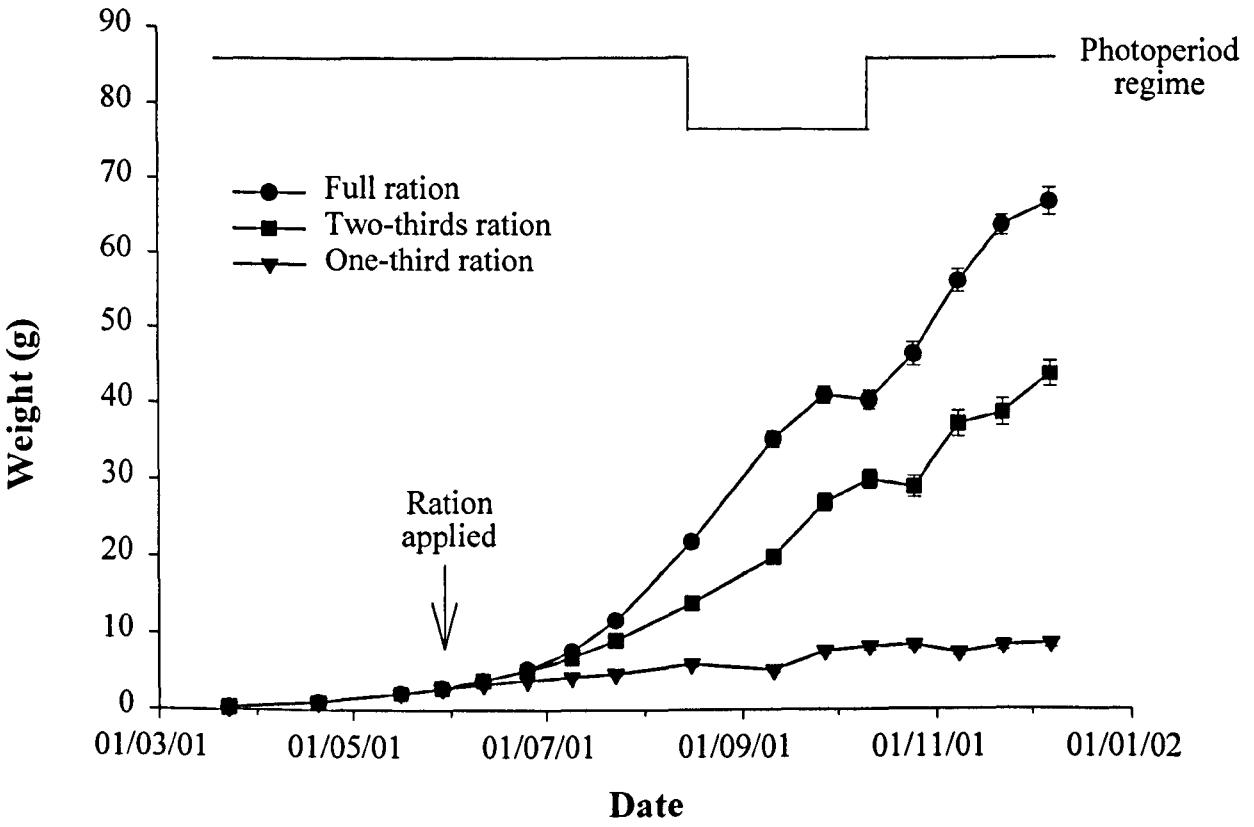


Fig. 4.16 Changes in weight (mean±S.E.M., n=100) of parr reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed.

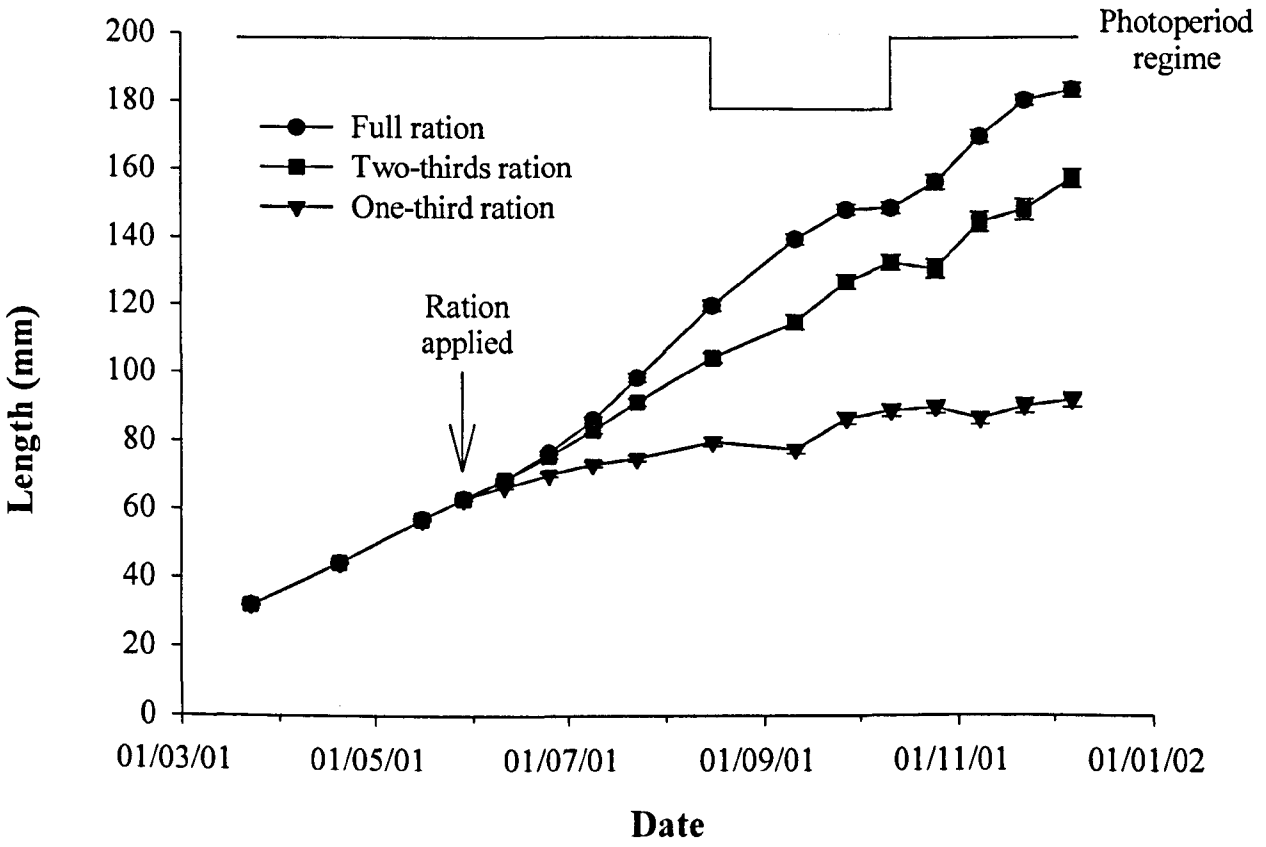


Fig. 4.17 Changes in length (mean±S.E.M., n=100) of parr reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed.

Prior to the application of the different rations the length of fish increased until 29th May. After rations were applied all groups had similar lengths on 11th June, although from 25th June and 9th July respectively until the conclusion of the experiment the full and two-thirds ration fish were longer than the one-third ration fish ($p<0.05$). The full and two-thirds ration fish had similar lengths until 16th August from which time the full ration fish were longer until the conclusion of the experiment ($p<0.01$).

Between consecutive sampling points the full and two-thirds ration fish increased in length until 11th and 27th September respectively ($p<0.05$) although no consistent increases were observed in the one-third ration group.

Condition factor

The different rations had distinct effects on the condition factor of individuals (Fig. 4.18). Prior to the application of rations CF increased until 16th May although such changes could not be analysed statistically.

After the application of rations the CF of the full ration fish increased ($p<0.01$) to peak levels on 11th September, with the two-thirds ration fish showing a peak on 27th September although overall increases up to this peak were not significant. The CF of the full and two-thirds ration fish subsequently declined to the conclusion of the experiment ($p<0.01$). The CF of the one-third ration group decreased over the experimental period ($p<0.01$).

The CF of the full and two-thirds ration fish was higher than the one-third ration fish

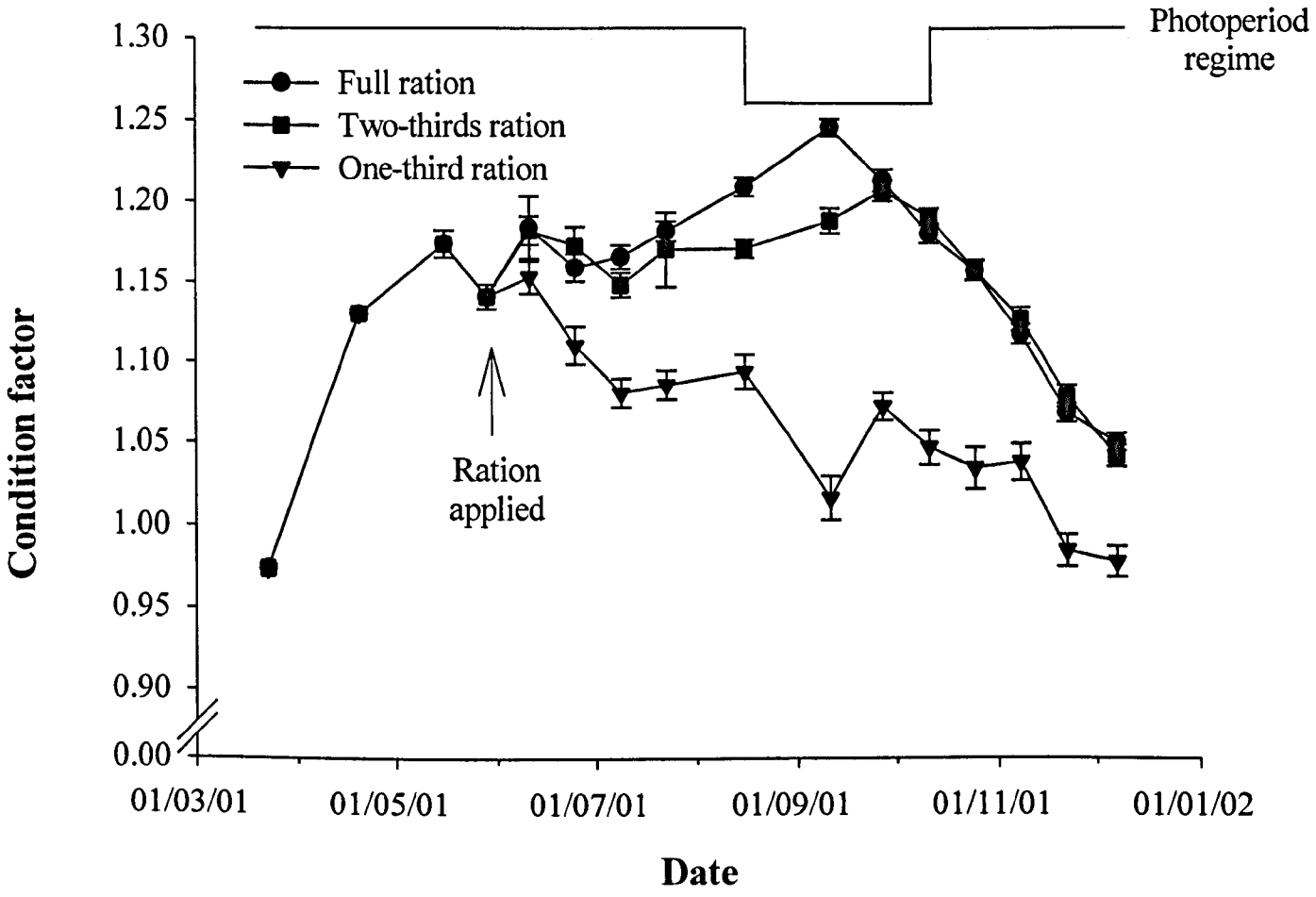


Fig. 4.18 Changes in condition factor (mean±S.E.M., n=100) of parr reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed.

from 25th June onwards ($p<0.01$) with the CF of the full and two-thirds ration groups similar throughout the experiment ($p>0.05$), with the exception on the 11th September sampling.

SGR

Because changes in SGR were calculated from the changes in mean weight statistical analyses could not be performed on the data. However, prior to the application of the different rations SGR declined rapidly (Fig. 4.19). The growth of the full ration group then increased to a peak on 23rd July after which growth rates declined. For the two-thirds ration group SGR remained fairly constant until 23rd July after which it declined. The growth of the one-third ration group decreased throughout the experiment. From September onwards all groups displayed variable, but low, growth rates.

Weight-frequency distribution

Differing rations of feed had effects on the weight-frequency distribution of the fish populations (Fig. 4.20). In both the full and two-thirds ration groups bimodality was evident by 16th August. However, in these two groups the structure of the respective modes differed slightly. The full ration group had less LM fish than the two-thirds ration population with the UM of the full ration group also covering a wider size range and containing larger fish than in the two-thirds ration group. For the one-third ration group a bimodal divide in the population did not occur at any point during the experiment.

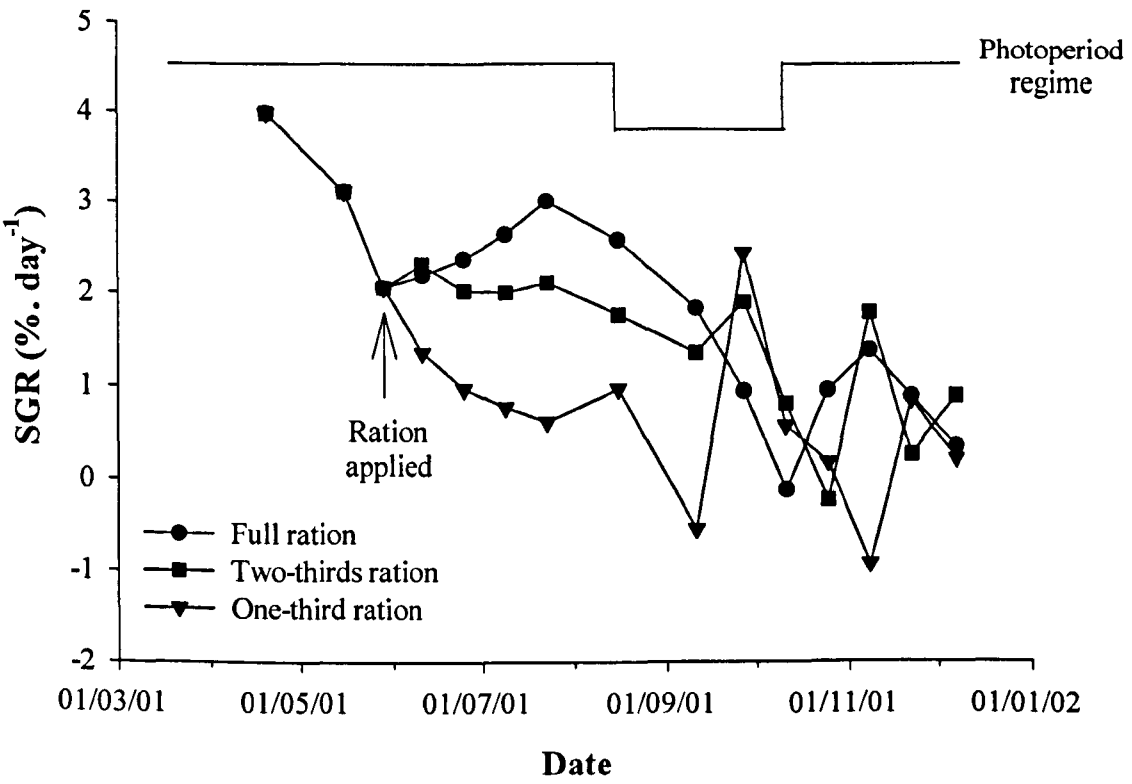


Fig. 4.19 Changes in SGR (mean±S.E.M., n=100) of parr reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed.

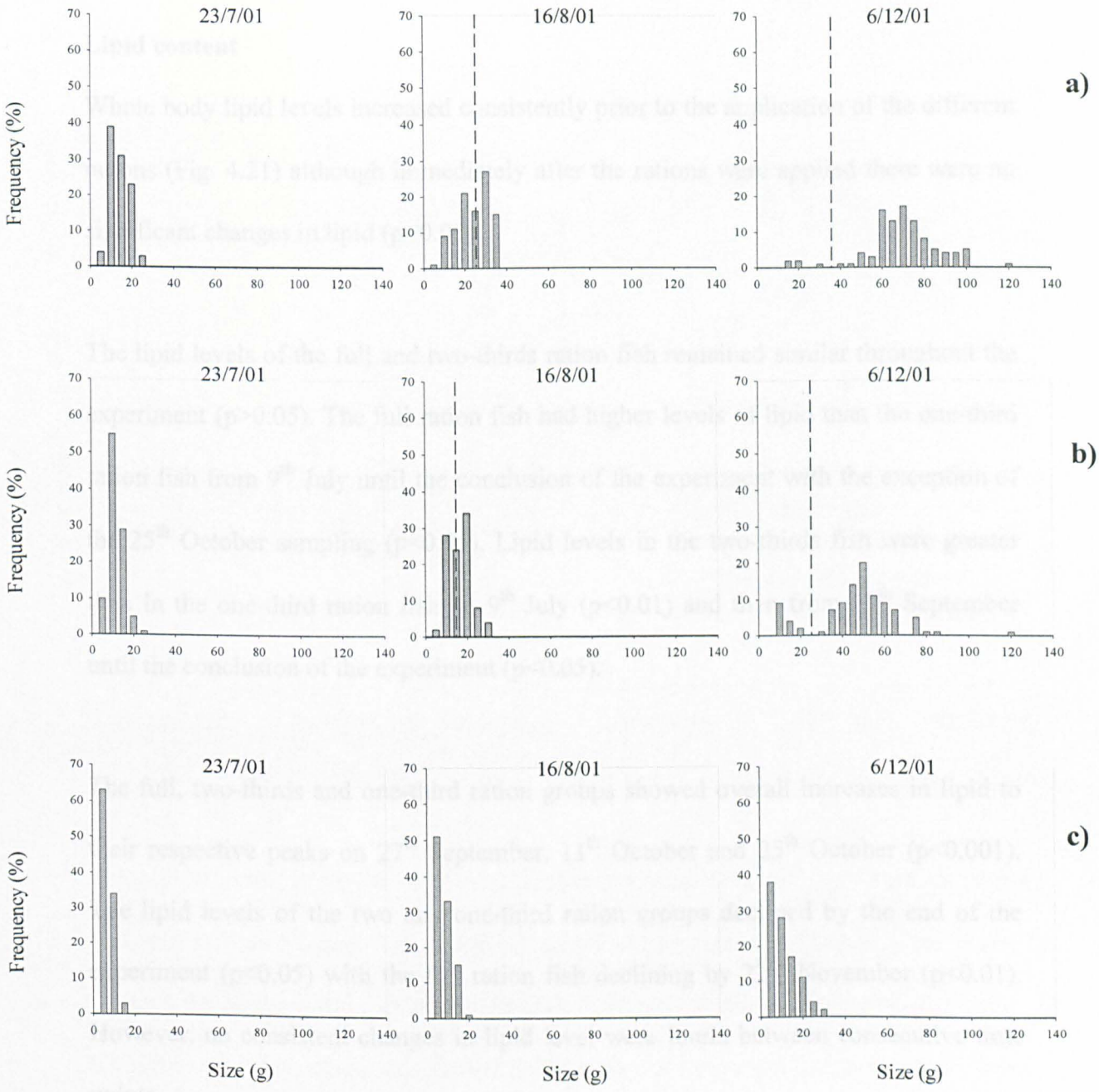


Fig. 4.20 The weight-frequency distributions of parr reared on different rations of feed, with groups grown under an 0+ photoperiod production regime (n=100). Plots represent the sample points just prior to, and at the emergence of bimodality, as well as at the final sample point. a) full ration, b) two-thirds ration, c) one-third ration.

4.3.2.2.2. Body composition

Lipid content

Whole body lipid levels increased consistently prior to the application of the different rations (Fig. 4.21) although immediately after the rations were applied there were no significant changes in lipid ($p>0.05$).

The lipid levels of the full and two-thirds ration fish remained similar throughout the experiment ($p>0.05$). The full ration fish had higher levels of lipid than the one-third ration fish from 9th July until the conclusion of the experiment with the exception of the 25th October sampling ($p<0.05$). Lipid levels in the two-thirds fish were greater than in the one-third ration fish on 9th July ($p<0.01$) and then from 27th September until the conclusion of the experiment ($p<0.05$).

The full, two-thirds and one-third ration groups showed overall increases in lipid to their respective peaks on 27th September, 11th October and 25th October ($p<0.001$). The lipid levels of the two and one-third ration groups declined by the end of the experiment ($p<0.05$) with the full ration fish declining by 22nd November ($p<0.01$). However, no consistent changes in lipid level were found between consecutive time points.

During the random sampling of fish for lipid determination one mature individual was identified in the full ration group. Although statistical analysis could not be performed it was clear that maturation had resulted in a 20% reduction in whole body lipid content compared to its full ration counterparts.

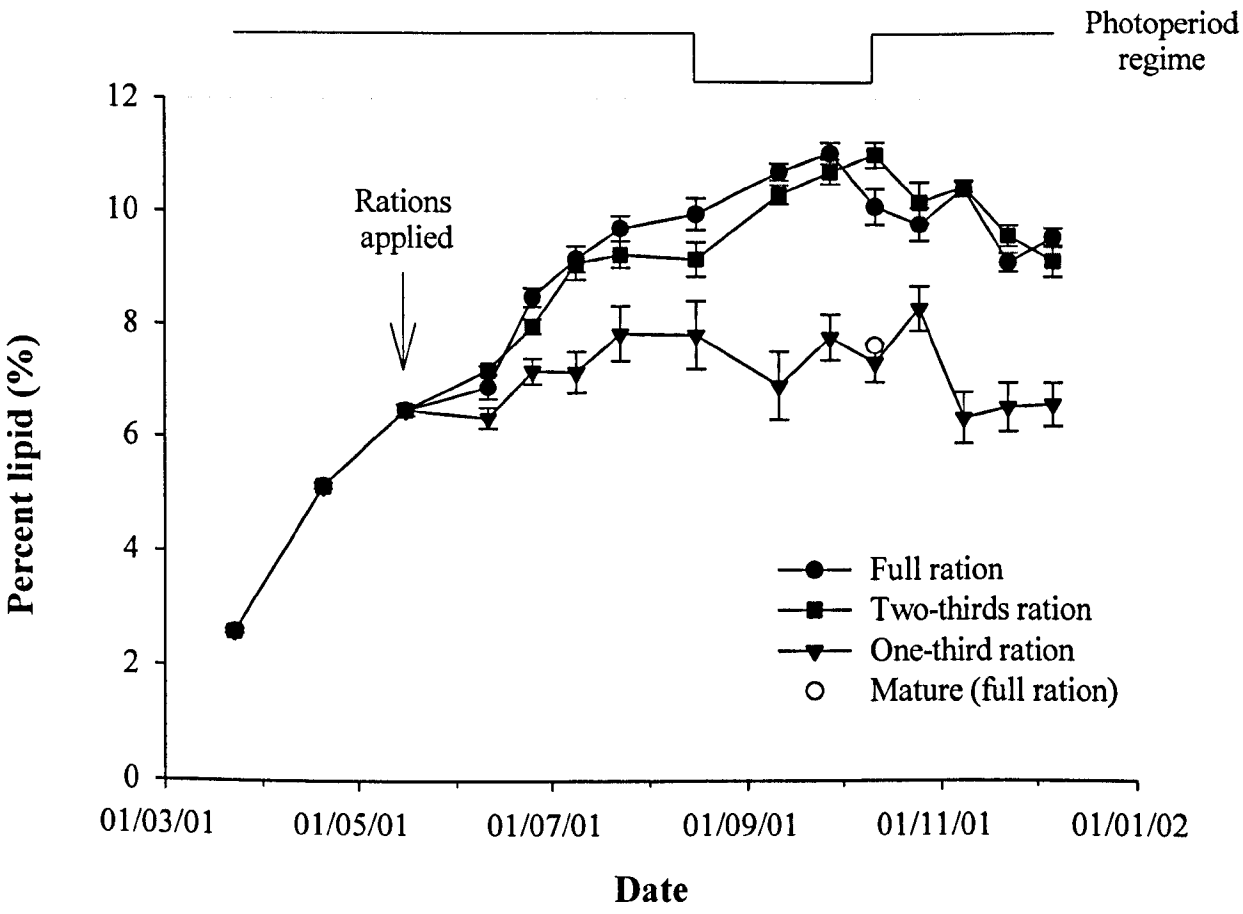


Fig. 4.21 Changes in whole body lipid content (mean±S.E.M., n=12) of parr reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed. Closed symbols represent the mean lipid content for the respective treatments, open symbols represent the lipid content of mature fish identified within the respective treatments.

Moisture content

Prior to the application of different rations whole body moisture levels declined (Fig. 4.22) although this decrease was only significant between 23rd March and 20th April ($p<0.05$).

The moisture content of the full and two-thirds ration fish remained similar throughout the experiment ($p>0.05$) with both groups having lower moisture contents than the one-third ration fish from 25th October until the conclusion of the experiment ($p<0.01$). The moisture content of the full ration fish was also lower than the one-third ration fish on 9th July ($p<0.05$).

The full, two-thirds and one-third ration groups showed overall decreases in moisture content to their respective minimum levels on 27th September, 11th October and 16th August respectively ($p<0.001$) although subsequent increases were not significant by the conclusion of the experiment. Between consecutive time points all groups showed a reduction in moisture content between 11th and 25th June ($p<0.01$) with the one-third ration group showing a significant increase between 25th October and 11th November ($p<0.05$).

The mature fish identified in the full ration group had a 5% increase in moisture content.

Lipid/Moisture correlation

When the lipid content of individual fish was plotted against their respective moisture content for each treatment but regardless of sampling time good linear regressions

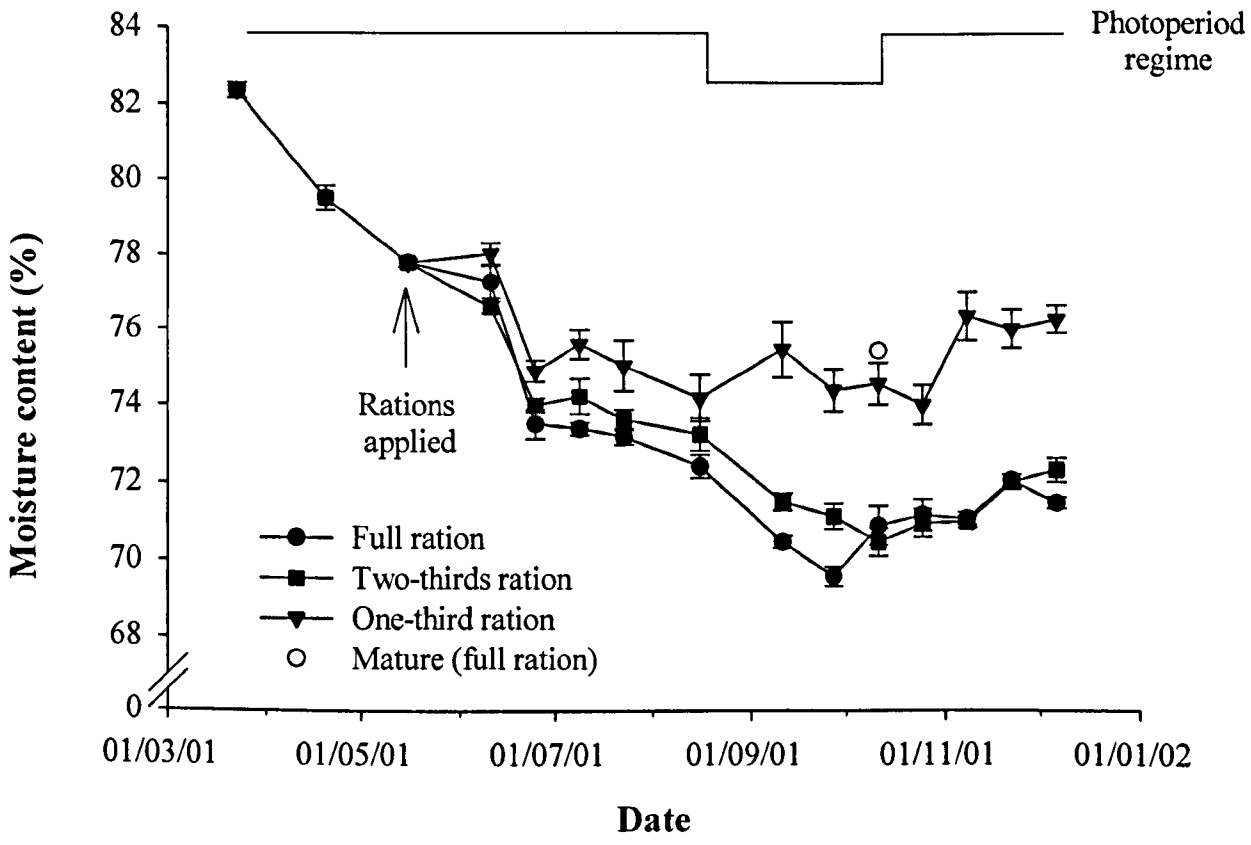


Fig. 4.22 Changes in whole body moisture content (mean±S.E.M., n=12) of parr reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed. Closed symbols represent the mean moisture content for the respective treatments, open symbols represent the moisture content of mature fish identified within the respective treatments.

were achieved (Fig. 4.23). Furthermore, for each ration group the correlation between lipid and moisture level was found to be highly statistically significant ($p<0.001$).

Lipid/Size correlation

The relationship between changing size and whole body lipid content was investigated in a similar way to that described in experiment IV. Therefore, the changing r^2 values of fish exposed to the different rations have been presented (Table. 4.5).

Highly variable r^2 values were found throughout the experiment for all ration groups with no consistent trends identified. However, the one-third ration fish generally displayed the highest r^2 values throughout the experiment.

4.3.2.2.3. Maturation

Incidence

Throughout the experiment the incidence of maturity was extremely low (≤1%) (Table 4.6) and consequently statistical analyses could not be performed. No mature female parr were found with mature males only present in the full and two-thirds ration groups. In the full ration treatment mature fish were first observed on 11th September and they remained until the conclusion of the experiment. In the two-thirds ration group one mature fish was identified on 25th October although mature individuals were only found until 25th November.

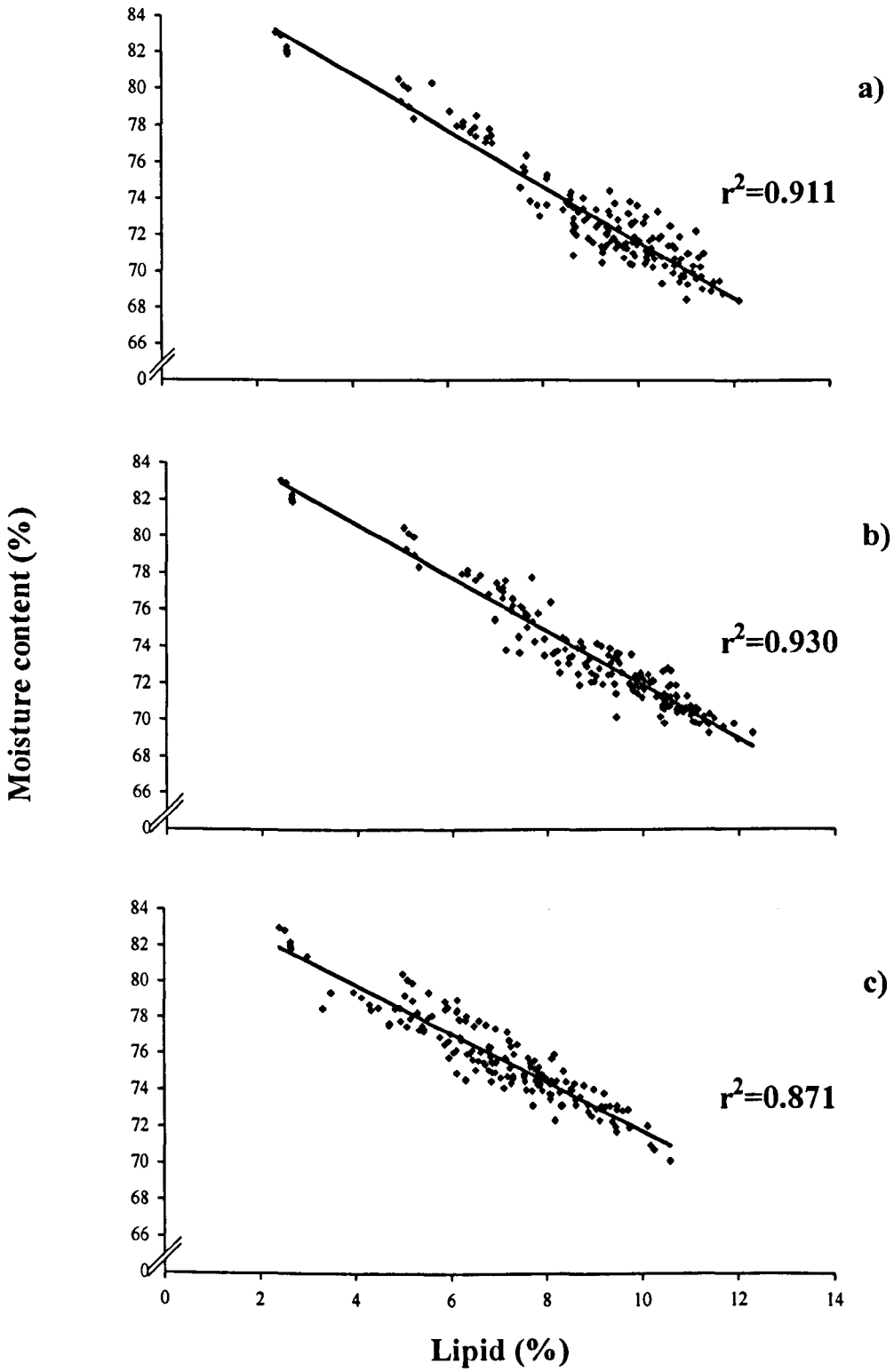


Fig. 4.23 The correlation between whole body lipid level and moisture content in parr reared on different rations of feed during the course of experiment Va (n=160). a) full ration, b) two-thirds ration, c) one-third ration.

Date	Ration of feed		
	Full	*Two-thirds*	*One-third*
11/06/01	0.418	0.140	0.570
25/06/01	0.166	0.660	0.338
09/07/01	0.322	0.650	0.766
23/07/01	0.755	0.156	0.519
16/08/01	0.516	0.492	0.836
11/09/01	0.013	0.455	0.593
27/09/01	0.375	0.317	0.673
11/10/01	0.327	0.309	0.702
25/10/01	0.280	0.667	0.598
08/11/01	0.133	0.389	0.478
22/11/01	0.280	0.072	0.499
06/12/01	0.231	0.141	0.726

Table 4.5 The changing r^2 values of linear regressions between the weight and whole body lipid level of parr, reared on different rations of feed during the course of experiment Va (n=12). Groups were grown under a 0+ photoperiod production regime.

Date	Incidence of maturation (%) *Ration of feed*		
	Full	*Two-thirds*	*One-third*
11/09/01	1.0	0	0
27/09/01	0.5	0	0
11/10/01	1.0	0	0
25/10/01	0.5	0.5	0
08/11/01	0	0.5	0
25/11/01	0	0.5	0
06/12/01	0.5	0	0

Table 4.6 The incidence of maturity of parr reared on different rations of feed, during the course of experiment Va (n=200).

4.3.2.2.4. Smoltification

Gill Na^+, K^+ -ATPase

During the period in which gill Na^+, K^+ -ATPase was measured it was possible to identify individuals destined to develop as smolts and those which would remain as parr (this prediction was based primarily on fish size). Consequently the gill Na^+, K^+ -ATPase profiles of fish destined to smolt and remain as parr have been plotted separately for each treatment group (Fig. 4.24).

All smolting fish showed an overall increase in gill Na^+, K^+ -ATPase over the experimental period ($p<0.05$) whilst those remaining as parr showed no overall increases ($p>0.05$). Between consecutive time points increases were only observed in the smolts from the full and two-thirds ration groups. Full ration smolts increased from 25th October onwards ($p<0.01$) with those from the two-thirds ration group increasing from 25th October until 22nd November ($p<0.05$).

The gill Na^+, K^+ -ATPase of all fish were initially the same but by the conclusion of the experiment on 6th December the levels found in the full ration smolts exceeded those of all other groups with the ATPase activities of the two-thirds ration smolts higher than the one-third ration smolts ($p<0.05$).

Serum osmolality

Serum osmolality was measured following a 24h seawater (35‰) challenge (Fig. 4.25a). However, it is important to first consider the survival rates of fish fed the different rations (Fig. 4.25b) in order that the changes in osmolality can be viewed in context. Seawater survivals in the full and two-thirds ration fish were similar

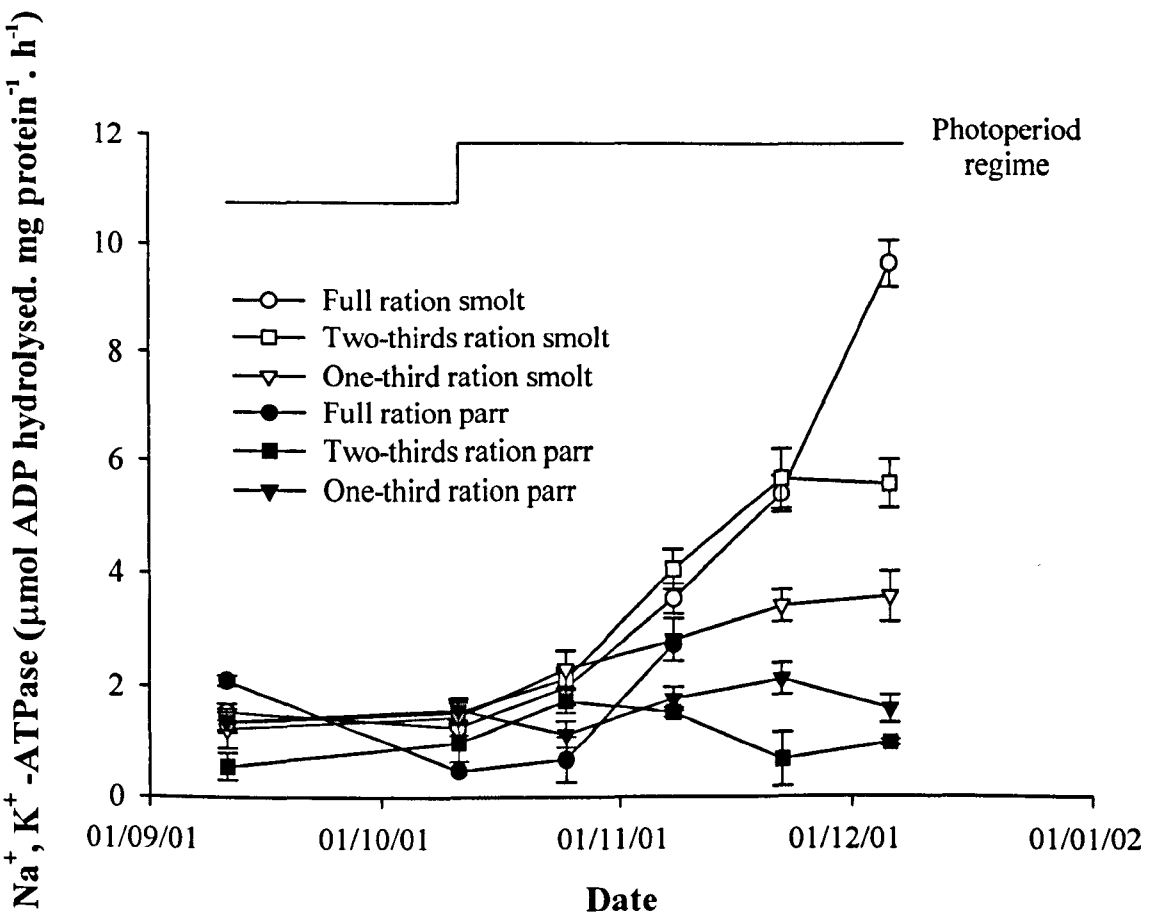


Fig. 4.24 Changes in gill Na^+, K^+ -ATPase (mean±S.E.M., n=20) of fish reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed. The gill Na^+, K^+ -ATPase of fish developing as smolts and those remaining as parr are plotted separately for each treatment group.

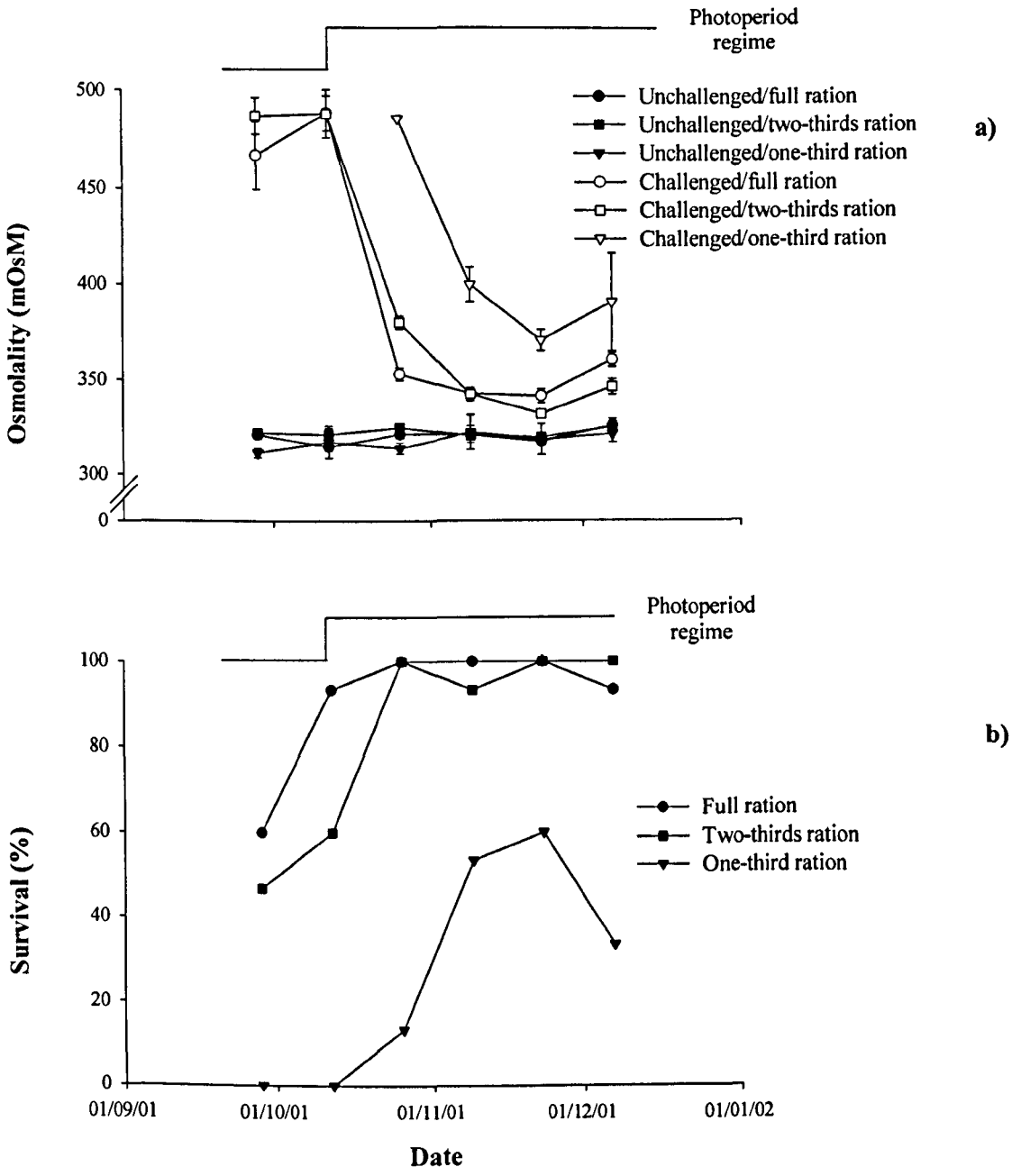


Fig. 4.25 Changes in serum osmolality (mean±S.E.M., n=1 to 15) (a) and the percentage survival (b) of fish following a 24h seawater (35‰) challenge. Individuals were previously reared on different rations of feed during the course of experiment Va. Groups were grown under a 0+ photoperiod production regime, with the regime of daylength displayed. Serum osmolalities were measured in fish surviving the 24h challenge. Due to the low survival rates of the one-third ration fish, some osmolality measurements are absent.

throughout the experiment and higher than that of the one-third ration group. Survival rates in these two groups were initially between 50 and 60% although levels rapidly increased to between 95 and 100% remaining so until the conclusion of the experiment. For the one-third ration fish surviving individuals were only present from 25th October with total survival rates never exceeding 60%. Due to this low survival of fish the subsequent analysis of the serum osmolality data must be viewed with caution.

The serum osmolality of challenged fish from the full and two-thirds ration groups was initially high (approximately 475mOsM) and significantly greater than unchallenged controls (approximately 320mOsM) ($p<0.05$). After the return to continuous light from the short day winter photoperiod the serum osmolality of challenged fish rapidly declined but remained higher than the control fish ($p<0.05$) until 22nd November. On 22nd November the challenged full and two-thirds ration fish had similar osmolalities to at least one of the control groups. By 6th December all challenged fish showed a slight increase in osmolality, although the two third ration fish had similar osmolalities to the unchallenged controls ($p>0.05$).

The serum osmolality of the one-third ration fish was only similar to controls on 6th December.

Smolt index

Ration of feed affected the smolt index scores that were recorded during the parr-smolt transformation (Fig. 4.26). The smolt index (see Section 2.8.4) ranged from 1 to 4, where smolt index 1 was a typical parr and smolt index 4 was a typical smolt.

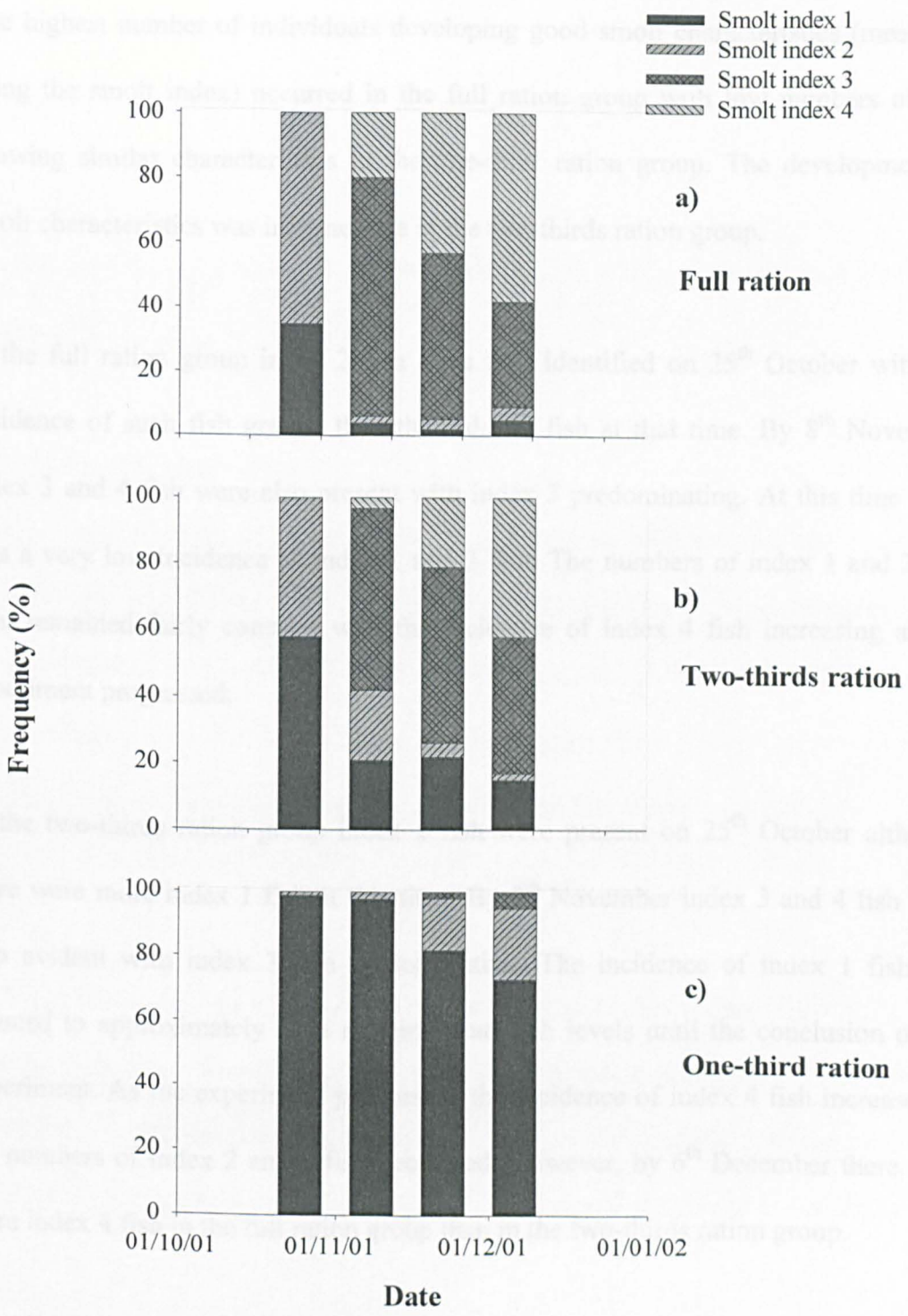


Fig. 4.26 Changes in the smolt index score of fish reared on different rations of feed during the course of experiment Va (n=100). a) full ration, b) two-thirds ration, c) one-third ration. Smolt index 1 = typical parr, with parr marks clearly visible, Smolt index 2 = parr marks visible, but some silvering, smolt index 3 = silvered with visible parr marks, Smolt index 4 = typical smolt, no parr marks visible.

The highest number of individuals developing good smolt characteristics (measured using the smolt index) occurred in the full ration group with low numbers of fish showing similar characteristics in the one-third ration group. The development of smolt characteristics was intermediate in the two-thirds ration group.

In the full ration group index 2 fish were first identified on 25th October with the incidence of such fish greater than the index 1 fish at that time. By 8th November index 3 and 4 fish were also present with index 3 predominating. At this time there was a very low incidence of index 1 and 2 fish. The numbers of index 1 and 2 fish then remained fairly constant with the incidence of index 4 fish increasing as the experiment progressed.

In the two-thirds ration group index 2 fish were present on 25th October although there were more index 1 fish at this time. By 8th November index 3 and 4 fish were also evident with index 3 fish predominating. The incidence of index 1 fish had reduced to approximately 20% remaining at such levels until the conclusion of the experiment. As the experiment progressed the incidence of index 4 fish increased as the numbers of index 2 and 3 fish decreased. However, by 6th December there were more index 4 fish in the full ration group than in the two-thirds ration group.

In the one-third ration group index 2 and 3 fish were first evident in low numbers on 22nd November, although the incidence of these fish had only increased slightly by the conclusion of the experiment. No index 4 fish were found in this group.

4.3.2.2.5. Population structure

At the conclusion of the experiment the total population structure was analysed:

Sex ratios

Similar numbers of males and females were found within each treatment ($p>0.05$) (Table 4.7).

Life history strategy

Similar high numbers of 0+ smolts were observed in both the full and two-thirds ration groups (95.8% and 87.0% respectively) (Table 4.7) with lower numbers in the one-third ration group (42.3%) ($p<0.05$). Furthermore, in both full and two-thirds ration treatments the numbers of 0+ smolts was significantly higher than parr ($p<0.05$) with significantly more parr present in the one-third ration group ($p<0.05$).

Survival

Different rations had an overall effect on the survival of individuals (Table 4.7). High survival rates were found in the full and two-thirds ration groups (93.5% and 93.8% respectively) with the one-third ration fish having a significantly lower survival rate (80.4%) ($p<0.05$). However, it was not possible to record the size of the mortalities.

Ration level	Sex (%)		Population structure (%)				Survival (%)
			0+ smolts		*Parr*		
	Male	*Female*	*imm*	*mat*	*imm*	*mat*	
Full	54.0 ^{Aa}	46.0 ^{Aa}	95.8 ^{Aa}	0.0 ^{Ab}	2.8 ^{Ac}	1.4 ^{Ac}	93.5 ^{A}
Two thirds	51.0 ^{Aa}	49.0 ^{Aa}	87.0 ^{Aa}	0.0 ^{Ab}	12.2 ^{Bc}	0.9 ^{Ad}	93.8 ^{A}
One third	51.0 ^{Aa}	49.0 ^{Aa}	42.3 ^{Ba}	0.0 ^{Ab}	57.7 ^{Cc}	0.0 ^{Bb}	80.4 ^{B}

Table 4.7 The population structure, sex ratio and survival of individuals recorded at the conclusion of experiment Va, where fish, under a 0+ production regime, were reared on different rations of feed (for population structure n=250-450, sex ratio n=100, survival n=750). *imm* denotes immature fish, *mat* denotes mature fish. Similar lettering denotes statistical similarity ($p<0.05$). Capital lettering denotes differences between treatment groups, lower case lettering denotes differences within treatments.

4.3.2.3. Summary of the results from Experiment Va.

- Fish fed full and two-thirds rations showed initial increases in length and weight becoming longer and heavier than the one-third ration fish soon after the different rations were applied. The full ration fish then became longer and heavier than the two-thirds ration fish.
- The CF of the full and two-thirds ration groups remained similar throughout the experiment increasing initially and subsequently declining. The lowest CF values were found in the one-third ration fish with condition declining throughout the experiment.
- Initially the SGR of the full ration fish increased with that of the two-thirds ration fish remaining stable and the growth of the one-third ration fish declining. From September all groups displayed variable but relatively unchanged growth rates.
- Full and two-thirds ration groups developed bimodal distributions at the same time although the proportion of LMG fish was higher in the two-thirds ration group. Fish fed one-third ration exhibited a unimodal distribution throughout the experiment.
- The whole body lipid level of all groups initially increased with a subsequent decline. The levels recorded in the full and two-thirds ration fish remained similar throughout with lower levels found in the one-third ration fish.
- Moisture levels initially declined in all groups with a subsequent rise. Full and two-thirds ration groups had lower moisture levels than the one-third ration fish.
- There was a significant negative correlation between whole body lipid level and moisture content in all groups.
- No clear relationship was found between whole body lipid level and fish size.

- Mature fish were only identified in the full and two-thirds ration group although levels of maturity were low throughout the experiment.
- All smolting fish showed an increase in gill Na^+, K^+ -ATPase. The full and one-third ration smolts had the highest and lowest enzyme activities respectively, with intermediate levels in the two-thirds ration smolts.
- Following a seawater challenge the one-third ration group had poor survival rates. The subsequent osmolalities of surviving individuals from all groups decreased to similar levels as unchallenged controls.
- At the conclusion of the experiment the full ration group had more fish displaying well developed smolt characteristics (i.e. smolt index 4 fish) than the two-thirds ration groups. One-third ration resulted in low numbers of fish that were showing some signs of developing smolt characteristics (smolt index 2 and 3) with such fish developing later than in the other treatment groups.
- At the conclusion of the experiment the full and two-thirds ration groups comprised of mainly 0+ smolts with the one-third ration group having more parr than 0+ smolts.

4.3.3. Experiment Vb. The effects of ration of feed on growth, maturation and smoltification in 1+ production fish.

4.3.3.1. Materials and Methods.

The experiment was started at Site 6 (Section 2.1.1). Ova from a low grilsing Scottish stock were fertilised and held in heated water (6.0±1.2°C) under darkness until hatching (28th February 2001). The fry were then held under a natural photoperiod in heated water (6.1±1.9°C) until first-feeding (22nd April 2001). At first-feed 2500 fish were placed into each of three 1m square, 0.4m^3 tanks and exposed to LD24:0, with water heated slightly above the natural temperature regime (Fig. 4.27). From first-feed fish were fed a commercial diet (EWOS; Scotland, UK) (Table 4.4) at either full, 2/3 or 1/3 of the manufacturers' recommended daily ration throughout the 24h light period, with the actual weight of feed given to each tank of fish re-calculated at weekly intervals. On 28th June 2001 fish were moved to Site 7 (Section 2.1.1) with each treatment group randomly divided into two, 1m square, 0.4m^3 tanks and exposed to an ambient temperature regime and a simulated natural photoperiod (Fig. 4.27). The duplicated treatments were maintained on their respective rations of feed with feed supplied during the light phase of the photoperiod (see Fig. 4.28 for experiment protocol).

On 20th April, 16th May and 11th June, 100 individual length and 6 batch weight measurements were made. Then at monthly intervals from 28th June 50 individual length and weight measurements were taken per tank.

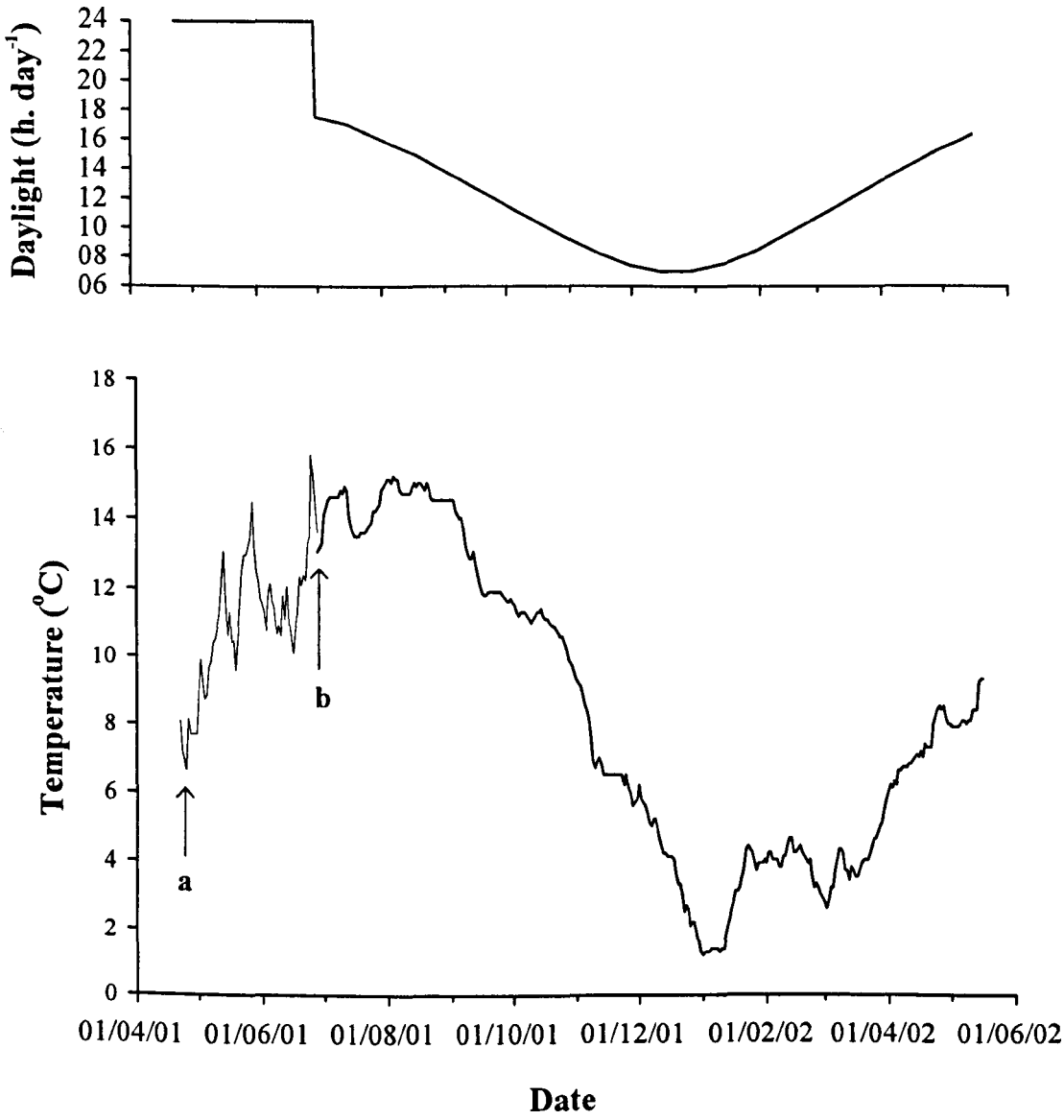


Fig. 4.27 Photoperiod, temperature and feed regime of parr during experiment Vb, where groups were fed different rations of feed. The time of first-feeding is denoted by 'a', ' b' denotes the time when fish were moved from Site 6 to Site 7. Between a and b the ambient temperature was raised by artificial heating.

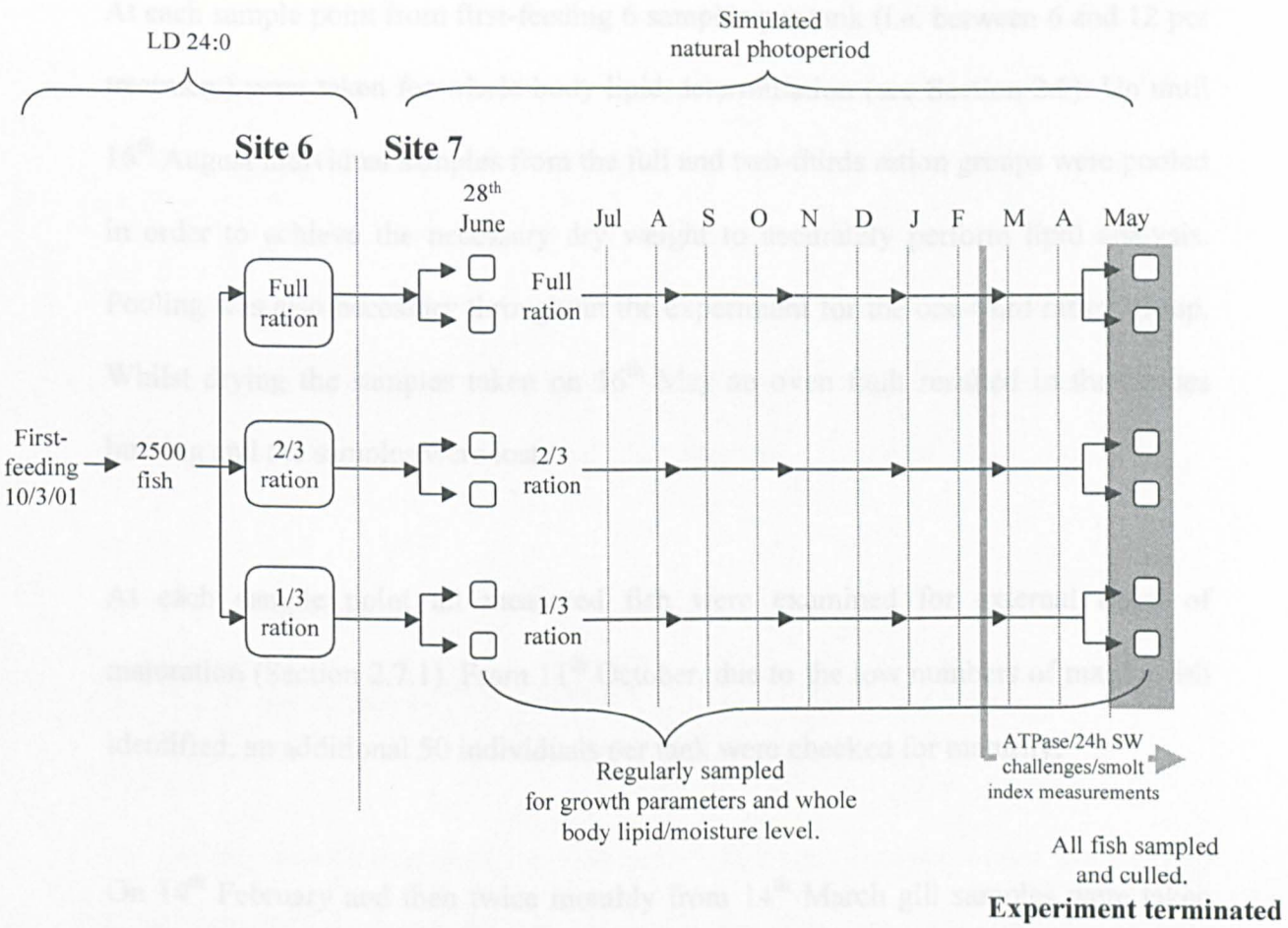


Fig. 4.28 The experimental protocol used during experiment Vb. For further details of the sampling regime refer to section 4.3.3.1.

At each sample point from first-feeding 6 samples per tank (i.e. between 6 and 12 per treatment) were taken for whole body lipid determination (see Section 2.9). Up until 16th August individual samples from the full and two-thirds ration groups were pooled in order to achieve the necessary dry weight to accurately perform lipid analysis. Pooling was also necessary throughout the experiment for the one-third ration group. Whilst drying the samples taken on 16th May an oven fault resulted in the tissues burning and the samples were lost.

At each sample point all measured fish were examined for external signs of maturation (Section 2.7.1). From 11th October, due to the low numbers of mature fish identified, an additional 50 individuals per tank were checked for maturity.

On 14th February and then twice monthly from 14th March gill samples were taken from 20 individuals at random per treatment for the determination of gill Na^+, K^+ - ATPase (Section 2.8.1), with seawater challenge tests also performed on 15 fish per treatment (Section 2.8.3). At twice monthly intervals from 14th February 100 fish per treatment were examined and classified for external smolt appearance using the smolt index (Section 2.8.4), with the range from index 1 to 4 representing the morphological change from a typical parr to a smolt.

On 15th May 2002 all fish were culled with the numbers of 1+ smolts and parr in each group recorded based on both size and the presence of external silvering. The total number of mature fish within each smolt class was also recorded. Furthermore, approximately 100 individuals per treatment were dissected with the individuals, sex and internal signs of maturation recorded.

Growth data, whole body lipid level, moisture content, gill Na^+, K^+ -ATPase and serum osmolality were compared using a General Linear Model (Section 2.11), although for changes in weight and length a natural log transformation was used to improve normality and homogeneity of variance. Pearson's product moment correlation was used to compare scatter plots of whole body lipid and moisture level. For the analysis of population structure, sex ratios, mortality and seawater survival, 95% confidence limits were calculated and compared.

4.3.3.2. Results.

4.3.3.2.1. Growth

Weight

All groups showed an overall increase in weight over the experimental period (Fig. 4.29) ($p<0.001$). Between consecutive time points increases were only observed for the full and two-thirds ration groups. Full ration fish increased between 28th June and 11th September ($p<0.01$) with the two-thirds ration fish increasing between 28th June and 16th August ($p<0.01$) and then between 11th September and 11th October ($p<0.05$).

Both full and two-thirds ration fish became heavier than one-third ration fish from 23rd July onwards ($p<0.05$) with those fed the full ration then heavier than the two-thirds ration fish from 16th August until the conclusion of the experiment ($p<0.01$).

Length

All groups showed an overall increase in length over the experimental period (Fig. 4.30) ($p<0.001$). Between consecutive time points all groups increased in length until 11th June ($p<0.01$). Subsequently, the length of fish increased from 28th June until 11th

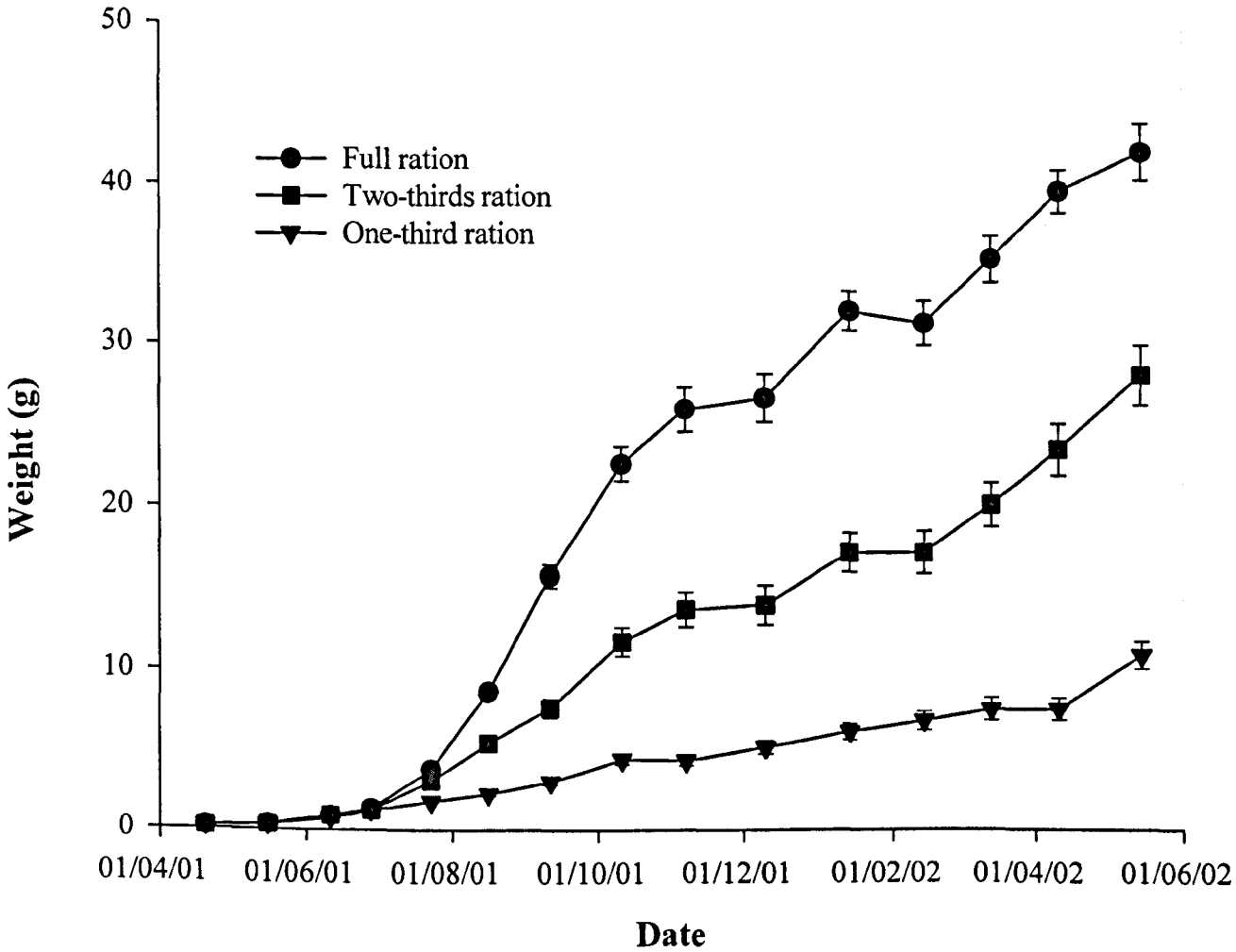


Fig. 4.29 Changes in weight (mean±S.E.M., n=100) of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime.

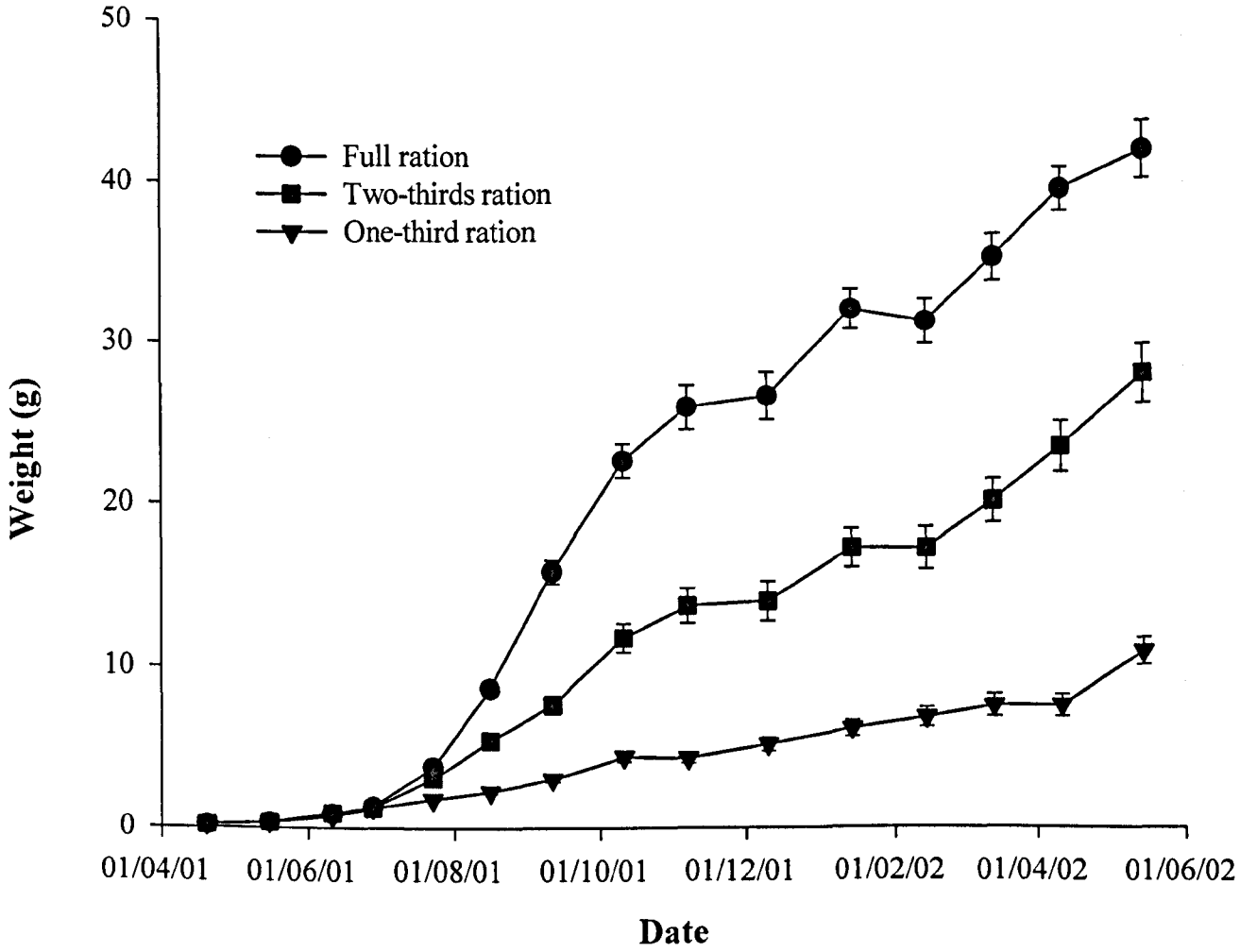


Fig. 4.30 Changes in length (mean±S.E.M., n=100) of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime.

September, 16th August and 23rd July for the full, two-thirds and one-third ration groups respectively ($p<0.01$), with the two-thirds ration fish also increasing between 11th September and 11th October ($p<0.01$).

Both the full and two-thirds ration fish were longer than one-third ration fish from 23rd July onwards with the full ration fish longer than the two-thirds ration fish from 16th August until the conclusion of the experiment ($p<0.01$).

Condition factor

Condition factor showed an initial increase in all groups peaking on 23rd July for the one-third ration fish and then on 16th August for the full and two-thirds ration groups ($p<0.001$) (Fig. 4.31). Subsequently the CF of all groups declined up until the conclusion of the experiment ($p<0.001$), although for the one-third ration group an increase in CF was observed between 11th April and 15th May ($p<0.01$).

The full and two-thirds ration fish had a higher CF than the one-third ration fish from 11th September until 7th November ($p<0.01$). Then on 10th December and from 14th March until 15th May the CF of the full ration fish was higher than the one-third ration fish although the CF of the two-thirds ration fish was only higher on 11th April ($p<0.01$). Finally, on 15th May the CF of the one-third ration fish had become higher than that of the full ration group ($p<0.05$).

SGR

Although differences in SGR could not be examined statistically a general trend in growth could be observed (Fig. 4.32). Initially all groups showed an increase in

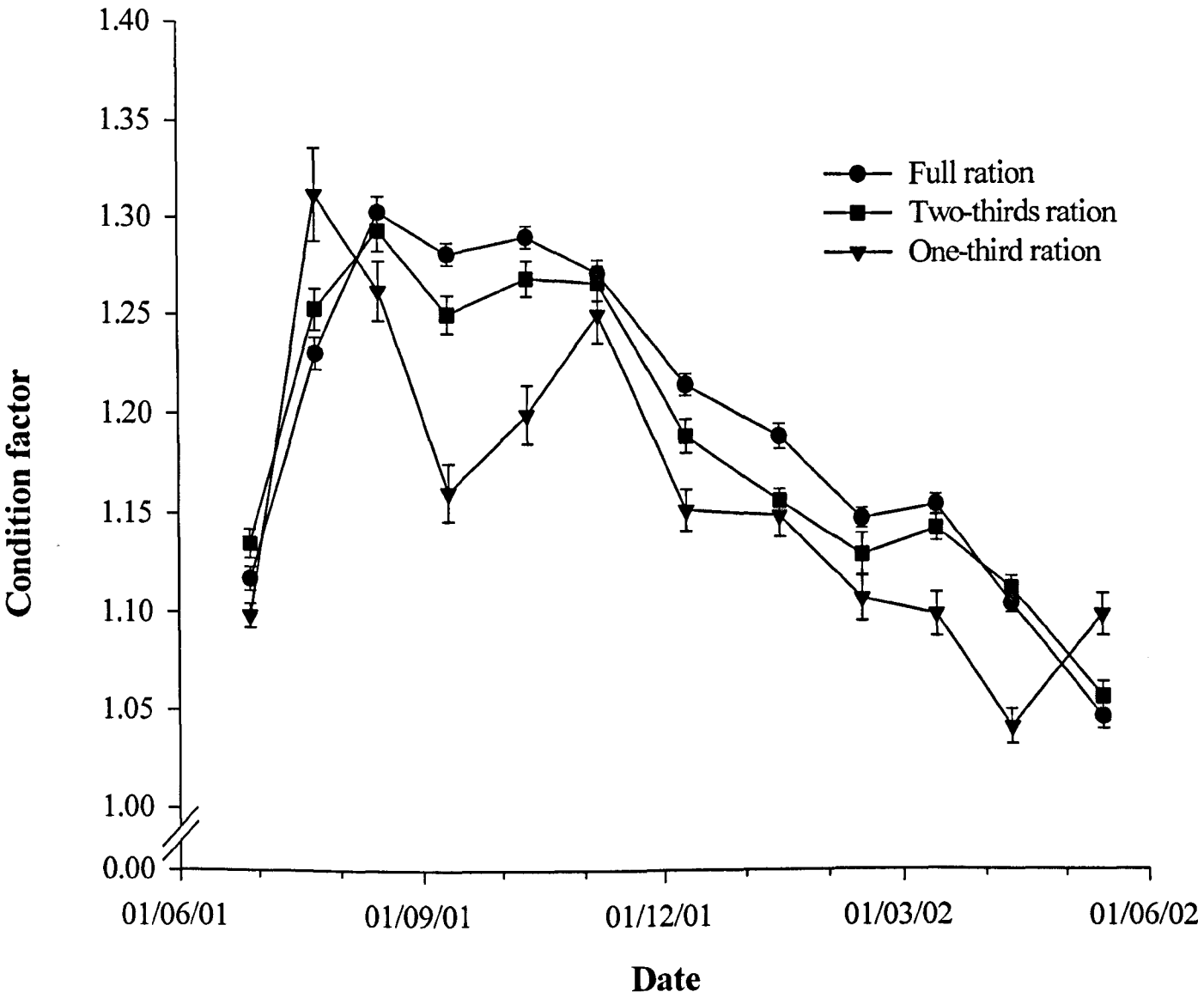


Fig. 4.31 Changes in condition factor (mean±S.E.M., n=100) of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime.

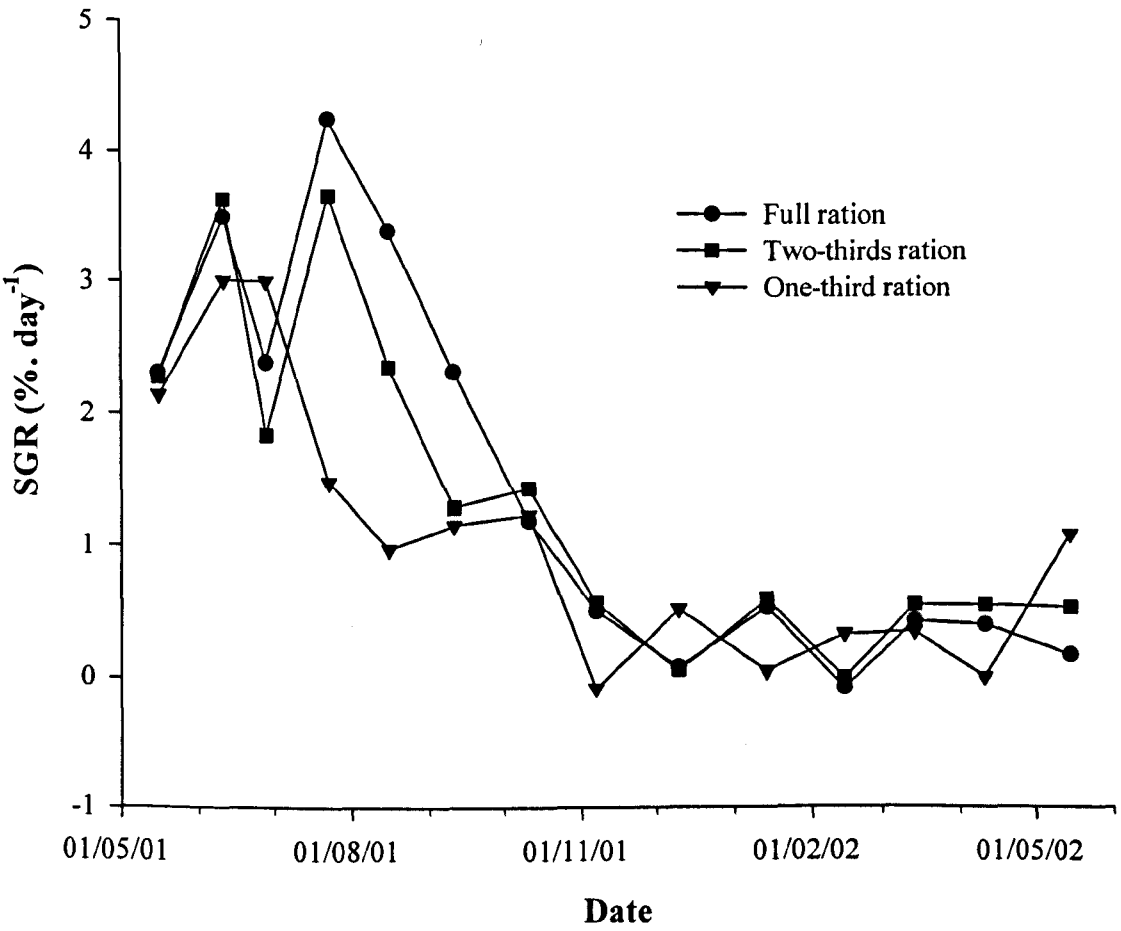


Fig. 4.32 Change in SGR of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime.

growth with that of the one-third ration fish peaking on 16th June. Between 16th and 28th June the growth of the full and two-thirds ration groups declined with a subsequent rise to peak levels on 23rd July. The SGR of all groups then declined until November from which time growth remained unchanged until the conclusion of the experiment.

Until November the SGR of the full and one-third ration fish was highest and lowest respectively with intermediate growth in the two-thirds ration group. From November onwards the SGR of the full and two-thirds ration fish remained similar although slight fluctuations in the growth of all groups could be seen.

Weight-frequency distributions

Different rations of feed affected the weight-frequency distribution of populations (Fig. 4.33). For the full and two-thirds ration fish bimodal divides developed by 11th September and 11th October respectively. By the conclusion of the experiment the full ration group contained more, and larger, UM fish than the two-thirds ration group although the size of the LMG fish was similar in both the full and two-thirds ration groups.

For the one-third ration group bimodality was weak throughout the experiment with the suggestion of a population divide by 14th March. However, the population was more skewed than bimodal throughout the experiment.

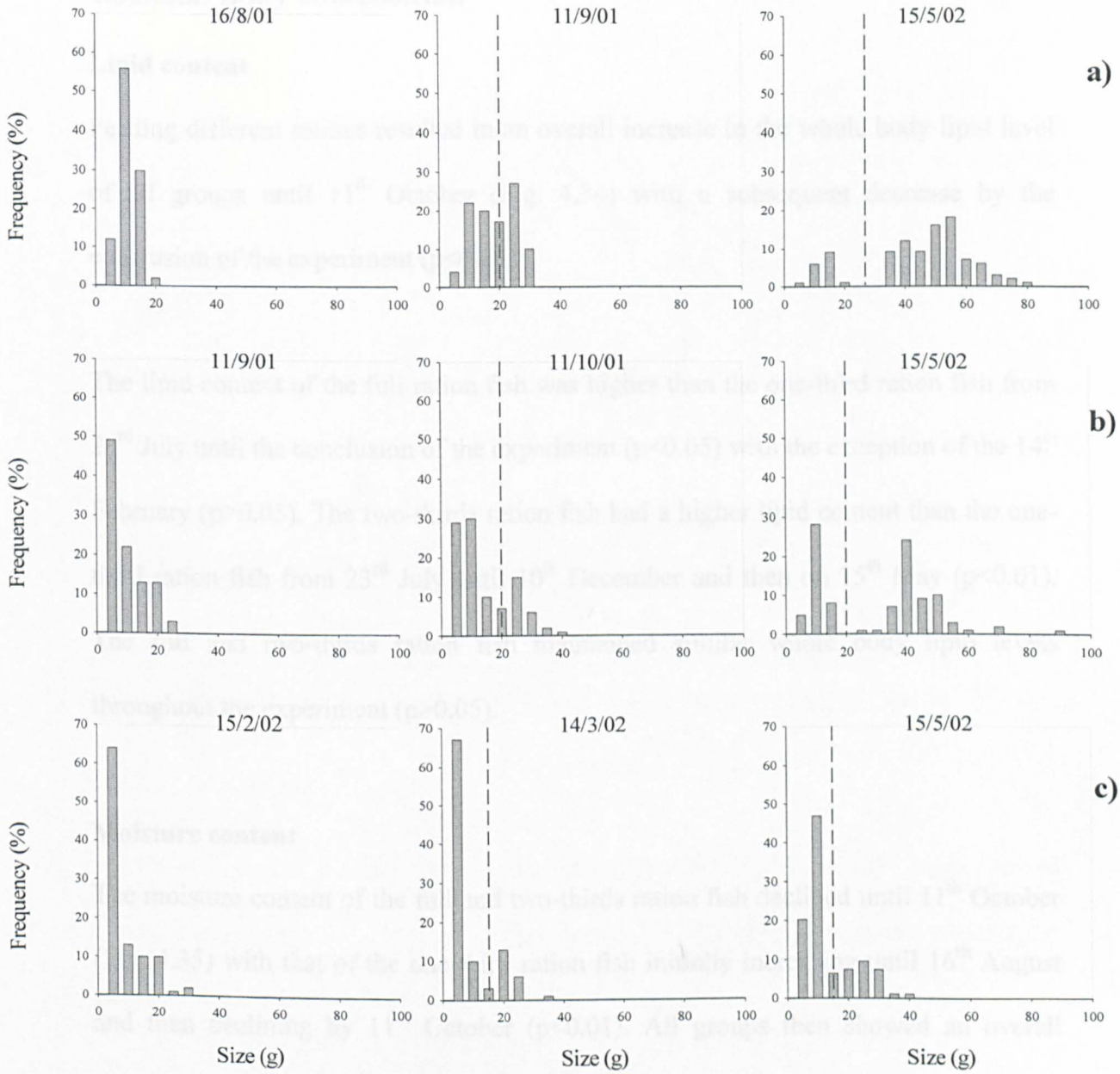


Fig. 4.33 The weight-frequency distributions of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime (n=100). Plots represent the sample points just prior to, and at the emergence of bimodality as well as at the final sample point. a) full ration, b) two-thirds ration, c) one-third ration.

4.3.3.2.2. Body composition

Lipid content

Feeding different rations resulted in an overall increase in the whole body lipid level of all groups until 11th October (Fig. 4.34) with a subsequent decrease by the conclusion of the experiment ($p<0.001$).

The lipid content of the full ration fish was higher than the one-third ration fish from 23rd July until the conclusion of the experiment ($p<0.05$) with the exception of the 14th February ($p>0.05$). The two-thirds ration fish had a higher lipid content than the one-third ration fish from 23rd July until 10th December and then on 15th May ($p<0.01$). The full and two-thirds ration fish maintained similar whole body lipid levels throughout the experiment ($p>0.05$).

Moisture content

The moisture content of the full and two-thirds ration fish declined until 11th October (Fig. 4.35) with that of the one-third ration fish initially increasing until 16th August and then declining by 11th October ($p<0.01$). All groups then showed an overall increase in moisture content by the end of the experiment ($p<0.001$).

The moisture content of the full ration fish was lower than the one-third ration fish from 23rd July until the conclusion of the experiment ($p<0.05$), with levels in the two-thirds ration fish lower from 23rd July until 14th January ($p<0.05$) and then from 14th March until the end of the experiment ($p<0.05$). The moisture content of the full and two-thirds ration fish remained similar throughout the experiment ($p>0.05$).

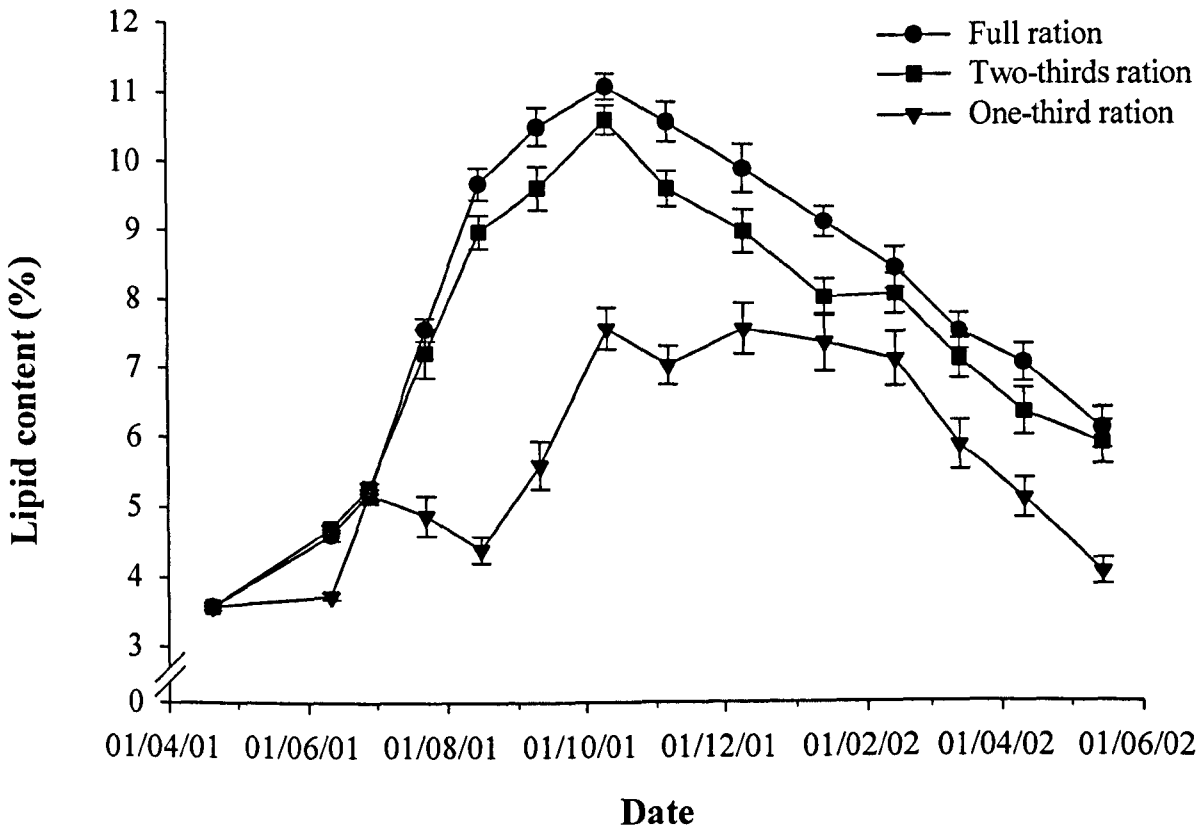


Fig. 4.34 Changes in whole body lipid content (mean±S.E.M., n=12) of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime.

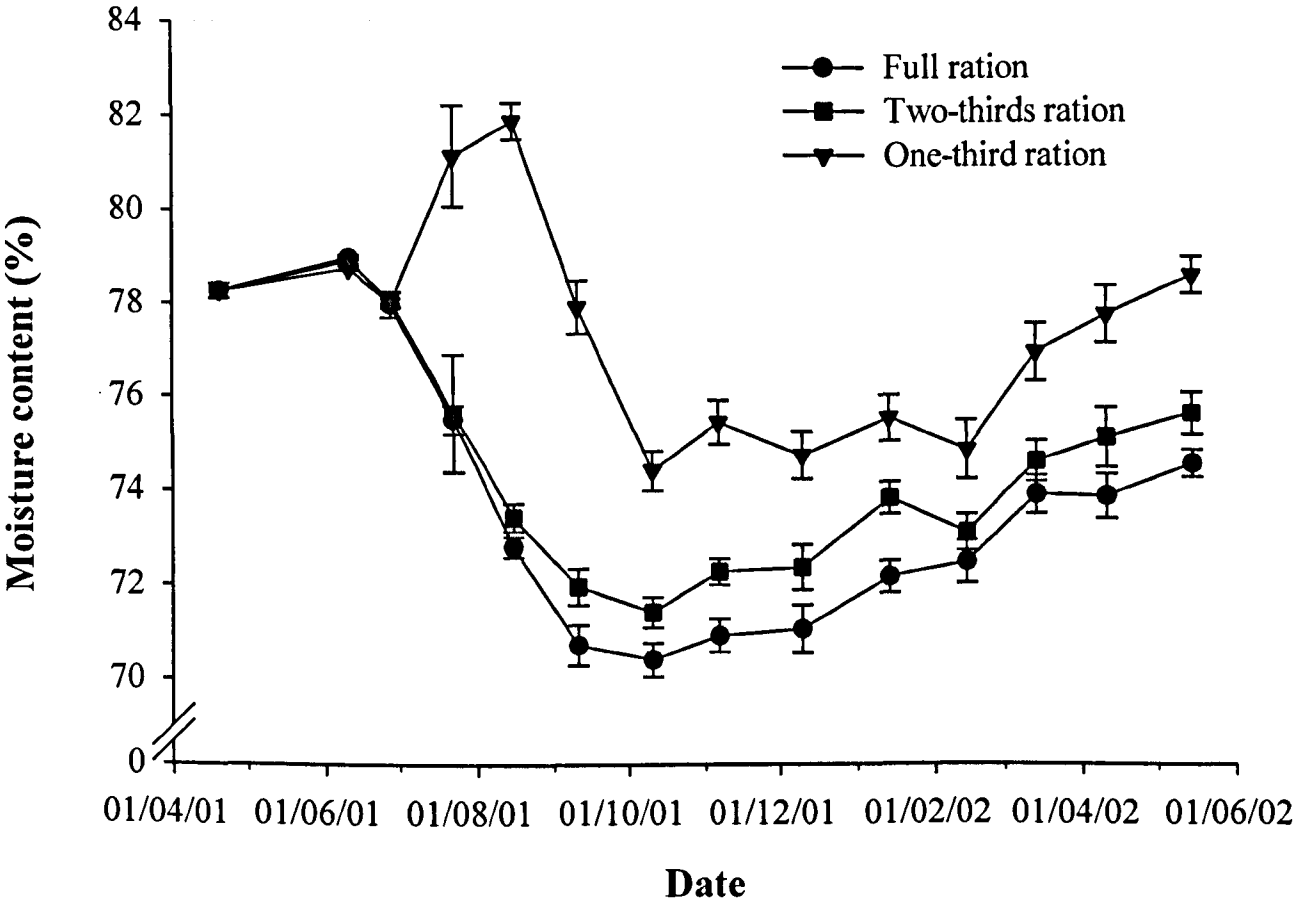


Fig. 4.35 Changes in whole body moisture content (mean±S.E.M., n=12) of parr, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime.

Lipid/Moisture correlation

When the lipid content of individual fish was plotted against their respective moisture content, for each treatment but regardless of sample time highly significant linear regressions were achieved ($p<0.001$) (Fig. 4.36).

Lipid/Size correlation

The relationship between changing size and body lipid content was investigated in a similar way to that described in experiment IV. Therefore, the changing r^2 values of fish exposed to the different rations have been presented (Table 4.8).

Highly variable r^2 values were found throughout the experiment for all ration groups with no distinct or consistent trends identified within each ration group.

4.3.3.2.3. Maturation

Incidence

No mature male or female fish were found at any time during the experiment.

4.3.3.2.4. Smoltification

Gill Na^+, K^+, ATPase

The gill Na^+, K^+ -ATPase profiles of fish which were liable to smolt and those remaining as parr are shown (Fig. 4.37).

The gill Na^+, K^+ -ATPase levels of all smolting fish showed an increase over the duration of the sampling period ($p<0.001$) with the enzyme activities of the parr remaining unchanged ($p>0.05$).

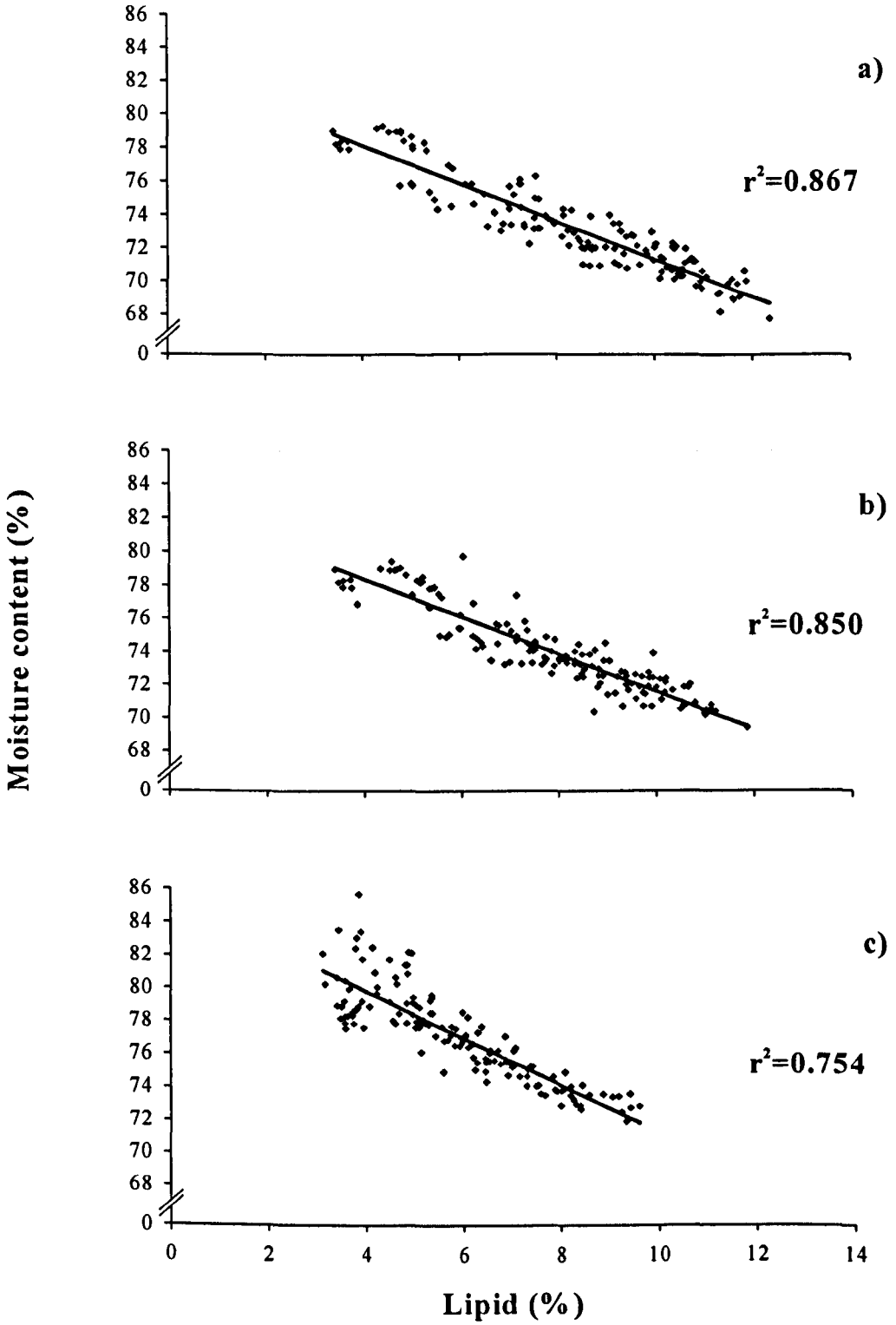


Fig. 4.36 The correlation between the whole body lipid level and moisture content of parr, reared on different rations of feed during experiment Vb (n=160). Groups were grown under a 1+ photoperiod production regime. a) full ration, b) two-thirds ration, c) one-third ration.

Date	Ration of feed		
	Full	*Two-thirds*	*One-third*
20/04/01	0.495	0.495	0.495
11/06/01	0.172	0.112	0.363
28/06/01	0.925	0.462	0.815
23/07/01	0.393	0.065	0.037
16/08/01	0.536	0.202	0.348
11/09/01	0.912	0.411	0.260
11/10/01	0.538	0.797	0.451
07/11/01	0.353	0.469	0.362
10/12/01	0.499	0.807	0.574
14/01/02	0.315	0.529	0.855
14/02/02	0.495	0.258	0.567
14/03/02	0.520	0.509	0.856
11/04/02	0.269	0.508	0.562
15/05/02	0.674	0.413	0.014

Table 4.8 The changing r^2 values of linear regressions between the weight and whole body lipid level of parr, reared on different rations of feed during experiment Vb (n=12). Groups were grown under a 1+ photoperiod production regime.

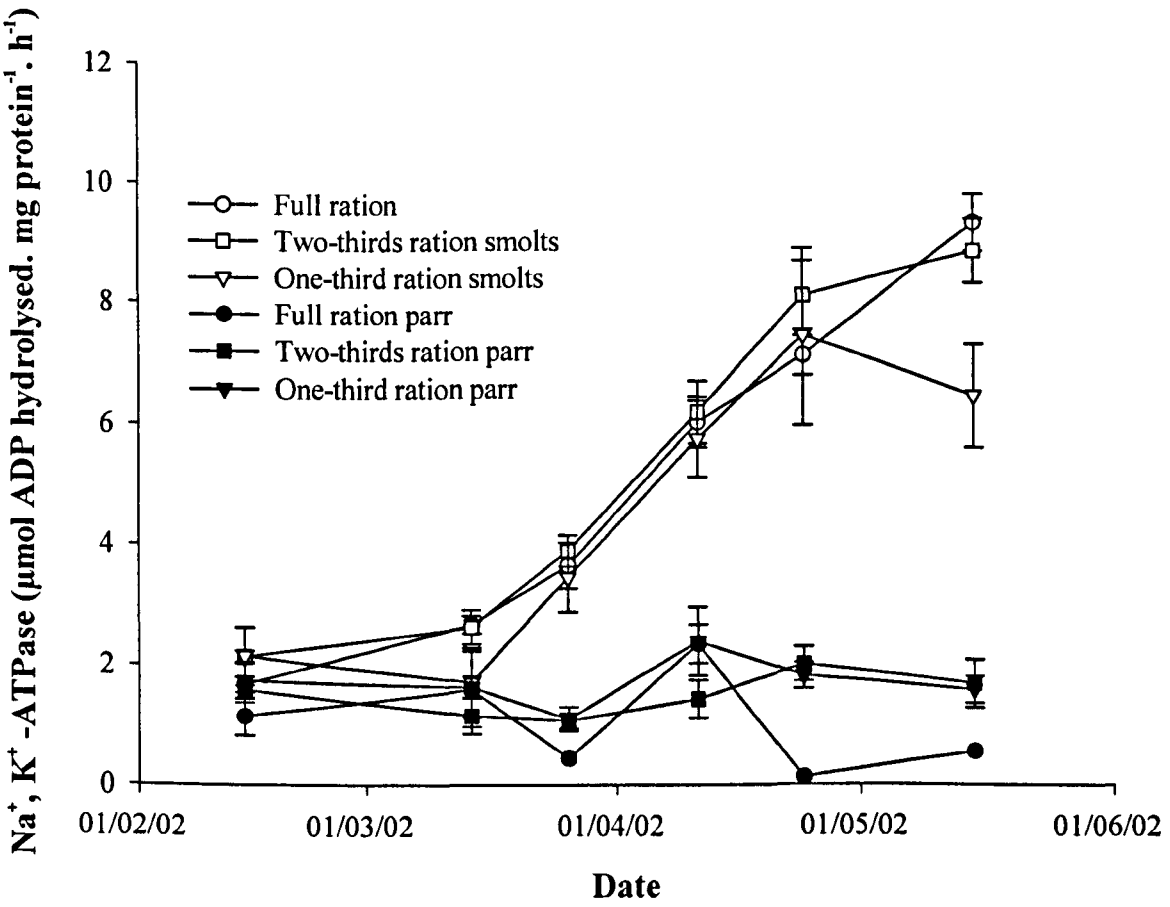


Fig. 4.37 Changes in the gill Na^+, K^+ -ATPase activity (mean±S.E.M., n=20) of fish, reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime. The gill Na^+, K^+ -ATPase of fish developing as smolts and those remaining as parr are plotted.

There were initially no differences between the gill Na^+, K^+ -ATPase levels of the ration groups. Then from 26th March until the end of the experiment both the full and two-thirds ration smolts had higher ATPase levels than the parr, with levels in the one-third ration smolts higher from 11th April ($p<0.01$).

Serum osmolality

Serum osmolality was measured following a 24h seawater (35‰) challenge (Fig. 4.38a). However, to accurately interpret the changes in osmolality the survival rates of the fish must first be considered (Fig. 4.38b).

On 14th February the survival of the full ration group was 70% being higher than that of both the two-thirds and one-third ration fish ($p<0.05$). Then on 14th March the survival of both the full and two-thirds ration fish increased to similar high levels (>90%) with lower levels in the one-third ration fish ($p<0.05$). A similar situation occurred on 11th April, although on 30th March and 25th April survival rates in the one-third ration fish were similar to those of the two-thirds ration fish. On 15th May the survival rates of all groups were similar.

The serum osmolality of challenged fish from the full and one-thirds ration group were initially high (approximately 440mOsM) and significantly greater than the unchallenged controls (approximately 320mOsM) ($p<0.05$), which had similar and unchanged osmolalities throughout the experiment. The osmolality of challenged fish subsequently declined remaining higher than the control fish ($p<0.05$) until 11th April when the challenged two-thirds ration fish had similar osmolalities to the unchallenged fish ($p>0.05$). On 25th April the challenged one-third ration fish had

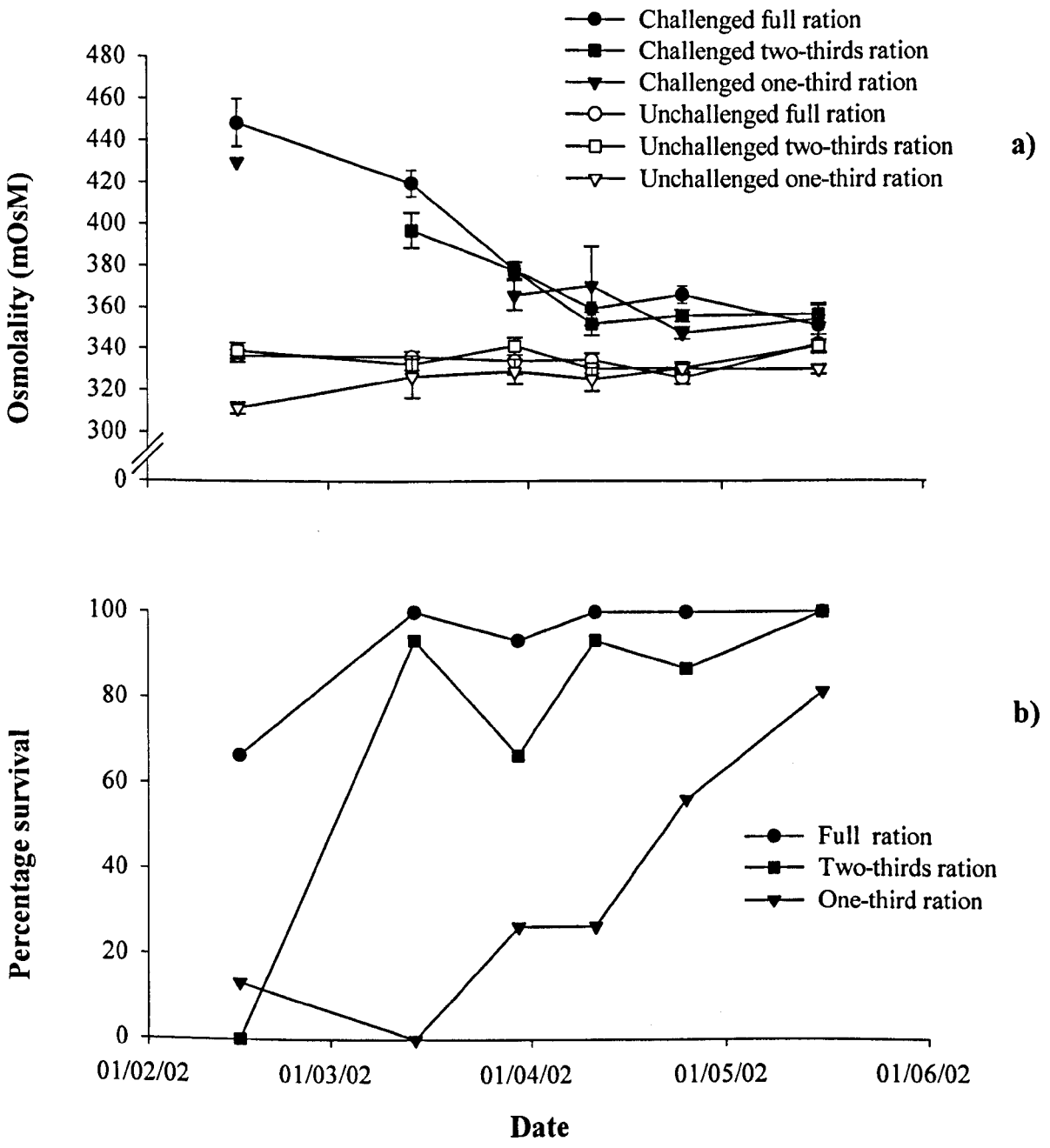


Fig. 4.38 Changes in serum osmolality (mean±S.E.M., n=1 to 15) (a) and the percentage survival (b) of fish following a 24h seawater (35‰) challenge. Individuals were previously reared on different rations of feed during experiment Vb. Groups were grown under a 1+ photoperiod production regime. Serum osmolalities were measured in fish surviving the 24h challenge. Due to the low initial survival rates some osmolality measurements are absent.

similar osmolalities to the unchallenged fish ($p>0.05$) and by the conclusion of the experiment the osmolality of all challenged groups had become similar to the unchallenged fish ($p>0.05$).

Smolt index

Different rations of feed affected the changes in smolt index that were recorded during the parr-smolt transformation (Fig. 4.39). The smolt index (see Section 2.8.4) ranged from 1 to 4, where smolt index 1 was a typical parr and smolt index 4 was a typical smolt.

The highest number of individuals developing good smolt characteristics (measured using the smolt index) occurred in the full ration group with low numbers of fish showing similar characteristics in the one-third ration group. The development of smolt characteristics was intermediate in the two-third ration group.

In the full ration group index 2 fish were first identified on 14th March with numbers increasing until 11th April. Index 3 fish were first identified on 11th April with numbers increasing as the incidence of index 2 fish decreased. By 25th April index 4 fish were also present. At the end of the experiment no index 2 fish were present with index 3 and 4 fish predominating and only low numbers of index 1 fish.

In the two-thirds ration group index 2 fish were first identified on 14th March with numbers increasing until 11th April. Index 3 fish were first identified on 25th April with index 4 fish identified on 15th May when all index 2 fish had developed into either index 3 or 4 fish. However, at this time although index 3+ fish predominated

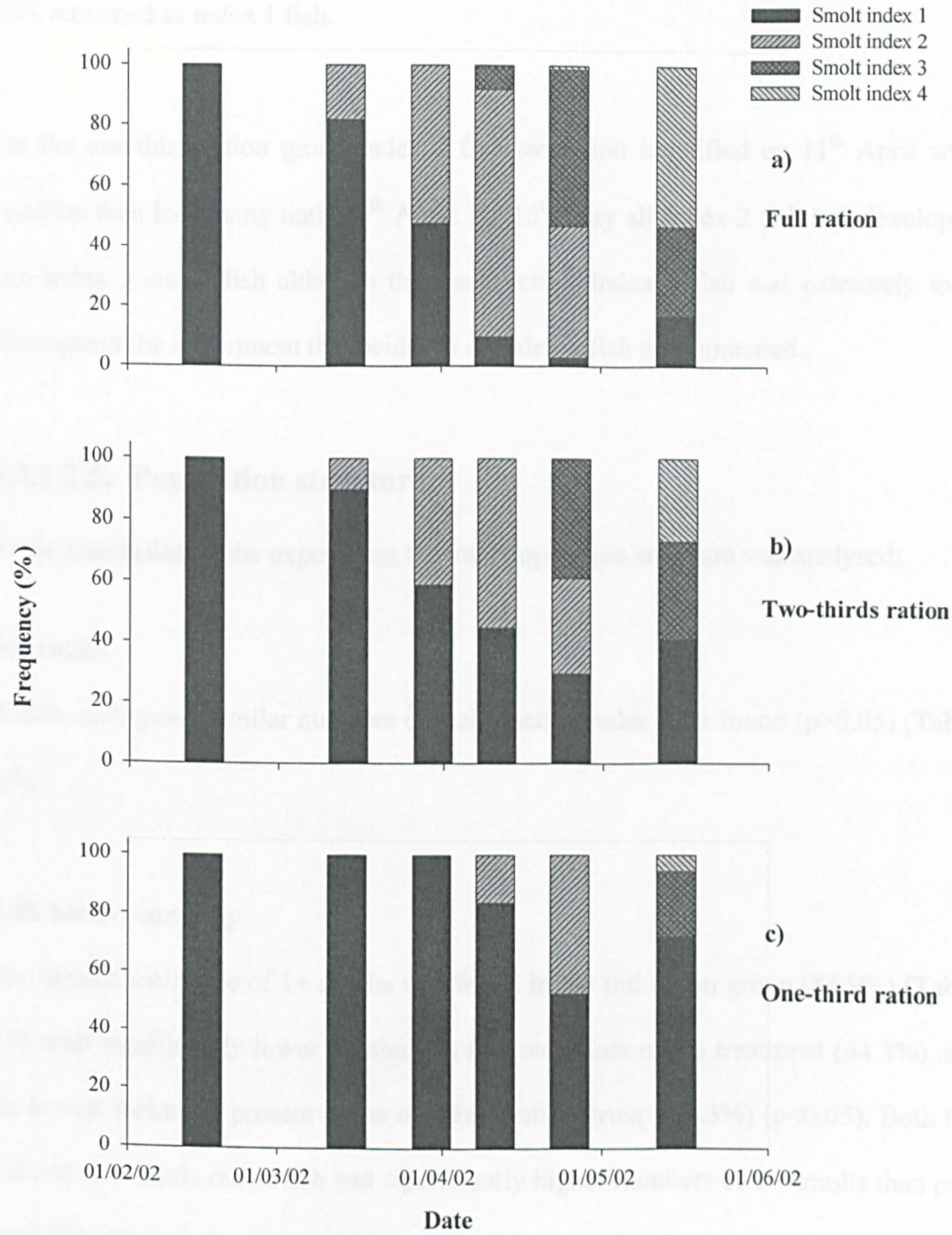


Fig. 4.39 Changes in the smolt index score of fish, reared on different rations of feed during experiment Vb (n=100). a) full ration, b) two-thirds ration, c) one-third ration. Groups were grown under a 1+ photoperiod production regime. Smolt index 1 = typical parr, with parr marks clearly visible, Smolt index 2 = parr marks visible, but some silvering, smolt index 3 = silvered with visible parr marks, Smolt index 4 = typical smolt, no parr marks visible.

40% remained as index 1 fish.

For the one-third ration group index 2 fish were first identified on 11th April with numbers then increasing until 25th April. By 15th May all index 2 fish had developed into index 3 and 4 fish although the incidence of index 4 fish was extremely low. Throughout the experiment the incidence of index 1 fish predominated.

4.3.2.2.5. Population structure

At the conclusion of the experiment the total population structure was analysed:

Sex ratios

Within each group similar numbers of males and females were found ($p>0.05$) (Table 4.9).

Life history strategy

The highest incidence of 1+ smolts was found in the full ration group (84.9%) (Table 4.9) with significantly lower numbers in the two-thirds ration treatment (64.3%) and the lowest incidence present in the one-third ration group (27.3%) ($p<0.05$). Both the full and two-thirds ration fish had significantly higher numbers of 1+ smolts than parr ($p<0.05$) although for the one-third ration fish more parr than 1+ smolts were found ($p<0.05$).

Survival

Varying the ration of feed had an overall effect on the survival of individuals (Table 4.9). The full ration fish had the highest survival (90.4%) with that of the two-thirds ration fish significantly lower (76.5%). The survival of the one-third ration group

	Sex (%)		Population Structure (%)		Survival (%)
Ration level	*Male*	*Female*	*1+ smolts*	*Parr*	
Full	53.0 ^{Aa}	47.0 ^{Aa}	84.9 ^{Aa}	15.1 ^{Ab}	90.4 ^{A}
Two thirds	50.0 ^{Aa}	50.0 ^{Aa}	64.3 ^{Ba}	35.7 ^{Bb}	76.5 ^{B}
One third	41.7 ^{Aa}	58.3 ^{Aa}	27.3 ^{Ca}	72.4 ^{Cb}	27.8 ^{C}

Table 4.9 The population structure, sex ratio and survival of individuals recorded at the conclusion of experiment Vb, where fish, under a 1+ production regime, were reared on different rations of feed (For population structure n=250-450, sex ratio n=100, survival n=450). Similar lettering denotes statistical similarity ($p<0.05$). Capital lettering denotes differences between treatment groups, lower case lettering denotes differences within treatments.

was the lowest of all groups (27.8%). However, it was not possible to record the size of the mortalities.

4.3.3.3. Summary of the results from Experiment Vb.

- Fish fed full and two-thirds rations showed initial increases in length and weight becoming longer and heavier than the one-third ration fish soon after the different rations were applied. The full ration fish then became longer and heavier than the two-thirds ration fish.
- The CF of all groups increased initially and then declined to the end of the experiment. The CF values of the full and two-thirds ration groups were similar throughout the experiment with lower CF values in the one-third ration fish.
- Initially the SGR of all fish increased with the growth of all groups subsequently declining until November from which time growth remained constant. The highest and lowest growth rates were found in the full and one-third ration fish respectively with the SGR of two-thirds ration fish intermediate.
- Full and two-thirds ration groups developed clear bimodal distributions although the timing of the emergence of modality differed. In the one-third ration fish bimodality was weak.
- Whole body lipid levels initially rose in all groups with a subsequent decline. The levels recorded in the full and two-thirds ration fish remained similar throughout with lower lipid levels found in the one-third ration fish.
- Moisture levels initially declined in all groups although the decrease of the one-third ration fish was preceded by a brief increase in moisture. Subsequently, the moisture levels of all groups rose. Full and two-thirds ration groups had similar moisture levels throughout that were lower those in the one-third ration fish.

- Whole body lipid levels were negatively correlated to moisture content in all groups.
- No clear relationship was found between whole body lipid level and fish size.
- No mature fish were found in any treatment group throughout the experiment.
- All smolting fish showed an increase in gill Na^+, K^+ -ATPase although no difference was found between the smolts from each treatment group.
- Following a 24h seawater challenge the one-third ration group had the poorest survival rates. The osmolalities of surviving individuals from all groups decreased to similar levels as unchallenged controls.
- At the conclusion of the experiment both the full and two-thirds ration groups had a high number of fish displaying well developed smolt characteristics (i.e. smolt index 4 fish), although the two-thirds ration fish still had high numbers of parr (i.e. smolt index 1 fish). One-third ration resulted in low numbers of fish displaying smolt characteristics (i.e. smolt index 2+ fish) with such fish developing later than in the other treatment groups.
- At the conclusion of the experiment full and two-thirds ration groups comprised mainly of 1+ smolts whereas the one ration group had more parr.
- The lowest and highest mortality rates were found in the full and one-third ration groups respectively with intermediate survival in the two-thirds ration group.

4.4. Discussion

The experiments detailed in this chapter have shown that changes in dietary lipid level and ration of feed exert profound effects on the growth and smoltification of Atlantic salmon parr. Unfortunately, levels of maturation were low in all of the experimental groups and as such it was difficult to correlate differences in maturation to the relative dietary regimes.

4.4.1. Growth

From the results of experiment IV it seems that differences in dietary lipid regime will not affect the growth of individuals. Regardless of dietary lipid inclusion both the weight and length of fish from the respective treatments were similar throughout the experiment. Previously, differences have been found in the growth of seawater reared salmon fed diets containing different lipid inclusions (Hemre and Sandnes, 1999; Torstensen *et al.*, 2001). However, although Refstie *et al.* (2001) found a slight effect of increased dietary lipid on growth in adult salmon, of the 122g difference in body weight between individuals fed either 32% or 39% dietary lipid for 235 days, 91g could be accounted for by increases in whole body lipid content. In support of this Shearer *et al.* (1997) suggested that ration level influences growth whereas dietary lipid determines adiposity. Therefore, it is possible that where dietary lipid induced differences in weight have previously been documented much of the weight differential may be accounted for by lipid deposition as opposed to skeletal or muscle growth.

It is interesting to note that where dietary lipid treatment has been found to influence changes in weight such findings have primarily been documented in adult salmonids.

In adults it is possible that increases in lipid accumulation are more significant than in juvenile fish with parr utilising body lipid accumulation for early organ development and physiologically demanding processes such as smoltification (Woo *et al.* 1978; Birt and Green, 1986; Helland and Grisdale-Helland, 1998). Reinitz (1983) found that dietary lipid did not affect growth when high rations of feed were fed to 2.1g rainbow trout although at low and medium ration levels a difference was noted. It is therefore likely that when fed to satiation (as in the current experiment) juvenile salmonids fed different dietary lipid inclusions will grow at a similar rate although it is probable that such a relationship only holds above a certain minimum lipid threshold that allows at least maintenance metabolic rates to be achieved.

The changes in SGR found in experiment IV also indicate that dietary lipid levels are ineffective in altering juvenile fish size. Following the change in diet of both the 25/12.5 and the 12.5/25 groups a large decrease in SGR was noted in the 25/12.5 fish suggesting that the dietary lipid regime had influenced growth. However, a decline in growth was observed in all groups in particular the 25/25 treatment indicating that the diet regime had not necessarily affected the growth of individuals. Indeed, it is more likely that the concurrent reduction in photoperiod (from a continuous light regime to the ambient summer photoperiod) resulted in this growth decline with similar reductions in growth due to photoperiod well documented (Saunders and Harmon, 1990; Skilbrei *et al.*, 1997; Duncan *et al.*, 1998; Duncan *et al.*, 1999).

In experiment V evidence was provided that ration affects growth. Both the 0+ and 1+ groups rapidly showed a division of size relative to the rations that they were fed. Previously, this finding has been well documented in both adults (Elliott, 1975b;

Storebakken and Austreng, 1987a; Johansson *et al.*, 1995; Hillestad et al., 1998) and juveniles (Storebakken and Austreng, 1987b; McCormick *et al.*, 1989; Stead *et al.*, 1996; Shearer *et al.*, 1997) and it is clear that a strong relationship exists between ration and growth.

However, it is possible that the magnitude of the response to ration will be affected by other factors. Elliott (1975b) found that for brown trout the optimum temperature for growth declined as ration was reduced. In experiment V the growth rate (SGR) of the 1+ fish became similar as the experiment progressed and it is possible that during autumn and winter the low water temperatures caused the growth of the one-third ration fish to increase relative to those of the full and two-thirds ration fish. In support of this both the weight and length of the full and two-thirds ration fish remained unchanged between consecutive time points from October suggesting that growth had been influenced by temperature in these groups. Further support can be found when the growth rates of the 0+ fish are considered. Again, until late September the growth rates of fish from the respective treatments remained different. Subsequently, the growth of all fish became erratic and no consistent differences between ration groups could be found suggesting an effect of temperature. However, it is also interesting to note that the decline in SGR of all groups during this stage was not of a similar magnitude to that of the 1+ fish. It is therefore possible that as well as temperature photoperiod plays some role in the growth response to ration given that continuous light regimes were applied to the 0+ fish during the time when a reduction in the seasonally-changing temperature occurred.

From the weight-frequency distributions it was clear that both dietary lipid inclusion and ration had affected the development of bimodality although the effect of ration appeared more influential. In experiment IV the 25/25, 25/12.5 and 12.5/25 dietary lipid regimes resulted in a similar timing of bimodal divide, although for the 12.5/12.5 fish an earlier divide occurred. Interestingly, this implies that dietary lipid inclusion will only influence the development of bimodality if it occurs for a long period of time possibly over a series of important developmental periods. Exposing individuals to low dietary lipid during either early (12.5/25 group) or late (25/12.5 group) development resulted in a similar timing of bimodal divide as fish maintained on the high dietary lipid throughout (25/25 group). For the fish fed low levels of dietary lipid throughout development (12.5/12.5 group) a different timing of bimodal divide resulted.

The results of experiment V indicate that ration also affects bimodality. Fish maintained on full rations had a larger UMG with the two-thirds ration fish containing slightly more LMG fish. Furthermore, the one-third ration generally resulted in only LMG fish. Previously, it has been found that feeding restricted rations to adult rainbow trout (Storebakken and Austreng, 1987a) and Atlantic salmon parr (Storebakken and Austreng, 1987b; Nicieza and Metcalfe, 1997) resulted in a greater percentage of lower modal group fish. Thorpe (1977) suggested that population bimodality resulted from genetic, social and environmental interactions and it would seem that the current study supports the suggestion by Storebakken and Austreng (1987b) that ration is also significant environmental factor affecting bimodality. The mechanisms by which this is achieved are not well understood.

However, the results of the current experiments may provide some insight into the mechanisms influencing growth, feeding and bimodality. Previously, it has been found that bimodality can result from differential feeding motivation and appetite in fish destined to enter the respective modes (Higgins and Talbot, 1985; Metcalfe *et al.*, 1986, 1988; Metcalfe and Thorpe, 1992) although this difference is not thought to be linked to food availability with an internal control mechanism postulated (Metcalfe *et al.*, 1986, 1988). It has been suggested that photoperiod would synchronise such an internal rhythm of appetite (Villarreal *et al.*, 1988). In the current experiments the full and two-thirds ration 0+ fish developed bimodal populations prior to any change in photoperiod whereas the 1+ fish that were fed the respective rations developed bimodality at different times during both the decreasing and increasing phases of the natural photoperiod. From this it may be suggested an endogenous rhythm of appetite and growth (Villarreal *et al.*, 1988) is not influential in bimodality. However, it may be that the respective rations mediated a differential response to the respective endogenous rhythms and ration may have interacted with the rhythms of growth and appetite to result in bimodality.

The current experiments also provide support for the influence of a developmental or size threshold in bimodality (Kristinsson *et al.*, 1985; Stewart *et al.*, 1990; Skilbrei, 1988). For the 0+ fish the continuous light regime (Komourdjian *et al.*, 1976; Lundqvist, 1980; Solbakken *et al.*, 1994; Sigholt *et al.*, 1995) as well as the high summer temperatures (Elliott, 1975a, b, 1976; Clarke *et al.*, 1978) would have resulted in the rapid growth of individuals allowing such a size or developmental threshold to be achieved at a similar date in both the full and two-thirds ration fish. The natural photoperiod regime experienced by the 1+ fish would have resulted in

reduced photoperiodic and temperature effects on growth so that ration would have been more influential in the attainment of such critical thresholds with a resultant differential in the timing of modality. It may be possible that changes in appetite and feeding motivation, previously suggested as a controlling mechanism in population structure (Higgins and Talbot, 1985; Metcalfe *et al.*, 1986, 1988; Metcalfe and Thorpe, 1992) may be a result of rather than an influencing mechanism in bimodality.

It is also important to note that in the ration experiments growth and indeed other parameters may have been influenced by the ration related mortality of individuals. For the 0+ regime a low rate of survival was found in the one-third ration fish whereas in the 1+ group this ration resulted in a very low survival rate with the survival of the two-thirds ration fish lower than that of the full ration fish. Clearly the 1+ regime had a greater effect on survival possibly linked to the length of time that rations were applied for combined with the low temperature regimes experienced by individuals during the natural winter period. High mortality rates have previously been found where restricted feed regimes were applied for long periods of time with such mortality linked to fish size (Storebakken and Austreng, 1987b). It is therefore possible that in the one-third ration groups of the 1+ photoperiod regime in particular size-dependant mortality occurred which subsequently affected population structure and growth, especially during the latter stages of the experiment.

However, it is important to note that in the current experiments the size of mortalities was not recorded and as such it is not possible to determine the precise effect of any size dependant mortality on the results found. It is certainly possible that smaller individuals within the population suffered high mortality rates due to a reduced

accumulation of lipid reserves that would have obviously resulted in their low growth rates and size. Alternatively, it may be that with time the smaller individuals reduced their activity and metabolic rates relative to their feed rates. This would have resulted in a reduction on energy requirements and a higher probability of survival when fed low rations. The larger individuals within the population may have maintained a high level of activity with high metabolic rates and as such they may have suffered more from the low feed rates than the smaller individuals within the population. Certainly given the importance of over-wintering survival rates future experiments should ensure that the size of mortalities is recorded, with consideration of the metabolic requirements of fish on different rations for long periods of time also important.

4.4.2. Lipid accumulation

Previously, it has been suggested that dietary lipid level determines adiposity whereas ration influences growth (Shearer *et al.*, 1997) and indeed there is much evidence in the literature suggesting that whole body lipid content is correlated to dietary lipid level. Although Hillestad *et al.* (1998) found a negligible effect of dietary lipid on cutlet or carcass fat levels in adult salmon a clear relationship has now been documented for both adults (Bjerkeng *et al.*, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001) and juveniles (Reinitz, 1983; Grisdale-Helland and Helland, 1997; Shearer *et al.*, 1997; Shearer and Swanson, 2000).

In experiment IV further support is provided that dietary lipid affects lipid accumulation with fish fed the 25% lipid diet consistently maintaining higher body lipid levels than for those fed the 12.5% diet. Over time all fish regardless of dietary lipid level had initial increases in body fat content followed by a period where levels

remained fairly constant. From December onwards a decline in whole body lipid level occurred. Therefore, although many studies investigating dietary lipid only measure body fat at the beginning and the end of the experiment (e.g. Bjerkeng *et al.*, 1997; Grisdale-Helland and Helland, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999) it appears that in juvenile salmonids unlike larger adults, (c.f. Bjerkeng *et al.*, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001), seasonal changes in lipid content will occur. Initially levels rise (Mørkøre and Rørvik, 2001; Shearer and Swanson, 2000; this study) to reach a point where levels are maintained at a constant level dependant on dietary lipid (Reinitz, 1983; Shearer *et al.*, 1997; this study) after which a decline will occur over winter (Mørkøre and Rørvik, 2001; Shearer and Swanson, 2000; this study). Furthermore, it would seem that in juveniles the winter reduction in body lipid content could be due to a number of reasons in particular temperature (Elliott, 1976; Saunders *et al.*, 1982) or physiologically demanding processes such as smoltification (Komourdjian *et al.*, 1976; Saunders and Henderson, 1978; Shearer, 1994) or maturation (Jonsson *et al.*, 1991; Rowe *et al.*, 1991; Kadri *et al.*, 1995).

In experiment IV it was evident that following the change in dietary lipid level individuals rapidly re-adjusted their body fat content to similar levels to fish which had been maintained on the same diet throughout development. Previously, Miglavs and Jobling (1989a) showed that following an 8 week period of restricted feeding juvenile Arctic charr recovered their lipid store after only 8 weeks of *ad libitum* feeding, with Morgan and Thorpe (2001) observing that body fat was replenished in two weeks by Atlantic salmon parr following a 6 week period where feed was only available on one day per week. Similarly, Metcalfe and Thorpe (1992) found that the

lipid deficit created by three weeks of starvation was replenished within 4 weeks of re-feeding in Atlantic salmon parr. It is therefore possible that rapid changes in body composition are primarily due to the small size of juvenile fish.

Given the findings of the current experiment it is likely that for small juvenile salmonids at least previous dietary lipid regime will have only a minimal effect on an individuals body fat content at a particular time with the current dietary regimes used having a greater influence. Unfortunately, there are few studies that incorporate a change in dietary lipid inclusion such as in experiment IV but it is important to note that there are many studies where experimental diets are used after a period of commercial diet application (e.g. Reinitz, 1983; Bjerkeng *et al.*, 1997; Grisdale-Helland and Helland, 1997). Therefore, in future some effort should be made to document the composition of the commercial diets used with information also necessary on the body lipid content of fish prior to the application of test diets.

It is well documented that ration size will affect whole body fat levels in both adults (Elliott, 1976; Storebakken and Austreng, 1987a; Johansson *et al.*, 1995; Hillestad *et al.*, 1998) and juveniles (Reinitz, 1983; Silverstein *et al.*, 1998). However, Shearer *et al.* (1997) found that ration level had no effect on lipid content with a review by Rasmussen (2001) also highlighting variable results related to ration induced changes in body fat content.

The current work as well as that of Storebakken and Austreng (1987b) help to explain the role of ration. Storebakken and Austreng (1987b) found effects of ration on body lipid content at low feed rates but at higher rates no differences were found. Similarly,

in experiment V the lipid content of both full and two-thirds ration fish (under both photoperiod regimes) remained similar with the fat levels of the one-third ration groups generally lower. Therefore, up to a certain level changes in ration will affect the whole body fat content of fish, but above this level adiposity is maintained at an internally determined point irrespective of additional feed. Indeed during experiments that have investigated the effects of different dietary lipid inclusions it has been found that above a certain level, increases in dietary lipid will not increase the whole body lipid content of individuals suggesting a maximum lipid attainment (Einen and Skrede, 1998; Hemre and Sandnes, 1999). In support of this during experiment V the full and two-thirds ration fish had similar body lipid contents despite there being distinct differences in size.

The current experiments therefore provide further support for the theory that salmonid growth is under lipostatic control. Previously, it has been found that following periods of starvation or restricted feeding individuals are able to fully recover their size compared to that of fish that were fed throughout (Weatherley and Gill, 1981; Reimers *et al.*, 1993; Hopkins and Unwin, 1997). However, it has also been shown that following periods of restricted feeding individuals may either become larger or remain smaller than fish that are maintained on full rations throughout (Weatherley and Gill, 1981; Dobson and Holmes, 1984; Miglavs and Jobling, 1989a; Nicieza and Metcalfe, 1997). Subsequently, Jobling and Johansen (1999), Silverstein *et al.* (1997) and Johansen *et al.* (2001) have suggested that the growth of salmonids is under lipostatic control such that increases in size are controlled by the maintenance of a distinct body fat content. In the current experiments it seems that the full and two-thirds ration fish achieved such a lipid level although for the one-third ration fish

differences were found between the two production regimes. Under the 0+ regime individuals could not achieve the lipostatic level regardless of the large reductions in size. However, for the 1+ fish similar lipid levels were achieved in the one- and two-third ration fish towards the conclusion of the study indicating that the one-third ration fish had achieved the lipid level that is maintained under lipostatic control.

O'Connor *et al.* (2000) found that when juvenile salmon were deprived of food their standard metabolic rate fell, with Elliott (1976) noting that the optimum temperature for growth declines with decreasing ration. It would therefore seem that in experiment Vb the lower metabolic rate of the one-third ration fish combined with the low winter temperatures experienced, resulted in these fish increasing their lipid deposition relative to that of the full and two-thirds ration fish thus allowing them to achieve the lipid level that individuals fed *ad libitum* maintain through lipostatic regulation.

During the current experiments a strong negative correlation was found between whole body lipid level and moisture content; such relationships are well documented in the literature (Reinitz, 1983; Elliott, 1976; Miglavs and Jobling, 1989a; Bjerkeng *et al.*, 1997; see reviews by: Shearer, 1994; Johansen *et al.*, 2001; Rasmussen, 2001). It seems the current study as well as the majority of the literature suggest that as fish become fatter moisture is replaced by lipid (Bjerkeng *et al.*, 1997). Furthermore, due to the strong negative correlation that was found between lipid and moisture content in the current study it is probable that whole body water levels can be used to accurately predict the body fat content of fish (Elliott, 1976).

For the interpretation of proximate composition Shearer (1994) suggested that it is important to incorporate fish size as a covariate within the analysis because changes in body composition are influenced by fish growth. Indeed there is reasonable support in salmonids that fat levels increase with increasing weight (Reinitz, 1983; Storebakken and Austreng, 1987b; Bjerkeng *et al.*, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001). In the current experiments poor lipid/size correlations were found throughout development in both ration experiments with high, although variable, r^2 values found during the early stages of development in the dietary lipid experiment. Similarly, Gardiner and Geddes (1980) found only slight increases in the water content of small fish when compared to larger individuals, with Vanstone and Markert (1968) only finding a lipid/size relationship in parr that were not undergoing exponential growth.

Interestingly, the majority of literature identifies such a relationship in adult salmon (e.g. Bjerkeng *et al.*, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Torstensen *et al.*, 2001) and where clear relationships have been found in juveniles they have been identified in studies which have focused on early development (Reinitz, 1983; Storebakken and Austreng, 1987b). Therefore, for juveniles it would seem that a correlation between body fat level and size may only occur during a brief period in early development. During the majority of development there will be little correlation with this possibly linked to the onset of physiologically demanding processes such as maturation (Jonsson *et al.*, 1991; Rowe *et al.*, 1991; Kadri *et al.*, 1995) and smoltification (Komourdjian *et al.*, 1976; Saunders and Henderson, 1978; Shearer, 1994). During the commercial production of juvenile salmonids, where freshwater development and smoltification are achieved in a short period of time,

correcting for size during the analysis of proximate composition data may result in a considerable loss of valuable information.

4.4.3. Maturation

It has been well documented that increases in both dietary lipid level (Hillestad *et al.*, 1998; Silverstein *et al.*, 1998; Shearer and Swanson, 2000) and ration (Storebakken and Austreng, 1987b; Clarke and Blackburn, 1994) result in increases in the number of fish choosing to mature. In the current studies low levels of maturation were observed throughout the year with no mature fish found during experiment Vb. Consequently, conclusions regarding the effects of ration and dietary lipid inclusion on maturation are difficult.

4.4.3.1. Incidence of maturation

During the dietary lipid experiment mature fish were only found in the 25/12.5 and 12.5/25 groups. However, it is unlikely that these findings and the lack of mature fish within either of the other treatment groups are linked to dietary lipid influences since these levels actually constituted only one or two fish per group.

From the results of experiment Va it may be possible to suggest a role for diet manipulation in parr maturation. Mature fish were identified within the full and two-thirds ration groups although no such individuals were found in the one-third ration treatment. Previously, it has been suggested that maturation is influenced by a lipid threshold (McCormick and Naiman, 1984; Simpson, 1992; Herbinger and Friars, 1992; Silverstein *et al.*, 1997) and it possible that fish fed either the full or two-thirds ration were able to achieve this threshold whereas those under the one-third ration

regime did not. Previously, Herbinger and Friars (1992) suggested that a fat threshold for male parr maturation may be very low and it would seem that such a threshold will be achieved by feeding between one-third and two-thirds of the suggested ration for maximum growth.

Regardless of the experimental regime applied the low levels of maturation found within the current experiments are interesting and there are a number of possible reasons for the lack of gonadal development. Firstly, it is possible that the levels of dietary lipid used were insufficient to generate a high incidence of maturation. In a parallel growth study performed with the same diets as those used in experiment IV, but with a different stock of fish, similar low levels of maturation were found (G. Bell, unpublished data) indicating a possible effect of the dietary inclusions used. However, it seems unlikely that the lack of maturation in both of these experiments was due to the insufficient accumulation of lipid within individuals. The 25% diet that was used contained a greater lipid level than most commercial diets and mature fish have been found in experimental groups where both higher and lower dietary inclusions have been used for long periods of time (Hillestad *et al.*, 1998; Silverstein *et al.*, 1998; Shearer and Swanson, 2000). Furthermore, during experiment V the feed rates of the full ration fish were similar to those used in commercial production where high levels of maturation are frequently recorded (D. Mitchell, A. Smart, G. Beaton, *pers comm*). Given that a lipid threshold for male parr maturation may be fairly low (Herbinger and Friars, 1992) it is extremely unlikely that the diets and regimes used in the current experiments can sufficiently explain the observed lack of maturation.

It is possible that temperature played an important role in the low maturational levels recorded in the present work. Although Herbinger and Friars (1992) found temperature to exert little effect on maturation growing evidence exists that maturation is affected in some way by temperature (Mackinnon and Donaldson, 1976; Berglund, 1995; Davies *et al.*, 1995; Duston and Saunders, 1997). Both growth (Elliott, 1975a, b) and body composition (Elliott, 1976) are affected by temperature and it is clear that either a direct role on maturation, or an indirect influence through the manipulation of the complex interactions between growth and body composition, may have occurred. In the current experiments the reservoir providing water for Site 7 is at a high altitude and water temperature fluctuations are modest. It is generally accepted that the water temperatures at Site 7 are colder than at many rearing sites (R. Murray, M. Porter, *pers comm*) such as Sites 1 and 2 where water is supplied from a shallow river. If the temperature at Site 7 were influential in the low incidence of maturation it might be expected that the 0+ groups of experiment Va would have high numbers of mature males due to the high summer rearing temperature. Indeed some mature fish were found in this group compared with the absence of maturation in the 1+ fish of experiment Vb suggesting a possible role of temperature. Previously, it has been shown that maturation will be influenced by growth potential during a critical period in spring (Thorpe, 1986; 1987b; Duston and Saunders, 1992; Metcalfe, 1998; Thorpe and Metcalfe, 1998) and it is therefore possible that instead of directly affecting maturation temperature affected the growth of the 0+ fish during their seasonally adjusted spring with a resultant increase in the incidence of maturation. However, it does seem that the evidence for an effect of temperature on maturation levels is at best circumstantial.

A more likely cause for the low levels of maturity may be the genetic influence of the stock used. Previously, a clear genetic component in both fresh- and seawater maturation has been documented (Naevdal, 1983; Thorpe *et al.*, 1983; Gjerde, 1984; Myers and Hutchings, 1986; Herbinger and Newkirk, 1990; Gjøen and Bentsen, 1997) although the genetic links between adult and parr maturity are poorly understood. Gjerde (1984) found that maturation in both fresh- and sea water were traits that were not inherited from one another. However, it is possible that early maturation, regardless of whether maturity occurs as a juvenile or adult, is of genetic importance with the underlying genetic components of growth (Thorpe *et al.*, 1983: Nilsson, 1990; Silverstein and Hershberger, 1994; Gjøen and Bentsen, 1997) also of importance in maturation. In the current experiments it is likely that the low grilsing Scottish stock used had an intrinsically low rate of parr maturation as well as a low rate of grilsing. As a result the incidence of maturation may not have been high enough to show between treatment variation. Indeed, the commercial production fish used at Site 7 are from the same stock as that used in the current study and maturation rates <1% are regularly noted under both 0+ and 1+ production cycles (A. McPhee, *pers comm*).

It is therefore likely that the genetic stock used in the current study was the main cause of the lack of maturity within groups. Clearly, care must be used in future experiments to ensure that the genetically determined rates of maturation in the test stock are high enough, as well as consistent enough, to allow the between treatment variation to be greater than the within stock variation.

4.4.3.2. Lipid accumulation

It is well documented that fish destined to mature show an increased accumulation of body fat followed by a reduction in lipid which occurs either slightly prior to, or during, gonadal development (Aksnes *et al.*, 1986; Jonsson *et al.*, 1991; Rowe *et al.*, 1991; Kadri *et al.*, 1996; Arndt *et al.*, 2000). In the current experiments only three maturing fish were found and it was not possible to follow the development of fat accumulation in these fish. However, in agreement with the literature all of these fish had much lower lipid contents than their immature counterparts highlighting the importance of lipid reserves in gonadal development.

Interestingly, in experiment IV the two mature fish that were identified had 4.4% and 3.0% body lipid compared to 7.8±0.3% and 7.7±0.4% for their respective immature counterparts. In experiment Va the mature fish found within the full ration treatment of the 0+ group contained 7.6% lipid compared to 10.1±0.3% for its immature counterparts. Similar variations were recorded by Shearer and Swanson (2000) when it was found that mature chinook salmon parr that were fed diets containing either 4 or 22% lipid contained 6.3±0.2% and 8.5±0.6% fat respectively whereas immature fish contained 5.2±0.4% and 10.1±0.4% lipid. Although care must be taken when comparing the lipid contents of individuals from different experiments, the variations in lipid content of mature and immature fish in the current experiments as well as those of Shearer and Swanson (2000) indicate that the fat threshold previously suggested for parr maturation (McCormick and Naiman, 1984; Simpson, 1992; Silverstein *et al.*, 1997) may not be of importance. However, it may be that a lipid threshold does influence maturation and that it is affected by environmental

parameters that influence the utilisation of energy such as temperature and photoperiod.

4.4.4. Smoltification

Throughout the literature various parameters have been used for the assessment of smoltification and in the current experiments a range of determinants were used.

Previously, a decline in condition factor (CF) has been indicative of the parr-smolt transformation (Solbakken *et al.*, 1994; Thrush *et al.*, 1994; Duncan and Bromage, 1998; Duncan *et al.*, 1998; Handeland and Stefansson, 2001). In experiment IV, all groups showed a peak in CF during September with condition subsequently declining in all groups to levels of around 1.1 in May indicating the progression of the parr-smolt transformation. However, it is important to note that Duncan and Bromage (1998) found the decrease in condition to be correlated with the decrease in autumnal temperature and it was questioned whether the decrease in condition was due solely to smoltification. In the current experiments the decline in CF occurred around the time of the autumn reduction in temperature although it should be noted that during spring the rise in water temperature did not result in an increase in condition. As such, a temperature/CF correlation does not hold throughout the parr-smolt transformation.

However, in experiment IV it was evident that whole body lipid levels showed a reasonable association with the decline in condition. Previously, although some contradictory evidence has been presented (Simpson *et al.*, 1992; Shearer and Swanson, 2000; Sutton *et al.*, 2000) good correlations have been found between condition and whole body lipid level (Herbinger and Friars, 1991). A decline in the

lipid level of fish undergoing the parr-smolt transformation has been found (Vanstone and Markert, 1968; Komourdjian *et al.*, 1976; Woo *et al.*, 1978; Helland and Grisdale-Helland, 1998) with such decreases ascribed to the increased metabolic constraints imposed by smoltification (Woo *et al.*, 1978; Saunders and Henderson, 1978; review by Shearer, 1994). Therefore, the results of experiment IV indicate that smoltification and CF may not be directly linked but that as the parr-smolt transformation progresses the utilisation of lipid reduces the condition of such individuals.

In experiment V a similar decline in CF was recorded as the parr-smolt transformation progressed in the 1+ fish and further supporting evidence is provided that whole body lipid levels were correlated with the decline. However, when the changes in condition of the 0+ fish were compared a different situation occurred. Although a clear decline in CF was found amongst the full and two-thirds ration fish the whole body lipid levels did not decline greatly and although the decline in CF has previously been correlated with temperature (Duncan and Bromage, 1998) during experiment Vb the decline in CF occurred some time after a decrease in temperature. Therefore, given that temperature controls the rate of growth response to photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994) the current study indicates that temperature will regulate the rate at which body lipid is utilised as opposed to directly cueing the timing of the decline in body fat or CF.

For a complete understanding of the interactions that occur during smoltification it is necessary to consider the effect of photoperiod. Photoperiod has an important role in smoltification (Duston and Saunders, 1992, 1995; Sigholt *et al.*, 1995; Duncan and Bromage, 1998; see chapter 3 for a detailed review). Therefore, by incorporating the

findings presented in this chapter it is likely that following the photoperiodic initiation of smoltification lipid reserves will be mobilised for the energetic process of hypo-osmoregulation although the rate with which this occurs will be determined by temperature. Condition factor will subsequently fall and although lipid levels may correlate well with this decline (especially in the case of 1+ fish) condition will not necessarily be a measure of whole body lipid status (Simpson, 1992; Shearer and Swanson, 2000; Sutton *et al.* 2000).

It is important to discuss the differences in condition and lipid content that occurred between the one-third ration fish from the 1+ and 0+ regimes. Under the 0+ photoperiod regime the one-third ration fish had a lower CF and lipid content than the full and two-thirds ration fish throughout the experiment although for the 1+ fish CF and lipid content were only lower until December. It is likely that temperature was influential in this between treatment difference. Previously, it has been found that the optimum temperature for growth and the accumulation of body lipid decreases with ration size (Elliott, 1975b, 1976) with the standard metabolic rate (SMR) of fish fed lower rations also shown to be reduced (O'Connor *et al.*, 2000). Therefore, during the colder months of winter growth and the accumulation of lipid in the one-third ration fish from the 1+ regime would have increased relative to the full and two-thirds ration fish. For the 0+ fish, rearing temperatures were high and as such the one-third ration fish did not experience an optimisation of growth at lower temperatures.

It is also interesting to note that a decrease in CF was observed in the one-third ration fish from both production regimes as the parr-smolt transformation progressed with this decline of similar timing and magnitude to that observed for the full and two-

thirds ration fish. Therefore, although the one-third ration fish were clearly under some level of nutritional stress they were still influenced by the relative smoltification cues with their growth and changes in body fat affected in a similar way to fish where full smoltification was likely.

During experiment IV gill Na^+, K^+ -ATPase levels were also recorded and used as a measure of smoltification (Johnston, 1983; McCormick *et al.*, 1987; Handeland and Stefansson, 2001). It should also be noted that in this experiment only the largest individuals were sampled thus those which were likely to undergo smoltification (Kristinsson et al, 1985; Thorpe, 1987a; Økland *et al.*, 1993). Differing dietary lipid level had no effect on the ATPase level of smolting fish until the final sample point when the levels recorded in the 12.5/12.5 group were lower than those of the 25/25 fish. Redell *et al.* (1988) previously reported that dietary lipid had no effect on smoltification in Atlantic salmon with Saunders *et al.* (1982) stating that high fat levels were not necessary for smolting. Incorporating the findings of the current experiment it would seem that dietary lipid plays only a minor role on smolt status.

In the current experiment although no consistent statistical differences could be highlighted the 12.5/12.5 regime appeared to result in a lower incidence of smolts compared to the other treatment groups although it should be noted that this distinction was made by external appearance (Johnston and Eales, 1970; Saunders and Henderson, 1978; Erikson and Lundqvist, 1982; Birt and Green, 1986; Tanguy *et al.*, 1994; Sigholt *et al.*, 1995), which is not necessarily a good measure of smoltification (Saunders *et al.*, 1985; Duncan and Bromage, 1998). Therefore, it seems that dietary lipid will have only a negligible effect on the incidence of smoltification.

Previously, feed restriction (Thorpe and Metcalfe, 1998) and starvation (Larsen *et al.*, 2001) during winter has been shown to have negligible effects on smoltification although unlike the current study there are no previous studies which have investigated the role of long-term rations of feed on smoltification. In experiment V, ration affected both the number of fish choosing to smolt, as well as the quality of such smolts. In both the 0+ and 1+ experiments feeding with full rations resulted in the highest numbers of smolts with high numbers in the two-thirds ration group but only low numbers in the one-third ration group. Thorpe and Metcalfe (1998) suggested that food restriction had an indirect role on smoltification by affecting the size of fish. Therefore, since ration will affect growth (Elliott, 1975b; Storebakken and Austreng, 1987a, b; McCormick *et al.*, 1989; Shearer *et al.*, 1997) the attainment of certain growth thresholds necessary for smoltification (Elson, 1957; Thorpe *et al.*, 1980; Skilbrei, 1988) will be achieved to a greater or lesser degree in the respective ration groups.

During experiment V it was evident that the 0+ fish had a higher incidence of smolts than their 1+ counterparts. Given the increased scope for growth under continuous light regimes (Higgins and Talbot, 1985; Saunders and Henderson, 1988; Villarreal *et al.*, 1988; Sigholt *et al.*, 1995; Taranger *et al.*, 1995) it is likely that the attainment of growth thresholds necessary for smoltification was achieved by a greater number of fish within the 0+ production regime especially if such growth advantages occurred during seasonally critical times when smoltification could be enhanced.

It is also important to note that the numbers of smolts found within the one-third ration group should be considered with some caution. High levels of mortality were

found amongst the low ration groups and it is likely that size selective mortality occurred within these populations (Storebakken and Austreng, 1987b). Since smoltification has been linked to size (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988; Økland *et al.*, 1993) it is likely that the percentage of smolts observed in this treatment was, to some degree, influenced by the survival of a particular size of individual. However, as mentioned earlier during the current experiments it was not possible to record the size of mortalities and as such it is difficult to determine the precise effects of any size-selective mortality. In particular, it is difficult to establish whether large or small individuals would have been more susceptible to mortality and consequently the effect of this on the smolting population.

Different rations of feed had variable effects on the hypo-osmoregulatory ability of smolting fish. For the 1+ fish neither the gill Na^+, K^+ -ATPase nor serum osmolarities of smolting fish showed differences between treatments with smolts from all groups attaining a good smolt status. For the 0+ fish the osmolarities of both the full and two-thirds ration fish decreased to similar levels as those of unchallenged individuals although the one-third ration fish did not show a decline of similar magnitude. Furthermore, at the final sample point, gill Na^+, K^+ -ATPase increased with increasing feed ration. Although the changes in serum osmolarity indicated a good smolt status in the full and two-thirds ration fish, with the differences in the ATPase level possibly negated in affecting ultimate seawater performance because ATPase levels have been shown to rise following seawater transfer (Saunders and Henderson, 1978; Solbakken *et al.*, 1994; Berge *et al.*, 1995), an effect of ration is likely in the 0+ fish.

Previously, Shearer (1994) suggested that a nutritional threshold was necessary for smoltification to progress. However, it is unlikely that the attainment of a nutritional threshold can account for the differential effects of ration on smoltification in the 0+ and 1+ fish of the current experiments since the levels of body lipid in the respective ration groups were similar regardless of photoperiod. Previous investigations have found good rates of survival following the out-of-season transfer of fish to sea (Duncan *et al.*, 1998) and as such understanding the mechanisms influencing the results of the current experiments is difficult.

It is possible that by adjusting the timing of the winter photoperiod the endogenous rhythms of smoltification had been disrupted (Clarke *et al.*, 1978; Eriksson and Lundqvist, 1982; Saunders and Harmon, 1990; Sigholt *et al.*, 1995; Duston and Saunders, 1995; Duncan and Bromage, 1998). Alternatively, it is possible that during the development of the 1+ fish the formation of osmoregulatory mechanisms utilised energy from both long and short term lipid stores. The liver is known as a short term energy store and Storebakken and Austreng (1987a) found that the weight of the liver increased with increased rations. Furthermore, Woo *et al.* (1978) found that both muscle and liver fat declined during smoltification, with Helland and Grisdale-Helland (1998) also finding that visceral fat declined during the parr-smolt transformation concluding that visceral lipids were the preferred energy source for smoltification. However, in the studies of Woo *et al.* (1978) and Helland and Grisdale-Helland (1998) a natural photoperiod regime was used. It is therefore possible that under accelerated photoperiod regimes fish may not be able to mobilise their preferred long-term energy stores (such as the viscera). As such, short-term reserves (i.e. the liver) may be utilised and those fish that have achieved enhanced

short-term lipid deposits (c.f. Storebakken and Austreng, 1987a) will develop an enhanced smolt status. In support of this the 0+ fish of the current study had only slight reductions in whole body lipid content, with Nordgarden *et al.* (2002) finding no changes in muscle or body lipid levels when a manipulated photoperiod regime was used suggesting that long-term energy reserves had not been mobilised. It is therefore clear that although a high smolt status can occur following photoperiod manipulation further study will be required into the changes in body composition that occur during the parr-smolt transformation before a clear understanding of variations in smolt status can be made.

In summary, it appears that dietary lipid will have only a minor effect on both the quality and incidence of smolts. The ration of feed, however, will have distinct effects on the number of fish choosing to undergo the parr-smolt transformation with some, although slight, effects on the quality of such fish. However, it is possible that whereas the effects of decreased ration may be more detrimental to smolt quality under advanced photoperiod production regimes such techniques may result in a reduced incidence of fish remaining as parr.

4.4.5. Conclusions

- Ration of feed has a primary affect on the growth of Atlantic salmon parr whereas dietary lipid exerts a greater influence on adiposity.
- The growth of parr appears to be under lipostatic regulation.
- Variable evidence exists that either maturation or smoltification are influenced by nutritional thresholds.
- Parr maturation may have a strong underlying genetic component, which could influence the environmental manipulation of maturity.
- Ration of feed affects the number of fish undergoing the parr-smolt transformation with dietary lipid having only a minor effect on the decision to smolt.
- The use of photoperiod manipulation to create out-of-season smolts may have detrimental effects on smolt quality especially where feed is limiting.

Chapter 5: General Conclusions and Future Work.

The present study aimed to investigate the role of photoperiod and diet on growth, maturation and smoltification in Atlantic salmon parr. By understanding how such environmental determinants and developmental processes interact their role in life history strategy could be investigated with such information utilised to increase the freshwater productivity of farmed Atlantic salmon.

5.1. Maturation

During the current investigation parr maturation was enhanced by a winter photoperiod applied during early development, i.e. in the May or June following first-feeding, in late March/April. This suggested that an early period during development was influential in the decision to mature (Saunders *et al.*, 1982; Saunders and Henderson, 1988; Thorpe, 1994b; Metcalfe, 1998) and it is probable that such a period was only influential during a strict developmental window, with extended exposure to the winter photoperiod during this period increasing the incidence of maturation. Continued exposure to this regime outside the developmental window would not necessarily cause increases in the incidence of maturation. It was also found that some of the mature parr were initially amongst the largest individuals providing further support for the importance of an early decision period in maturation.

Given that commercial production utilises increasingly early winter photoperiods for the year round supply of smolts (Thrush *et al.*, 1994; Duncan *et al.*, 1998) it may be possible that such regimes will influence the seasonal timing of the decision to mature and subsequently the incidence of maturation in populations. Between stock variation

in the timing of such a decision period may also result in an increased incidence of maturation in photoperiodically manipulated stocks. Furthermore, during the current investigation it was not possible to conclude whether the decision to mature was influenced at a particular chronological age of the fish or during a specific time of the year. Therefore further work will now be required to investigate the role of this early decision period in order to avoid increased rates of maturation following the out-of-season production of smolts.

During the current experiments there was the indication that a winter photoperiod later in the year (during either August or September) influenced a period when maturation was suppressed. Therefore further research will be required to identify if stocks with previously high levels of maturation can have their rates of maturation suppressed by photoperiod treatment during late summer.

During investigations into the effects of diet on freshwater development a clear lack of maturation was observed. Although some tentative suggestions were drawn from the mature fish that were identified it was evident that the between treatment variation in the incidence of maturation had been considerably lower than the natural variation within the test stock. Dietary, photoperiodic, and thermal regimes all had negligible effects on maturation and as such it was suggested that parr maturation may be influenced by a strong genetic component, which overrides environmental influences. In further support, when fish from the diet treatments were compared to groups exposed to similar experimental regimes during the photoperiod experiments (e.g. the August photoperiod treatment of experiment I) it could be seen that there was a clear difference in the incidence of maturation, with the photoperiod groups recording

higher rates of parr maturation. Given that the fish used in the photoperiod experiments were from a high grilsing stock, with the diet experiments utilising a low grilsing stock, it is possible that stocks with a high rate of grilsing also have an intrinsically high rate of parr maturation. It may therefore be more appropriate to consider stocks in terms of their rates of early maturation as opposed to their rates of grilsing.

5.2. Smoltification

Smoltification was dependant on the attainment of a particular size/developmental threshold either prior to, or during, a stimulatory winter photoperiod regime (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988). However, it was evident that for individuals which had not achieved these thresholds following exposure to an 8 week winter photoperiod smolt status and incidence could be enhanced by exposure to a 12 week winter photoperiod. From these findings it was suggested that a critical size threshold influenced recruitment to the smolting population during, and not solely prior to, the winter photoperiod (Duston and Saunders, 1997). However, it was also suggested that the magnitude of the critical size for smoltification was influenced by the duration of the winter photoperiod. As such further work will be required to identify whether a model can be provided that will give the most appropriate duration of winter photoperiod to stimulate smoltification for the size of a particular fish. Such a model may also allow the increasingly early production of smolts by exposing individuals traditionally considered too small to undergo smoltification to extended winter photoperiods regimes. However, it is also important that when investigating such a model

consideration is made to the effects that such photoperiod regimes might have on parr maturation and the obvious effects that this will have on seawater adaptability.

Dietary lipid level had no effect on the incidence of smoltification (Redell *et al.*, 1988) with the smolt status of individuals only slightly reduced by long-term exposure to low lipid inclusions (12.5%) over a range of developmental periods. As such, it is clear that a high lipid threshold is not necessary for smoltification (Saunders *et al.*, 1982) and it is likely that this was due to the different dietary lipid inclusions having no effect on growth. It is therefore probable that the commercial goals of freshwater development (i.e. good growth and a high incidence and quality of smolts) would not be affected by a reduction in the current dietary lipid inclusions used during juvenile production. However, further investigations will be required to elucidate the effects of freshwater dietary lipid inclusion on long-term survival and harvest quality in individuals following seawater transfer. Only after such research would it be possible to confirm the profitability of reducing the dietary lipid levels fed to juveniles.

Ration of feed was correlated with the incidence of smoltification with some reduction in the hypo-osmoregulatory ability of smolting individuals also found in fish fed the lower rations of feed. These results implied that such effects occur through ration mediated growth (Storebakken and Austreng, 1987b; McCormick *et al.*, 1989; Shearer *et al.*, 1997) highlighting the importance of a size threshold in determining smoltification (Elson, 1957; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988). Therefore research is necessary to identify the most productive rations of feed to maximise smolt production. It is also important that this research

takes into consideration interactions that occur between photoperiod, temperature and feed regime, in order to maximise productivity.

Under a 0+ photoperiod regime smolt status was affected by ration of feed with the 0+ treatment also resulting in a higher incidence of smolts compared to the 1+ photoperiod. These results indicated that the use of long periods of constant LD24:0, prior to short day treatment, enhanced growth (Saunders and Henderson, 1988; Stefansson *et al.*, 1989; Solbakken *et al.*, 1994) allowing a large proportion of the population to reach the threshold required for smoltification. It was also suggested that during the manipulated photoperiod regimes individuals could not utilise long-term energy reserves in order to undergo the morphological, behavioural and physiological changes required for full smolt development. As such short-term energy stores (e.g. the liver) may have been utilised as the primary energy source, which could have subsequently led to incomplete smolt development. Therefore, individuals under nutritional stress may not necessarily achieve a good hypo-osmoregulatory ability. Further research will be required to elucidate the role of ration of feed in smoltification and in particular it will be important to identify how energy reserves are mobilised and used during the development of smoltification. Such studies will provide important information regarding an individuals' energetic requirements during different developmental periods, in particular where manipulated photoperiod regimes are used to produce out-of-season smolts.

5.3. Maturation and smoltification interactions

Maturation and smoltification were not found to be completely mutually exclusive processes as previously suggested by Thorpe and Morgan (1980), Thorpe (1986,

1987a) and Herbinger and Friars (1992). Some smolting fish were found to be undergoing some level of gonadal maturation and since maturation is thought to be the preferred developmental route it is likely that mature fish subsequently developed to smolts. It may therefore be possible for commercial farms to use photoperiod treatments in order to recondition maturing parr and transfer them to sea shortly after gonadal maturation. In the light of the current findings further research will be required to elucidate the scope that such photoperiod treatments can provide for transferring previously mature parr to sea. Additional work will then be required to establish the potential for seawater growth in such individuals and whether they will be more susceptible to maturation following transfer. Subsequent cost-benefit analysis will also be necessary to establish whether it is financially advantageous to transfer mature parr to sea or whether it is more beneficial to cull the individuals from population, as is currently undertaken.

5.4. Endogenous rhythms

The results of the current experiments imply that growth (Clarke *et al.*, 1978; Duncan and Bromage, 1998; Duncan *et al.*, 1999), maturation (Whitehead *et al.*, 1978; Bromage *et al.*, 1984; Elliott *et al.*, 1984; Duston and Bromage, 1986, 1987, 1991) and smoltification (Clarke *et al.*, 1978; Erikson and Lundqvist, 1982; Stefansson *et al.*, 1989; Sigholt *et al.*, 1995) are all under some degree of endogenous control. However, the importance of such mechanisms may vary for the respective developmental strategies. Furthermore, although maturation occurred in the absence of photoperiodic changes when winter photoperiods were applied at different times of the year a phase shift in the timing of gonadal recrudescence was not observed. It is therefore important that further research is conducted into the role of endogenous

developmental rhythms in juvenile salmonids. Indeed, given the current commercial use of long periods of continuous light as well as winter photoperiods at increasingly early times of the year it will be important to establish how production regimes influence endogenous cycles of development.

5.5. Lipid accumulation

Whole body fat content was found to be correlated with dietary lipid inclusion (Reinitz, 1983; Shearer *et al.*, 1997; Shearer and Swanson, 2000) although no such correlation was found for fish growth. However, long-term variations in ration of feed did affect the growth of parr and although whole body lipid levels were reduced for those fed low rations of feed (i.e. one-third ration) at higher rations (i.e. full and two-thirds ration) whole body fat levels were maintained. This maintenance of body fat status through reductions in size provided support for the theory that growth is under lipostatic control (Silverstein *et al.*, 1997; Jobling and Johansen, 1999; Johansen *et al.*, 2001).

5.6. Summary

In commercial aquaculture freshwater productivity focuses on achieving good growth rates and a high incidence of fully competent smolts. Such goals are increasingly achieved through the manipulation of environmental parameters but the effects of such regimes should be considered in depth before such manipulations are used on a commercial scale.

From the investigations detailed in the current thesis it is likely that in the longer term genetic manipulations will provide the most effective method of limiting parr

maturation. Environmental influences, in particular photoperiod, will continue to be used to control maturation (Berg *et al.*, 1994; Taranger *et al.*, 1998; Porter *et al.*, 1999a; Duston and Saunders, 1995) but it is likely that a strong genetic component underlies such parameters. However, in order to further reduce levels of maturation through environmental manipulation more research will be necessary. Furthermore, it is clear that whether environmental or genetic manipulations are to be used some investigation will be required into the links between the age at maturity of both juvenile and adult fish.

Although the manipulation of photoperiod and nutrition is extensive within salmonid aquaculture the effects of temperature on salmonid development are less well documented. Although it has been suggested that temperature affects the rate of response to particular environmental factors such as photoperiod (Clarke *et al.*, 1978; Solbakken *et al.*, 1994) a clear understanding of its role in particular with regards to its seasonality is required. The control of temperature in experimental and production facilities can prove problematic (primarily due to the costs of heating and chilling water) but through either direct temperature manipulation, or through the out-of-season use of experimental parameters to utilise different components of the yearly temperature profile, some investigation into thermal influences is required.

Similarly, during future experiments it is important that there is consideration of the effects that different rearing conditions have on growth and development. During the current experiments it was not possible to standardise factors such as tank size, flow rates and water quality throughout all of the experimental treatments. Indeed this is frequently the case in both production and research populations. The influence of such

factors is not currently well understood and it is important that in future studies there is a better standardisation of rearing conditions. Furthermore, future research is required to elucidate the effects that such factors have on fish development.

Finally, it is evident that the way in which current salmon research is undertaken needs to be built upon. Typically, experiments focus on either freshwater or marine development without considering interactions that occur between the two phases. For example many of the suggestions for further study detailed in this chapter should only be conducted if the long-term effects on subsequent adult development are properly considered. With the now increased understanding of developmental strategies it is important to consider the wider picture and assess investigations throughout the life of the salmon.

This thesis has extended our understanding of freshwater development and the interactions which occur to determine the life history strategies undertaken by juvenile Atlantic salmon. It has provided useful information to aid both aquaculture and fisheries enhancement programmes although it is clear that substantial further research will be required before a more complete understanding of parr growth, maturation and smoltification might be provided.

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Appendix 1.

Research publication

Photoperiodic effects on precocious maturation, growth and smoltification in Atlantic salmon, *Salmo salar* .

Available online at www.sciencedirect.com
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Aquaculture 222 (2003) 239–252
www.elsevier.com/locate/aqua-online

Photoperiodic effects on precocious maturation, growth and smoltification in Atlantic salmon, *Salmo salar*

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Received 31 October 2002; accepted 18 December 2002

Abstract

. Current Atlantic salmon farming practice induces early smoltification with artificial photoperiod regimes, however the importance of these photoperiods on parr maturation and interactions with smoltification are poorly understood. These questions were addressed in the present investigation, which examined the effects of photoperiod manipulation on the development, maturation and smoltification of individually tagged parr.

Approximately 9000 salmon parr from a high grilsing stock were exposed to continuous light (LL) from first feeding. Three sub-groups of 2400 parr, each sub-group in triplicate tanks, were then exposed to an 8-week "winter photoperiod" (LD 10:14) starting on either the 18th May, the 9th August or the 20th September (defined, respectively, as the May, August and September groups). Following the artificial winter, each group was returned to LL. A fourth group of 1600 fish was maintained in replicate tanks on LL throughout.

The highest levels of maturation (approx. 20%) were recorded in the May group. August and September groups showed low levels of maturity (<5%) with constant LL throughout resulting in intermediate levels (<9%). However, only groups exposed to the August photoperiod showed high levels of smoltification.

It is concluded that the photoperiod to which parr are exposed early in their life acts as an important trigger for precocious maturation but does not necessarily phase shift the endogenous rhythm which is thought to control its timing. Smoltification is strongly influenced by the timing of

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0044-8486/03/$ - see front matter
doi:10.1016/S0044-8486(03)00125-X

exposure to winter photoperiod with clear evidence indicating that maturation and smoltification are not mutually exclusive processes.

Keywords: Atlantic salmon; Parr; Maturation; Smoltification; Photoperiod

1. Introduction

Understanding the plasticity of the Atlantic salmon, *Salmo salar*, life cycle (Thorpe, 1994a; Fleming, 1998; Metcalfe, 1998) is an important determinant of the success of its culture. Of particular importance to growth and smoltification is the "precocious" maturation of a proportion of parr in fresh water. Early maturation, although rare in females (cf. Bagliniere and Maisse, 1985; Hindar and Nordland, 1989) is commonplace among males under both wild (Dalley et al., 1983; Myers, 1984; Bagliniere and Maisse, 1985; Whalen and Parrish, 1999) and farmed conditions (Thorpe et al., 1990; Rowe and Thorpe, 1990a; Duston and Saunders, 1992, 1997). However, the environmental, physiological and genetic interactions which result in precocious maturation are poorly understood.

Early maturing fish are initially among the fastest growing individuals within the population (Saunders et al., 1982; Rowe and Thorpe, 1990a). However, somatic growth then decreases in favour of gonadal growth. Population bimodality may occur as a consequence of such growth differentials related to life history strategy (Thorpe, 1977; Bailey et al., 1980; Thorpe et al., 1980; Porter et al., 1998). Various thresholds of size, growth rate and energetic status suggested for smoltification (Elson, 1957; Thorpe et al., 1980) and maturation (Berglund, 1992; Herbinger and Friars, 1992; Whalen and Parish, 1999; Porter et al., 1999) are important in determining when smoltification and maturation are initiated. Thorpe and Morgan (1980) and Thorpe (1986) suggested that smolting and maturation are mutually exclusive and that smoltification is the result of a fish failing to mature (Thorpe, 1994b; Thorpe and Metcalfe, 1998). However, Saunders et al. (1982), Myers (1984), Bagliniere and Maisse (1985) and Kristinsson et al. (1985) have all described mature fish which smolt in the subsequent spring suggesting that the two are not mutually exclusive.

The manipulation of environmental parameters, such as temperature (Adams and Thorpe, 1989), photoperiod (Adams and Thorpe, 1989) and feed availability (Rowe et al., 1991; Berglund, 1995) at seasonally critical times, has resulted in reduced parr maturation. Photoperiod manipulation is the tool most used by farms to control growth, reproduction and smoltification (Hansen et al., 1992; Thrush et al., 1994; Duncan et al., 1998; Porter et al., 1999; Endal et al., 2000). However, the effects of photoperiod on parr maturation (e.g. Lundqvist, 1980; Saunders and Henderson, 1988) are still largely unknown and are further addressed in the present study.

2. Materials and methods

2.1. Fish stock and rearing conditions

Experimental fish were of Loch Lochy stock, maintained at the Buckieburn Freshwater Research Facility, Scotland (56°N) under ambient water temperatures (Fig. 1). From first feeding on 29th March, 800 fish were placed into each of 11 2-m^2 tanks which were constantly illuminated (LL) by 500 W halogen lights providing 3800 lx at the water surface and 1200 lx at the tank floor (0.3 m) (photometric sensor, Skye Instruments, UK). Flow rates were 1 l s^{-1} and oxygen levels remained above 8 mg l^{-1}. Feed was supplied at the manufacturer's recommended rate (Trouw Aquaculture) and was distributed evenly throughout the light phase.

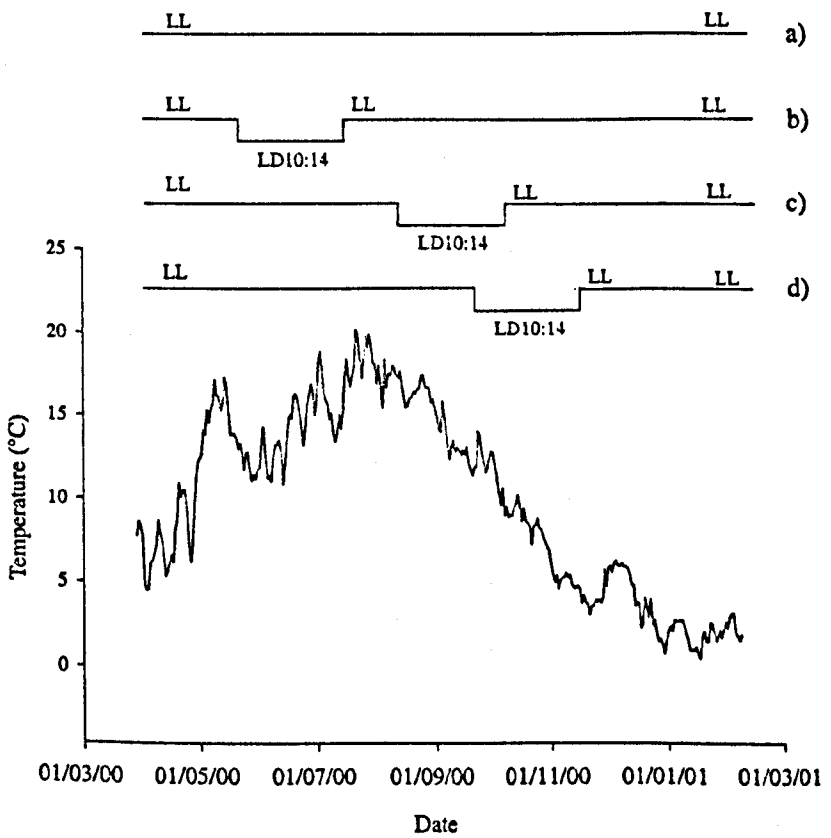


Fig. 1. Ambient water temperature relative to the four experimental photoperiod regimes. (a) Constant illumination (LL), (b) May photoperiod, (c) August photoperiod, (d) September photoperiod.

On 18th May, four experimental treatments were created (Fig. 1) within the 11 tanks as follows:

- May winter photoperiod—Triplicate tanks with an 8-week winter photoperiod (LD 10:14) starting on 18th May. LL thereafter.
- August winter photoperiod—Triplicate tanks with an 8-week winter photoperiod (LD 10:14) starting on 9th August. LL thereafter.
- September winter photoperiod—Triplicate tanks with an 8-week winter photoperiod (LD 10:14) starting on 22nd September. LL thereafter.
- Constant light (LL)—Duplicate tanks exposed to LL throughout.

On 25th July, 100 individuals from each tank were PIT tagged (AVID tags, Norco, CA, USA) and the adipose fin removed. Size at tagging was approximately 4 g and mortality <5%. Individuals from the May photoperiod group were not tagged as they were too small.

2.2. Sampling regime

From 25th July, individual fork lengths (±1 mm) and weights (±0.1 g) were recorded, under anaesthesia, twice monthly in all groups to ensure the identification of first maturation and the timing of growth divergences between cohorts. Condition factor was calculated as: weight (g) × fork length $(cm)^{-3} \times 100$. At each sampling, all non-PIT tagged fish were assessed for maturity, i.e. the presence of running milt.

At 2-week intervals, from 4th October, 15 randomly selected individuals per treatment were exposed to a 96-h seawater (37.5 ppt) tolerance test (Saunders et al., 1985) and mortalities recorded.

On 4th January 2001, 100 non-tagged individuals per treatment group were killed and dissected to quantify internal signs of maturation, i.e. enlarged gonadal tissue. The tagged fish from all groups were then randomly divided into two 2-m^2 tanks and maintained on LL until 7th February 2001 at which point they were measured for fork length and weight; sacrificed and maturity assessed by internal examination.

At the conclusion of the experiment, fish were classified into five cohorts based on morphology (Birt and Green, 1986) as follows:

1. Smolts: Fully silvered fish with no parr marks and black margins on the fins.
2. "Large" smolts: Fully silvered fish with no parr marks with black margins on the fins. These fish were significantly larger than the smolts described above (i.e. >100 g).
3. "Silvered" parr: Fish that were partially silvered with parr marks that were obscured but still visible.
4. Parr: Fish showing no signs of silvering and with the presence of distinct parr marks.
5. Small parr: Fish showing no signs of silvering, with the presence of distinct parr marks but that were significantly smaller than the parr described above (i.e. <10 g).

2.3. Statistical analysis

Data were analysed using Minitab v13.1. Changes in weight and condition factor were compared using a General Linear Model. Residual plots were used to confirm normality and homogeneity of variance. A significance level of 5% was applied to statistical tests (Zar, 1999).

3. Results

3.1. Maturation

The four photoperiod regimes had clear effects on maturation (Fig. 2). Maturing fish were first observed in early October and continued to be identified until the conclusion of the experiment in all groups. In the May photoperiod group, the percentage of mature males rose sharply between early and mid-November with levels reaching approximately 20% of all fish by December and remaining above 20% until February. Under constant light, the percentage of maturing fish increased to 8% during early November and remained unchanged through to February. August and September treatments resulted in maturity levels of approximately 3% from October onwards.

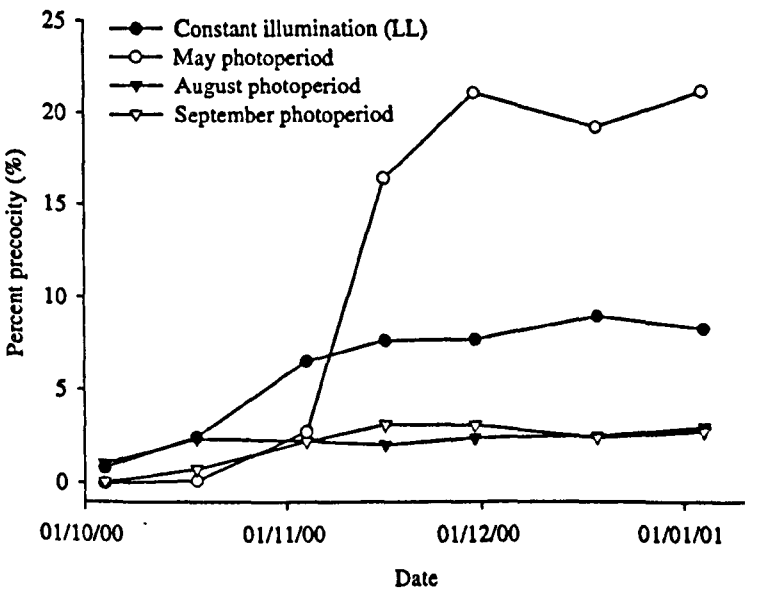


Fig. 2. Cumulative percentages of precocious males in the four experimental treatments. Values were for all non-tagged individuals within the population with maturity based on the presence of running milt.

3.2. Growth

Under LL, fish destined to become small parr were significantly smaller than all other cohorts in August (Fig. 3a). Smolts were significantly larger than mature parr by mid-September ($p<0.05$) with immature parr differing from smolts by early October. However, it was not until mid-October that the parr cohort showed significant differences between immature and mature fish ($p<0.05$).

In the August photoperiod group, all cohorts except small parr remained of a similar size until 16th November (Fig. 3b). Fish destined to mature as parr were significantly larger than small parr by July ($p<0.05$), whereas remaining cohorts did not differ significantly until August. In mid-November, smolts were significantly larger than precocious parr ($p<0.05$). Immature parr only differed significantly from the smolts and precocious parr from late November ($p<0.05$).

All the cohorts except small parr in the groups under the September photoperiod remained of similar size until mid-December (Fig. 3c). Immature parr diverged from small parr in early August with smolts larger by mid-August and mature parr heavier by early September ($p<0.05$). Smolts and parr had similar weights until mid-December when smolts were heavier than mature parr. In early January, the weights of all groups were statistically different ($p<0.05$). However, by the end of the experiment, in early February, the weights of immature parr and smolts were similar ($p>0.05$).

In the May photoperiod group, only the growth of immature or mature fish could be studied (Fig. 3d). However, no significant differences in weight were observed between immature and mature fish ($p>0.05$).

Under LL, both immature and mature parr showed initial increases in condition factor (Fig. 4a) with immature, mature and small parr showing an overall decline in CF, from approximately 1.25 to 1.15, by January ($p<0.05$). However, with the exception of immature and mature parr, which were significantly different from late September onwards, no consistent differences occurred between cohorts throughout the experiment.

In the August photoperiod, CF initially rose in smolt, immature parr and small parr groups (Fig. 4b) with all cohorts showing an overall decline in CF by January ($p<0.05$). Smolts also showed a significant decline during October although no consistent differences were observed between cohorts.

Smolts, immature parr and small parr all showed initial increases in CF under the September photoperiod (Fig. 4c) with only the condition factor of immature parr significantly decreasing by January ($p<0.05$). Again, no consistent differences were observed between cohort groups.

A May photoperiod resulted in an initial rise in the CF of immature fish (Fig. 4d) with an overall decrease by January ($p<0.05$). However, the CF of mature fish did not decline or differ significantly with the CF of immature parr throughout the experiment ($p>0.05$).

Between treatment, differences in CF only occurred in immature parr and smolt cohorts. For immature parr, the CF of LL and August photoperiod groups remained similar, with the CF of both groups higher than that of the immature parr from the September photoperiod. These differences remained from July until late September for the LL group and throughout the experiment for the August photoperiod fish. The CF of smolts only differed between August and September photoperiod groups with August

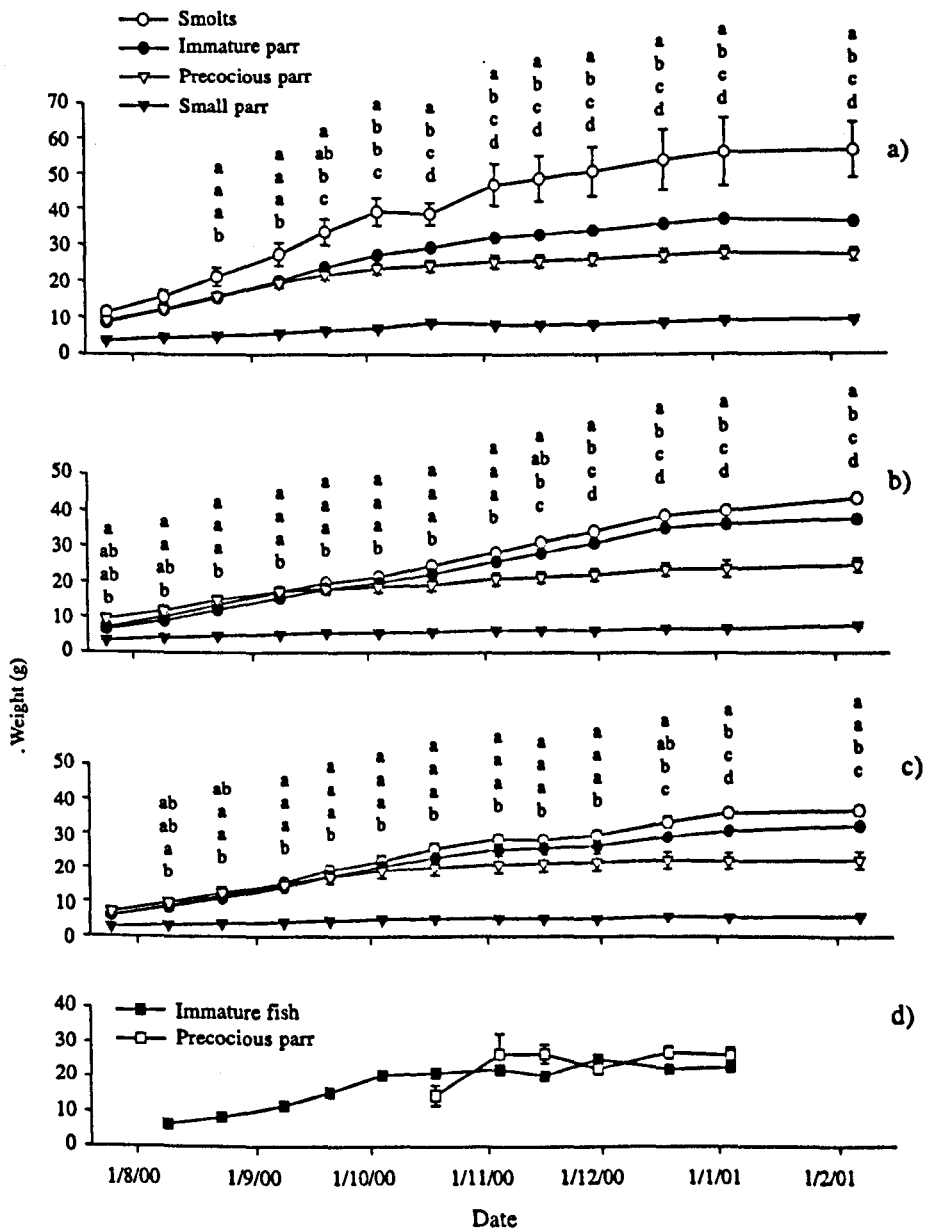
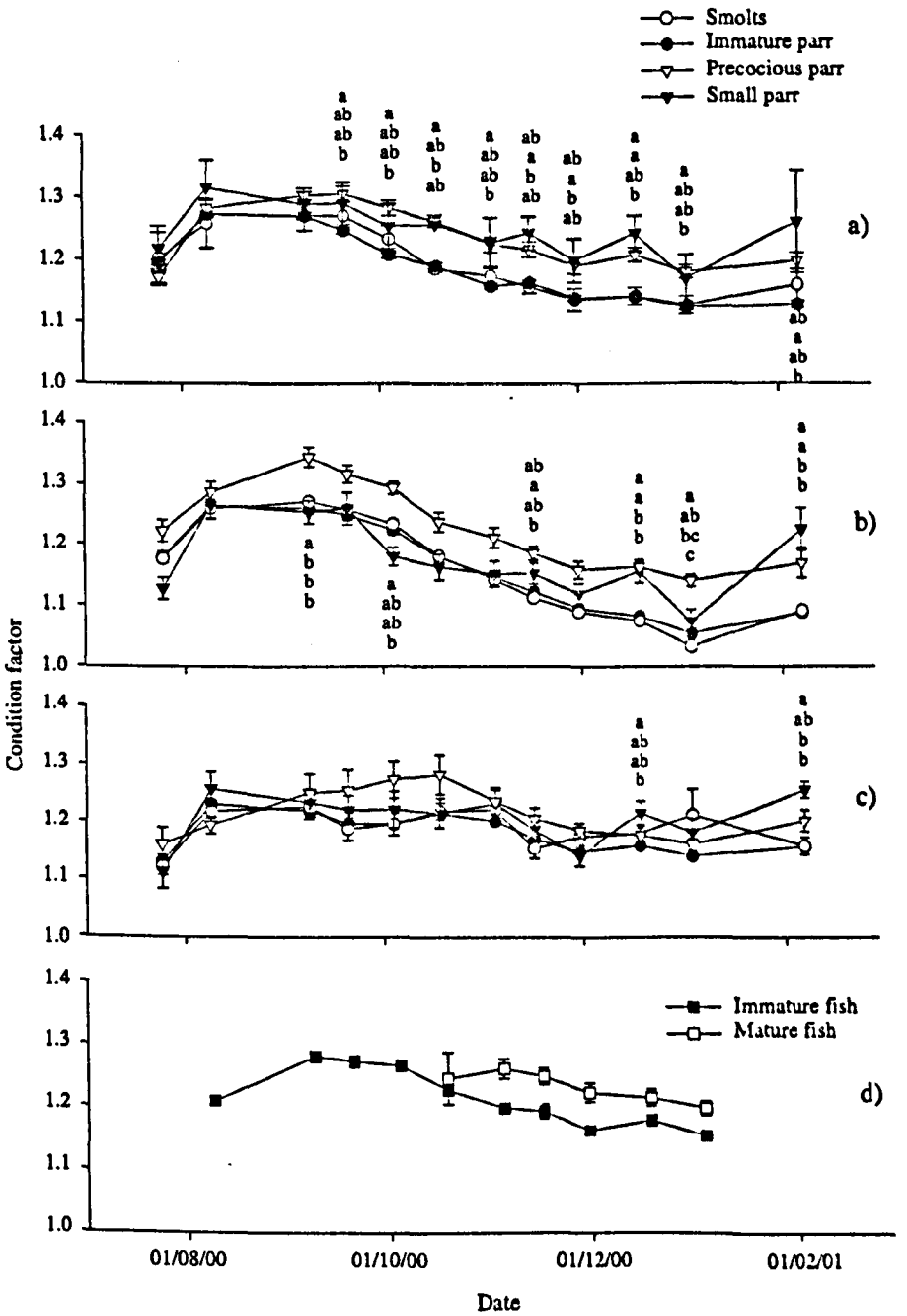


Fig. 3. Changes in weight of the four cohorts of individually PIT tagged fish following exposure to constant illumination (LL) (a), August photoperiod (b), September photoperiod (c) and May photoperiod (d) regimes (mean ± S.E.M., $n = 100$ for constant illumination, August photoperiod and September photoperiod groups, $n = 30$ for May photoperiod fish). For the May photoperiod group, only mature and immature fish are shown due to the absence of tagging in that group. Values with different letter labels are significantly different ($p < 0.05$). Lettering has been stacked in the same order as the graph lines.


Smolts
Immature parr
Precocious parr
Small parr
Immature fish
Mature fish
a)
b)
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d)
Condition factor
Date
01/08/00
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01/12/00
01/02/01

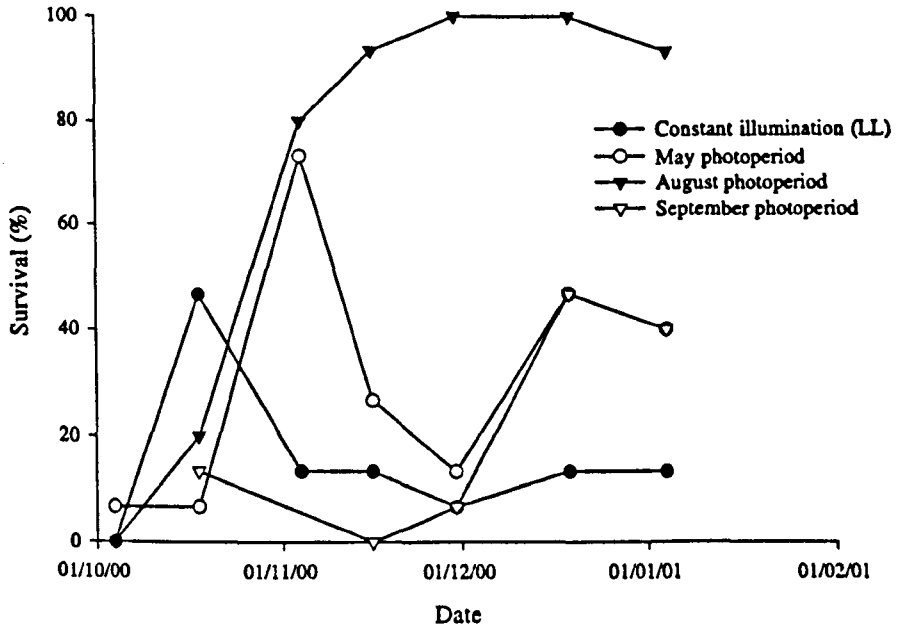

Fig. 5. Percentage survival following a 96-h seawater (37.5 ppt) tolerance test for fish exposed to the four photoperiod regimes.

photoperiod smolts having a higher CF from November until the end of the experiment ($p<0.05$).

3.3. Seawater tolerance

Survival rates following seawater exposure showed variable results in the LL group as well as in the May and September photoperiod groups throughout the experiment (Fig. 5). However, fish exposed to an August winter photoperiod showed increases in survival from 4th October, reaching 100% during late November, before declining slightly in early January.

3.4. Cohort structure

Photoperiod manipulation resulted in distinct differences in population structure (Table 1). LL resulted in 92% of the population remaining as parr, including 10% that matured.

Fig. 4. Changes in condition factor of the four cohorts of individually PIT-tagged fish following exposure to constant illumination (LL) (a), August photoperiod (b), September photoperiod (c) and May photoperiod (d) regimes (mean ± S.E.M., $n=100$ for constant illumination, August photoperiod and September photoperiod groups, $n=30$ for May photoperiod fish). For the May photoperiod group, only mature and immature fish are shown due to the absence of tagging in that group. Values with different letter labels are significantly different ($p<0.05$). Lettering has been stacked in the same order as the graph lines.

Table 1
The effects of varying the timing of exposure to an 8-week winter photoperiod on the cohort structure (based on external appearance) and internal signs of maturation of non-tagged individuals within the population at the conclusion of the experiment (4th January 2001)

	Constant illumination		May photoperiod		August photoperiod		September photoperiod	
	Imm (%)	Mat (%)	Imm (%)	Mat (%)	Imm (%)	Mat (%)	Imm (%)	Mat (%)
"Large" smolts	–	–	14	4	–	–	–	–
Smolts	–	–	1	1	19	1	–	–
Silvered parr	6	–	10	3	30	7	28	–
Parr	82	10	38	11	13	7	50	9
Small parr	2	–	13	5	21	2	12	1

Refer to Materials and methods for details of cohort nomenclature. Imm denotes immature fish. Mat denotes mature fish.

The May photoperiod treatment caused 49% of the population to develop as parr. The remainder of the population included fish from all cohort classes and it was only in this group where the presence of "large" smolts was observed. Every cohort in this group exhibited mature individuals. A winter photoperiod in August provided the highest percentage of immature smolts (19%), silvered parr (30%) and small parr (21%). Again, all cohort classes included maturing fish. A winter photoperiod in September resulted in the majority of fish remaining as parr (59%) with 28% appearing as silvered parr. Small parr were also observed (13%) but the incidence of maturing individuals was restricted to parr (9%) and small parr (1%).

4. Discussion

Varying the time of exposure of Atlantic salmon parr to 8 week periods of short days resulted in significant effects on both smoltification and maturation with early exposure resulting in the highest levels of maturation.

The timing of maturation in salmonids is said to be most stimulated by an initial period of long days followed by a period of short days (Bromage et al., 1984; Elliott et al., 1984; Takashima and Yamada, 1984). In the present work, high levels of maturation were observed in the May photoperiod group confirming the importance of a reduction to short days in the control of maturation in parr development. However, the absence of high levels of maturing fish in the two groups exposed to winter photoperiods in August and September indicates that a period of short days is not necessarily required for maturation to be completed. Eriksson and Lundqvist (1980) noted that a sudden change from long to short days did not necessarily induce maturation in Baltic salmon parr. However, Berg et al. (1994), reported similar results to the present study, with a 7-week period of LD 14:10 resulting in high levels of maturation in Atlantic salmon parr. The early period of reduced daylength may initiate reproductive development or phase shift the reproductive cycle (Duston and Bromage, 1986). It has been shown that photoperiod manipulation (Porter et al., 1999; Taranger et al., 1999) and feeding restriction (Rowe and Thorpe, 1990a; Berglund, 1995;

Hopkins and Unwin, 1997) at seasonally critical times, can suppress maturation with springtime being suggested as the critical period (Rowe and Thorpe, 1990a; Berglund, 1995; Taranger et al., 1999). However, this implies that the developmental choice to mature has already been taken and it may be that it is not the timing that is as important as the developmental stage of the fish. Furthermore, it is well documented that maturing fish are initially among the fastest growing individuals within a population (Saunders et al., 1982; Dalley et al., 1983; Rowe and Thorpe, 1990b; Berglund, 1992) and it seems from the present work that the period, shortly after first feeding, may be an important one in the decision to mature.

Under LL, maturation still occurred indicating that maturation is controlled by an endogenous rhythm, entrained by photoperiod, as suggested by Eriksson and Lundqvist (1982), Bromage et al. (1984), Elliott et al. (1984) and Duston and Bromage (1986). However, the timing of maturation between treatment groups was similar, therefore a phase shift of the rhythm had not occurred.

Previously, Thorpe and Morgan (1980) and Thorpe (1986) suggested that smoltification and maturation were mutually exclusive and smolting occurred as a consequence of failing to mature (Thorpe, 1994b; Thorpe and Metcalfe, 1998). The results presented here, as well as those of Bagliniere and Maisse (1985), Whalen and Parrish (1999) and Utrilla and Lobón-Cerviá (1999) show that these processes are not exclusive. Salmon need to attain a threshold size before they can either mature (Berglund, 1992) or smolt (Elson, 1957; Skilbrei, 1988) and Saunders et al. (1982) suggested that the maturation threshold is lower than that for smoltification. Furthermore, the reduced growth rate of maturing fish (Rowe and Thorpe, 1990b) may preclude such individuals from smolting. In the current study, the May and August photoperiods were proceeded by long periods of constant light and under such conditions of good growth it has been suggested that certain fish may first attain a suitable size to mature, and then continue to grow such that smoltification is also possible (Villareal et al., 1988; Solbakken et al., 1994). Furthermore, temperature is an important factor in growth (Herbinger and Friars, 1992; Duston and Saunders, 1997) and as such can be a determinant in the decision to both mature (Adams and Thorpe, 1989; Solbakken et al., 1994) and smolt (Solbakken et al., 1994; Duston and Saunders, 1997). In the May photoperiod group, the period of increased ambient temperature, prior to the application of the winter photoperiod, as well as elevated temperatures during the applied winter and spring/summer may have enhanced the number of fish choosing to mature. For August photoperiod fish, it is possible that the decline in temperature following the return to LL may have resulted in fish opting to undertake smoltification as opposed to maturation. For September photoperiod fish, it seems that the winter photoperiod and subsequent LL occurred at temperatures which were too low to greatly enhance the numbers of either mature or smolting fish.

Finally, the feeding regime applied to treatment groups may have influenced the decisions to both mature and smolt. All groups were fed at the same rate throughout the respective light phases of the specified photoperiods. Higgins and Talbot (1985) noted that photoperiod was influential in regulating food intake, and indeed in the current study fish exposed to winter photoperiod regimes were fed over a shorter period of time (although total feed rates were not reduced). During artificial winter

photoperiods, growth is always suppressed and therefore it is unlikely that the feeding regime curtailed growth rates.

In conclusion, the current study shows that photoperiod has a major influence on the incidence of precocious maturation as well as smoltification in Atlantic salmon parr. It also showed that some individuals were able to mature and then undergo smoltification showing that the two processes are not mutually exclusive. A period of short days, early in development, increased the percentage of the population which showed early maturation. These results suggest that under current farming conditions the use of increasingly early winter photoperiods, to further advance smoltification, may result in increased incidences of precocious maturation.

Acknowledgements

The authors would like to thank the staff at the Buckieburn Freshwater Research Facility, and Billy Robertson for advice on fish rearing and PIT tagging. This work was supported by a NERC CASE award to IB and NERC ROPA and Marine Harvest Scotland grants to NB and MP.

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