ENVIRONMENTAL REGULATION OF SMOLTING AND MATURATION IN ATLANTIC SALMON (*Salmo salar* L.) PARR.

Thesis

submitted for the degree of

Doctor of Philosophy

in the

Faculty of Science

at the

University of Glasgow

by

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January, 1988.

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ACKNOWLEDGEMENTS

This work was carried out at the University Field Station, Rowardennan and at the D.A.F.S. Almondbank Hatchery. I would like to thank Prof. R.S. Phillips for his support in the provision of facilities at the U.F.S. and Mike Miles, Steve Keay and Jim Muir who provided invaluable husbandry and technical assistance at Almondbank.

I should also like to express my gratitude to my supervisors, Dr J.E. Thorpe, who initially interested me in this project and carefully guided me through the research, and Dr R. Tippett who provided considerable support throughout.

This work benefitted greatly from discussion with Dr Neil Metcalfe, especially on statistical matters and from technical assistance from Derek Adams, David Brown, Janette Crawford and Rab McMath.

SUMMARY

Fish successfully exploiting rapidly changing environments must be capable of short-term adjustments to their life history strategy. Such a fish is the Atlantic salmon (Salmo salar L.).

Salmon developmental rates (growth, smolting and sexual maturation) vary greatly between populations and families and are known to be heritable. However expression of genetically controlled developmental rate may be modified by environmental conditions. Thorpe in 1986 published a model linking parr growth, smolting and maturation rates. The model predicts that parr during their first year may be twice faced with distinct developmental pathway options, of which there are 3 phenotypic outcomes: sexually mature parr, capable of breeding during the following winter; immature parr which shut down growth over winter and appear as the lower mode of a bimodal size distribution in late autumn and do not emigrate to sea the following spring; smolts, which continue growth into their first winter, appearing as the upper mode in a bimodal size frequency distribution in autumn and emigrate to sea the following spring. The model predicts that the decisions are made during two discrete periods and these are temporally separate and that the developmental outcome of each is dependent on the rate of

acquisition of energy during these periods.

To test these predictions, sibling populations of underyearling parr, hatched early using heated incubation water and hatched at ambient water temperature, were exposed to simulated natural photoperiod regimes out-of-phase with controls (up to $\pm$ 3 months) and elevated water temperatures (up to 5°C above ambient) and to constant photoperiod and temperature regimes. Growth rates were measured monthly and population smolting rates and reproductive investment measured during the first winter.

Cyclic photoperiod regimes out-of-phase with ambient were found to influence the timing of the smolting decision, indicated by a change in the timing of segregation of the bimodal distribution in autumn. However, extreme photoperiod phase changes did not reflect identical phase changes in segregation. In addition modal segregation was still found to occur under constant light regimes. This supports the hypothesis that photoperiod is acting as a synchronisor of an endogenous seasonal timing rhythm (Chapter 2).

Photoperiod and temperature were both found to affect growth (Chapter 3). Parr were shown to have reduced food intake during low light intensity periods (Chapter 5), so daylength affects food intake. Temperature affects

activity, appetite and assimilation efficiency. An index, the "thermal-sum" combining both of these factors is proposed as an indicator of the growth opportunity afforded by these abiotic factors. Confirming the model's predictions growth opportunity, especially in July and August, was found to correlate well with smolting rate, suggesting that it is during this period when the developmental decision to smolt is taken.

Good growth opportunity in early spring was found to induce male sexual maturity in 0+ parr. The population exposed to the best growth conditions at this time also showed high rates of smolting as did a reciprocal population that did not receive early spring photoperiods, however the latter population did not show any male sexual maturation, indicating that the timing of the maturity decision is influenced by photoperiod and that it occurred prior to the smolting decision between 1st February and 1st May, in these populations.

No females matured, all oocytes were found to be in the primary oocyte stage of development. However growth opportunity was found to influence the relative investment in ovary. Elevated temperature and longer growth period prior to the breeding season, as determined by perceived photoperiod, increased the investment in ovary for any given

size of fish. This increased investment took the form of larger oocytes in most groups, however in two of the fastest growing groups evidence of increased oocyte numbers is presented. It is suggested that good growth opportunity at this early stage of oocyte development may affect the background fecundity level, which will be modified in the light of subsequent growth opportunity.

Social rank is suggested as a possible biotic regulator of developmental rate. This was investigated from behavioural analysis of individually marked fish held under conditions where competition for feeding territories was high. However no link between social status and smolting rate was established.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The Atlantic salmon (Salmo salar L.) is a fish species with unequalled public status. This partly stems from its often spectacular migratory behaviour and partly from its reputation as a "clean water" fish.

It is also a commercially important species. In Scotland, traditional netting of migrating salmon, yielded over 1000 tonnes of salmon in 1984 (Russel et al. 1986). In addition, the salmon farming industry is becoming increasingly important as a rural employer and as source of table salmon to the consumer. Scottish farms yielding around 4000 tonnes in 1984, and a predicted 20,000 tonnes in 1988 (Laird and Needham 1986).

Scottish tourism benefits from an abundance of good salmon angling rivers, which attract foreign visitors. It has been estimated that salmon fishing may directly and indirectly contribute as much as £100 million per annum to the Scottish economy through tourism (Smith 1986).

Biologically salmon have a number of features that are of

special interest. Their anadromous life-cycle requires their physiology to cope with the change from a hypotonic to a hypertonic medium and vice versa, without excessive disruption to their hydromineral balance.

Atlantic salmon also show an enormous amount of flexibility in their life history. Growth rates, the timing of emigration to sea, the timing of their return and their age at first maturity all vary greatly between and within populations. In recent years much ecological interest has been focussed on the theory of life history strategies, specifically how different strategies between and within species can provide behavioural answers to particular ecological problems. As a result of the plasticity of life history strategy, Atlantic salmon provide a particularly attractive species for evaluating such theories.

It is the life history strategy of underyearling parr, specifically the timing of emigration to sea and the age of first sexual maturation, with which this thesis is concerned.

1.2 Life cycle of Atlantic salmon

The life cycle of the Atlantic salmon can be classified into a series of developmental stages which are more or less

well defined (Fig. 1.1) (For detailed description of the life-cycle see Jones 1959 and Mills 1986).

1.2.1 OVA

In the U.K. the cycle begins in November or December when the eggs are laid in a depression in gravel, constructed by the female in the spawning bed (the redd), normally in a rapidly flowing shallow stream. They are immediately fertilized by a male, who has been in close association throughout the construction of the nest. The eggs are then covered by the debris displaced from the excavation of the next nest, normally immediately upstream from the first. Several nests may be constructed in this way until all the eggs, up to 20,000, have been shed.

The incubation period of the ova is very temperature dependent and thus will vary between years and rivers. However, normally the eggs will hatch around March or April in Scotland.

1.2.2 ALEVINS

The alevins which emerge from the eggs remain under the gravel of the spawning bed, utilizing reserves from their yolk-sac as an energy source. As the yolk-sac is used up, the alevins start to move up through the gravel to open water where they will take their first food.

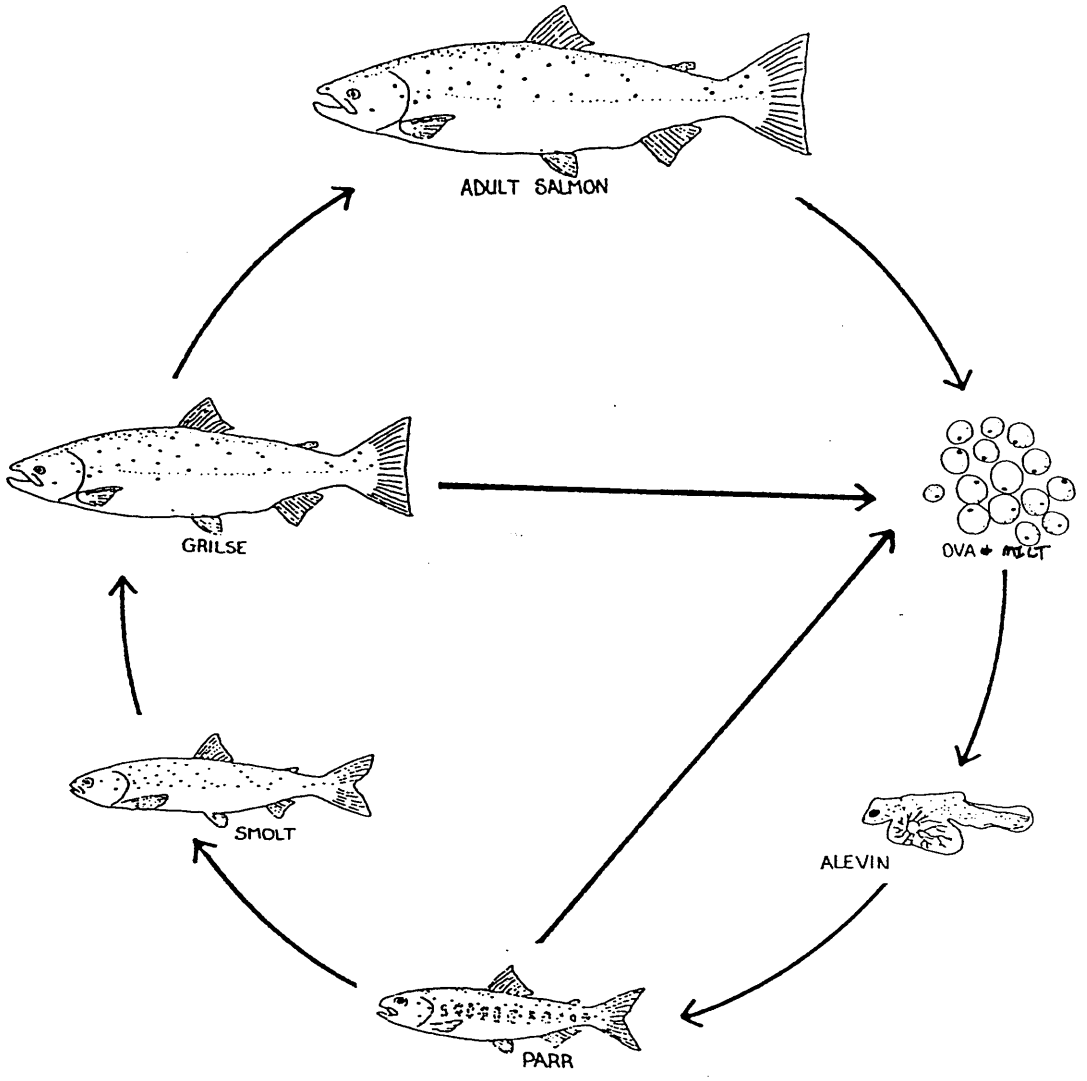


Figure 1.1 The life-cycle of the Atlantic salmon.

1.2.3 FIRST-FEEDING (SWIM-UP) FRY

The period during which fry must find and consume their first food is critical during the life-cycle. Once all remaining yolk has been utilized the fry has only a short period in which to take in food, if it is unable to do so quickly then energy reserves may become too low to enable digestion of any food subsequently ingested.

1.2.4 PARR

Under normal conditions the juvenile fish continue to grow rapidly throughout their first summer, being known as parr after first feeding. During this period parr normally defend feeding territories in rapidly flowing shallow nursery streams, or riffles of the headwaters of larger rivers (Kalleberg 1958). They maintain station close to the substrate in the feeding territory and make rapid lunges and short forays from their position to seize mainly current borne prey (Keenleyside 1962, Wankowski and Thorpe 1979).

1.2.5 SMOLTS

After a period of time in the river, which varies greatly, parr undergo a complex series of morphological, physiological and behavioural changes that together constitute the metamorphosis from parr to smolt. The process of smolting is a general term used to cover all the specific

changes of behaviour and physiology necessary for migration to sea.

During smolting, the characteristic dark "parr marks" on the flank of the fish become obliterated by the deposition of silvery purine crystals in the superficial layers of the dermis. Fish become more streamlined due to an increase in the length/weight ratio. The pectoral fin area decreases and the fins become darker. There is a markedly improved tolerance to salt water, coupled with an increased ability to regulate ionic balance despite external ionic fluctuation. Higgins and Talbot (1985) have shown that smolting salmon have higher lipid stores, higher flesh calorific value, a lower proportion of water in tissues and a proportionally lower ash content, when compared with parr of the same age.

In addition, during smolting, fish abandon their territorial, bottom feeding behaviour and aggregate in surface waters. Station maintenance is abandoned and smolts drift downstream. Thus seaward emigration is thought to be mainly passive (Thorpe 1982).

(See Langdon (1985) and Hoar (1976) for general review of smolting).

1.2.6 SALMON AT SEA

Large numbers of salmon, from Europe and North America are known to feed off the coast of Greenland, where a substantial offshore fishery for this species has developed. There they grow rapidly, feeding mainly on sand launce, capelin and amphipods (Christensen and Lear 1980).

It is known that fish that mature as grilse (after 1 sea winter) make little contribution to the Greenland fishery. It has been suggested (Gardiner 1976) that some British salmon spend their first sea winter feeding off the coast of the Faroe Islands; they then either return to freshwater to mature as grilse or migrate to more distant feeding grounds off Greenland and mature as salmon (i.e. more than one winter at sea). The evidence for this is limited and is based on a relatively small number of tag returns. However it is clear that there is some seasonal migration of salmon between feeding grounds.

1.2.7 SEAWATER-FRESHWATER MIGRATION

It is well known that sea-run salmon and grilse return to the river of their origin to breed. Fish may ascend the river at any time during the 12 months prior to the breeding season. In general sea-run salmon remain in the relatively deep water of the lower reaches of river systems until some days or weeks prior to spawning. At this time they move

upstream during high water flows to spawning redds, where nest construction and spawning takes place.

1.2.8 EARLY MATURATION IN PARR

It has long been recognised that parr, especially males can under certain circumstances become sexually mature without having first migrated to sea. These have been call paedogenic or precocious males (Jones and Orton 1940) and dwarf males (Osterdahl 1969).

Gesner in 1558 recognised that parr, Salmo paruus as he called them, could become mature. At the time however, it was generally believed that parr represented a separate species of fish from the sea-run salmon, thus parr sexual maturity was not considered unusual.

It was almost 3 centuries later that any systematic examination of maturity in parr was made. In a series of 3 papers (1836, 1838 & 1840) John Shaw, a Dumfriesshire water bailiff, provided some remarkably astute observations on the early life history of salmon. He noticed the abundance of male parr which would exude milt (spermatic fluid) when handled and their close association with sea-run females on the spawning redds of the River Nith. He suspected that these mature parr may be contributing to the fertilization of eggs from large sea-run females (he could find no mature

female parr). Shaw tested this by obtaining a large female of 14 LBS (6.4 kgs) before spawning, he removed the ova and then collected milt from a 1.5 oz (43 g) mature male parr. He mixed the two and planted the ova in a stream with no access to the open sea.

His attempts at *in vitro* fertilization were successful. He was able to observe the growth and development of the progeny of this pairing until smolting and concluded that there was no difference between the development of these fish and the progeny of a more conventional pairing.

Despite the fact that Shaw replicated his painstaking observations several times, the importance of his work was not fully recognised. Critics at the time believed that he had simply produced a hybrid, it was still not universally accepted that parr were juvenile salmon.

It was almost a century later that attempts were made to establish whether the observations of Shaw, made on the Nith, were more widely valid.

Orton, Jones and King (1938) examined the incidence of parr sexual maturity in the Dee (Cheshire). They found that 40% of 2 and 3 year old males were in the final stages of maturity. When they extended their survey to 20 other British rivers (Jones and Orton 1940) they found that overall, 75% of male parr were either ripe or spent. Not one

of 1500 females examined during this study showed signs of maturity. Thus maturity in male salmon parr was shown to be normal, not exceptional as was previously thought.

Jones and his co-workers went on to repeat the artificial fertilization experiments of Shaw and were able to confirm that male parr sperm is capable of fertilizing eggs from a sea-run female. In addition they examined the behaviour of mature male parr at the breeding site. Using an observation tank they found that at the pairing of a male and female sea-run salmon, a mature male parr would take up position behind and below the vent of the female. Often this was in the bottom of the nest as construction neared completion (Jones 1959).

Myers and Hutchings (1985) extended this observation by demonstrating that a sea-run female could construct a nest and spawn in the absence of male sea-run salmon, in an observation tank. When only mature male parr were present, the ova were fertilized and the proportion of viable ova was no different from those of a conventional, sea-run pairing.

Mitans (1973) and Thorpe and Morgan (1980) have examined the fate of mature male parr and have found that they can and do smolt in subsequent years, migrate to sea and return as full size spawners.

Since the initial studies of Jones and his co-workers in the U.K., there have been a large number of observations, from many countries of high incidences of maturity in male parr, indicating that this is a widespread phenomenon (see Tchernavin (1938) for historical review). In addition there have been a small number of reports of maturing female parr (Regan 1938, Prouzet 1981, Bagliniere & Maisse 1985). Maturity in female parr from anadromous populations would seem to be exceptional. However several workers have examined female sexual maturity in landlocked populations of Salmo salar. Dahl (1928) found an large population of landlocked Atlantic salmon in Lake Byglandsfjord, Norway. Fish grew to 30 cms long and females spawned at 25 cms. Barbour et al. (1979) examined a similarly landlocked population in Newfoundland. They found that salmon grew slowly to a maximum length of 25 cms and they retained juvenile parr characteristics, despite the fact that males normally matured at age 4 and females at age 5 years.

It is now known that there are many viable, isolated populations of non-migratory Atlantic salmon, where males and females mature at small size. There is a large body of evidence that shows that sea water growth and large size are not essential to successful breeding. In anadromous populations males frequently and females rarely become

mature prior to migration to marine feeding grounds. Fish that adopt a strategy of early maturation as parr are not precluded from subsequent maturity as full size spawners.

1.3 SMOLTING

The idea that the metamorphosis from parr to smolt is solely determined by age has been rejected by several workers. Jones (1959) reports that 90% of wild smolts in the Hampshire Avon were yearlings (known as S1's), whereas in the Welsh Dee and the Derwent, 90% migrated to sea at 2 years old (S2's). In Scandinavia parr may remain in the river for up to 7 (S7) or 8 (S8) years.

Bailey *et al.* (1980) demonstrated variation in the smolting rate between populations (61-89% S1's) from different New Brunswick rivers. Thorpe (1977) showed variation between families from the same stock, the River Almond (from 20 to 94% potential S1's). Even within families variation in smolting rate has been observed. Thorpe *et al.* (1980) found differences in proportions of S1's in populations of siblings held under different conditions.

1.3.1 Growth, Size and Smolting

In the search for explanations for the the observed variation in smolting rate between groups, growth rate and size have been considered important.

It has been suggested that in salmon populations with good river growth conditions e.g. high water temperatures or high nutrient loading, parr will smolt earlier. Jones (1959) for example, comments on the relationship between increasing latitude and increasing age at smolting. He implies that this is due to a general decreasing temperature trend.

Elson (1957) concluded that parr that have reached a critical size of 10 cms at the end of one growing season are likely to become smolts the following spring. The idea of a critical size threshold, above which smolting will occur has been taken up by others. Evropeytseva (1963) has stated her belief that smolting takes place in Baltic salmon (Salmo salar L.) only on reaching a critical size. Knutsson (1979) noted that Norwegian salmon farmers use 10cms as a critical size during the winter, to estimate which fish will smolt the following spring and thus may be transported to seawater.

1.3.2 The Phenomenon of Bimodality

It is now well established that during the first year of growth in hatchery conditions, a population of sibling parr (i.e. all progeny from a single mating) will split into two clearly defined sub-populations. These sub-populations become evident as a bimodal length-frequency distribution in

early autumn. This becomes more pronounced, until by early winter the two growth modes are completely separate.

This phenomenon was first reported in hatchery reared Scottish parr (Simpson and Thorpe 1976, Thorpe 1977, Thorpe and Morgan 1978, 1980). Bailey et al. (1980) and Kristinsson et al. (1985) found North American stock similarly developed bimodal length frequency distributions, as did Knutsson and Grav (1976) working on Norwegian stock. Bimodality has also been reported in wild populations (Bagliniere and Maisse 1985).

Thorpe (1977) showed that bimodality is not the result of differing growth rates between sexes nor between mature and immature parr (see also Bailey and Saunders 1978 and Villarreal and Thorpe 1985).

Simpson and Thorpe (1976) showed that separation of the two modes is due to a reduction in the growth rate of fish destined to become lower mode (LM) fish, while upper mode (UM) fish continued to grow into the autumn. Higgins (1985) and Higgins and Talbot (1985) have shown that, despite excess food availability, lower mode fish consistently ate less (as a proportion of body weight) throughout the late autumn and winter of their first year. Metcalfe et al. (1986) reported a steady reduction in food intake rate in

individually tested fish, which eventually entered the LM growth group, over the period July to September, indicating that social factors preventing feeding opportunity are unlikely to be the principal cause of reduced intake. They have further shown that over the same period the mean distance over which parr move to intercept passing food items is reduced and that food once attacked is more likely to be rejected in September, than in July. Reduction in food intake they conclude, is due to general loss of appetite in lower mode fish which declines over the period July to August and occurs independently of food availability, competition or decreasing water temperature.

Evidence from the literature seems to support the conclusion that bimodality results from physiological processes acting on appetite.

The eventual fate of fish which continue growth into the winter of their first year has been investigated by many workers. Simpson and Thorpe (1976), Thorpe (1977), Thorpe and Morgan (1980) and Baglinere and Maisse (1985) (and others) have all provided data that establishes that fish found in the upper mode in winter of one year will smolt and migrate to sea during the following spring. Thorpe et al. (1980) have shown that in a population of hatchery reared parr, those found in the upper mode during their first

winter represented fish smolting at 1+ years old, those in the lower mode at this time smolted at 2+ years old. In general, Thorpe (1977) suggests that subpopulations in the upper and lower modes represent fish that will smolt at ages t and t+1 years respectively.

This growth check phenomenon found in Salmo salar has also been found in Masu salmon (Oncorhynchus masou) in Japan reared in aquaria (Hirata in press.) and in the wild (Hirata et al. 1986) and in hatchery reared Coho salmon (Oncorhynchus kisutch) (Clarke and Shelbourn 1986).

1.3.3 Critical Periods in Development

Bateson (1979) argues that the characteristics of many behavioural systems are determined at particular stages in the development of an animal. However the mechanisms generating those changes can often be operating throughout the life of the animal. He suggests that there are specific periods of life during which an animal may be sensitive to external stimuli and outside which, the same stimuli have a different or no effect. Giving examples ranging from bird song development to mother-child receptivity, he suggests that this is a very widespread phenomenon. Although Bateson was principally concerned with the development of learning, he suggests that learning sensitive periods are the same as physiological sensitive periods, e.g the masculinisation

effect of rodents by androgen which is effective only within certain periods.

Thorpe (1986) postulates that a sensitive period (window) exists in the development of parr when, during the summer of their first year, some parr take a "physiological decision" to reduce food intake via appetite. Decision in this context is used to describe the resulting observed developmental outcome of two or more possible physiological pathways and does not imply any conscious thought.

The timing of this critical period during which the decision is made to reduce food intake has been considered by Thorpe et al. (1980). They have shown that the timing of of divergence of upper and lower length frequency modes differed slightly between families. As divergence of modes necessarily occurs some time after a reduction in the feeding rate by a proportion of the population, they estimate the timing of the feeding reduction by backward extrapolation of growth curves, as occurring in June-July. This is supported by data from Metcalfe et al. (1986) who found a reduction in the feeding rate in lower mode fish between July and August, thus indicating a time for the feeding reduction switch as prior to August.

Further evidence for the timing of the physiological

decision to reduce food intake comes from Villarreal (1983), who found clear relationships between specific growth rates and RNA:DNA ratios of parr muscle during early (unimodal) growth and after bimodality was evident. However around the time of modal separation, the relationship was disrupted. This disruption started around the beginning of July.

1.3.4 Smolting Critical Size Threshold

It has been clearly established that bimodality of length frequency distributions in the winter prior to smolting, is normal in populations of salmon parr in hatcheries. It is also well established that upper mode fish become smolts in the following spring. Thus the physiological decision to continue growth into autumn effectively precludes remaining as parr for a further year. At least in the ideal conditions of the hatchery the decision to maintain growth into winter is a decision to smolt the following spring. This moves the timing of the smolting decision from autumn, as suggested by Elson (1957), to June - July (section 1.3.3 and Thorpe 1986, 1987a & 1987b) 9 - 10 months prior to emigration to sea. This makes the idea of a simple size threshold less likely.

Thorpe *et al.* (1980) noted that if such an absolute size threshold does exist, then the critical size for smolting must differ between stocks. Fish reared at St. Andrews, Canada, emigrated at lengths of >14 cms, whereas smolts of

the same age emigrate at 9 - 13 cms in Scottish rivers, the size of lower mode Canadian fish of the same age.

Further supportive evidence comes from Hirata (in press). His work on individually marked hatchery reared Masu salmon, clearly showed that entry into the upper mode was independent of absolute body size during the period prior to modal separation.

The importance of attainment of a critical body size has been questioned by Thorpe (1986) on adaptive grounds. He argues that absolute size is a measure of past performance, giving no information on current growth rates. It is not inconceivable that given the sudden loss of an abundant food source a rapidly growing large fish is faced with maintenance ration. Thus Thorpe advocates that some measure of instantaneous growth rate is likely to be a better criterion on which to base the developmental decision to smolt or remain in freshwater.

It is likely that when workers suggested critical size as a determinant for the onset of smolting they were in fact observing the consequences of a physiological smolting decision, taken earlier than originally thought, resulting in continued growth in potential smolts and arrested growth for fish remaining as parr. Thus if growth is important in

determining the outcome of the smolting decision then it is growth prior to and during early summer that is likely to be critical.

1.3.5 Genetic Influences on Smolting Rates

There is substantial evidence that smolting rates are heritable. Thorpe (1977) showed differences between proportions of yearling smolts (S1's) in different families reared under identical conditions. Thorpe and Morgan (1978) further established that variation in smolting rate, although influenced by both parents, was more influenced by the male than the female parent. In support of this finding, Bailey et al. (1980) working on North American stock showed that high proportions of S1's in a population were correlated with early smolting in male parents.

1.3.6 Environmental Influences on Smolting Rates

Thorpe et al. (1980) found that the width of a shelter ring in tanks of hatchery reared salmon parr, affected growth rates. Wider rings produced better growth and increased the proportion of one group of a sibling population smolting as S1's compared with another group grown in tanks with narrower rings.

In addition Thorpe and Wankowski (1979) found that manipulating tank flow rates consistently altered the

percentage in the upper mode in sub-populations of sibling parr exposed to a variety of flow rates.

In the wild Kuzmin and Smirnov (1982) attributed the large variation in age at smolting (2+ to 7+ years) between rivers of the Kola peninsula, U.S.S.R. to differences in "natural climatic conditions" of the rivers. Annual variation in smolting rate were explained by annual variation in thermal conditions and similar trends were found throughout the peninsula between years.

Clarke and Shelbourne (1986) working on Coho salmon parr found that increased culture temperatures allowed a higher proportion of fish to enter the upper mode of the bimodal distribution found in late August in this species.

1.4 MATURATION

1.4.1 Growth and Maturation

Alm in 1959 was the first to highlight a relationship between growth and maturation in fish. Since then many authors have commented on this relationship. Thorpe in a review (1986) cites 30 authors covering 12 salmonid species, where a positive correlation between growth rate and maturation rate was found.

Rates of maturation are known to vary considerably between rivers, years, stocks, and families (Jones and Orton (1940), Dalley et al. (1983), Schaffer and Elson (1975) - rivers; Saunders and Sreedharan (1977) - stocks; Thorpe (1975), Glebe et al. (1978), Bailey et al. (1980) - families).

These observed differences may be at least partly, due to differences in growth rates. Parr that become mature are initially amongst the fastest growers. In laboratory studies of tank held populations several workers (Leyzerovich 1973, Bailey et al. 1980, Saunders et al. 1982, Thorpe et al. 1983) have shown high maturation rates are linked to initially rapid growth rates.

Likewise in the field Dalley et al. (1983) found, in an examination of Newfoundland rivers, that it was the fastest growing males that became mature. Bagliniere and Maisse (1985) found parr maturing as underyearlings, only in the lower reaches of the River Scorff, France, where higher growth rates were apparent. However once sexual maturation commences, growth rates decline rapidly (Saunders and Sreedharan 1977, Eriksson et al. 1979, Saunders et al. 1982, Myers et al. 1985) this may be, at least partly, due to reallocation of resources to sexual development.

1.4.2 Genetic Influences on Maturation Rate

Thorpe et al. (1983) have found that maturation rate is heritable. Progeny of early maturing parr grew faster and matured earlier than offspring of later maturing parents. In addition, selecting for early maturity over two generations increased the percentage of male parr maturing at age 1+ years from 7 to 30%. This finding is supported by Gjerde (1984) who also found that age at sexual maturity could be altered by selection and is heritable.

1.4.3 Environmental Influences on Maturation

The influence of environmental factors on the rate of parr maturity in Atlantic salmon has been commented on by several workers.

Saunders et al. (1982) associated high water temperatures at the Mersey Fish Culture Station with high percentages of mature male parr. This was contrasted to the Mactaquac Fish Culture Station which produced relatively lower proportions of mature male parr and had lower temperatures.

Bagliniere and Maisse (1985) have shown that maturation rates differed within the River Scorff. Lower, highly productive reaches of the river produced up to 5% male parr maturing at 0+ years. Observations of 0+ years mature parr

are infrequent in natural situations and are only found in hatcheries with very good growth conditions (Bailey et al. 1980). In an area of very high production, due to light organic pollution, Bagliniere and Maisse (1985) captured a small number of 1+ years mature female parr. These have only very rarely been recorded elsewhere (Regan (1938), Tchernavin (1938)). Growth rate in the area of capture was very high indeed.

1.4.4 Maturation Critical Sizes

Myers et al. (1985) proposes a critical size threshold above which parr will become mature. Similar adaptive arguments can be levelled against attainment of an absolute critical size for maturation as have been for a smolting critical size. However as these authors suggest there may be a lower size limit below which it is not feasible to invest energy in gonad without serious survival consequences.

1.5 SMOLTING AND MATURATION - DEVELOPMENTAL CONFLICT

It is clear from the literature that there is a relationship between growth and the age at metamorphosis from parr to smolt. Equally clearly there is much variation in the age at which this transformation takes place, from 1+ to 7+ years.

It is also well established that there is a correlation

between high growth rates and early maturity and that given optimum growth conditions sexual maturation is possible at age 0+. It is thus conceivable that, under good conditions of growth, an underyearling parr could both mature and smolt within the first 18 months of life. However there is evidence to suggest that this is unlikely.

Maturation requires that adaptations to life in freshwater are retained for breeding to be successful, smolting necessitates their loss. Logically maturation and smolting developmental pathways are mutually exclusive, at any one time.

Thorpe (1986) showed that, within sibling populations, parr that smolted at 1+ years, did not mature until 2.5 years (as salmon). Smaller fish that did not smolt at 1+ years, were able to mature at 1.5 years, without having migrated to sea. In this case it seemed that smolting inhibited maturation.

If, as has been argued, continued growth into autumn reflects a developmental decision taken in June or July to commence the physiological and behavioural processes that constitute smolting, then the smolting decision is taken prior to the breeding season for that year (November - January). It seems unlikely that a smolting decision could

be triggered at a second period of the year, after the breeding season in time for smolt migration in May. There is some evidence to support the idea that maturation inhibits smolting. Langdon and Thorpe (1985) found that parr maturing at 1.5 years, failed to undergo complete smolting changes at age 2 years. Aida et al. (1984) working on Masu salmon (Oncorhynchus masou), a pacific salmon commonly yielding high percentages of mature male parr, showed that underyearlings that had been completely castrated in late autumn, after having matured in early autumn, were capable of smolting the following spring. Sham operated, previously mature males did not smolt.

It has been argued that smolting and maturation may be incompatible processes within any one year. It seems clear from the literature that the developmental pathway outcome of physiological decisions to smolt and to mature are both influenced by similar parameters, namely some aspect of growth. As these developmental pathways are very distinct and do not result in two different expressions of a similar overall phenomenon, then for a single stimulus to influence both physiological decisions, the decision making processes must be different. They may differ in two ways.

The two physiological decisions may differ in threshold

level of response. Thorpe et al. (1980) and Saunders et al. (1982) have examined this possibility and have argued that populations with high proportions of large upper mode fish in a bimodal population in autumn of their first year would yield a higher percentage of mature males than populations with smaller upper mode fish. Thorpe (1986) shows that small S1 smolts are produced at the Almondbank hatchery, Scotland and maturation does not occur at this age, under normal hatchery conditions. In contrast smolts at the St. Andrews hatchery, New Brunswick, were much larger and maturation at 0+ years did occur.

Alternatively, the physiological decisions may be made at different times. This hypothesis predicts two distinct sensitive critical periods, during which maturation and smolting decisions are taken. This has been explored by Bailey et al. (1980) who show that 0+ years maturing males, reduce growth and are found in both modes of a bimodal distribution in November. They suggest that the maturation decision must be taken early in the season to enable parr to participate at spawning and that maturation and smolting decisions are taken separately.

1.6 A MODEL OF ENVIRONMENTAL EFFECTS ON SMOLTING AND MATURATION RATES

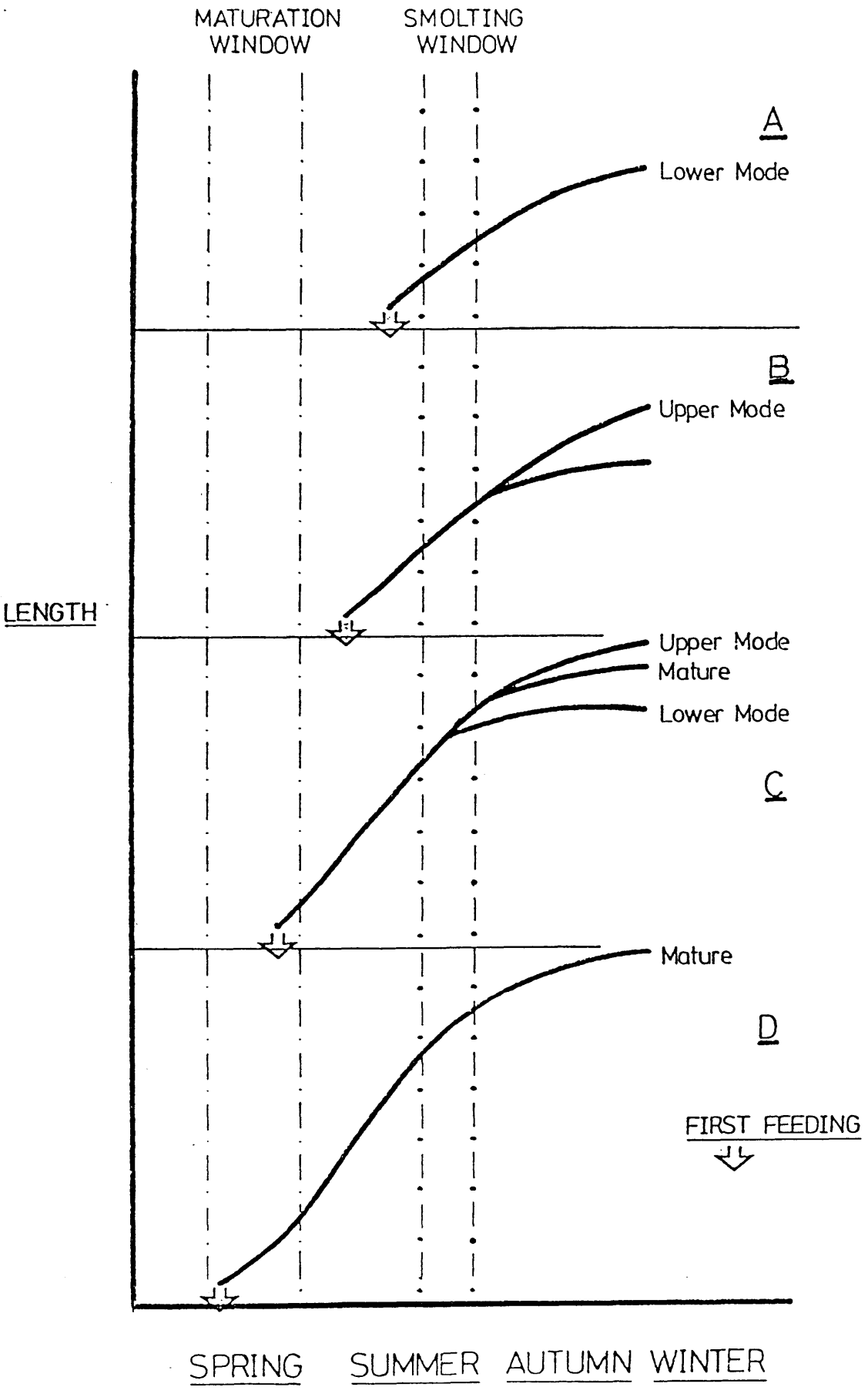
Thorpe reviewing the status of knowledge on early life-history choices in Atlantic salmon (1986 & 1987b) has formalised the relationship between growth, maturation and smolting of any one year class into a model (Fig. 1.2) Thorpe considers two extremes and two intermediates of environmentally influenced growth conditions.

Situation A - Very poor growth coupled with late hatching results in no individuals taking the option to smolt that year. Length frequency distributions remain unimodal (all fish in LM) fish essentially arrest development (growth, smolting and maturation) and remain as parr until subsequent years.

Situation B - Fair growth conditions coupled with late first feeding results in a bimodal distribution in autumn, upper mode fish continue development and will smolt the following spring.

Situation C - Good growth conditions coupled with early hatching. Fish are subject to maturation sensitive period (window), the faster growers commence the maturation process and reduce somatic growth. The remainder remain immature as

Figure 1.2 Thorpe's (1986) unified model of growth, smolting and sexual maturation in salmon parr (see section 1.6 for detailed description).


MATURATION WINDOW
SMOLTING WINDOW
A
Lower Mode
B
Upper Mode
LENGTH
Upper Mode
Mature
Lower Mode
C
Mature
D
FIRST FEEDING
SPRING
SUMMER
AUTUMN
WINTER

parr, however the fastest growing immature fish, take the physiological decision to smolt and continue growth into the autumn. Mature fish, although fastest growing originally are overtaken in size by immature, potential smolts. Immature, non-smolting fish, remain as parr and appear as the smallest size group of fish of that age-class.

Situation D - Population shows very high developmental rate. Very good growth conditions in early spring during maturation sensitive period result in all fish males and females, becoming mature. No fish remain immature and thus no fish become smolts in this year-class.

Thorpe predicts that the actual proportions of any age class found in the three possible developmental stages (i.e. immature parr, mature parr, or smolts) will vary between stocks, between families and between years.

It has been shown that percentages in each developmental stage (immature parr, smolt and mature parr) may by modified by both genetic and environmental factors. Whether age at entry into successive developmental stages is inherited directly or indirectly through growth rates is unclear from the literature. However it is suggested that inherited developmental rates may be altered by environmental conditions, especially those which modify growth.

Clearly defined, testable predictions about population growth, smolting and maturation rates may be drawn from this model. This treatise is primarily concerned with testing some of these predictions by examining the environmental effects of water temperature and photoperiod on the growth, smolting and maturation rates in cohorts of hatchery reared, sibling Atlantic salmon during their first year.

CHAPTER 2

FISH STOCK AND CULTURE CONDITIONS

2.1 STOCK SOURCE

Fish stock used throughout this work were obtained from one source, namely the River Almond, a tributary of the River Tay. The Tay system drains a very large area of Eastern Scotland, from the Grampian Highlands in the west and north, to the Ochil hills to the south, into the Firth of Tay near Perth.

Ripe, sea-run, Atlantic salmon were obtained from the River Almond by electrofishing during the winters of 1984-85 and 1985-86. The ova and milt (spermatic fluid) were obtained by hand-stripping from suitable male and female fish and the ova fertilized using standard salmonid hatchery techniques (Huet 1972).

The parental stock of the study group examined from spring 1985 until March 1986 was taken from fish breeding in the River Almond during the winter of 1984-85. The parental male was a 78 cm. adult, sea-run, fish which had spent 2

years in freshwater and 1 year at sea (2.1), as determined by scale readings. The milt from this male was used to fertilize approximately 4000 ova obtained from a 85 cm. female, which had spent 2 years in the river and 2 years at sea (2.2), on the 7th December 1984. The resulting family from this pairing will be subsequently referred to as family 4/84.

The parental stock stripped on the 28th November 1985, provided the family 4/85. The female was a 88 cm fish which had spent 2 years in freshwater followed by 2 years at sea. Approximately 5000 ova obtained from this female were fertilized by the milt from several 4 year old mature male parr, that had never been to sea, being held at the Department of Agriculture and Fisheries for Scotland, Almondbank hatchery, Perthshire. This family formed the study group examined from spring 1986 until March 1987.

2.2 HATCHING CONDITIONS

Incubation of all eggs was carried out at Almondbank, using the horizontal-flow trough incubation system, with corrugated bottom trays, (see Thorpe 1981 for details of this standard system).

After fertilization each batch of eggs was divided into approximate halves. One half was incubated at ambient River

Almond water temperature. The other group was incubated in heated water at c. 10°C, to accelerate incubation. Incubation was carried out in very low intensity red light, the wavelength used being outwith the wavelength range of behavioural response (Thorpe 1981). However to prevent possible perception of a daily rhythm the light was kept on constantly. This light was necessary to facilitate removal of dead ova to prevent infection. After hatching, alevins were kept in hatching trays, in the hatchery, until the yolk-sac had been absorbed and the fry were ready to accept their first food. At first-feeding the fry were moved to tanks at one of two sites, where they were held throughout these experiments.

2.3 ROWARDENNAN HOLDING FACILITIES

During the winter and early spring of 1985-86, fish rearing facilities were installed at the University Field Station, Rowardennan, on the east shore of Loch Lomond.

2.3.1 Water Supply

The water system consisted of a pumped supply, obtained from Loch Lomond and delivered to two header tanks, with a total capacity of 6800 l, on a rise to the east of the main Field Station buildings. The supply pump installed was a Desmi S70/50, with a discharge of 250 l/min at 30 metres

head. The pump was run intermittently, controlled by a float switch in the header tank. From the central header tanks, water was distributed as required to various sites around the Field Station.

2.3.2 Recirculation System

In order to hold fish at uniform temperature, within two constant temperature rooms situated at the Field Station it was necessary to design and construct two small recirculation systems. These allowed the water in the system to be maintained at the required temperature with minimal fluctuations. The recirculation system used was as follows.

Water was pumped by a small Berisford PV 22 , 240 volt pump,(see Fig. 2.1) from a 340 l. sedimentation tank and discharged into the radial flow holding tank above, at the rate of 20 l/min. A small amount of water was removed from this main supply, to wash pelleted food from the food hopper. This water (plus food) was returned to the main supply prior to its discharge into the holding tank. Water entered at the centre the tank, close to the floor, and was drained at all points around the outer circumference of the floor of the tank. A central ring provided shading for the fish, under which they remained almost exclusively when undisturbed. Radial flow tanks have been designed to provide

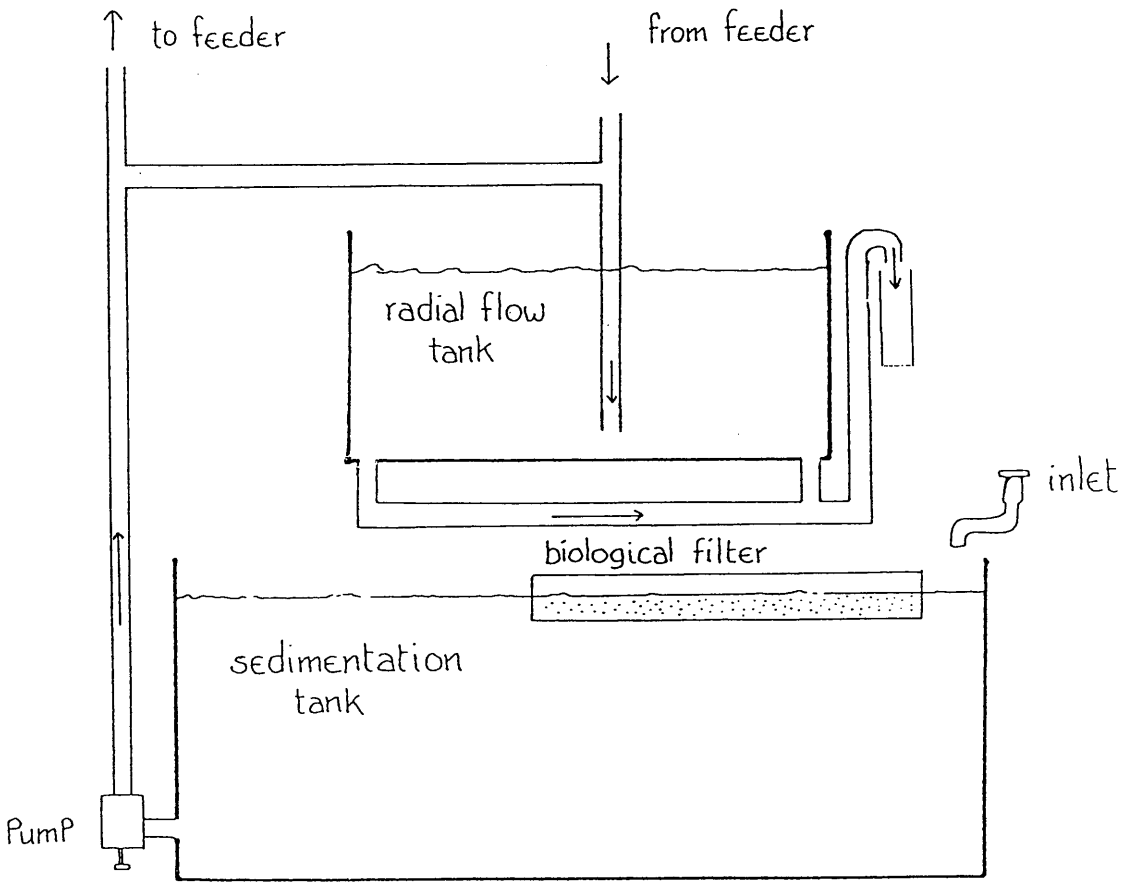


Figure 2.1 The recirculating fish holding facility designed for use within constant temperature rooms at Rowardennan (see section 2.3.2 for detailed description).

abundant feeding opportunity and to reduce fighting frequency and hence increase uniformity of population growth (Thorpe and Wankowski 1979). The possible effects of agonistic activity on smolting are explored in Appendix 4.

The waste water from the radial flow tank flowed from the stand-pipe (used to control water level in the tank) through a screen to remove coarse particulate matter and was then returned to the sedimentation tank.

2.3.3 Water Quality

Early pilot tests of the recirculation system with salmon parr showed that the microbial breakdown of waste food and excreta resulted in an unacceptably high build-up of nitrogenous compounds, especially salts of ammonium (NH_4^+), after a period of a few days. For this reason a biological filter was incorporated into the system between the radial flow holding tank and the sedimentation tank. This filter consisted of a large tray, the lower half of which was submerged below the surface of the water in the sedimentation tank and filled to water level with marble (calcium carbonate) chips. Water discharged from the radial flow tank passed through the fine screen and into the filter tray through which it leached relatively slowly.

The marble chips had a twofold function. They provided a large surface area for the growth of nitrifying organisms

which, once the filter had become conditioned (Spotte 1970), reduced ammonium levels dramatically. In addition the calcium carbonate helped to buffer pH fluctuations that may have occurred due to the build up of organic matter in the system.

A trickle flow from the main header tanks of around 1.5 l/min was added to the system. It was found that this small input was not enough to influence the water temperature in the recirculation system within the constant temperature rooms. With approximately 350 l. in the sedimentation tanks it was estimated that the turnover time for water in the system was around 18+ minutes.

The physical breakdown of food used throughout these experiments posed the constant threat of a build-up of suspended solids. This problem was countered by daily cleaning of the radial flow tanks to remove excess food. Similar weekly cleaning of the sedimentation tanks to remove fine particulate matter also proved necessary. With regular maintenance this system performed well and water quality remained high.

2.3.4 Emergency Backup System

The system described above relies heavily on an uninterrupted power supply, as this is not always guaranteed

at this site, especially during the winter months, a backup system was installed. This consisted of a 24 volt d.c. battery powered, self-priming, Jabsco "Water Puppy" pump. This drew water from both sedimentation tanks and returned it to the radial flow tanks. With a discharge of 30 l/min, it could supply 15 l/min to each tank. Enough battery power was available to run this pump for around 25 hours continuously. The backup system was connected via a 240 volt relay, enabling the backup pump as the mains (240 volt) system failed.

2.3.5 Feeding

Fish were fed automatically using adapted "Caddymatic" dispensers. The push-pull mechanism of the caddy was actuated by a 12 volt d.c. solenoid (Fig. 2.2). The solenoid was switched by a custom built timer linked to 240/12 volt transformer (Fig. 2.3). The system was set to deliver 4 meals per hour for 24 hours in every day. Each meal consisted of approximately 1 gram/tank (this figure was slightly reduced during the first few weeks of feeding when requirement was lower). Food used throughout was Ewos pelleted salmon food, produced by Ewos-Baker, Bathgate, Scotland. Pellet sizes ranged from size 0, at first-feeding, to size 3 at one year old. This followed manufacturers guidelines.

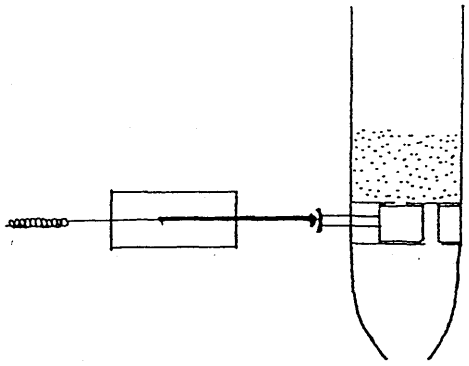


Figure 2.2 A solenoid operated "Caddymatic" fish food dispenser.

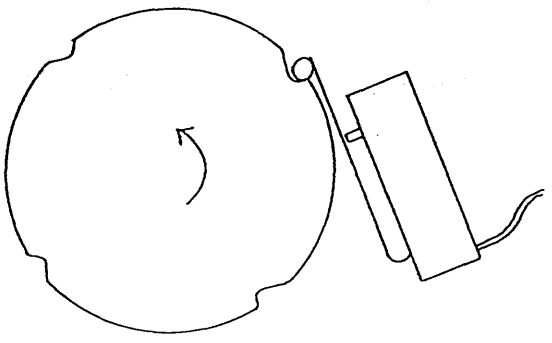


Figure 2.3 A timing device used to control feeding interval. The cam was constantly driven at 1 rev/hr. by a geared electric motor. Feeding frequency was altered by changing the number of notches in the cam.

2.3.6 Lighting Systems

To manipulate photoperiod cycles it was necessary to reproduce natural daily and annual photoperiod rhythms under controlled conditions. Because there is very little known about what aspects of the photoperiod cycle animals use as cues to time cyclical events, it was important to simulate as exactly as possible, all aspects of the photoperiod cycle. For this reason a system was developed, tested and compared to natural light conditions as follows.

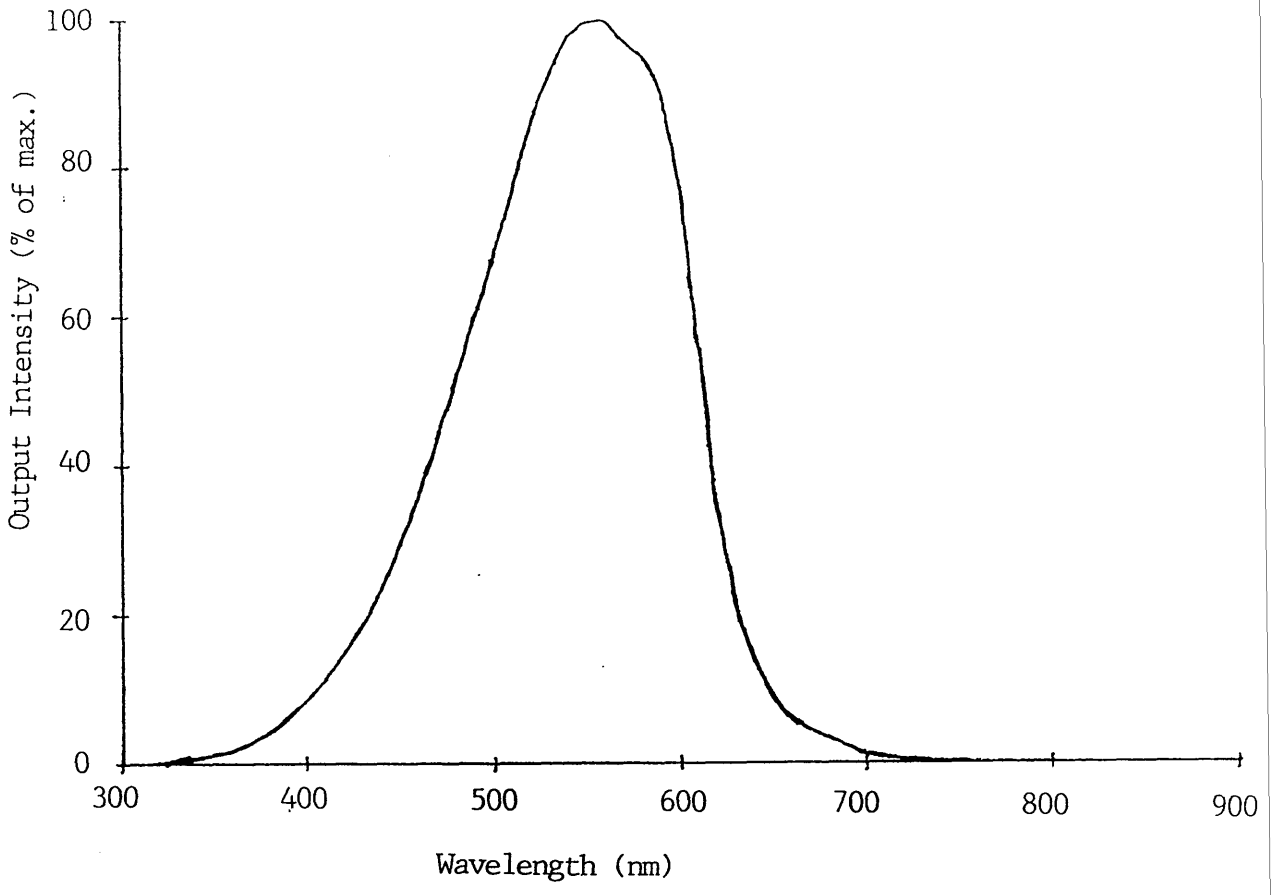
A 100 Watt incandescent, tungsten bulb suspended approximately 1 metre above the centre of the radial flow tank was found to illuminate the tank evenly at an adequate light intensity of around 25 lux (Li-cor LI 185B meter). The spectral output from a tungsten bulb was analysed on an amplifying spectrophotometer (Zeiss Monochromator M4Q111) (see Fig. 2.4). A comparison of these data with published data for sunlight (Fig. 2.5) shows that over the visible wavelengths from 400 - 700 nm, there are minimal differences in wavelength quality.

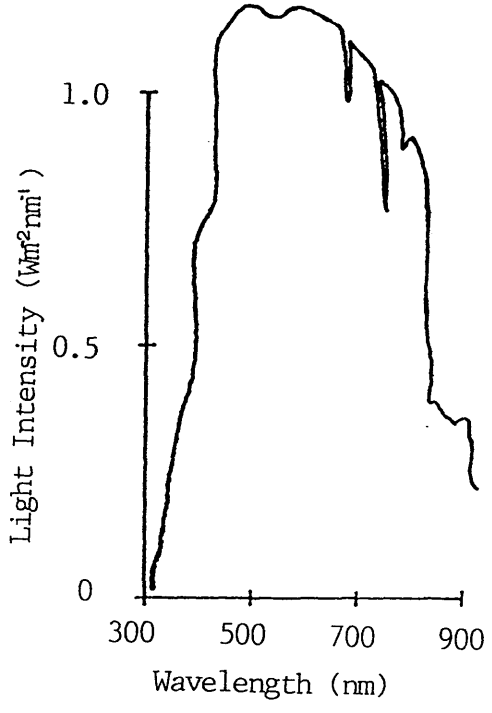
Light intensity control

The greater the latitude, the greater the variation in the length of twilight, that diffuse light persisting after sunset and before sunrise, during different periods of the year. Twilight is caused by the scattering of sunlight by atmospheric molecules, this is then reflected down on to the

Figure 2.4 The spectral quality of a 100 watt tungsten bulb. Output expressed as a percentage of maximum output.

Figure 2.5 The spectral quality of sunlight at ground level under cloudy conditions. Light intensity expressed as watts/m^2/nm (after Wilson 1979).


100
80
60
40
20
0
Output Intensity (% of max.)
300
400
500
600
700
800
900
Wavelength (nm)


1.0
0.5
0
Light Intensity (Wm²nm¹)
300
500
700
900
Wavelength (nm)

earth by dust and moisture droplets (Strahler 1971). Thus the length of twilight is related to the angle at which the sun sinks below the horizon. Assuming constant atmospheric conditions, the length of twilight is cyclical and predictable, and thus may provide seasonal information. Thus twilight changes that would be experienced under ideal visibility conditions were simulated. Appendix 1 shows the annual changes in time of sunset and sunrise and the time at which astronomical twilight (the period during which any sunlight can be detected) commences and ends at the 56°N latitude line (data extracted from Whitakers Almanac 1985) which lies approximately 12 km south of Rowardennan.

Figure 2.6 shows the apparatus designed to simulate the natural photoperiod and twilight cycle in holding tanks at Rowardennan. The clear perspex disc rotated with a 24 hour cycle. At sunset the blind on the disc started to cover the light columnator. As the blind continued to rotate, so the light intensity in the tank dropped. When the blind completely covered the columnator, at the end of evening twilight, the bulb was switched off by a time-switch. In the morning this process was reversed. By varying the distance from the centre point of pivot of the twilight control disc, to the columnator, the speed at which the blind crossed the columnator (and hence the length of twilight) could be altered. Figure 2.7 shows an example of the reduction in the light intensity achieved at the center of the tank over the

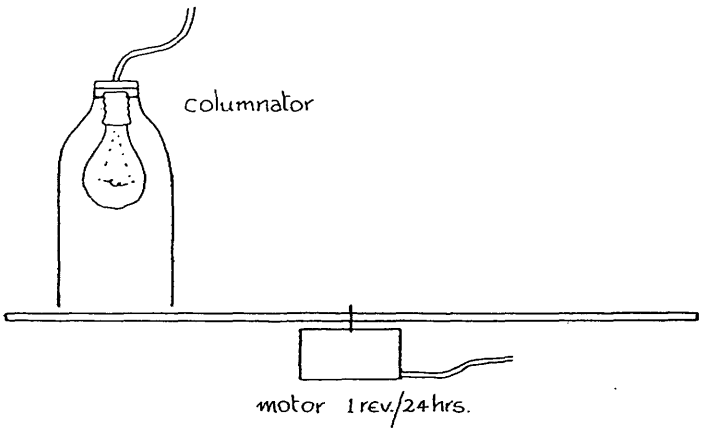


Figure 2.6 A natural twilight simulator.
Above: Elevation Below: Plan
(See section 2.3.6 for detailed explanation of operation).

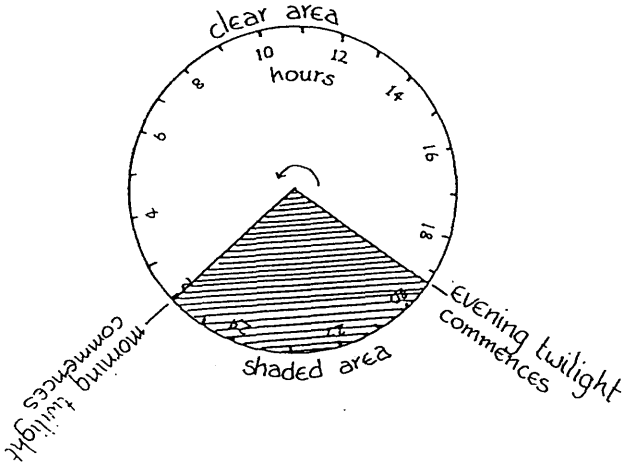
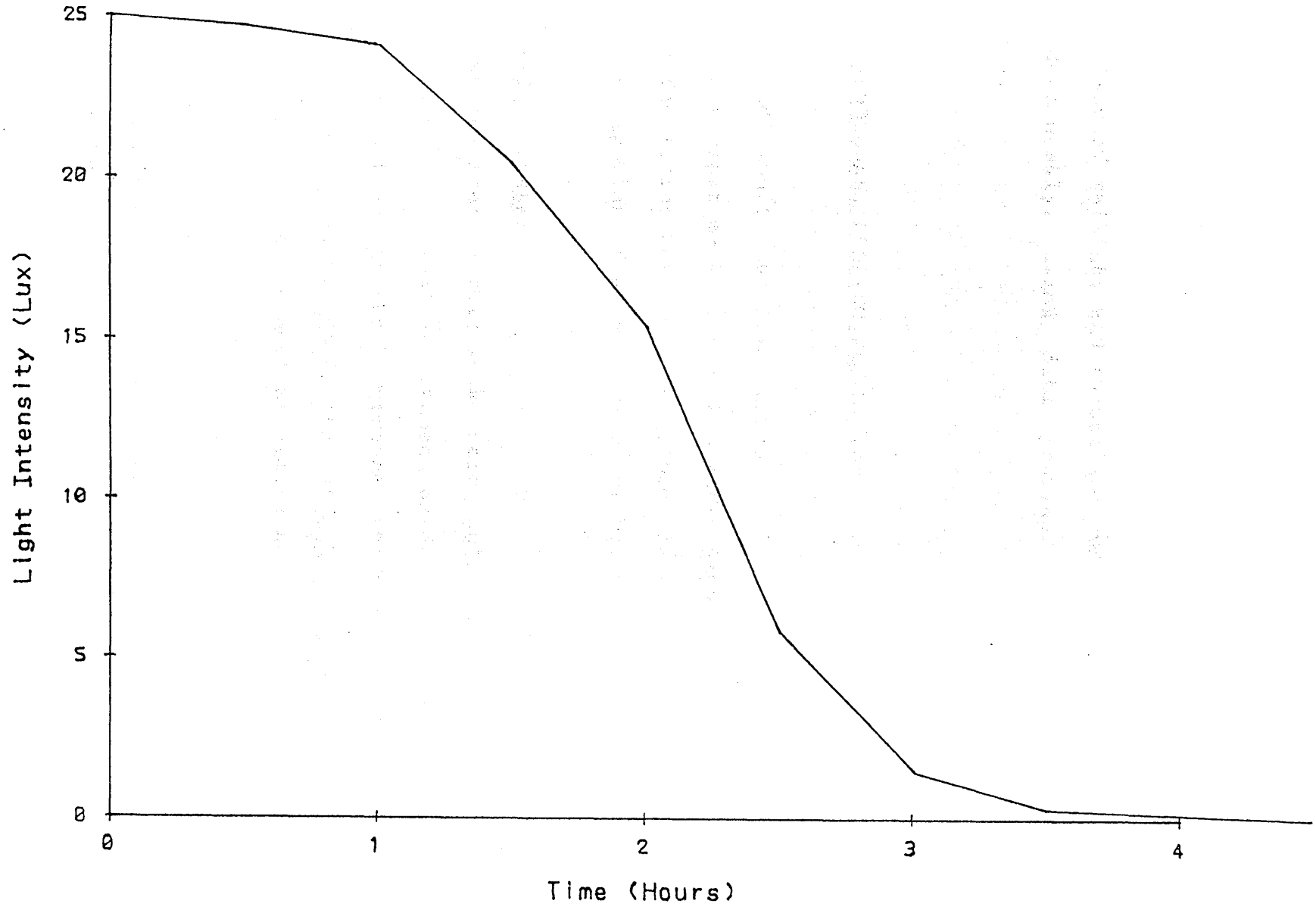


Figure 2.7 The reduction in light intensity following simulated sunset, corresponding to the 17th May, at water level in tanks at Rowardennan.


25
20
15
10
5
0
Light Intensity (Lux)
0
1
2
3
4
Time (Hours)

evening twilight period during on the 17th May. The main disadvantage of this system was the need to alter the length and times of twilight and sunset and sunrise, manually. This was done weekly, using data provided in Whitakers Almanac (1985).

2.4 ALMONDBANK HOLDING FACILITIES

In addition to the Rowardennan facilities, fish were also held for experiment at the D.A.F.S. Almondbank Hatchery. These facilities have been described elsewhere (Minaur 1973) however important features are described below.

2.4.1 Tank system

Fish involved in this work were held in 1 metre radial flow tanks identical to those used at Rowardennan (Fig. 2.1). In contrast to the Rowardennan recirculation system, a flow-to-waste system, utilizing water drawn from the River Almond, was used throughout at Almondbank.

2.4.2 Water Quality

Water quality in the tanks reflected the water quality in the river. Thus during spates high suspended solids in the river was obvious as water discolouration in the tanks. However in general River Almond water is of high quality

(M. Miles pers. comm.) with a pH range of 6.5 - 7.8.

2.4.3 Environmental Control

To enable manipulation of the environmental conditions (temperature and light) of a number of underyearling parr populations, each tank was enclosed in black polythene sheeting to form a "controlled environment cell". Within the cell light regimes could be controlled without interference between tanks or from natural daylight. As a result of the flow-to-waste water system the temperature of water in each tank could also be controlled without interference between tanks.

Temperature control

Throughout these experiments two elevated temperature regimes were used, ambient water temperature plus 5°C and ambient plus 2°C. Fish exposed to these regimes experienced normal daily and annual temperature fluctuations, elevated by either 2 or 5°C. This effect was achieved by using a through-flow immersion heater system (Fig. 2.8). The increase in temperature of the water discharged from the heater was determined by the power of the immersion heater and the rate of water flow past the immersion element. Thus with a 3 kw heater and a flow rate of 24 l/min (reduced from an average of 34-36 l/min, in tanks with no heating) an additional 2°C was added to the ambient water temperature

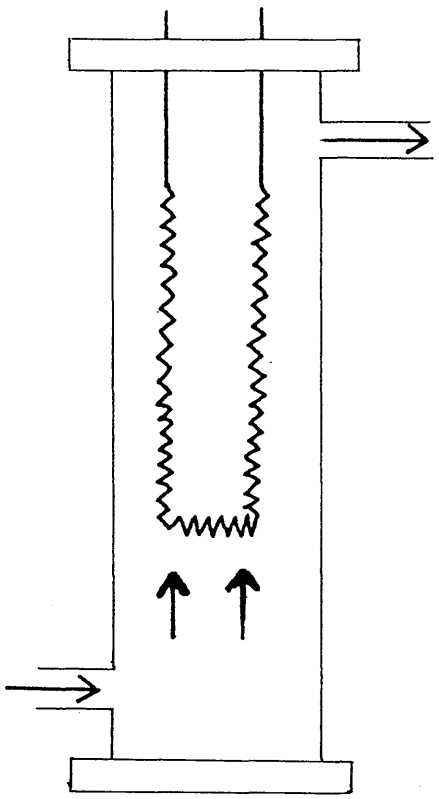


Figure 2.8 The flow-to-waste immersion heater.

discharging into the tank. With a 6 kw immersion heater and mean flow rates of 20 l/min, additional heating to plus 5°C was achieved. Both heaters were thermostatically controlled to cut out if water temperatures exceeded 20°C, as they might in midsummer. Temperatures greatly in excess of 20°C are likely to be lethal to young salmon parr, whilst being well fed. The slightly lower flows in the tanks with heated water, did not seem to hinder tank operation or water flow structure in these tanks in any way.

One problem encountered using this heating system was that of gas bubble disease (Roberts and Shepard 1979). This condition is caused when cool water with a high dissolved air content (i.e. mainly nitrogen) is heated. The warmer water is not capable of carrying as much dissolved gas at the higher temperature and in this system of water heating, as there is no escape for excess gaseous air before entering the tank, discharged water is supersaturated. During periods of low ambient water temperature, when the dissolved gas load was high, the problem was at its greatest. This condition only became a problem in tanks heated to plus 5°C in mid-winter and resulted in a slightly elevated mortality rate at this time.

2.4.4 Light Quality

Each tank was illuminated by 4 x 45cm, 15 watt "Sylvania Warmwhite" fluorescent tubes. This bulb is recommended by

the manufacturer as one which simulates closely the natural daylight spectral output. Figure 2.9 shows the spectral output of this tube measured on a Zeiss Monochromator M4Q111 amplifying spectrophotometer. The output is displayed as a percentage of the maximum intensity, thus the total area under the graph represents the total light output over the whole spectrum. Although the spectral emission lines of the tube gas are clearly visible (at 367, 408 and 440 nm) the output from these wavelengths represents a very small percentage of the total. Thus the overall quality of the light output is not greatly affected by these peaks. Nonetheless it can be seen that there is a tendency for the red end of the visible spectrum (longer wavelengths) to be slightly over-represented in the total output when compared with natural sunlight (Fig. 2.5).

Villarreal et al. (in press) examined the effects of this simulated natural lighting system. They found that, over the first six months, no growth differences could be found between sibling groups held under simulated-natural and natural lighting.

2.4.5 Photoperiod Control

The light intensity of the tank lights at Almondbank was controlled by a system of motorised blinds. A central processing unit was programmed to simulate the natural daily

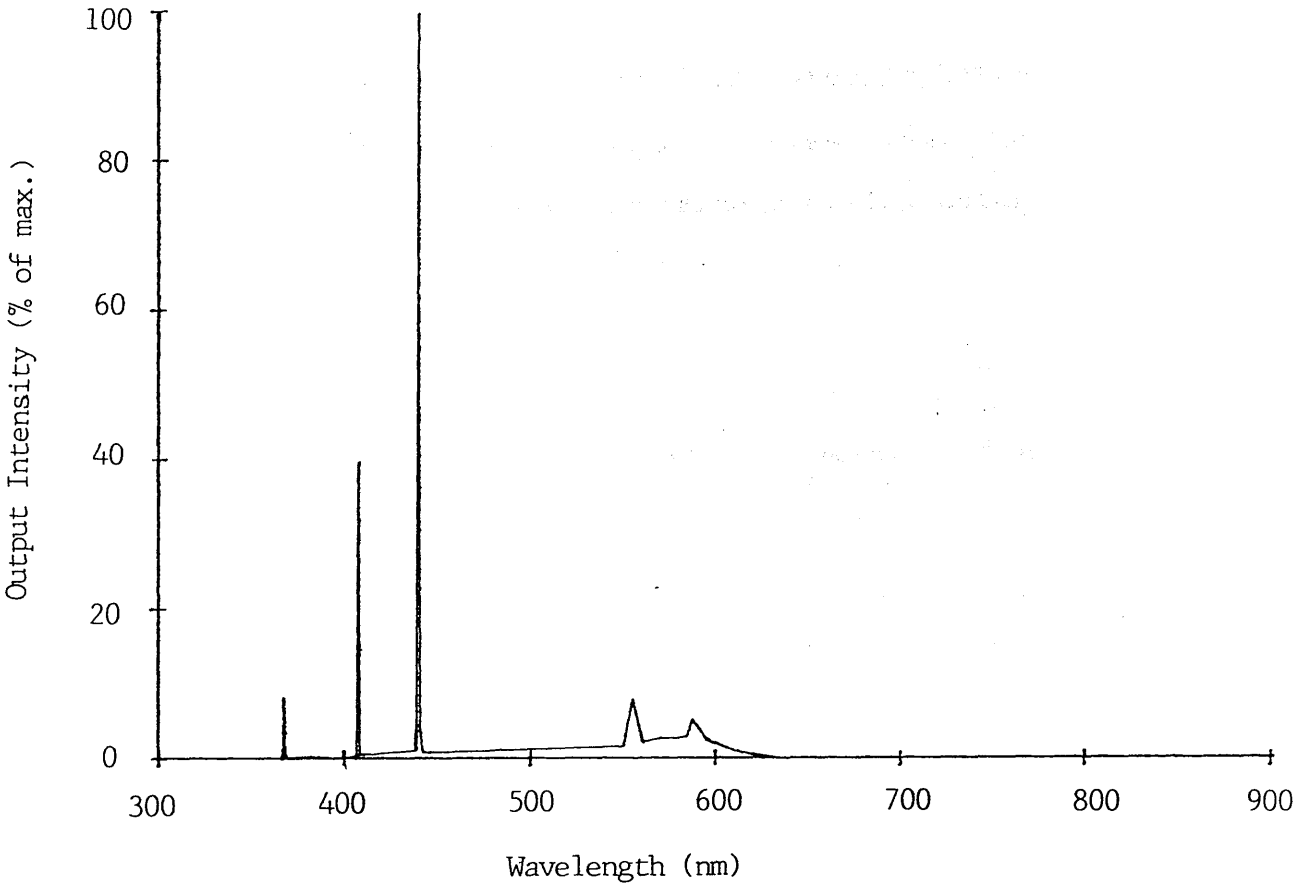


Figure 2.9 The spectral quality of a 15 watt "Sylvania Warmwhite" fluorescent tube. Output expressed as a percentage of maximum intensity.

light intensity changes, at 56° 20mins N. at morning and evening twilight, in ten tanks independently. Photoperiod cycles were moved out of phase from natural photoperiods in some tanks, thus although daily changes remained natural, (as shown in Appendix 1) photoperiod starting dates differed between tanks.

2.4.6 Feeding

Feeding was automated using "Caddymatic" feeders (as described earlier, Fig. 2.2) controlled from a central point. Feeding was in excess, at approximately 1 gram/tank fed 3 times per hour at first, increased to 6 times per hour as the fish grew. The food used was identical to that used at Rowardennan, (Ewos salmon food), sizes ranging from 0 to 3 as per manufacturers recommendations.

CHAPTER 3

THE EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON GROWTH AND SMOLTING

3.1 INTRODUCTION

Thorpe's model (1986 & Section 1.6) defines two distinct periods during the annual cycle when the outcome of a developmental pathway choice is decided. The model predicts that, if the rate of acquisition of energy is above a genetically predetermined level during the maturation window, then the physiological pathway leading to sexual maturation is likely to be triggered.

Similarly, it is predicted that high energy acquisition above a genetically determined threshold during the second window will lead to triggering of the smolting developmental pathway.

Clearly the most obvious sign of high energy acquisition is high growth rate. Thorpe's model predicts that factors that may affect growth rate during these sensitive window periods, will influence parr maturation and smolting rates. Such factors may be: biological - such as food availability, foraging efficiency and social interactions; genetic - e.g. the threshold level above which acquisition of energy triggers the onset of a particular pathway may be

genetically controlled or physical - such as water temperature and photoperiod.

In addition the model predicts that any change in the timing of the critical window periods, such that growth potential during these periods change, will lead to changes in maturation and smolting rates.

Although it is the effect of the physical factors, photoperiod and water temperature on the timing of the smolting decision, growth and smolting rates that are considered here, a pilot study of the possible effects of social interaction on smolting rate is described in Appendix 4.

3.1.1 The Timing of Successive Developmental Stages.

Cyclic Activity:

Much activity of both plants and animals is cyclic in nature. Breeding, migration, flowering, feeding, movement, dispersal, and many other activities may show peaks of intensity within well defined periods.

Rhythmic activity is a very widespread phenomenon and has been observed at all levels of organisation, from cells to populations (Cloudsley-Thompson 1980). Activities or

processes that occur only once in an individuals' lifetime are often seen as cyclical, when viewed from the population standpoint.

The timing of rhythmic activities is likely to be adaptive. For example, to have feeding young in the nest at the time of maximum food availability the optimum timing of egg formation and laying, in birds, must be synchronised by proximate cues. Daylength is one such cue known to be used by birds (Perrins and Birkhead 1983).

Timing Cues in Fish

The environmental cues used to determine time in fish, have come under scrutiny from a number of authors. Scott (1979) in a review of the control of teleost reproduction, suggests that in most fish it is based on an annual cycle. He concludes that photoperiod is likely to be the most important stimulus influencing the timing of fish breeding. However he suggests that in some species temperature may replace photoperiod as the principal timing cue. In Pacific salmon Clarke et al. (1978) noted that temperature governed the rate of physiological response, but itself was not a seasonal cue. Villarreal et al. (in press) point out that temperature, although it shows an annual and daily periodicity, is subject to much more unpredictable variation

than photoperiod and would therefore be much less reliable as a timing cue.

3.1.2 Photoperiod and Growth

In addition to its effect on the timing of life history decisions, photoperiod is likely to have a direct effect on growth. Higgins and Talbot (1985) have shown that feeding in Atlantic salmon parr is greatly reduced during darkness hours. In addition Villarreal et al. (in press) showed a direct relationship between growth and daylength in salmon parr.

3.1.3 Temperature and Growth

Water temperature may influence growth in fish in several ways. Temperature can influence food intake. Higgins and Talbot (1985) examined the relationship between food intake and water temperature in parr. They found that intake (expressed as a proportion of body weight) increased linearly with increasing temperature.

Temperature can influence assimilation efficiency. Weatherley (1972) shows (using data from Warren and Davis 1967) that growth increases to an optimum at 28° C, in young Cichlasoma, despite uniform food consumption. Above 28° C, growth declines again with temperature, for a given food consumption.

Temperature can influence activity. Hergenrader and Hasler (1967) tracked schools of Yellow Perch (Perca flavescens) in Lake Mendota, USA, using sonar. They were able to correlate mean swimming speed of fish with temperature. Increased activity is likely to have two opposing effects on growth of fish. It will increase energy expended in movement, reducing energy available for growth. However it may also result in increased food intake, as foraging intensity increases with activity.

The aim of this work is to test the null hypothesis that photoperiod and temperature have no influence on smolting rate in genetically homogeneous, underyearling Atlantic salmon populations.

3.2 MATERIALS AND METHODS

3.2.1 Experiment 1 - Accelerated Incubation

On the 18th February 1985 the accelerated incubation period ova of family 4/84 (Section 2.1) started to hatch. By the 29th March, the alevins had absorbed their yolk sacs and were ready to take their first food. On this date this sibling group was divided into 4 smaller groups of approximately 400 individuals and placed in 1 metre radial-flow tanks in controlled environment cells at Almondbank. The date of first feeding marked the first exposure of these fish to light.

Environmental Conditions: Each group was exposed to a simulated natural annual and daily photoperiod regime for 56° 20' N. However these regimes were moved out-of-phase from ambient in experimental tanks.

Two groups experienced photoperiods corresponding to the 8th March at first feeding, thus the photoperiod phase was retarded by 21 days. This photoperiod regime is nominally "ambient photoperiod" (designated AP) for experiment 1. The reciprocal pair of groups experienced a photoperiod corresponding to the 7th June at first feeding. This regime shall be called "Ambient + 3 months photoperiod" (designated +3P). From the date of first feeding until the

termination of the experiment all groups experienced the normal annual daylength and twilight changes shown in Appendix 1.

One group from each like photoperiod pair was exposed to heated water, temperatures being elevated by 5°C above ambient, (to a maximum of 20°C) i.e. experiencing normal annual and daily fluctuations around a mean 5°C higher than ambient (designated +5T). The reciprocal pair received ambient River Almond water temperatures (AT).

Table 3.1 summarizes experimental photoperiod and temperature regimes in tanks in experiment 1.

3.2.2 Experiment 2 - Standard Incubation

On the 25th March 1985, the second batch of eggs from sibling group 4/84 started hatching. On the 6th May this batch, incubated on ambient temperature River Almond water, was divided into 4 groups of approximately 400 fish and transferred to radial flow tanks in controlled environment cells at Almondbank, to take their first food. This date represents the first exposure of this group of fish to light.

Environmental conditions: Two of the four groups first

TANK DESIGNATION	PHOTOPERIOD REGIME	TEMPERATURE REGIME
AP/+5T	Ambient	+5° C
+3P/+5T	+3 months	+5° C
AP/AT	Ambient	Ambient
+3P/AT	+3 months	Ambient

TABLE 3.1 The photoperiod temperature regime combinations of Experiment 1 (Accelerated incubation, first fed March 1985).

TANK DESIGNATION	PHOTOPERIOD REGIME	TEMPERATURE REGIME
AP/+2T	Ambient	+2° C
-2P/+ 2T	-2 months	+2° C
AP/AT	Ambient	Ambient
-2P/AT	-2 months	Ambient

TABLE 3.2 The photoperiod temperature regime combinations of Experiment 2 (Standard incubation, first fed May 1985).

feeding on the 6th May, experienced simulated ambient daylength and twilight for that date, henceforth receiving the ambient annual photoperiod cycle (designated AP). The other pair were given a photoperiod corresponding to the 8th March i.e. 2 months out-of-phase (retarded) with the ambient photoperiod regime (-2P).

One group from each photoperiod pair, received ambient River Almond water temperatures (designated AT). The reciprocal 2 groups received additional heating of water to 2°C above ambient (to a maximum of 20°C) (+2T).

Photoperiod and temperature regimes of the 4 groups that constitute experiment 2, are summarized in Table 3.2.

Choice of photoperiod and temperature regimes:

Food intake and digestion is severely limited below about 5°C (Higgins and Talbot 1985). River Almond water is normally below 5°C during February and March when accelerated incubation fry were first-feeding. For this reason a significant addition to the water temperature (+5°C) was chosen to allow growth from first-feeding in accelerated incubation fish. Standard incubation groups experience higher water temperature at first-feeding, thus a lower temperature supplement was chosen for these groups.

There are 2 periods of the year when photoperiod changes are at a maximum, namely at the two equinoxes. It is reasonable to suspect that detection of these photoperiods is easier than most others, and thus they may be important as timing cues. For this reason the choice of photoperiod regimes was made in each experiment such that one photoperiod group received the possible cues offered by a spring equinox photoperiod while the other group did not.

3.2.3 Experiment 3

On the basis of the results from experiments 1 and 2 it was decided to essentially replicate, but also refine and extend these experiments with another sibling group over the following year (1986 - 1987). In an attempt to separate the effects of photoperiod and temperature, groups were held at constant temperature and at constant daylength. In addition, using comparable photoperiod regimes for both accelerated and standard incubation groups allowed a greater degree of comparison between groups.

Accelerated Incubation Period

The sibling ova from family 4/85, exposed to elevated water temperatures to accelerate incubation, hatched on the 20th January 1986. By the 6th March 1986 these fish were ready to accept their first food. On this date approximately 400 fish from this accelerated group were transferred into

each of 5 radial flow tanks, within enclosed environment cells at Almondbank. This date represents the first exposure of these fish to light.

Environmental conditions: Two of these 5 groups were exposed to a simulated natural daylength and twilight regime, retarded out-of-phase by 33 days. Thus the photoperiod at first feeding corresponded to that of the 1st February. From this date the photoperiod regime followed the natural annual cycle, as indicated in Appendix 1. For ease of notation this photoperiod regime will henceforth be called the "ambient photoperiod regime" (designated AP) within the context of this experiment. Two reciprocal tanks were placed on a daylength and twilight regime 56 days advanced out-of-phase (corresponding to the 1st May). This photoperiod regime shall nominally be called "+ 3 months" out-of-phase (designated +3P).

From each pair of homologous photoperiod regimes, one tank received 5°C additional heating (to a maximum of 20°C) above ambient River Almond water temperature (designated +5T). The remaining pair received River Almond water at ambient temperature (designated AT).

The fifth tank in the series was exposed to a constant 12 hours light, 12 hours dark photoperiod, without any twilight or annual daylength changes (designated 12P). This

population received ambient river water temperatures.

Standard Incubation Period

The other half of the sibling group of ova, family 4/85, incubated at ambient river water temperature, hatched on the 17th March 1986. On the 16th May, they were ready to receive their first food.

This batch was split into 7 groups, of approximately 400 fish. Two groups were placed on a simulated photoperiod regime, 16 days retarded out-of-phase, so that at first feeding they experienced a daylength and twilight corresponding to the 1st of May. Two other groups received photoperiod regimes retarded 105 days, so that at first feeding they experienced a daylength and twilight corresponding to the 1st February. These two photoperiod regimes will nominally be, "ambient photoperiod" (retarded 16 days)(designated AP) and "-3 months photoperiod" (retarded 105 days) (designated -3P) within the context of this experiment.

One group from each homologous photoperiod pair received ambient River Almond water temperature. The other from each photoperiod pair, received additional heating, 2°C above ambient, to a maximum of 20°C.

A fifth group was placed on a 12 hours light 12 hours dark photoperiod regime, with no twilight nor any annual photoperiod changes, on ambient River Almond water temperature (12P and AT).

The remaining 2 groups were removed to Rowardennan, to constant temperature facilities, where both groups were exposed to constant water temperatures of 15°C (+/- 0.25°C) with no annual fluctuations (designated 15T). One group received a -3 months photoperiod regime (retarded 105 days), the other an ambient photoperiod regime (retarded 16 days).

The photoperiod and temperature regimes of the 12 groups that comprised Experiment 3 are summarized in Table 3.3.

3.2.4 Experimental Protocol

To monitor growth patterns of fish exposed to differing temperature and photoperiod regimes, fork length was chosen as the most suitable size parameter (Lagler 1978). Fork length in salmon is a clearly defined in fish above 20 mm., its measurement is simple, allowing large numbers of fish to be monitored efficiently and it was found to be very closely related to body weight.

TANK DESIGNATION	PHOTOPERIOD REGIME	TEMPERATURE REGIME	INCUBATION PERIOD
AP/+5T/A	Ambient	+5°C	Accel.
+3P/+5T/A	+3 months	+5°C	Accel.
AP/AT/A	Ambient	Ambient	Accel.
+3P/AT/A	+3 months	Ambient	Accel.
12P/AT/A	12 hrs light	Ambient	Accel.
-3P/+2T/S	-3 months	+2°C	Stand.
AP/+2T/S	Ambient	+2°C	Stand.
-3P/AT/S	-3 months	Ambient	Stand.
AP/AT/S	Ambient	Ambient	Stand.
12P/AT/S	12 hrs light	Ambient	Stand.
-3P/15T/S	-3 months	15°C	Stand.
AP/15T/S	Ambient	15°C	Stand.

TABLE 3.3 The photoperiod and temperature regime combinations of Experiment 3. Accelerated incubation group 1st fed March '86 standard incubation group 1st fed May '86.

Measurements of fork length of all fish from every group anaesthetised with "Benzocane" (Stephenson 1980) were normally made during the last week of every month.

During the initial period, when fish were small (until June 1985 - experiment 1, July 1985 - experiment 2, June 1986 - experiment 3) only a sample of each population was measured using a photographic technique. Anaesthetised fish were placed directly on to black and white, photographic printing paper, in the dark. The paper was then exposed to a bright light from an electronic flash gun, positioned directly above. The fish were returned to fresh water to recover and the photographic paper, developed immediately. The negative image of the fish was visible as a distinct "shadow". The image fork length was measured directly, using a graphic digitizer linked to an Apple micro computer. For very small fish enlargement of the image, helped to increase accuracy of measurement. This technique was found to be quicker for small fish than direct measurement, thus reducing the time fish spent out of water.

This method was compared to direct measurement of fish on a measuring board (Lagler 1978). Differences of +/- 0.4% between means of the data from the two techniques, were obtained indicating the suitability of this technique for

measuring small fish.

Sample sizes necessary to obtain an estimate of mean fork length of each population to within +/- 0.5mm with 99% confidence were calculated from the "t" statistic, using a method described in Snedecor (1950). Minimum sample sizes were exceeded by a factor of at least two.

Fork length monitoring of larger fish was by direct measurement of all fish from each group, on a measuring board. Again all fish were anaesthetised prior to measurement. Fish with obviously misleading fork lengths e.g. spinal deformities were not included in any results.

Statistical methods:

Mean lengths of populations were compared using the Students t-test. Numbers entering the upper modal group were compared using a Chi squared test. Percentage values of the proportion in the upper modal growth group were calculated from the bimodal distribution by a method described by Cassie (1954). Percentages were stabilised using the arcsine transformation where necessary, before statistical testing.

3.3 THE EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON GROWTH

Figures 3.1-3.3, 3.4-3.7 and 3.8-3.19 show fork-length percentage frequency histograms for populations over the study periods of experiment 1, 2, and 3 respectively.

Population AP/+5T (Experiment 1) was lost during a spate on the 15th August 1985, when silt blocked the water flow to the tank. Only minimal results from this population will be presented here. Similarly, on the 12th of September and the 22nd of November 1987 populations AP/15T/S and -3P/15T/S respectively were lost, mainly due to a failure of the plumbing system. Results for these two tanks prior to these dates for these populations will be discussed here.

General Trends in Fork-length frequency histograms

From Figures 3.1 - 3.19 it is clear that there are a number of characters common to the pattern of fork-length frequency changes of all populations.

Rapid fork-length increase during spring and early summer

with uniform, normal distributions around the mean is typical. In mid-summer to autumn, this pattern changes. The normal distribution spreads, resulting in an increased standard deviation and subsequently a bimodal length frequency distribution develops.

The timing of the separation into two modes, the proportions of the population in each mode, the extent of separation of the two modes, the mean fork-lengths of the two sub-populations and the extent of growth arrest over winter in the two modes, varies between groups held under different environmental conditions.

3.3.3 EXPERIMENT 1 - ACCELERATED INCUBATION

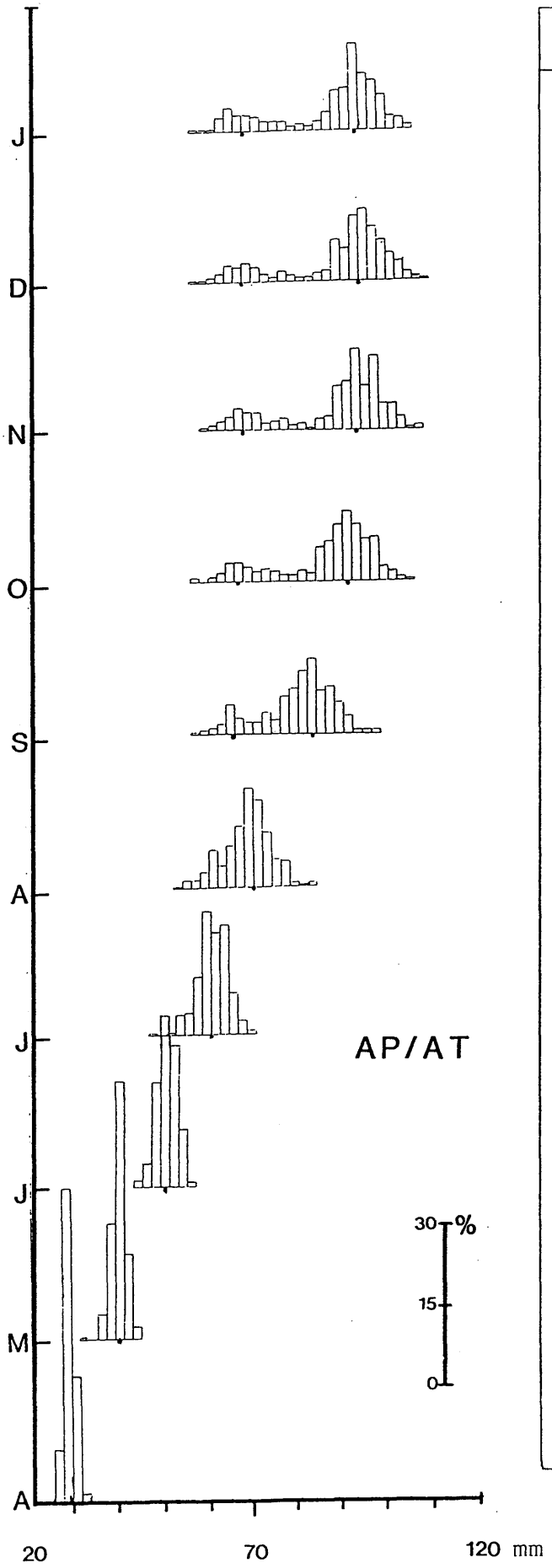
Control Population: AP/AT Figure 3.1

Fig. 3.1 shows the fork-length percentage frequency distribution changes for the control population AP/AT for experiment 1.

Initial growth from first feeding to July was rapid. The fork-length frequency histograms show a unimodal distribution, spreading slightly around the mean.

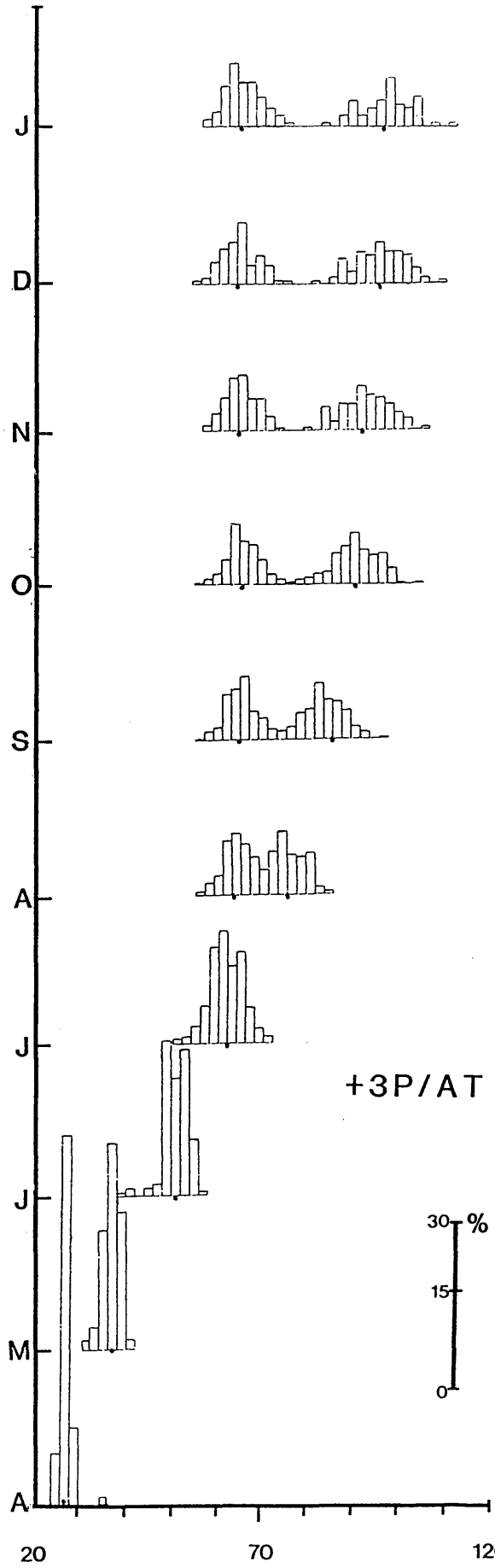
By August this broadening of the distribution became more

Figures 3.1 - 3.3 Monthly fork-length (mm), percentage frequency histograms for the accelerated incubation populations of Experiment 1 over the study period March 1985 - January 1986. Associated tables show monthly mean fork-lengths ($\bar{L}$) (also indicated on histograms), standard errors (S.E.), the number of fish monitored (N) and percentage in the UMG.



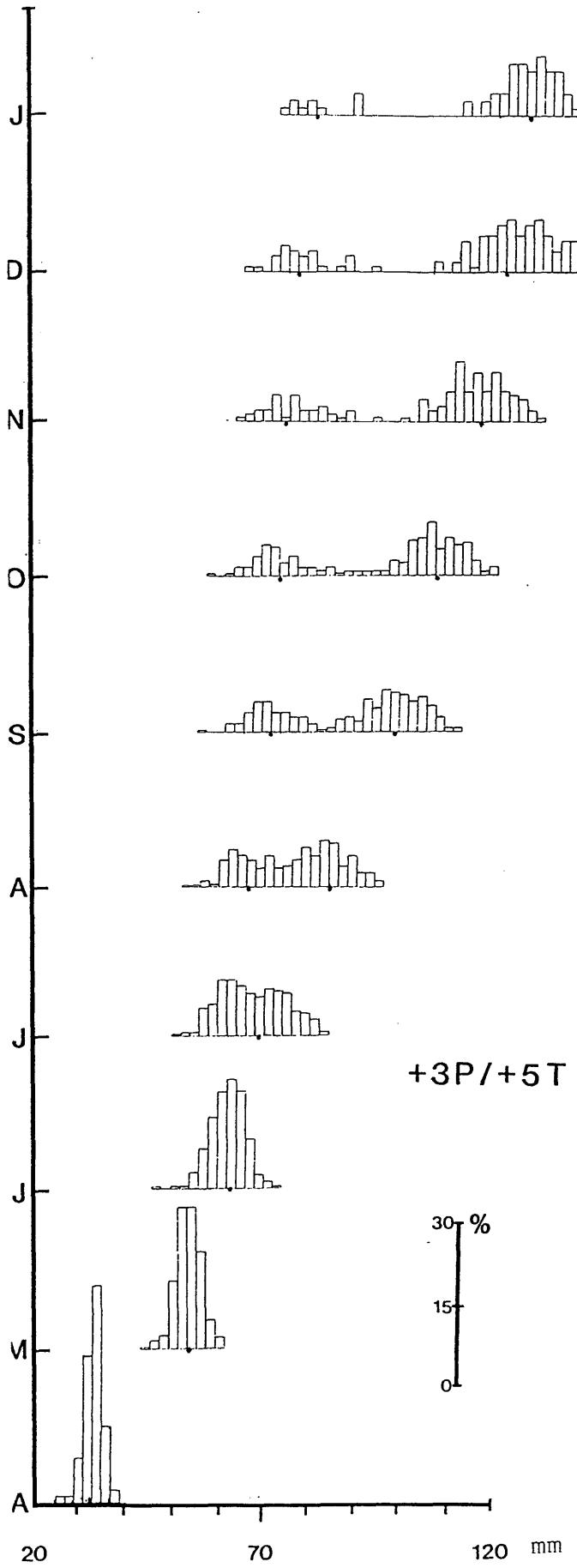
LMG 21.2%			UMG 78.8%		
$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
69.6	0.67	50	94.9	0.40	165
70.0	0.68	50	96.2	0.40	186
70.1	0.62	49	95.2	0.39	185
69.6	0.67	51	92.7	0.37	191
68.2	0.55	53	84.6	0.34	200
72.0	0.33	253			
61.8	0.21	250			
51.4	0.17	151			
38.7	0.11	199			
27.5	0.11	68			

Figure 3.1



	LMG 48.5%			UMG 51.5%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
J	66.5	0.46	75	97.7	0.72	60
D	66.0	0.47	76	96.6	0.60	84
N	66.1	0.40	85	93.4	0.56	88
O	67.0	0.40	134	91.2	0.37	142
S	65.5	0.29	135	83.6	0.36	146
A	64.3	0.25	133	76.0	0.29	145
J	63.3	0.23	284			
J	53.0	0.25	126			
M	38.8	0.25	126			
A	27.1	0.14	80			

Figure 3.2



	LMG 36.1%			UMG 63.9%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
J	80.1	1.97	10	126.9	0.81	57
D	76.4	1.25	27	121.6	0.77	70
N	74.9	1.08	41	115.7	0.63	92
D	72.3	0.79	82	106.3	0.48	145
S	70.0	0.63	87	97.0	0.49	149
A	66.6	0.44	88	84.3	0.46	152
J	69.2	0.41	275			
J	63.2	0.27	250			
M	52.6	0.18	199			
A	32.1	0.12	255			

Figure 3.3

conspicuous and by September had become clearly bimodal. Separation of the modes became more distinct between September and November, due to reduced growth rates of fish in the lower modal group (LMG) compared with fish in the upper modal group (UMG). This is indicated by a lower proportionate rate of increase in the mean fork-length ($\bar{L}$) over this period (LMG 2.8% cf. UMG 12.5% increase in $\bar{L}$).

From November until the termination of the experiment at the end of January, there was total growth arrest in LMG fish. In contrast to this the UMG showed a continued increase in mean length until December. The apparent reduction in mean length from December to January in the UMG is likely to be the result of the mortality of 21 fish from this group over this period.

The best estimate of the proportion of the population maintaining growth into the autumn, and so entering the UMG, was made in December prior to these mortalities, as 79% of the total population. A sample of fish from this population was sacrificed for histological examination (see Chapter 4) after termination of the experiment and did not affect the results presented here.

The Effect of Advanced Photoperiod at Ambient Temperature:

Experimental group: +3P/AT Figure 3.2

Control population for photoperiod: AP/AT Fig.3.1

Figure 3.2 shows the length percentage frequency histograms for the experimental group +3P/AT. As with the control group for this experiment (Fig.3.1), initial population growth over spring and early summer was typified by rapid fork-length increase with a unimodal distribution about the mean. By June, the longer days of an advanced photoperiod phase (this experimental tank would have received longer days until the 2nd June) had resulted in a mean fish length of 63.3 mm compared with 61.8 mm in the control ($p<0.001$).

Clear bimodality of length frequency distributions first appeared in August, one month earlier than in the control group. In September and October the experimental LMG and UMG had a consistently lower mean fork length than the controls, despite an initial size advantage at the end of the unimodal growth phase (e.g. Oct. $p<0.01$). During this period the control group was receiving longer days than the experimental group.

Non-random removal of fish from the experimental group between the months of October and November for histological examination is the most likely explanation for the fluctuation in mean length between these months. As with the control the LMG shows no growth over the period November to December. The UMG continued to show an increase in mean length over the period November to January.

The best estimate of the proportion in the UMG (52%) was made in October.

The Effect of Elevated Temperature at Advanced Photoperiod

Experimental Population: +3P/+5T Figure 3.3

Control group for temperature: +3P/AT Fig. 3.2

Growth over the initial unimodal phase to July was higher at elevated temperature, than at ambient temperature. By July fish on elevated temperature had reached a mean length of 69.2 mm compared with 63.3 mm in the control ($p<0.001$).

Clear bimodality was first evident in August, as it was in the control. Growth in the LMG of the experimental population showed no sign of total growth arrest at any point before the termination of the experiment, however this

is possibly due to deaths of the smaller fish (see below). Growth in the UMG remained high until the end of January, resulting in the complete segregation of the two modes.

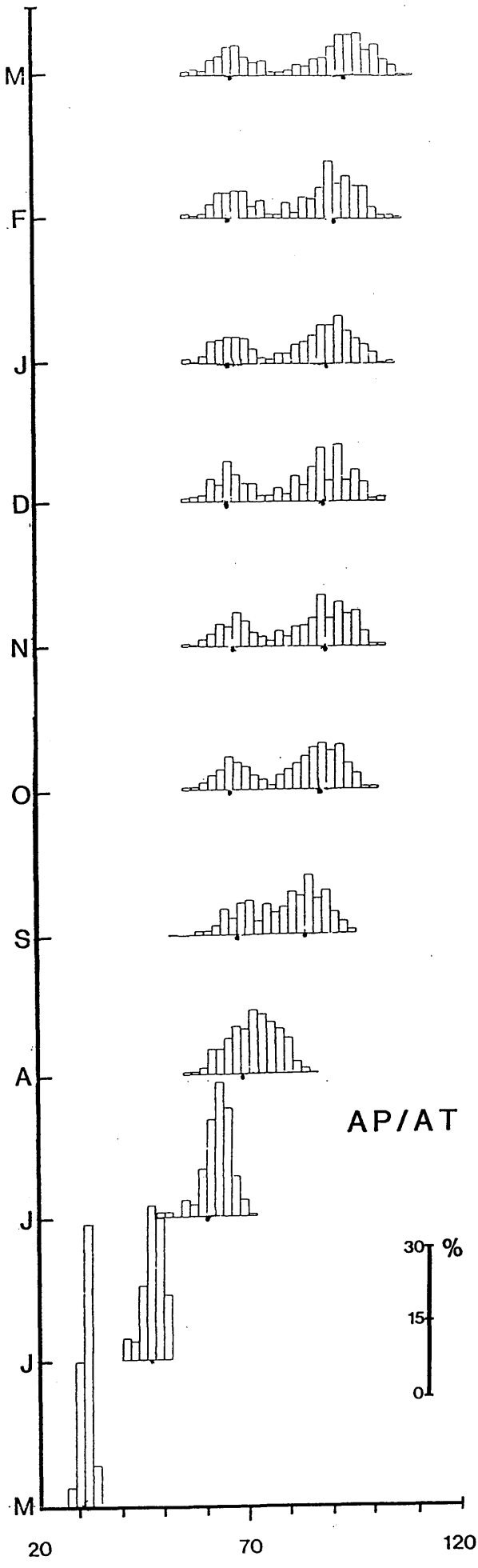
The best estimate of the proportion in the UMG (64%) was made in October. Fish were removed from this population for histological examination between October and November monitoring dates. Relatively high mortality occurred throughout late autumn and winter, caused by gas bubble disease. (see section 2.4.3 for details).

3.3.2 EXPERIMENT 2 - STANDARD INCUBATION

Control Population: AP/AT Figure 3.4

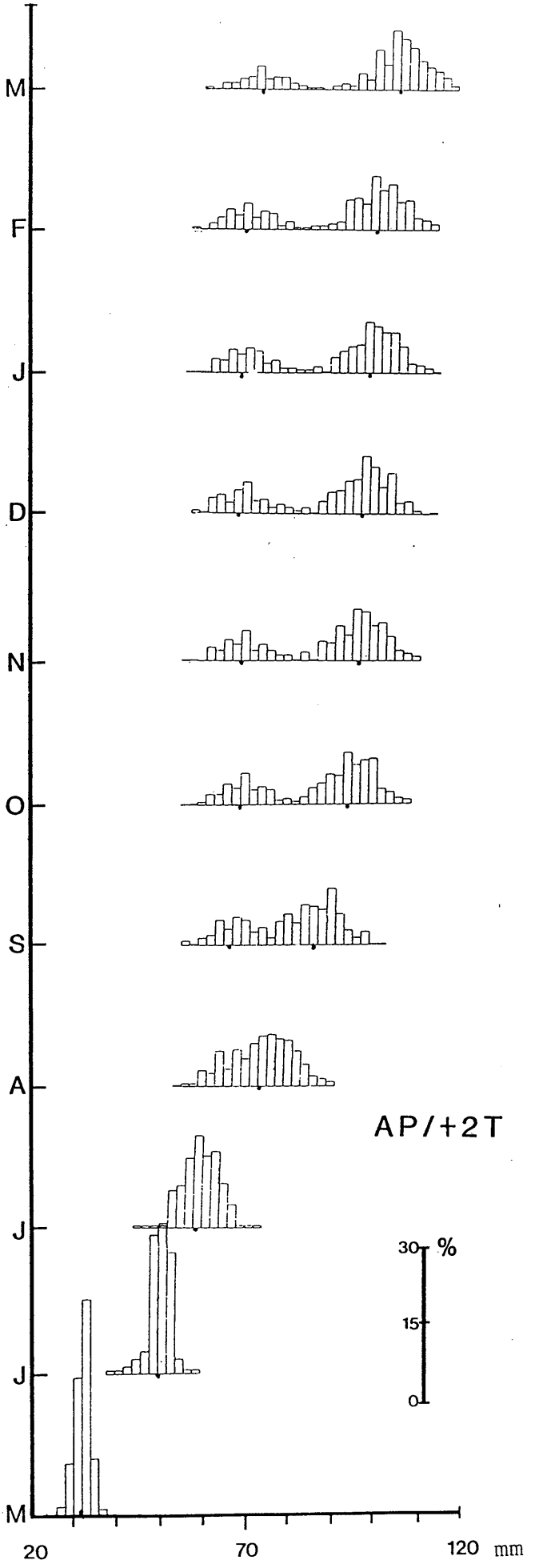
As with other groups, an initial, rapid increase in fork-length with the population normally distributed about the mean is evident after first feeding. There is a broadening of the distribution by August and by September a bimodal distribution is apparent, this becoming clearly defined in October. There was only a minimal increase in mean length in the LMG between October and November and no growth between November and January. There is possibly some evidence for a reduction in the mean fork length of this group during this period. In the UMG the fork length increased until November and growth arrest occurred from

Figures 3.4 - 3.7 Monthly fork-length (mm), percentage frequency histograms for the standard incubation populations of Experiment 2 over the study period May 1985 - March 1986. Associated tables show monthly mean fork-lengths ($\bar{L}$) (also indicated on histograms), standard errors (S.E.), the number of fish monitored (N) and the percentage in the UMG.



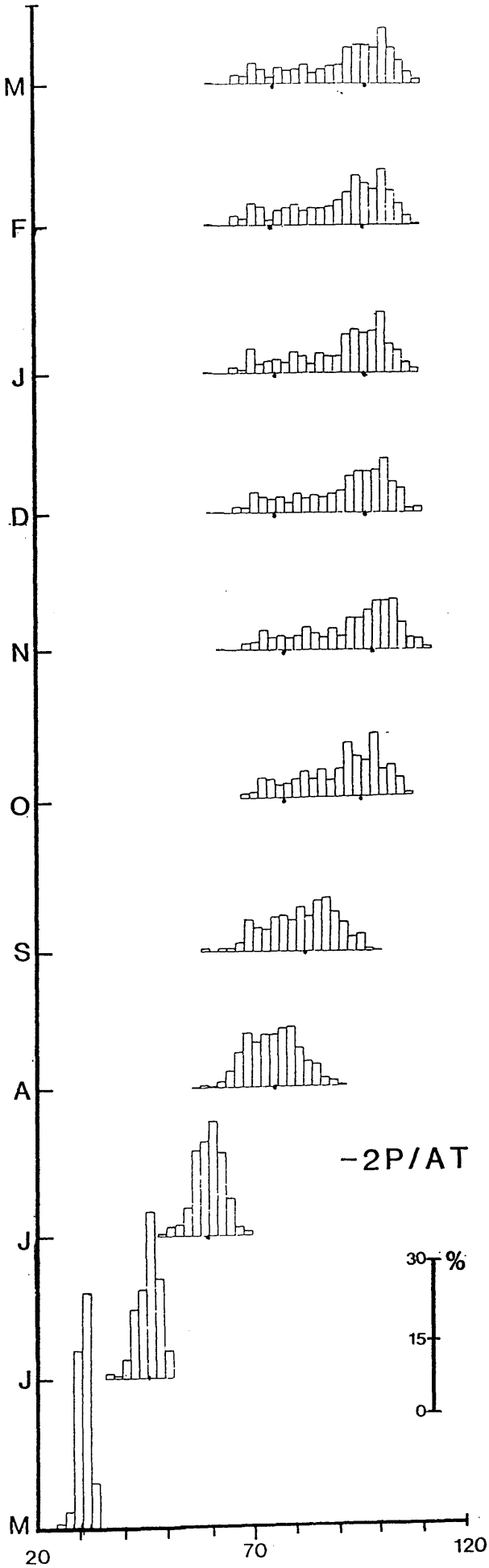
	LMG 33.7%			UMG 66.3%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	67.6	0.50	83	94.1	0.47	166
F	67.9	0.49	83	91.9	0.44	165
J	66.8	0.40	107	90.3	0.41	211
D	66.9	0.40	108	89.7	0.40	210
N	67.9	0.41	110	89.9	0.38	217
O	67.3	0.40	108	88.1	0.35	220
S	66.3	0.39	119	82.0	0.31	230
A	70.5	0.31	351			
J	60.2	0.23	205			
J	47.4	0.19	155			
M	30.4	0.10	135			

Figure 3.4



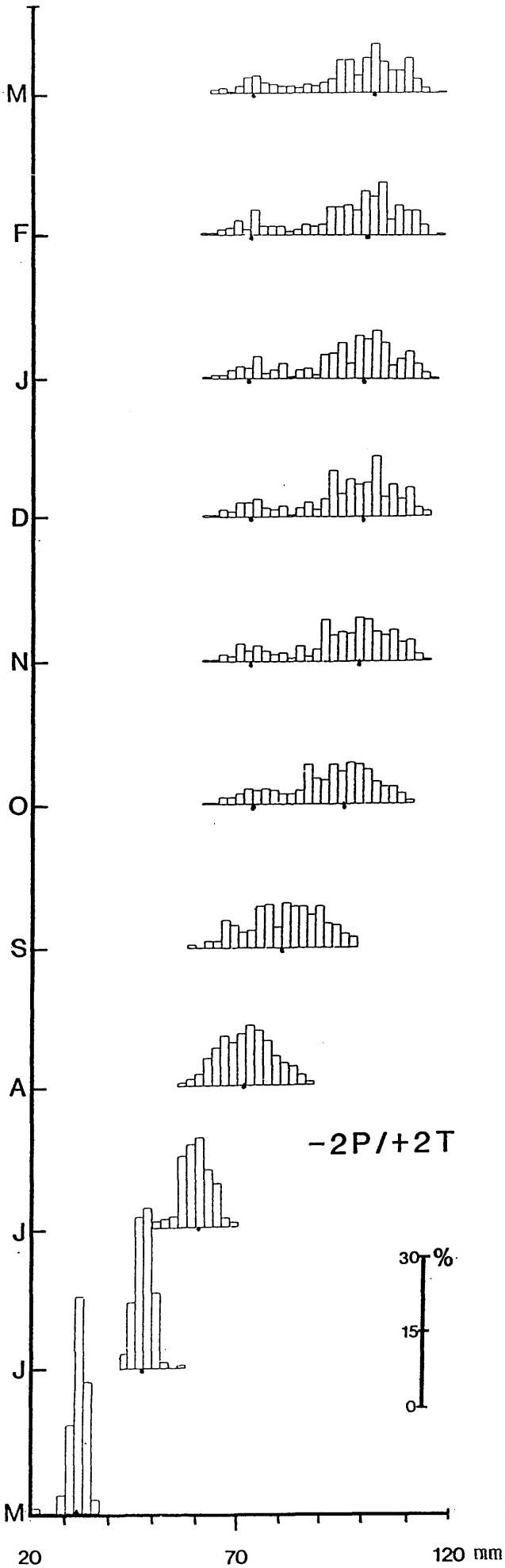
	LMG 30.1%			UMG 69.9%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	71.5	0.75	53	103.8	0.45	165
F	69.0	0.62	70	99.6	0.46	169
J	67.3	0.55	94	97.4	0.39	218
D	67.2	0.50	94	95.9	0.42	219
N	68.0	0.53	96	95.5	0.39	218
O	67.5	0.47	96	93.1	0.41	222
S	65.4	0.44	96	85.0	0.39	224
A	72.4	0.41	320			
J	61.2	0.30	205			
J	50.5	0.25	127			
M	32.2	0.16	110			

Figure 3.5



	LMG 27.7%			UMG 72.3%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	77.6	0.60	87	99.1	0.37	226
F	77.6	0.60	87	98.4	0.37	227
J	78.6	0.61	87	99.1	0.35	221
D	77.8	0.58	90	98.6	0.36	237
N	78.1	0.58	88	98.3	0.35	226
O	77.9	0.50	88	95.6	0.35	225
S	83.0	0.44	328			
A	74.5	0.34	326			
J	59.5	0.24	218			
J	46.3	0.19	173			
M	30.3	0.11	152			

Figure 3.6



	LMG 19.1%			UMG 80.9%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	72.2	0.72	51	100.7	0.50	217
F	71.9	0.66	51	99.4	0.50	221
J	71.4	0.65	52	98.2	0.50	221
D	71.5	0.65	51	97.7	0.48	221
N	71.5	0.67	51	96.8	0.49	218
O	71.8	0.61	55	93.2	0.47	217
S	80.1	0.43	277			
A	71.6	0.38	277			
J	58.7	0.27	204			
J	45.9	0.17	153			
M	31.0	0.17	108			

Figure 3.7

November to December, thereafter growth recommenced.

The best estimate of the proportion in the UMG (66%) was made in January. Fish for histological examination were removed from the population between January and February monitoring dates.

The Effect of Elevated Temperature at Ambient Photoperiod

Experimental Population: AP/+2T Figure 3.5

Control group for temperature: AP/AT Fig. 3.4

Elevated temperature resulted in increased growth of this population during the unimodal growth phase. Thus by August the mean length of fish in the experimental group was 72.4 mm compared with 70.5 mm in the control ($p<0.001$).

Clear bimodality first appears in the September distribution, as it does in the control. UMG fish appear to have continued growth throughout the winter, showing no evidence of complete growth arrest. The slightly elevated temperature of the experimental group had no discernible effect on the LMG growth arrest period (November to January). As in the control group, there is some evidence of a slight reduction in mean fork-length in the LMG over

the period December to January.

The best estimate of UMG percentage (70%) was made in January.

Fish were removed for histological examination before monitoring of fork-lengths in February.

The Effect of Retarded Photoperiod at Ambient Temperature

Experimental population: -2P/AT Figure 3.6

Control for photoperiod: AP/AT Fig. 3.4

Initially shorter days resulted in slightly slower growth over the period up to June in this experimental group on retarded photoperiod (June: $\bar{L}$ = 46.3mm retarded photoperiod cf. $\bar{L}$ = 47.4mm control $p<0.001$). However longer days experienced by this group after July resulted in the size differential being eroded, until by August the experimental population were longer, on average ($\bar{L}$ = 74.5mm) than the control group ($\bar{L}$ = 70.5mm $p<0.001$).

Retarded photoperiod delayed the onset of bimodality, the separation of the modes appearing in October (cf. September in the control population).

There was no significant increase in mean fork-length in the LMG from October to March in the experimental population. The UMG however, showed consistent but very slight increases in length from October until January, (resulting in a 3.7% length increase ($p<0.001$) over this period) thereafter there was no evidence of growth before the termination of the experiment.

The best estimate of the proportion in the UMG (72%) was made in February. Fish for histological analysis were removed after the termination of the experiment.

The Effect of Elevated Temperature and Retarded Photoperiod

Experimental population: -2P/+2T Figure 3.7

Control for photoperiod: AP/+2T Fig. 3.5

Control for temperature: -2P/AT Fig. 3.6

Initially size was consistently, but only slightly, lower after first feeding, in the experimental group than in the control at ambient water temperature. This difference is not significant and is not consistent with the results of other groups at elevated temperatures. The size difference remained with the LMG throughout the study period, and with the UMG until January then the experimental UMG overtook the

control in February and March.

The pattern of development of bimodality in this group was consistent with that of the control for temperature, that is clear signs of bimodality were delayed slightly (c. 1 month) when compared to the control group for photoperiod.

As with the other group on 2 months retarded photoperiod, growth was retarded over the unimodal growth phase by shorter days (July $\bar{L}$= 58.7mm - experimental and $\bar{L}$= 61.2mm - photoperiod control $p<0.001$).

Growth in the LMG was arrested after October and did not recommence before the end of the experiment. In the UMG there was slight continuous growth throughout the winter, except during the period December - January.

The proportion in the UMG was estimated in February at 81%. Histological samples were removed after the termination of the experiment.

The Effect of Accelerated Incubation

Comparison of group AP/AT (Fig. 3.1) experiment 1, accelerated incubation, with AP/AT (Fig. 3.4)experiment 2 standard hatching, gives an indication of the effects of accelerated incubation period on fish from the same sibling stock.

With an extra 43 days growth period advantage the accelerated hatching group maintained a slightly larger mean length throughout the unimodal growth phase. By August the accelerated group had a mean length of 72.0mm cf. 70.5mm in the control population. ($p<0.002$).

Bimodality first appears in September in both groups. The initial size advantage of the accelerated incubation population continues into the bimodal phase with LMG mean length in December 70.0mm cf. 66.9mm in the control ($p<0.001$) and with the UMG mean length 96.2mm cf. 89.7mm ($p<0.001$) in the control at this time. This difference was maintained throughout the course of the experiment.

3.3.3 EXPERIMENT 3

Figures 3.8 to 3.19 show fork-length percentage frequency histograms of the populations of Experiment 3 (see table 3.3) over the study period April '86 to March '87.

STANDARD INCUBATION PERIOD

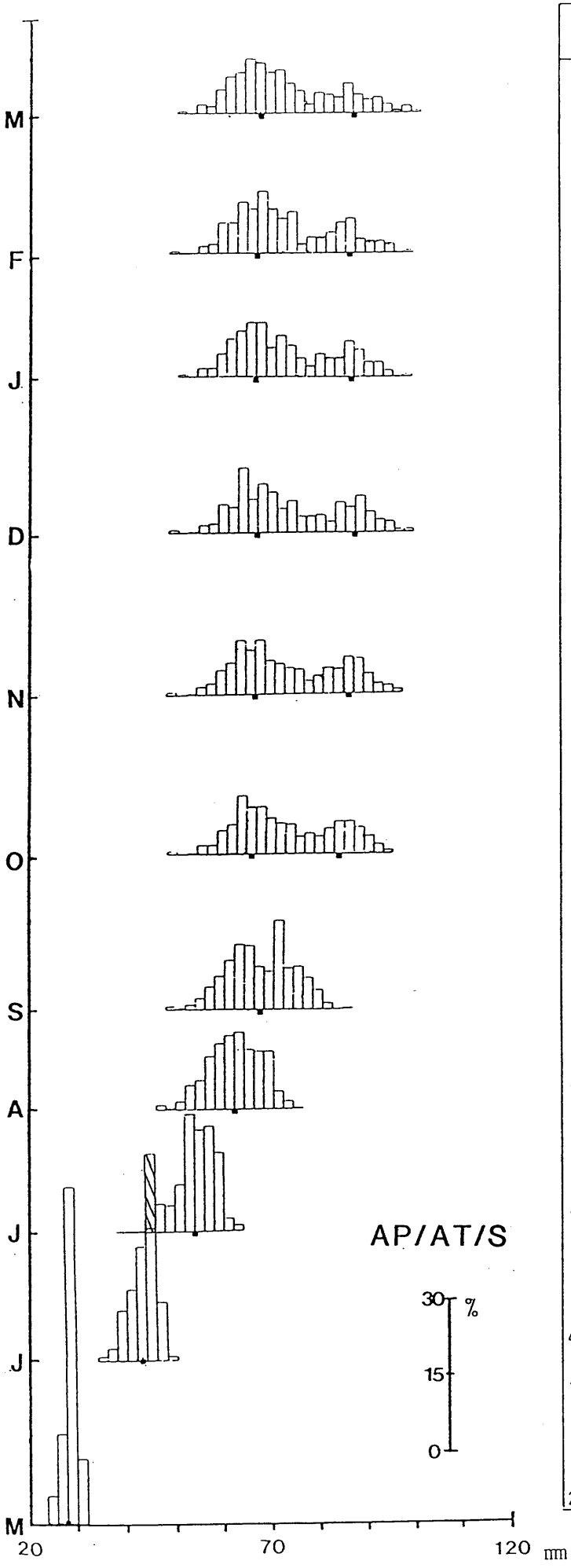
Control Population: AP/AT/S Figure 3.8

Figure 3.8 shows monthly fork-length frequency histograms for the control group on ambient photoperiod and temperature regimes, hatched at ambient temperature.

This population followed similar growth patterns observed in experiments 1 and 2. Rapid unimodal growth from first feeding until the end of August can be seen in length frequency histograms. Over the period September to November the unimodal distribution broadens. By October the bimodal distribution appears.

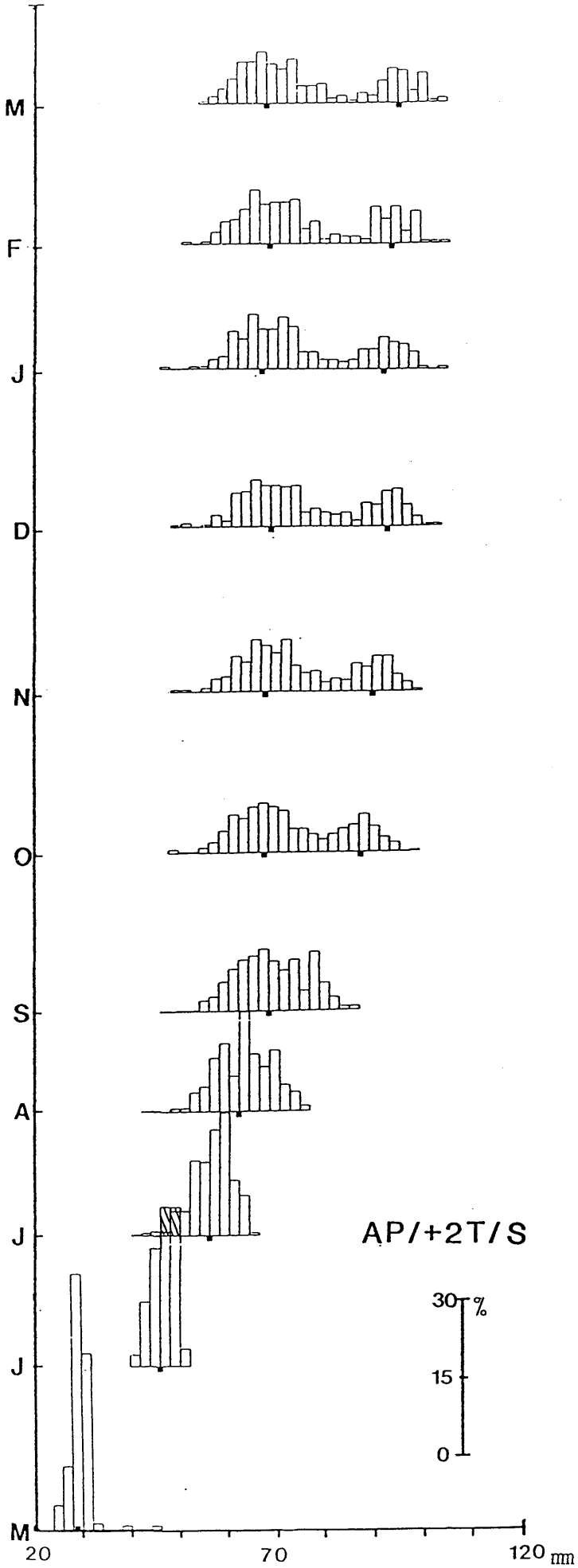
Growth of fish in the LMG ceased after November. However in the UMG there is evidence of continued growth until December, leading to clear separation of the modes.

Figures 3.8 - 3.19 Monthly fork-length (mm), percentage frequency histograms for the accelerated and standard incubation populations of Experiment 3 over the study period March 1986 to March 1987. Associated tables show monthly mean fork-lengths ($\bar{L}$) (also indicated on histograms), standard errors (S.E.), the number of fish monitored (N) and the percentage in the UMG.



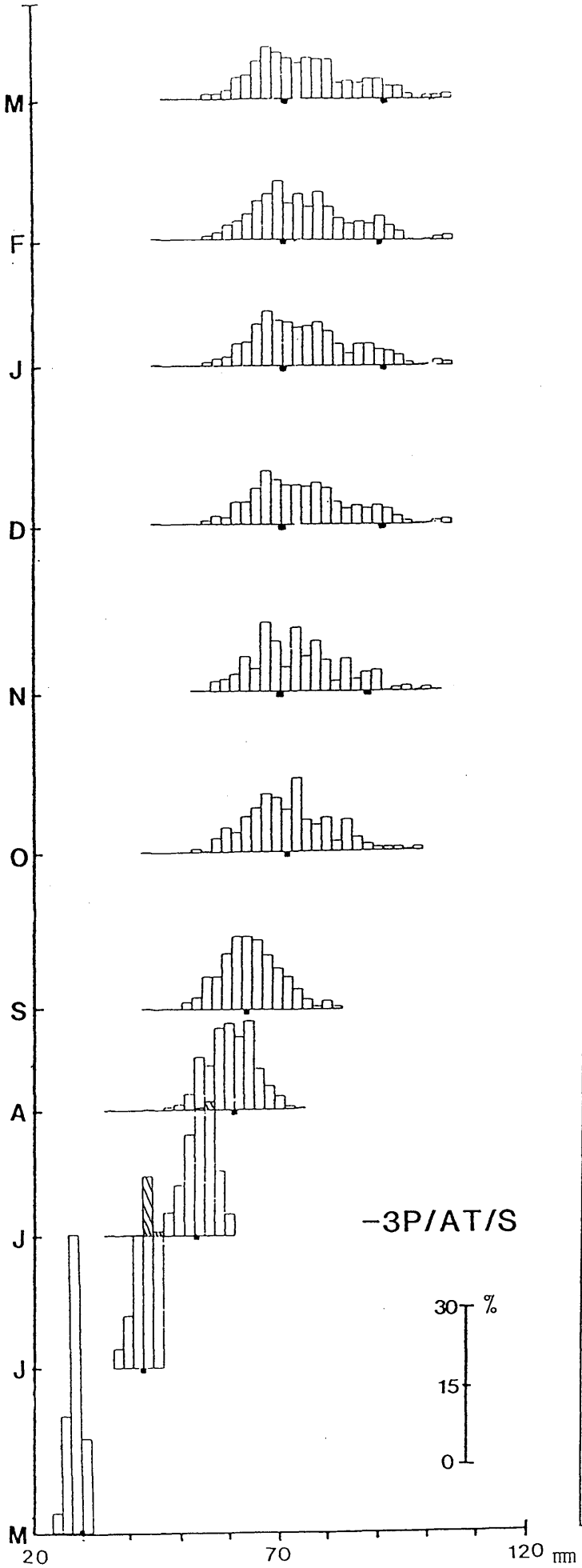
	LMG 67.0%			UMG 33.0%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	66.6	0.45	172	86.2	0.62	58
F	66.4	0.44	172	85.3	0.51	58
J	65.8	0.40	175	84.7	0.48	77
D	65.6	0.39	219	85.6	0.41	108
N	65.6	0.38	225	84.8	0.37	112
O	65.1	0.36	219	83.3	0.38	118
S	66.7	0.37	337			
A	61.7	0.29	336			
J	54.0	0.20	378			
J	42.9	0.24	124			
M	28.2	0.11	167			

Figure 3.8



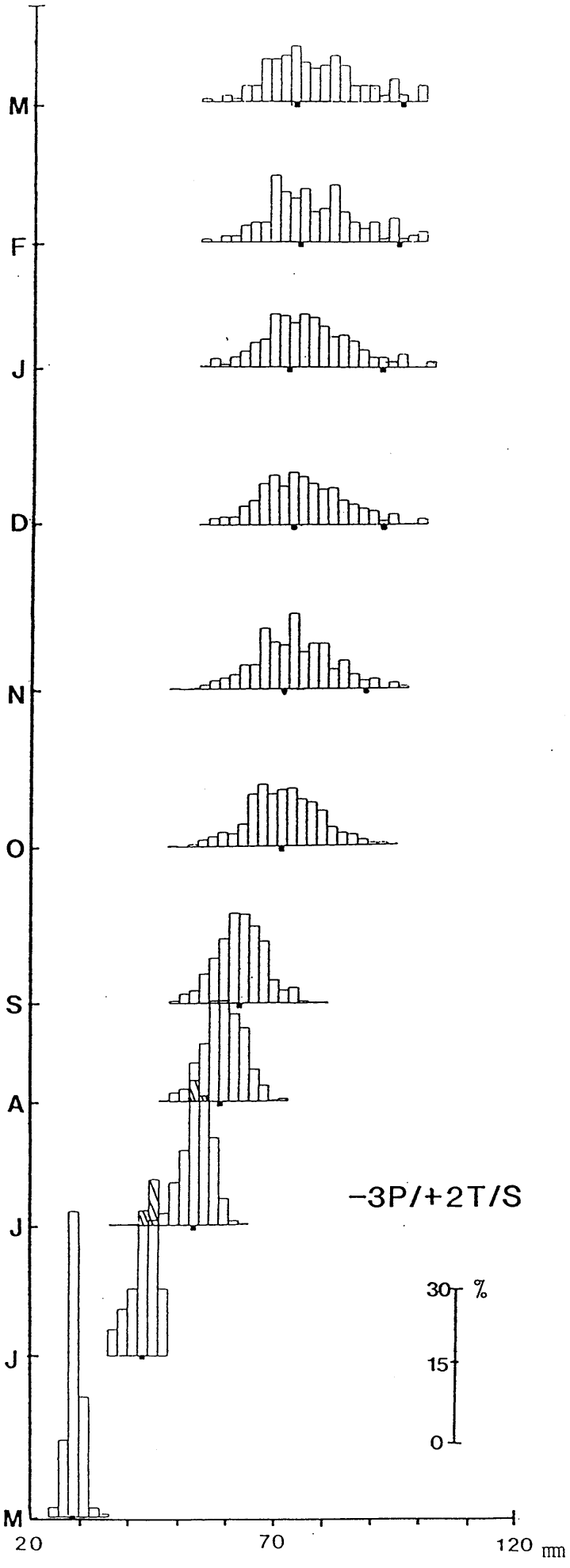
	LMG 69.9%			UMG 30.1%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	68.1	0.50	150	93.8	0.50	64
F	67.6	0.51	154	92.0	0.47	66
J	67.4	0.49	153	90.9	0.52	66
D	68.1	0.49	191	91.0	0.38	81
N	67.3	0.44	203	88.4	0.36	87
O	66.5	0.43	208	86.0	0.38	87
S	67.9	0.41	295			
A	62.3	0.33	294			
J	56.1	0.21	379			
J	46.2	0.24	90			
M	28.9	0.16	178			

Figure 3.9



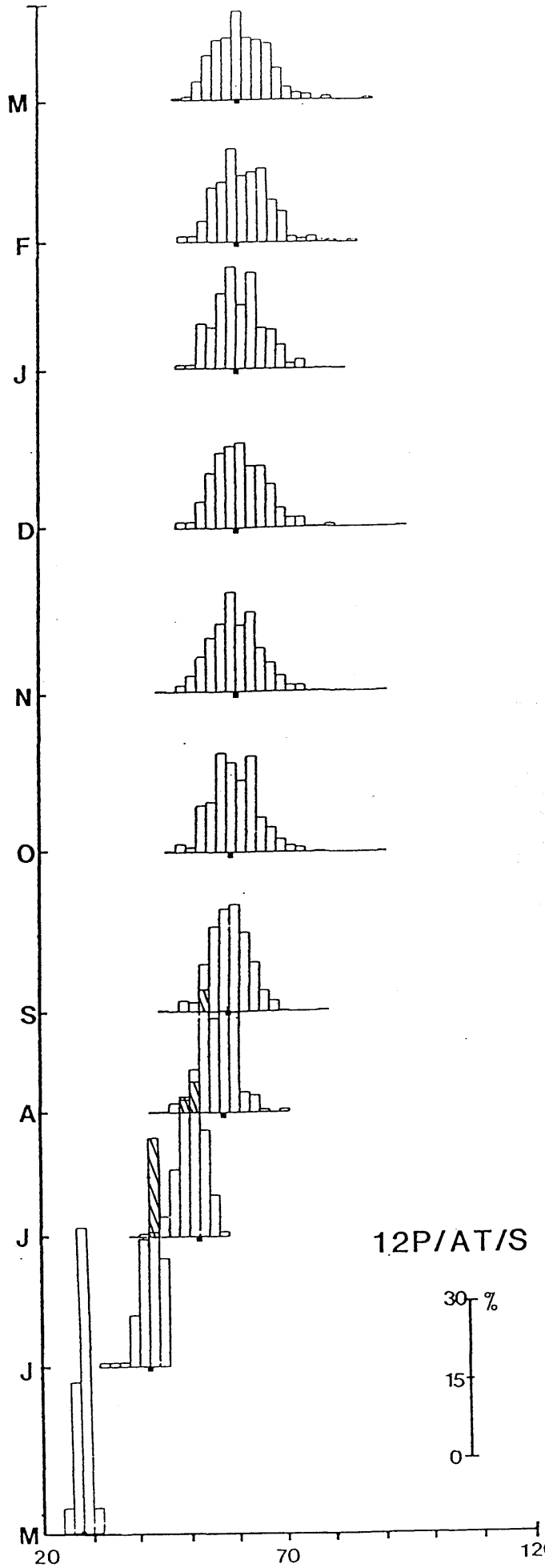
	LMG 81.1%			UMG 18.9%		
$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
70.3	0.43	241	90.0	0.68	56
70.0	0.43	241	89.1	0.68	56
70.1	0.43	243	89.5	0.66	57
70.2	0.42	248	89.5	0.68	57
69.7	0.39	252	87.0	0.61	58
70.7	0.48	309			
63.2	0.34	313			
58.9	0.28	316			
52.4	0.19	320			
41.9	0.39	112			
28.8	0.13	140			

Figure 3.10



	LMG 91.0%			UMG 9.0%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
M	74.5	0.62	146	94.9	0.75	14
F	74.3	0.60	147	94.4	0.75	14
J	71.5	0.39	335	91.0	0.66	33
D	72.8	0.38	341	91.3	0.66	34
N	70.9	0.36	343	87.1	0.61	33
O	70.7	0.38	377			
S	61.8	0.26	383			
A	58.4	0.21	384			
J	53.1	0.17	399			
J	42.8	0.43	102			
M	28.7	0.12	170			

Figure 3.11



	LMG 98.9%			UMG 1.1%
	$\bar{L}$	S.E.	N	
M	61.4	0.31	277	UMG mean not calculated.
F	60.8	0.31	280	
J	60.3	0.30	296	
D	60.3	0.28	327	
N	59.5	0.27	332	
O	59.2	0.25	333	
S	57.7	0.21	340	
A	55.1	0.20	334	
J	49.9	0.15	354	
J	41.8	0.21	112	
M	27.8	0.10	148	

Figure 3.12

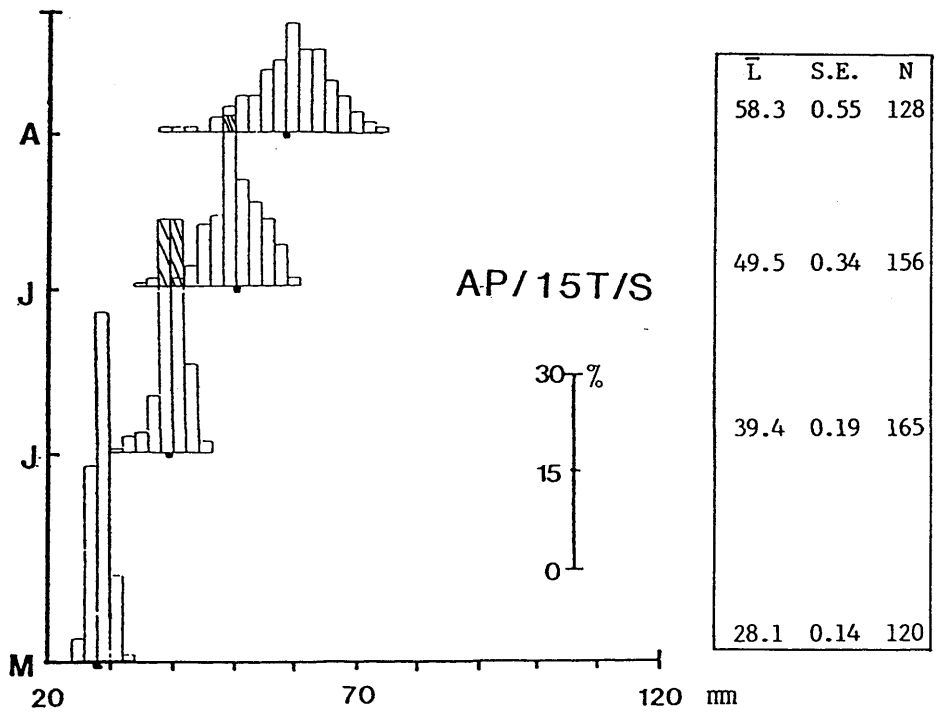
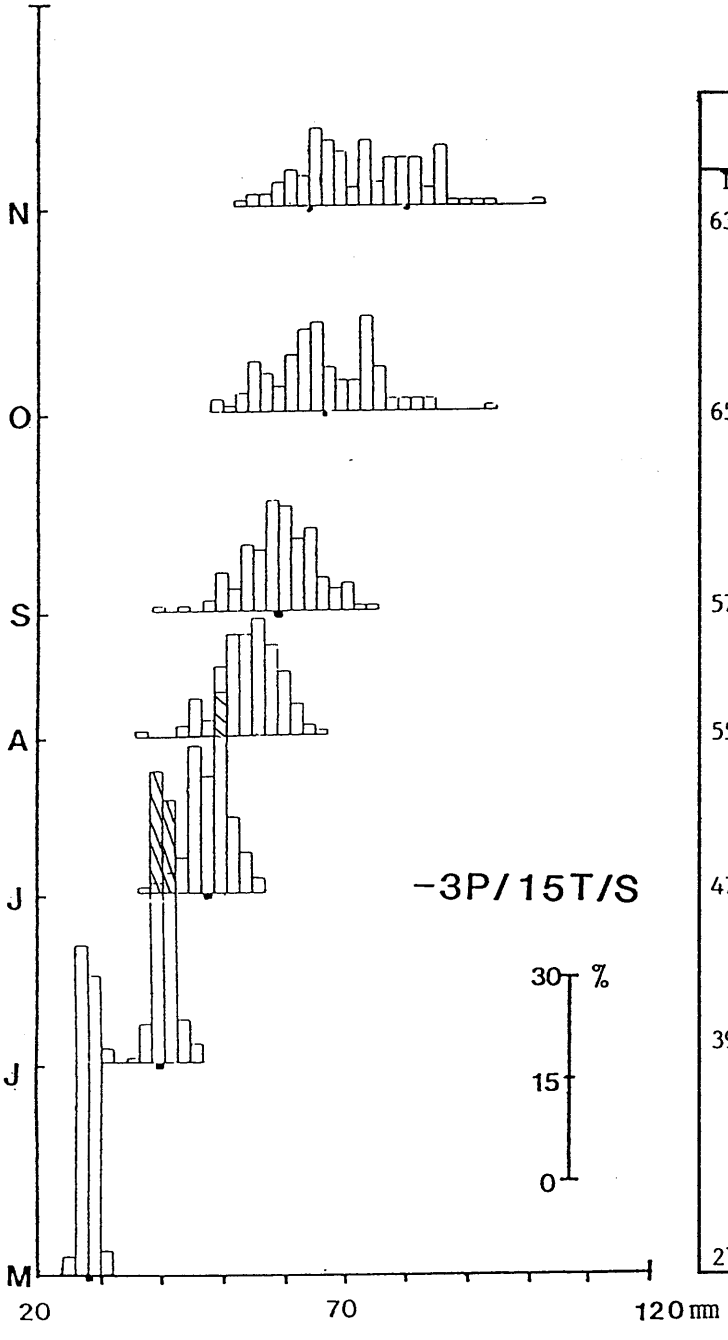
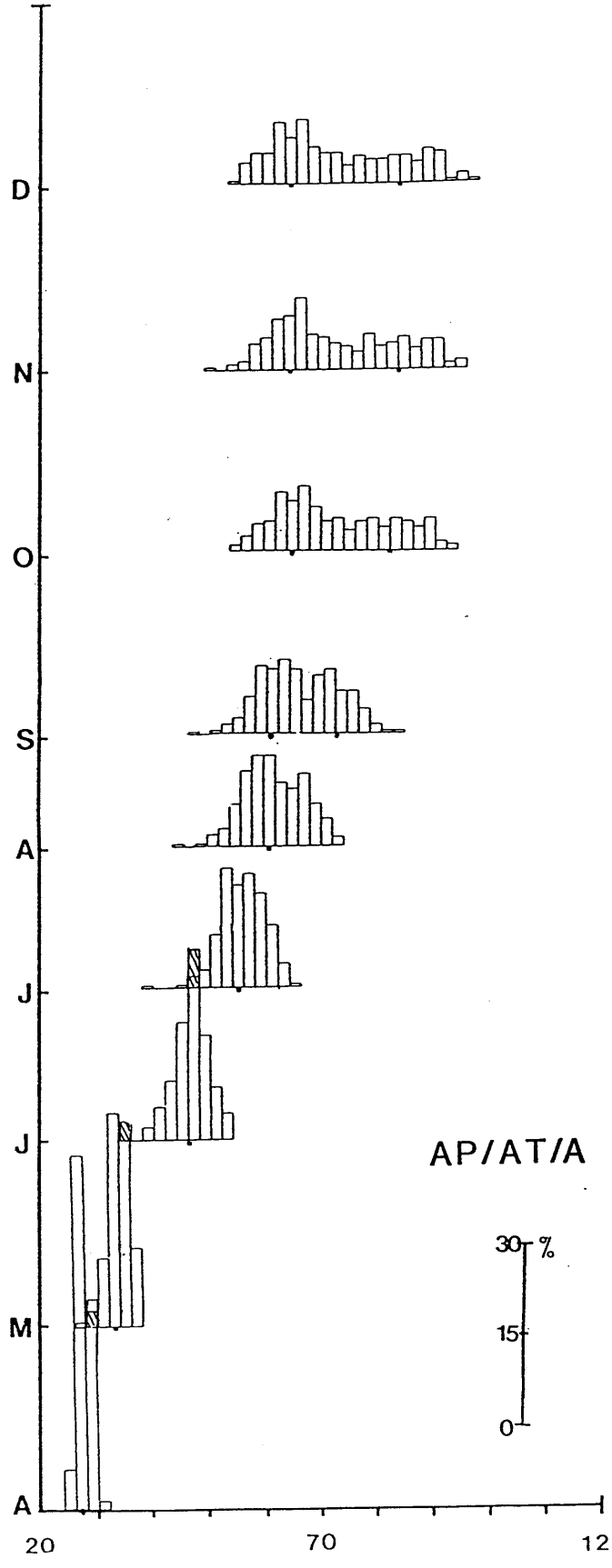

A
J
J
M
20
70
120 mm
AP/15T/S
30
%
15
0
L̄ S.E. N
58.3 0.55 128
49.5 0.34 156
39.4 0.19 165
28.1 0.14 120

Figure 3.13



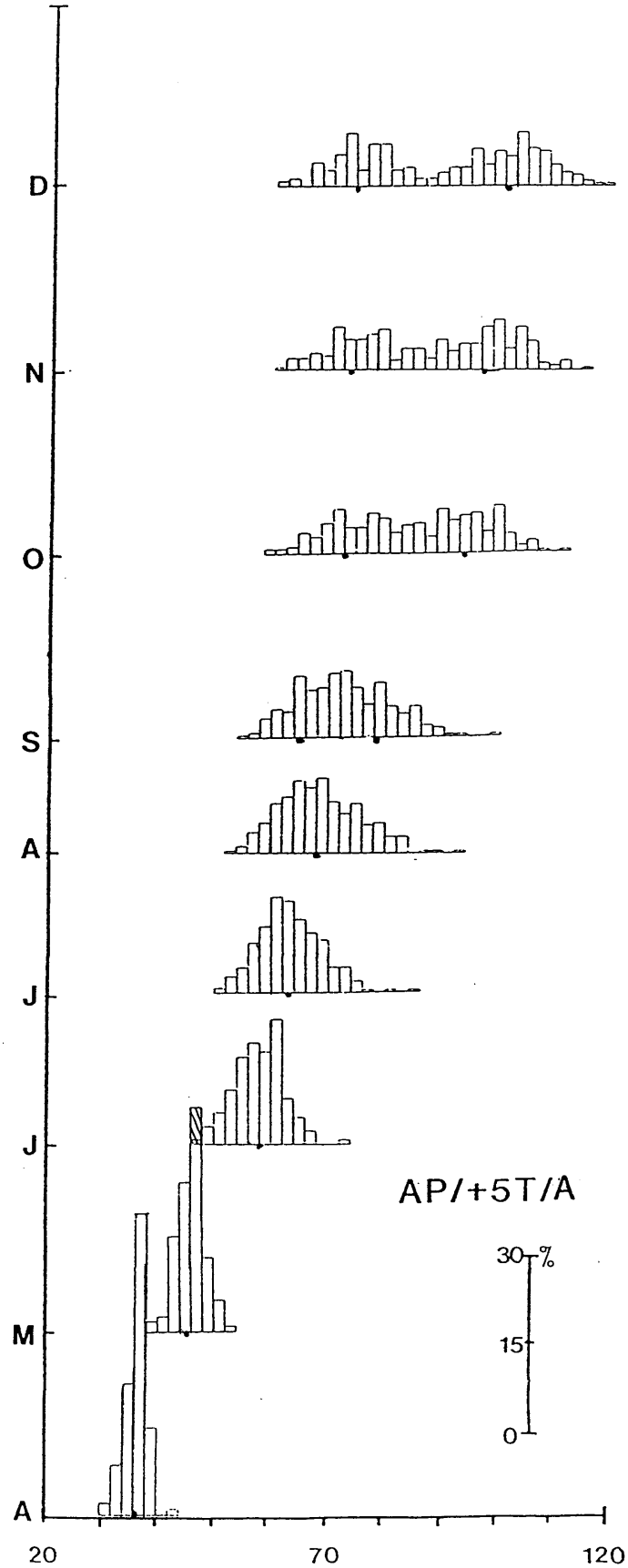
	LMG 46.9%			UMG 53.1%		
	L	S.E.	N	L	S.E.	N
N	63.6	0.56	53	79.0	0.77	60
O	65.5	0.76	118			
S	57.8	0.53	122			
A	55.0	0.43	124			
J	47.1	0.29	132			
J	39.4	0.18	141			
M	27.5	0.11	120			

Figure 3.14



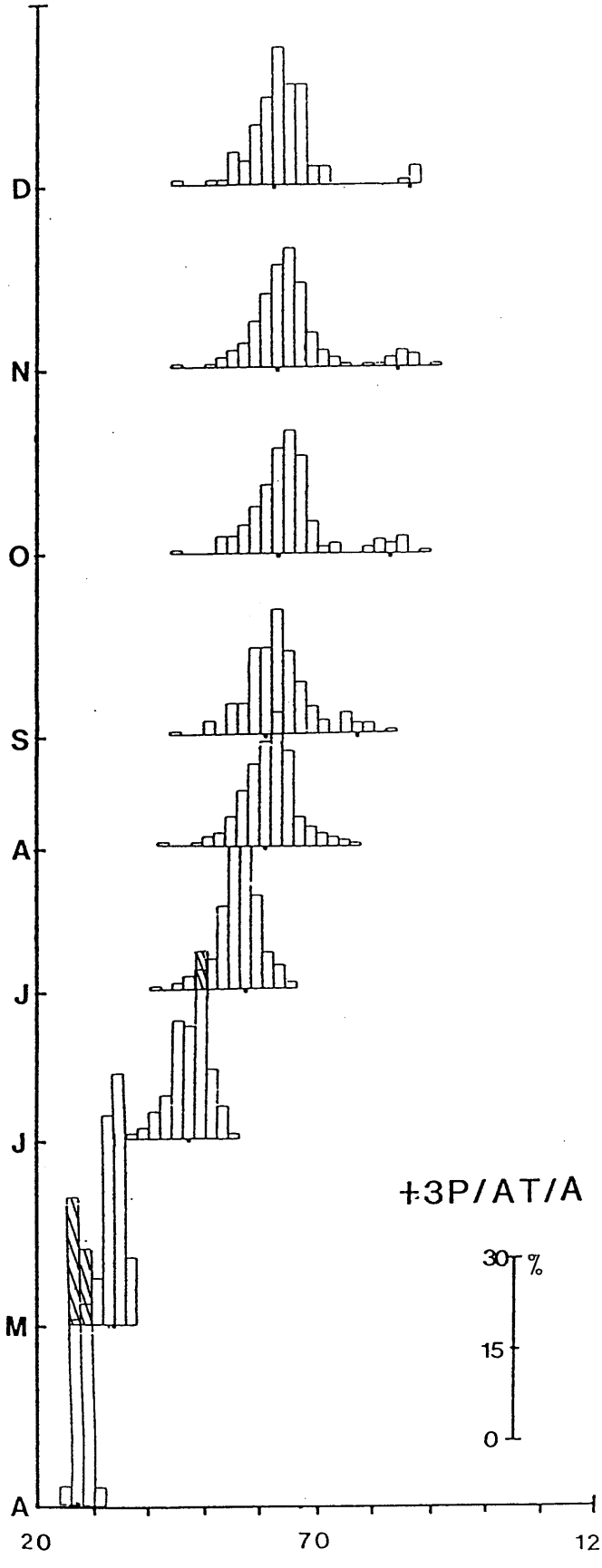
	LMG 58.8%			UMG 41.2%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
D	64.7	0.43	116	83.7	0.63	82
N	65.0	0.43	119	83.7	0.60	84
O	65.0	0.41	121	82.3	0.55	81
S	60.9	0.89	120	72.2	0.36	81
A	61.2	0.37	201			
J	55.3	0.27	207			
J	45.8	0.38	94			
M	33.2	0.19	129			
A	27.1	0.13	76			

Figure 3.15



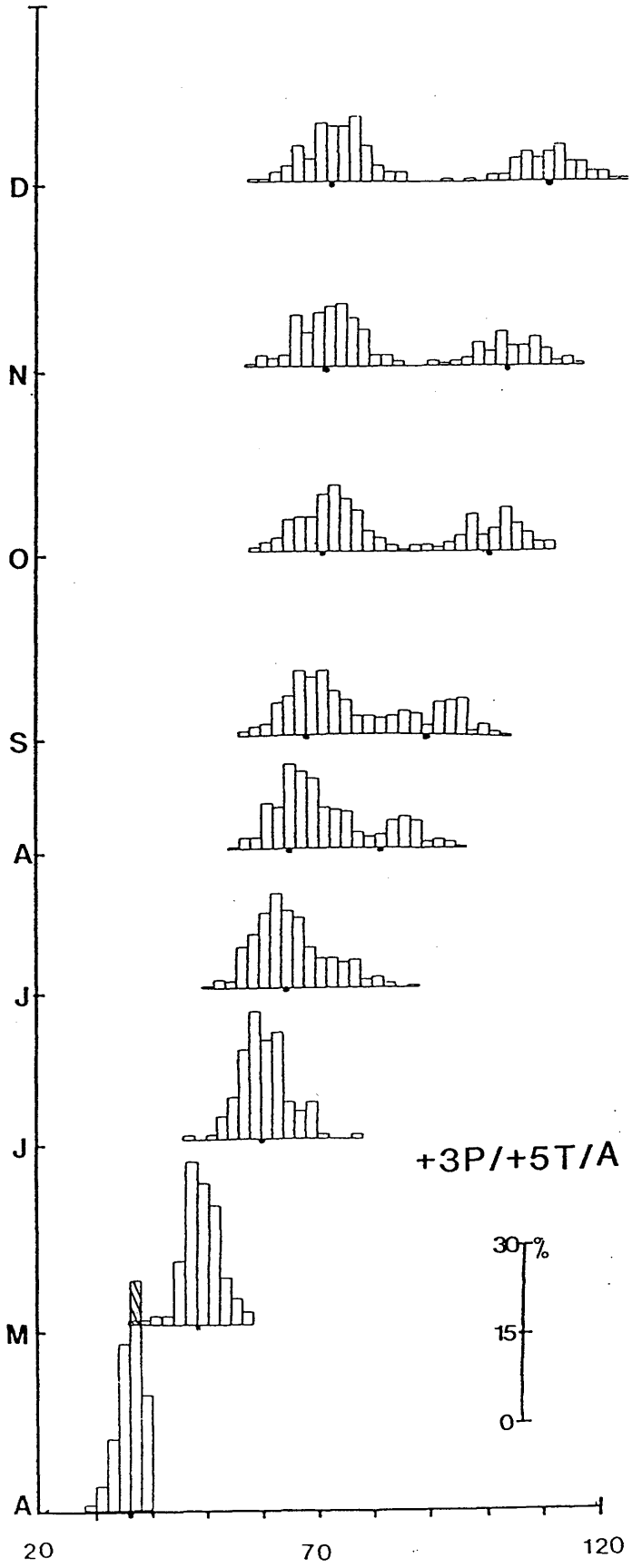
	LMG 42.6%			UMG 57.4%		
$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
73.8	0.52	103	100.4	0.61	139
73.2	0.51	110	96.4	0.57	148
72.0	0.50	115	92.3	0.52	148
64.9	0.35	112	77.6	0.45	150
68.2	0.44	264			
62.9	0.34	264			
57.5	0.38	132			
45.6	0.23	114			
36.0	0.19	99			

Figure 3.16



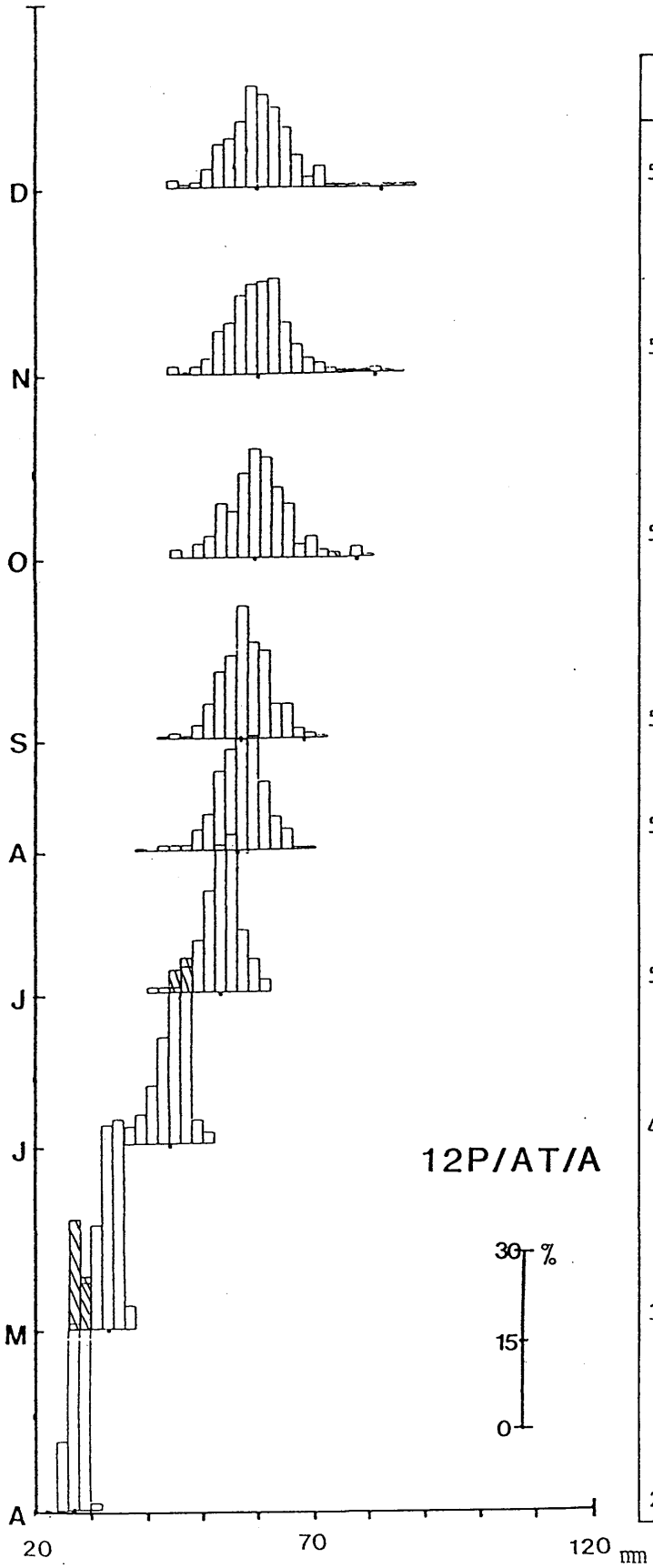
LMG 91.3%			UMG 8.7%		
$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
62.3	0.38	129	86.0	0.50	4
63.0	0.37	157	84.4	0.75	15
62.7	0.35	155	82.6	0.70	15
61.5	0.35	160	76.5	0.69	13
61.3	0.36	183			
55.4	0.28	182			
46.7	0.31	114			
33.5	0.19	115			
27.4	0.14	66			

Figure 3.17



	LMG 63.2%			UMG 36.8%		
	$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
D	73.0	0.48	122	110.5	0.72	69
N	72.3	0.44	144	104.1	0.70	69
O	71.4	0.41	170	100.1	0.57	99
S	68.3	0.35	165	89.0	0.61	101
A	65.1	0.28	169	80.5	0.62	100
J	65.2	0.39	284			
J	60.1	0.40	129			
M	48.3	0.27	140			
A	35.6	0.19	128			

Figure 3.18



LMG 97.7%			UMG 2.3%		
$\bar{L}$	S.E.	N	$\bar{L}$	S.E.	N
59.6	0.37	219	81.7	1.83	5
59.5	0.36	223	81.3	1.03	5
58.8	0.34	225	76.9	0.40	5
56.8	0.28	225	68.1	0.76	5
56.2	0.30	231			
52.8	0.23	235			
44.1	0.29	102			
32.6	0.21	102			
27.1	0.13	88			

Figure 3.19

Mean lengths of both LMG and UMG subpopulations from January to March seem to indicate some recommencement of growth, however this is more pronounced in the UMG ($\bar{L}$ increase 1.5mm Jan. - March) than the LMG ($\bar{L}$ increase 0.8mm Jan. - March).

The best estimate of the proportion in the upper mode was 33% made in December. Fish for histological analysis were removed from the population between the monitoring dates for November and December.

Effect of Elevated Temperature at Ambient Photoperiod

Experimental Population: AP/+2T/S Figure 3.9

Control for temperature: AP/AT/S Fig. 3.8

The elevated water temperatures experienced by this group resulted in a slightly higher growth rate (not significant) over the initial unimodal growth phase.

In September the unimodal distribution broadened and by October, as in the control, a bimodal distribution was established, with 30% in the UMG (estimated in January).

The slight mean size advantage of this group on elevated temperature was maintained and increased during the bimodal growth phase until by March the mean length differential between the experimental group and the control was 1.5mm in the LMG ($p<0.05$) and 7.6mm in the UMG ($p<0.001$).

Fish were removed for histological examination before monitoring in January.

Effect of Retarded Photoperiod at Ambient Temperature

Experimental Population: -3P/AT/S Figure 3.10

Control for temperature: AP/AT/S Fig. 3.8

Growth during the unimodal phase of the population on retarded photoperiod was found to be significantly lower than the control. Shorter days experienced by this population up to mid August resulted in a mean length of 58.9mm in August, compared with 61.7mm in the control ($p<0.001$).

Mode separation was not as clear in the experimental group as in the control. The normal distribution appearing markedly broad in October and continuing to spread over the

winter and into the spring without the two modes becoming totally distinct.

UMG growth continued until December, as it did in the control group.

Fish for histological examination were removed after the termination of the experiment. The best estimate of the percentage in the UMG (19%) was made in March.

The Effect of Elevated Temperature and Retarded Photoperiod

Experimental Population: -3P/+2T/S Figure 3.11

Control for temperature: -3P/AT/S Fig.3.10

Control for photoperiod: AP/+2T/S Fig.3.9

Temperature Effects:

Comparison of the mean fork-lengths of this experimental group (Fig. 3.11) with those of the control on ambient temperature (Fig. 3.10) shows no consistent length differences over the unimodal growth phase despite elevated water temperatures.

Development of bimodality occurred with a spreading of the normal distribution in October in both the experimental

and control group on retarded photoperiod. Both groups on this photoperiod regime show poor separation of the two modes before termination of the experiment. Elevated temperature resulted in a slight, consistent difference in the mean fork-length in winter of both LM and UM groups (not significant).

Due to an accident during routine tank cleaning between the monitoring dates of January and February, 55% of the total population were lost.

Delayed Photoperiod Effects:

Comparing the experimental population with the control population on ambient photoperiod at elevated temperature, it can be seen that the experimental group show consistently lower mean fork-lengths (e.g. September: $\bar{L}$ = 61.8mm retarded photoperiod, $\bar{L}$ = 67.9mm control $P<0.001$) over the unimodal growth phase.

Development of bimodality was delayed by approximately one month (November cf. October in control) by retarding the photoperiod cycle.

Despite being considerably smaller at the end of the unimodal growth phase ($\bar{L}$ = 61.8mm cf. 67.9mm control) the photoperiod retarded group had the advantage of longer days during the winter and quickly decreased the size

differential, eventually outstripping the control group in size, until by January LMG fish are slightly larger and UMG fish similar, in size, to the control.

The best estimate of the UMG percentage (9%) was made in February by including mortalities that occurred in late January.

Samples were removed for histological analysis after the termination of the experiment.

Effect of Constant Daylength

Experimental Group: 12P/AT/S Figure 3.12

Control for photoperiod: AP/AT/S Fig. 3.8

Mean fork-length of this experimental group on fixed daylength remained consistently lower than that of the control group over the unimodal growth phase. The disparity increases with time, until by September the difference in mean length is 9mm ($p<0.001$).

99% of the population arrest growth and appear as lower mode fish by October. The LMG showed only minimal growth from October to January, with complete growth arrest between December and January. Only 3 fish from the population of

335 were estimated as UMG fish in November. Because of this low number, mean fork-lengths of the UMG are not shown on Fig. 3.12.

Fish were removed for histological examination after length frequency monitoring in November.

Effect of Constant Temperature

Experimental Population: AP/15T/S Figure 3.13

Control for temperature: AP/AT/S Fig. 3.8

The very limited data presented in Figure 3.13 for this group shows growth only over the unimodal phase.

Growth of the experimental group on fixed 15°C water temperature was depressed over this period compared with the control population held on ambient river water temperature (August: $\bar{L}$=58.3mm - experimental, $\bar{L}$=61.7mm - control $p<0.001$)

Effect of Retarded Photoperiod and Constant Temperature

Experimental Population: -3P/15T/S Figure 3.14

Control for temperature: -3P/AT/S Fig. 3.10

Control for photoperiod: AP/15T/S Fig. 3.13

Comparing the unimodal growth phase of the experimental group with that of the control for photoperiod (Fig. 3.13), it can be seen that growth was depressed on retarded photoperiod, because of shorter days until mid-August. Thus by August mean length was 55.0mm on retarded photoperiod compared with 58.3mm in the control group ($p<0.001$).

As with the other population on fixed temperature growth over the unimodal growth phase was depressed by temperature (September: $\bar{L}$= 57.8mm experimental group cf. 63.2mm in the control on ambient temperature. $p<0.001$).

In October the unimodal distribution broadened and by November a bimodal distribution has developed. The timing of this is similar to the temperature control on a similar photoperiod (-3P/AT/S Fig. 3.10) that is, approximately one month delayed compared with the population on ambient photoperiod (AP/AT/S Fig.3.8).

The best estimate of percentage in the UMG was made in November, at 53%.

ACCELERATED INCUBATION PERIOD

Control Group: Accelerated Incubation AP/AT/A Figure 3.15

Population AP/AT/A represents the control group for populations with an accelerated incubation period.

As with other populations this group showed rapid growth with a unimodal distribution over the spring and early summer. During the late summer the normal distribution broadened and by October a bimodal distribution had developed. After October there was no evidence of further growth in the LMG. In the UMG, growth continued into November, before growth arrest occurred.

The best estimate of the proportion of fish entering the UMG was made in December as 41%. Fish for histological examination were removed from the population after the length frequency monitoring in December.

Effects of Accelerated Incubation Period:

Comparison of this accelerated incubation population with the control, standard incubation group AP/AT/S (Fig. 3.8) reveals that the longer growth period (72 days) in the accelerated group results in larger fish initially. In May the mean length is 33.2mm in the accelerated group compared with 28.2mm in the control ($p<0.001$). The size advantage of the accelerated group however, is quickly eroded, by higher growth rates in the standard incubation group. By August the standard group is marginally (but not significantly) larger on average ($\bar{L}$ =61.7mm) than the accelerated group ($\bar{L}$ =61.2mm).

Effect of Elevated Temperature at ambient photoperiod

Experimental Population: AP/+5T/A Figure 3.16

Control for temperature: AP/AT/A Fig. 3.15

Examination of the length frequency changes of the experimental group shows greatly increased growth rates over the unimodal growth phase, of fish on elevated temperature over those in the control on ambient water temperature, up to August (August: $\bar{L}$ =68.2mm experimental, and 61.2mm control $p<0.001$).

A bimodal distribution first appeared in September and was clearly defined by October in the experimental group, the timing of the bimodal split being the same as that of the control. However unlike the control, it is clear that the elevated temperature prevented a complete growth check in the LMG as was evident in the control from October. Rather the separation of the two modes was due to continued high growth rates in the UMG fish until the termination of the experiment.

The best estimate of the proportion of the population in the UMG was made in December at 57%. A sample of the population was removed for histological examination after the termination of the experiment.

Effect of Advanced Photoperiod at Ambient Temperature

Experimental Population: +3P/AT/A Figure 3.17

Control for photoperiod: AP/AT/A Fig.3.15

Longer days from first feeding until the end of May, experienced by the experimental group had little effect on the growth of the experimental population, during the unimodal growth phase, as indicated by virtually identical

mean lengths in August.

Despite a photoperiod phase difference of +3 months in the experimental group over the control there is no obvious acceleration of the timing of the bimodal split, however this may be due to the difficulty in detecting bimodality early with such a small proportion in the UMG (9% as estimated in November).

A sample of fish were removed from the population after the November monitoring.

Effects of Advanced Photoperiod and Elevated Temperature

Experimental Population: +3P/+5T/A Figure 3.18

Control for temperature: +3P/AT/A Fig. 3.17

Control for photoperiod: AP/+5T/A Fig. 3.16

Temperature Effects:

Elevated temperature increased initial unimodal growth rates over the control on ambient temperature. By July the mean length was 65.2mm in the experimental group compared with 55.4mm in the control ($p<0.001$).

Growth continued in both the UMG and the LMG throughout

the course of the experiment without a complete growth check. Higher UMG growth resulted in distinct separation of the two modal groups.

The UMG proportion of the population was estimated in October at 37% in the experimental group.

Advanced Photoperiod Effects:

Longer days until the end of May in the experimental group on advanced photoperiod resulted in larger fish by this time (May: $\bar{L}$ =48.3mm - advanced photoperiod cf. 45.6mm ambient - photoperiod $p<0.001$).

Development of bimodality started in July and was well defined by August in the advanced photoperiod group, compared with September in the control.

Continued growth throughout the bimodal phase was apparent in both the UMG and the LMG in the experimental and control groups. The initial size advantage was maintained in the UMG, by December the mean length of fish in the UMG was 110.5mm in the experimental group compared with 100.4mm in the control group ($P<0.001$). In contrast there is virtually no difference in the mean length in December of the LMG at 73.0mm and 73.8mm in the experimental and control LMG's respectively.

Effect of Constant Daylength

Experimental Population: 12P/AT/A Figure 3.19

Control for photoperiod: AP/AT/A Fig. 3.15

Fish held on a constant 12 hours light, 12 hours dark photoperiod regime grew less well over the unimodal growth period to August than the control group on ambient photoperiod.

Only a very few fish (c.2%) entered the UMG. Growth in the remainder was very slow from October and virtually ceased after November.

3.4 PHOTOPERIOD AND TEMPERATURE EFFECTS ON THE RATE OF ENTRY TO UPPER MODAL GROWTH GROUP

In an attempt to separate the effects of photoperiod phase and temperature on growth and smolting rate, a detailed examination of the size distributions was made on certain key dates when all groups within experiments had been subject to equal periods of total daylight.

3.4.1 Experiment 2 - Standard Incubation

The four populations of experiment 2 were examined on the 26th September 1985. On this date each population had received either 100 ± 6 days of increasing daylength followed by 44 ± 6 days decreasing daylength or the reciprocal of 44 days increasing plus 100 days decreasing daylength.

Figure 3.20 shows the fork-length percentage frequency distributions of the 4 populations on this date. These data show that populations that had experienced a long period of increasing daylength (long spring (-2P)) (right hand column) had a less clearly developed bimodal distribution than those populations experiencing a relatively short period of increasing photoperiod (short spring (AP)).

At ambient photoperiod (short spring) the slightly elevated temperature significantly increased the mean size of UMG fish ($p<0.001$) however this effect was not evident in the LMG.

In the retarded photoperiod groups (long spring) the effect of elevated temperature on growth is not seen (the mean fork-length being slightly greater in both modal groups). This is likely to be the result of an increase in the

Figure 3.20 Fork-length (mm) percentage frequency distributions of standard incubation populations (Experiment 2) after 144 days growth. Inserts show mean fork-length ($\bar{L}$) (mm) on that date and eventual proportion in the UMG.

Top row: Ambient temperature

Bottom row: Ambient +2°C

Left column: Short spring photoperiod (AP)

Right column: Long spring photoperiod (-2P)

	Population	Annual data
Top left	AP/AT	Fig. 3.4
Top right	-2P/AT	Fig. 3.6
Bottom left	AP/+2T	Fig. 3.5
Bottom right	-2P/+2T	Fig. 3.7

Figs following page 63.

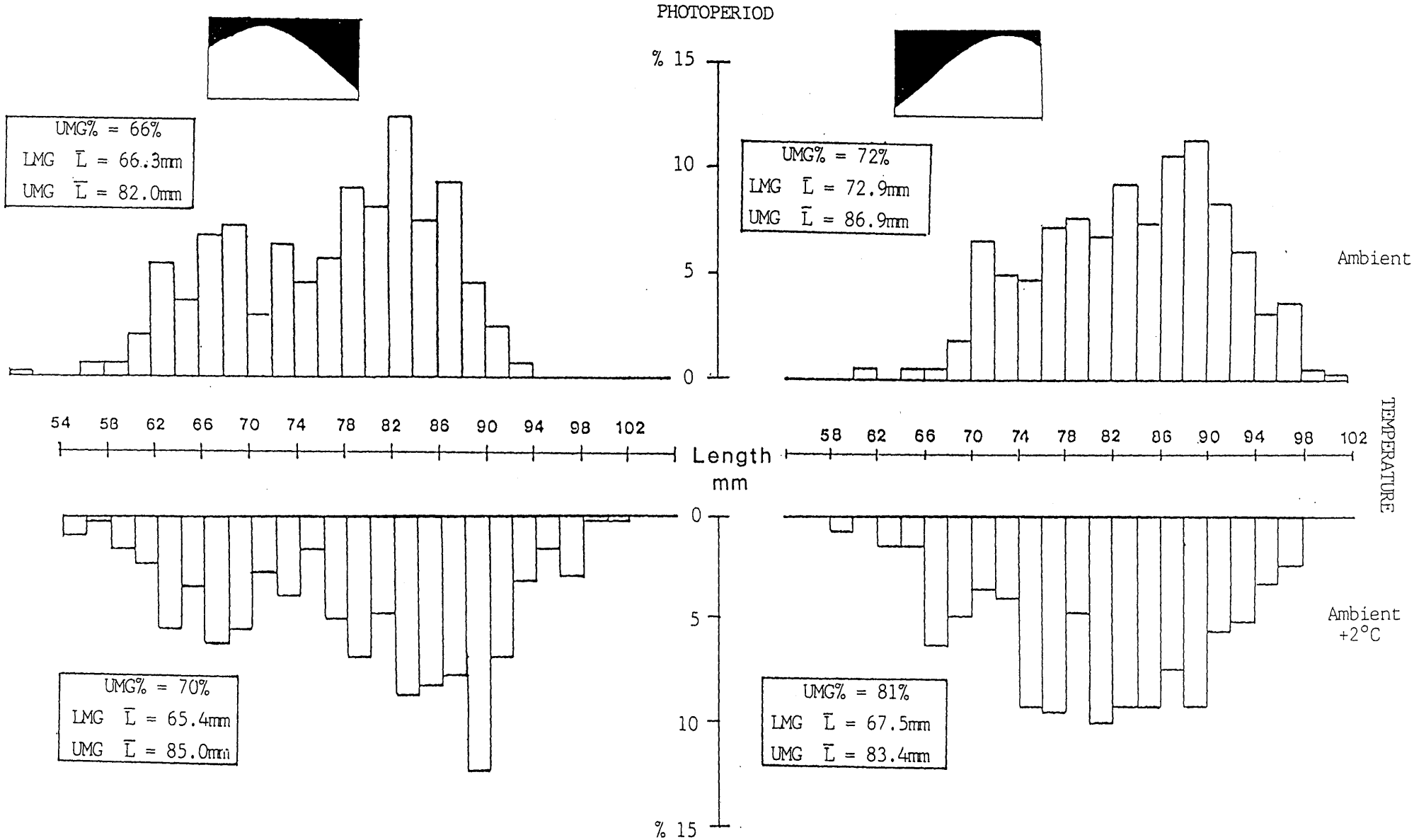


Figure 3.20

GROWTH PERIOD: 6th May - 26th September 1985.

proportion entering the UMG at elevated temperature (9% more $p<0.02$) in the heated water population. Elevated temperature also increased the proportion entering the UMG at ambient photoperiod (4% increase) however this difference is not significant.

These data also point towards the proportion in the upper mode being greater in the groups experiencing a long spring photoperiod (-2P)(comparing between columns: bottom row 11% greater $p<0.01$, top row: 6% not significant). Table 3.4 shows a summary of these results.

3.4.2 Experiment 1 - Accelerated Incubation

On the 25th July 1985, 123 days after first feeding all the groups in this experiment had received equal hours of daylight with 106 $\pm$ 2 days of increasing photoperiod and 17 $\pm$ 2 days decreasing photoperiod, or the reciprocal (17 days increasing and 106 days decreasing).

Owing to the loss of population AP/+5T, a complete analysis of the proportion of parr entering the UMG is not possible. However it is possible to examine the remaining groups.

STANDARD INCUBATION				
Population	LMG $\bar{L}$ mm	UMG $\bar{L}$ mm	UMG %	Month of Modal Segregation
AP/AT	66.3	82.0	66	Sept.
AP/+2T	65.4	85.0	70	Sept.
-2P/AT	72.9	86.9	72	Oct.
-2P/+2T	67.5	83.4	81	Oct.

TABLE 3.4 Summary of the effects of temperature and photoperiod on fork-length ($\bar{L}$) after 144 days growth, and UMG % of standard incubation populations of Exper.2.

ACCELERATED INCUBATION				
Population	LMG $\bar{L}$ mm	UMG $\bar{L}$ mm	UMG %	Month of Modal Segregation
AP/AT	unimodal $\bar{L}$ = 61.8		79	Sept.
AP/+5T	Pop. lost			
+3P/+5T	59.9	73.2	64	Aug.
+3P/AT	60.2	66.1	52	Aug.

TABLE 3.5 Summary of the effects of temperature and photoperiod on fork-length ($\bar{L}$) after 123 days growth, and UMG %accelerated incubation populations of Exper. 1.

Figure 3.21 shows that growth at elevated temperature was greater over this 123 day period. Group AP/+5T (distribution not shown) had a mean length of 68.5mm (standard error = 0.39), this was greater (10.8%) than the mean length for the control on ambient temperature (61.8mm) on a similar photoperiod regime ($p<0.001$). Similarly elevated temperature increased the mean length in the advanced photoperiod pair by 10.6% ($p<0.001$) in the UMG, however the difference in the LMG was not significant.

Proportion in the Upper Mode:

Advanced photoperiod reduced the proportion of the population entering the UMG at ambient temperature by 27% ($p<0.001$).

Elevated temperature (+5°C) increased the percentage in the UMG by 12% ($p<0.001$). A summary of these results is presented in Table 3.5.

3.4.3 Experiment 3:

All groups from this experiment were examined after the first 193 days of growth, on the 15th September 1986 for the accelerated incubation populations and on the 24th November 1986 for the standard incubation populations. On these dates

Figure 3.21 Fork-length (mm) percentage frequency distributions of accelerated incubation populations (Experiment 1) after 123 days growth. Inserts show mean fork-length ($\bar{L}$) (mm) on that date, eventual proportion in the UMG and the photoperiod cycle.

Population	Annual data
+3P/+5T	Fig. 3.3
AP/AT	Fig. 3.1
+3P/AT	Fig. 3.2

Figs following page 59.

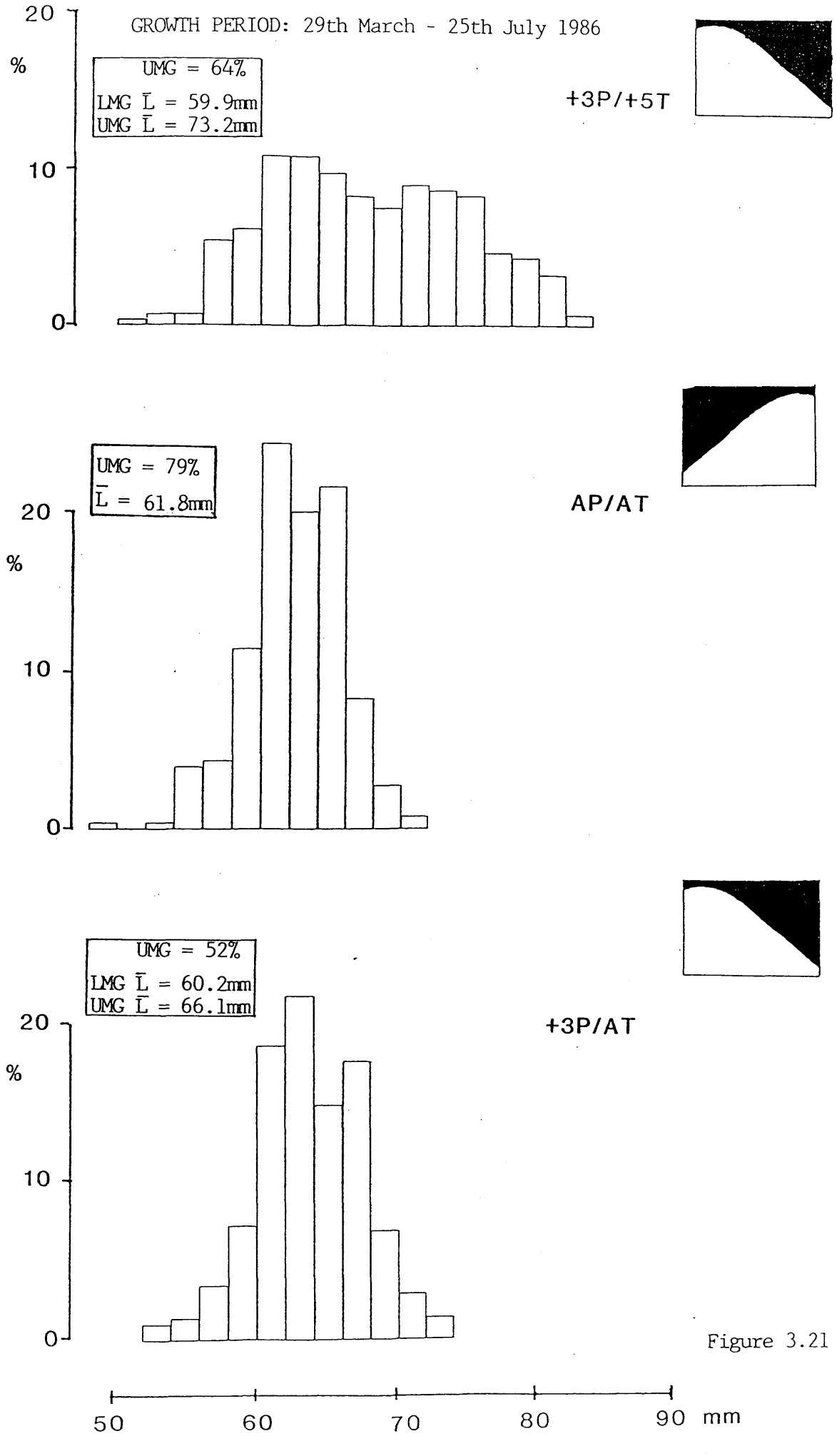

GROWTH PERIOD: 29th March - 25th July 1986
UMG = 64%
LMG $\bar{L}$ = 59.9mm
UMG $\bar{L}$ = 73.2mm
+3P/+5T
UMG = 79%
$\bar{L}$ = 61.8mm
AP/AT
UMG = 52%
LMG $\bar{L}$ = 60.2mm
UMG $\bar{L}$ = 66.1mm
+3P/AT
%
20
10
0
50
60
70
80
90 mm

Figure 3.21

populations had been subject to either 141 days of increasing daylength, followed by 52 days decreasing, or the reciprocal of 52 days increasing plus 141 days decreasing. On one of these dates all groups on photoperiod regimes had received equal periods of total daylight.

Accelerated Incubation

Figure 3.22 shows the fork-length frequency distributions for the 4 accelerated incubation populations on changing daylength.

Bimodality was more highly developed in groups on short spring photoperiod (i.e. advanced photoperiod cycle) (comparing between columns).

Growth:

Fish on supplemented temperature (+5°C) were consistently and significantly longer (6.6 - 16.3%) in both lower mode and upper mode groups, under both short spring (+3P) and under long spring photoperiods (AP) ($p<0.001$).

On short spring photoperiod (+3P) fish grew longer (1.0 - 14.7%) than those on ambient photoperiod (long spring photoperiod) in all LM and UMG's ($p<0.001$) except the LMG on ambient temperature, where the difference was not

Figure 3.22 Fork-length percentage frequency distributions of accelerated incubation populations on cyclic photoperiod and temperature regimes (Experiment 3) after 193 days growth. Inserts show mean fork-length ($\bar{L}$) (mm) on that date and eventual proportion in the UMG.

Top row: Ambient temperature

Bottom row: Ambient +5°C

Left column: Short spring photoperiod (+3P)

Right column: Long spring photoperiod (AP)

	Population	Annual data
Top left	+3P/AT/A	Fig. 3.17
Top right	AP/AT/A	Fig. 3.15
Bottom left	+3P/+5T/A	Fig. 3.18
Bottom right	Ap/+5T/A	Fig. 3.16

Figs following page 69.

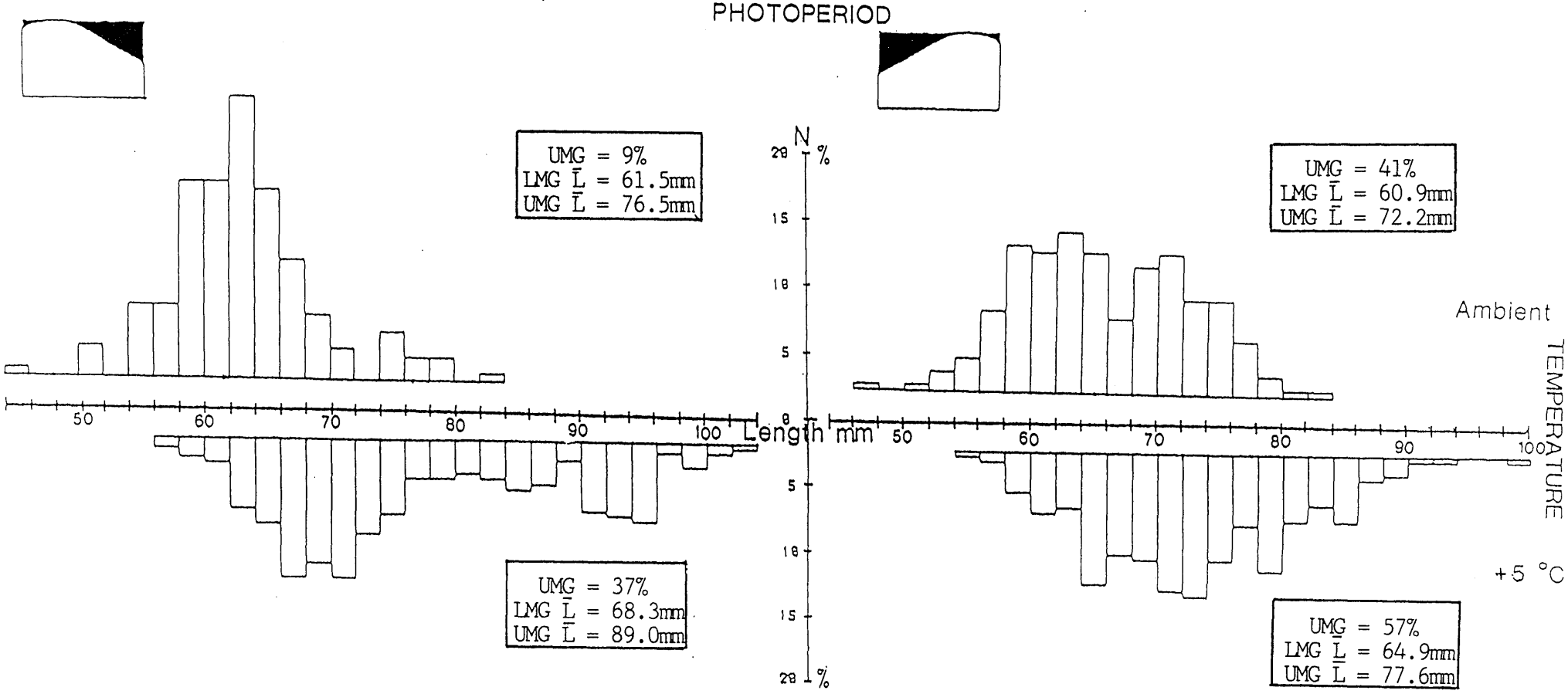


Figure 3.22 GROWTH PERIOD : 6 Mar. - 15 Sept. 1986

significant. A summary of these results is given in Table 3.6.

On fixed 12hours daylight, (Fig. 3.23) fish in the LMG and UMG were significantly smaller than all other groups ($p<0.001$).

Proportion in the upper modal group:

The proportion in the UMG was 16.2 to 28.1% greater in populations with supplementary heating on a long spring (ambient) photoperiod and on a short spring (advanced) photoperiod ($p<0.001$).

The proportion of the population entering the UMG was 20 & 30% greater in populations receiving a long spring (AP) photoperiod ($p<0.001$) over both temperature regimes (see Table 3.6 for summary.

Only 2.3% of fish from the population exposed to fixed 12 hours daylight (Fig. 3.23) were found in the UMG this was significantly less than all other groups ($p<0.01$)

Standard Incubation Populations

On the 24th November 1986 the standard incubation groups on changing photoperiod regimes, had received either 141 days of increasing daylength plus 52 days of decreasing

Figure 3.23 Fork-length (mm) percentage frequency distributions of populations from Experiment 3 on non-cyclic photoperiod or temperature regimes, after 193 days of growth. Inserts show mean fork-length ($\bar{L}$) (mm) on that date, eventual proportion in the UMG and photoperiod.

Population	Annual data
-3P/15T/S	Fig. 3.14
12P/AT/S	Fig. 3.12
12P/AT/A	Fig. 3.19

Figs following page 69.

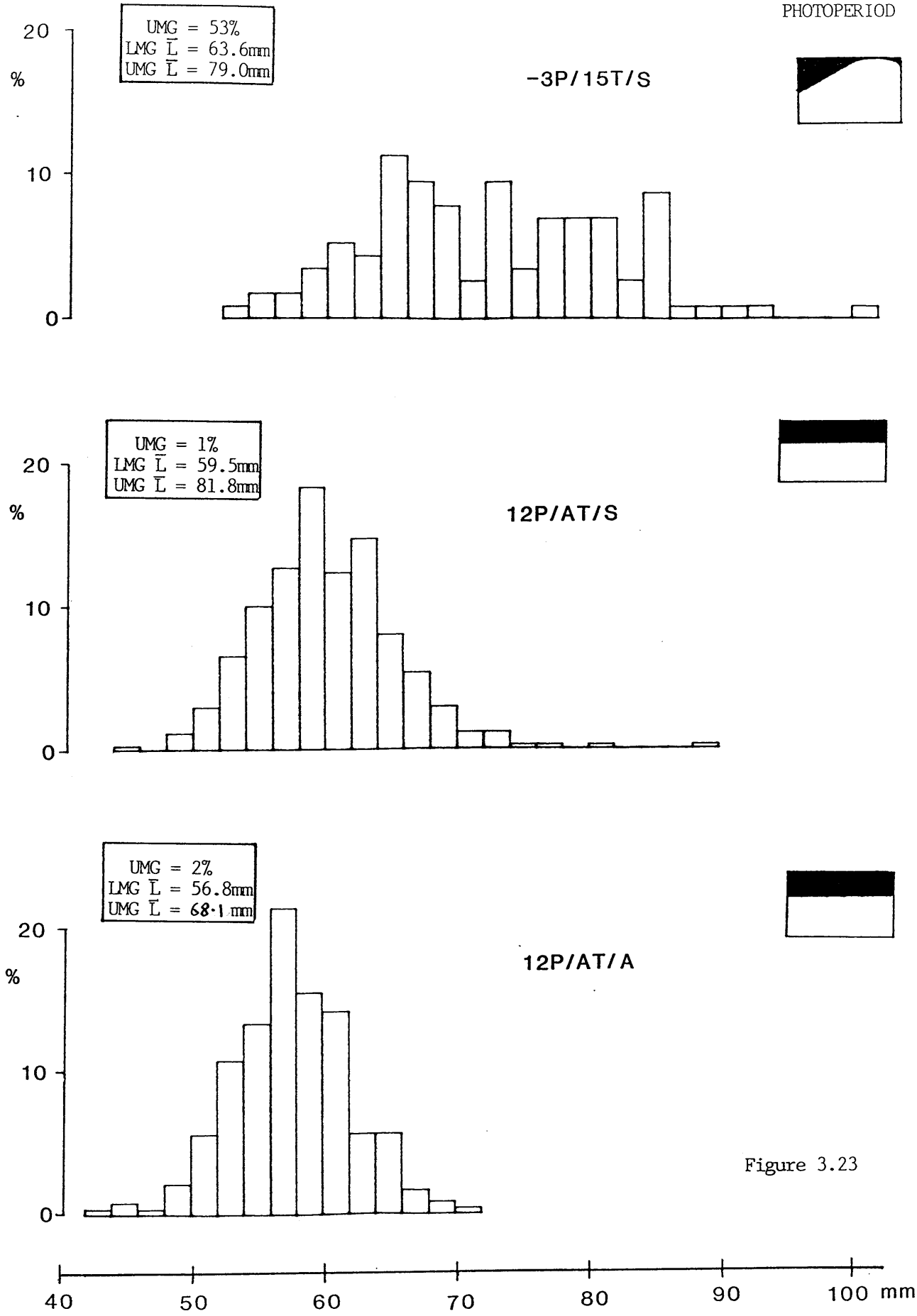

UMG = 53%
LMG L̄ = 63.6mm
UMG L̄ = 79.0mm
-3P/15T/S
20
%
10
0
UMG = 1%
LMG L̄ = 59.5mm
UMG L̄ = 81.8mm
12P/AT/S
20
%
10
0
UMG = 2%
LMG L̄ = 56.8mm
UMG L̄ = 68·1 mm
12P/AT/A
20
%
10
0
40
50
60
70
80
90
100 mm

Figure 3.23

ACCELERATED INCUBATION					STANDARD INCUBATION				
Population	LMG $\bar{L}$ mm	UMG $\bar{L}$ mm	UMG %	Month of Modal Segregation	Population	LMG $\bar{L}$ mm	UMG $\bar{L}$ mm	UMG %	Month of Modal Segregation
AP/AT/A	60.9	72.2	41	Sept.	-3P/AT/S	69.7	87.0	19	Nov.
AP/+5T/A	64.9	77.6	57	Sept.	-3P/+2T/S	70.9	87.1	9	Nov.
+3P/AT/A	61.5	76.5	9	Sept.	AP/AT/S	65.6	84.8	33	Oct.
+3P/+5T/A	68.3	89.0	37	Aug.	AP/+2T/S	67.3	88.4	30	Oct.
12P/AT/A	56.8	76.9	2	Sept.	12P/AT/S	59.5	81.8	1	Oct.
					-3P/15T/S	63.6	79.0	53	Nov.

TABLE 3.6 Summary of the effects of temperature and photoperiod on fork-length ($\bar{L}$) after 193 days growth, and UMG% in populations of Exper.3.

daylength, or the reciprocal of 52 days increasing followed by 141 days decreasing daylength. Figure 3.24 shows the length frequency distributions of populations receiving cyclic photoperiod and temperature regimes on this date.

Growth:

Fish in the lower mode and upper mode of all populations on elevated water temperatures showed better growth (1.7 - 5.1% mean length increase) up to this date than groups on ambient water temperature regardless of photoperiod regime ($p<0.02$ except in the UMG of the retarded photoperiod group, where the slight length increase (0.1%) was not significant).

Fish grew larger (2.6 - 6.3%) in all groups on long spring (-3P) photoperiod ($p<0.01$), except in the UMG of the population on a long period of rising daylength, where the fish were slightly smaller (see Table 3.6 for summary of results).

LMG fish on constant 12 hours daylight (Fig.3.23) were significantly smaller than all other groups ($p<0.001$). UMG fish were also smaller (1.8 - 6.5%) than all other UMG's on cyclic photoperiod regimes, these differences were not significant due to very small numbers in the UMG of this population.

Figure 3.24 Fork-length (mm) percentage frequency distributions of standard incubation populations on cyclic photoperiod and temperature regimes (Experiment 3) after 193 days of growth. Inserts show mean fork-length ($\bar{L}$) (mm) on that day and eventual proportion in the UMG.

Top row: Ambient temperature

Bottom row: Ambient +2°C

Left column: Short spring photoperiod (AP)

Right column: Long spring photoperiod (-3P)

	Population	Annual data
Top left	AP/AT/S	Fig. 3.8
Top right	-3P/AT/S	Fig. 3.10
Bottom left	AP/+2T/S	Fig. 3.9
Bottom right	-3P/+2T/S	Fig. 3.11

Figs following page 69.

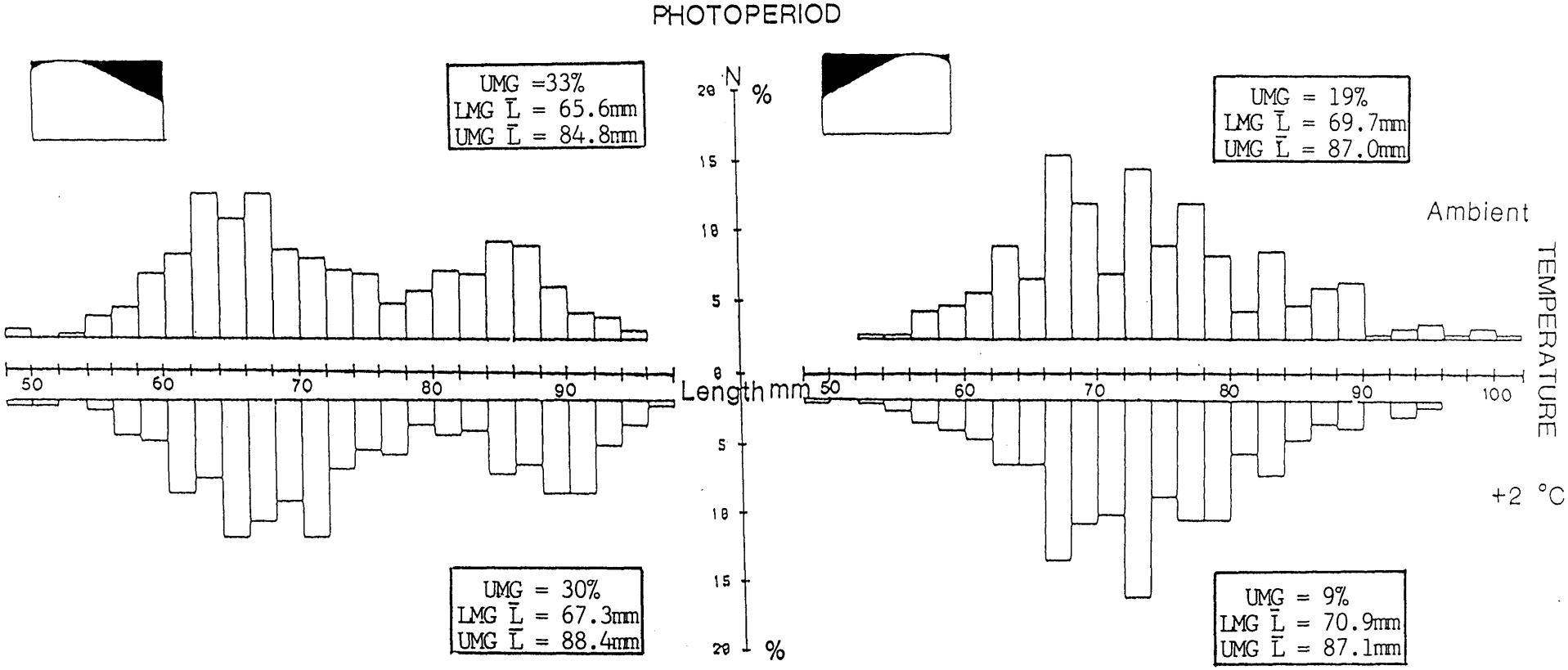


Figure 3.24 GROWTH PERIOD : 16 May - 23 Nov. 1986

The LMG of the population on retarded photoperiod (long spring photoperiod) (Fig. 3.23) and constant 15°C water temperature were significantly smaller (2.4 - 11.5% $p<0.02$) than all other groups on changing photoperiod regimes on this date. Similarly UMG fish were found to be significantly smaller (5.4 - 10.3%) than all other UMG's on changing photoperiod regimes ($p<0.001$).

Proportion in the upper modal group:

Unlike the equivalent accelerated incubation groups, the proportions in the UMG in populations with slightly elevated water temperatures (Fig. 3.24) were lower (2.9 - 9.9%) than those on ambient water temperature ($p<0.001$)

Populations on long spring photoperiod showed a reversal of the trend in the accelerated incubation group and had 21.1 - 14.1% fewer fish in the UMG than those on short period increasing photoperiod ($p<0.001$).

As with the accelerated incubation group the proportion of fish entering the UMG in the population held under constant 12 hours light photoperiod (Fig. 3.23) was lower than that of all other populations ($p<0.001$). A summary of results is given in Table 3.6.

The population held at constant 15°C temperature and long rising period of daylength, had a larger proportion in the UMG than all other groups ($p<0.001$).

Effects of Accelerated Incubation:

Ambient temperature:

Growth of accelerated incubation groups on ambient temperature was lower (6.3 - 17.0% smaller mean length) over the first 193 days ($p<0.001$) comparing between groups on equivalent periods of spring photoperiod length (summary - Tables 3.4 & 3.5).

The accelerated incubation group on long spring photoperiod (AP) had a larger proportion of fish in the UMG (8% more) than the group on equivalent long spring photoperiod in the standard hatched group ($p<0.001$).

However the accelerated incubation population on short spring photoperiod (AP) produced less (24% $p<0.001$) in the UMG than the standard incubation group on the equivalent photoperiod cycle (+3P), summary of results in Table 3.6.

Elevated temperature:

The accelerated hatching populations on elevated (+5°C) water temperatures produced more fish in the UMG than the comparable groups on +2°C elevated temperature and similar photoperiod regimes ($p<0.001$).

Within each incubation group and temperature regime the populations on an ambient photoperiod cycle (long spring photoperiod - accelerated incubation group and short spring photoperiod - standard incubation group) produced a larger proportion of fish in the UMG (14 - 32% $p<0.001$).

One explanation for the apparent discrepancy between accelerated and standard groups in the daylength phase that gives rise to the maximum proportions in the UMG, at ambient temperature (i.e. short spring - standard and long spring accelerated incubation groups) is given by an examination of the temperature cycle over the 141 day growth periods for the two incubation groups.

Figure 3.25 shows monthly mean temperatures over this initial 141 day period of growth for standard and accelerated incubation period populations on ambient water temperature. Comparing temperature regimes with photoperiod regimes shown on Figures 3.22 and 3.24, it can be seen that of the groups on ambient temperature, it is the groups which

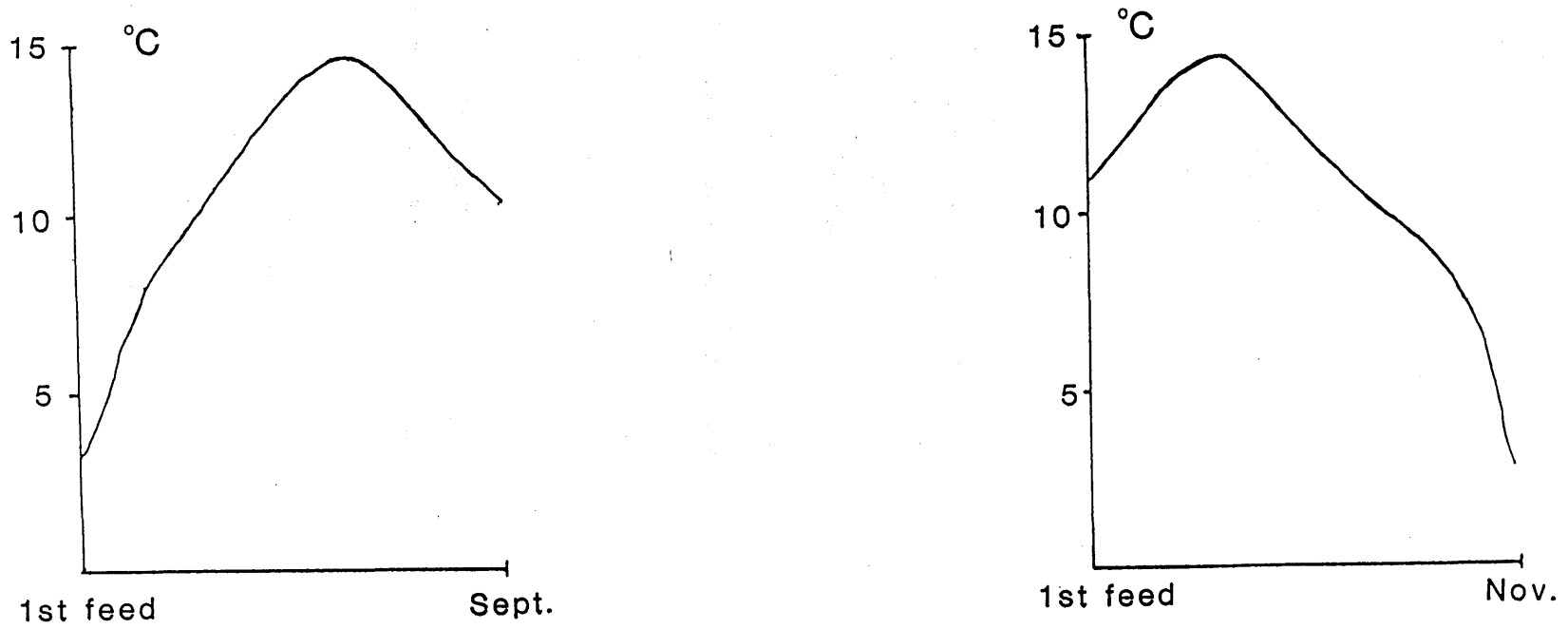


Figure 3.25 Ambient temperature (monthly mean) at Almondbank over the first 193 days of growth for Accelerated incubation (left) and Standard incubation groups (right.

have experienced temperature and photoperiod profiles in-phase, that produced the largest percentages in the UMG, (i.e. ambient photoperiod populations in both incubation groups).

This suggests that combinations of low temperature despite long days and short days despite high temperatures, were limiting the proportions entering the UMG.

To examine the proposal that both daylength and temperature can influence the proportion entering the UMG, possibly through growth rates an index combining these two parameters was devised.

The thermal sum is defined as:

TS = HD X MT

where HD = the number of hours of daylight (sunrise to sun set) and MT = the median temperature on that day.

This index was devised as a measure of growth opportunity. To test its validity it is necessary to establish whether observed growth rate is dependent on it. Mean daily TS values were calculated for each of the 10 Almondbank populations, for each of the intervals 29th May - 1st July 1987, and 1st July - 28th July 1987, as were

specific growth rates (G) defined as:

$$G = (\text{Ln } \frac{FL2 - \text{Ln} FL1}{T2 - T1}) \ 100$$

Where FL1 and FL2 are fork-lengths at time T1 and T2.
As specific growth rate is known to decline with fish size in any given season (Brett 1979), G was regressed jointly on TS and mean fork-length l_m for the appropriate interval, giving a least squares plane of:

$$G = 2.364 - 0.0423 \ (l_m) + 0.00189 \ (TS)$$

(data in Table 3.7: ANOVA in Table 3.8 (refers to June and July growth periods))

As predicted, growth rate was significantly dependent on both mean size ($p<0.001$) and thermal sum ($p<0.01$), which between them accounted for 92.7% of the observed variation.

To look for differential effects of growth opportunity (as estimated by thermal sum) at different periods during the first year, the thermal sum was calculated for individual months. In addition the thermal sum was calculated for subjective months (the month as defined by perceived photoperiod) and for the period of increasing daylength, for each population.

Treatment	Specific growth Rate (G)	Mean fork-length (lm) (mm)	Mean daily thermal sum (TS)
29th May - 1st July 1987			
AP/+5T/A	0.708	51.6	281
+3P/+5T/A	0.662	54.2	295
AP/AT/A	0.975	39.5	205
+3P/AT/A	1.007	40.1	215
12P/AT/A	0.916	38.4	163
-3P/+2T/S	1.221	35.8	164
AP/+2T/S	1.442	37.6	251
-3P/AT/S	1.136	35.4	143
AP/AT/S	1.271	35.6	217
12 P/AT/S	1.234	34.8	163
1st - 28th July 1987			
AP/+5T/A	0.332	60.2	342
+3P/+5T/A	0.302	62.7	263
AP/AT/A	0.698	50.6	258
+3P/AT/A	0.633	51.1	198
12P/AT/A	0.667	48.5	183
-3P/+2T/S	0.799	48.0	224
AP/+2T/S	0.719	51.2	291
-3P/AT/S	0.828	47.2	198
AP/AT/S	0.852	48.5	258
12P/AT/S	0.656	45.9	183

TABLE 3.7 Specific growth rate (G), mean fork-length (lm) and mean daily thermal sum (TS) for experiment 3, Almondbank populations.

ANOVA

Source of variation	DF	Sum of squares	Mean squares	Variance ratio	p<
Regression	2	1.5712	0.7856	108.53	0.001
Error	17	0.1231	0.0072		
Total	19	1.6943			

REGRESSION COEFFICIENTS

Variable	Coefficient	Standard error	t value	p<
intercept	2.3639	0.10893	21.702	0.001
lm	-0.0423	0.00343	-12.324	0.001
TS	0.0019	0.00054	3.520	0.01

TABLE 3.8 Multiple regression of specific growth rate (G) on mean fork-length (lm) and thermal sum (TS), experiment 3 populations, (data in TABLE 3.7): analysis of variance (ANOVA) and regression coefficients.

These thermal sums were then regressed against the proportion in the UMG for each population in a stepwise multiple regression. The multiple regression was part of a commercial statistical package (called SPSS) designed for use on IBM computers. This regression selects the independent variable (X), in this case thermal sum, that when regressed against the dependent variable (Y), best accounts for the variation in Y, the UMG percentage (arcsine transformed). The independent variable that minimises the residual variance is then selected and the combined regression calculated. This continues reducing the residual variance in steps.

Multiple Regression - Experiment 3

The 11 populations from which it is possible to estimate the proportion in the UMG from experiment 3 constitute the best data for a stepwise multiple regression.

Examination of the 8 groups on changing photoperiod and temperature regimes showed that the thermal sums of the months of June and July were significantly correlated with the proportion in the UMG (arcsine transformed) ($r= 0.76$ $p<0.02$ and 0.85 $p<0.01$ respectively). In addition, TS's for their counterpart subjective months of June and July were correlated but to a lesser degree (subjective June $r= 0.76$ $p<0.02$, July $r=0.81$ $p<0.01$).

Stepwise multiple regression showed that TS for the month of July explained the most variance in the data (72% $p<0.02$) and that no other measurement of thermal sum was able to explain a significant proportion of the residual variation.

Addition of the groups on fixed photoperiod regimes to the test necessitated the removal of all measures of thermal sums from subjective months (fixed photoperiod populations had no subjective months as defined by photoperiod). This was deemed justified on the evidence of the first analysis which showed TS for subjective months June and July to be of lesser importance in explaining the variance in the proportion in the UMG than their real month counterparts.

When the data from all populations from experiment 3 were included in the analysis, thermal sum for July although significantly correlated with UMG% ($r=0.69$ $p<0.02$) was relegated to lesser significance than August thermal sum ($r= 0.78$ $p<0.01$). September was also found to be slightly but significantly correlated ($r= 0.61$ $p<0.05$).

The stepwise multiple regression showed that August thermal sum explained 60% of the variance and that no other measure of thermal sum could explain a significant proportion of the remaining variance.

Multiple regression Experiments 1 & 2

A similar stepwise multiple regression was performed on the data from experiments 1 & 2.

Both the TS over the real month of July and the total thermal sum over the period of increasing daylight were highly correlated with the UMG%. (July $r=0.83$ $p<0.01$ and increasing daylength $r=0.90$ $p<0.01$). No measure of thermal sum from subjective months was correlated with UMG%.

The multiple regression showed that with 81% of the variance in the data explained by the thermal sum of the increasing daylength period, no other measure of thermal sum could significantly contribute to an explanation of the residual variance.

3.5 DISCUSSION

The 18 underyearling populations examined during the study period 1985 - 1987 typically showed rapid unimodal growth, followed by a distinct broadening of the length frequency distribution during summer and bimodality by late autumn. This pattern of growth has been well documented in many 0+ salmon parr populations (Thorpe 1977, Thorpe *et al.* 1980, Bailey *et al.* 1980), however it is notable that bimodality was found to occur in populations exposed not only to cyclic photoperiod and temperature regimes but also to constant daylength and to constant temperature.

Large differences between the two years, in the proportion entering the upper modal group may be attributable to genetic differences between families (Thorpe and Morgan 1978) as well as to slight environmental differences.

Bimodality in general results from a reduction in growth during the early summer of a proportion of the population (these become the LMG parr). Although the timing of separation of the modal groups under ambient conditions is a very crude *a posteriori* indicator of the time of onset of growth reduction, data presented here are consistent with

Metcalfe et al.'s (1986) finding of a reduction in appetite after July in LMG parr. It is likely that the apparent slight difference between years in the timing of the modal separation is due to the difficulty in detection of the much smaller proportions entering the UMG during the autumn of 1986, as well as to genetic differences in developmental timing (Thorpe et al. 1980).

It is apparent that a total arrest of growth in winter is not confined to LMG fish but also occurs in UMG fish under ambient conditions. Winter bimodality of length frequency distribution appears to result at least partly, from the growth arrest occurring earlier in fish entering the LMG, than those entering the UMG. Further separation of the modes in spring is likely to result from the later recommencement of growth in LMG fish the following spring.

TEMPERATURE

Growth:

Not surprisingly elevated temperature has a clear effect on growth, resulting in larger fish during the unimodal growth phase, which is continued into the bimodal phase. Growth of a wide variety of fish species has been found to be characterised by a temperature optimum (Windell 1978). Elevated water temperatures used in these experiments were

unlikely to be above that which may inhibit growth (Bardach et al. 1972). The effects of ambient temperature raised by 5°C was found to be much more marked than ambient +2°C.

Ambient +5°C eliminated the total winter growth arrest in both the LMG's and the UMG's, modal separation being due to differing growth rates over the autumn/winter period. Additional +2°C heat appeared to be insufficient to achieve this in the LMG, however there is some evidence that it may have further delayed complete growth arrest in UMG fish and hasten recommencement of growth in spring.

Growth at constant 15°C was depressed over the unimodal growth phase, compared with groups on ambient temperature regimes. This may have resulted from fish on ambient temperature regimes experiencing temperatures at or above 15° C for part of the day (during daylight hours) throughout most of June and July.

Proportion of the population entering the upper modal growth group:

Temperature affects the proportion of the population entering the upper modal growth group.

All groups receiving elevated (+5°C) water temperature showed larger proportions in the UMG. At +2°C the groups in experiment 2 showed an increase in the UMG proportion, whereas similar groups in experiment 3 showed a slight decrease. Why a UMG proportion decrease should occur in

these groups, despite better growth (longer fish after 141 days growth) remains unclear.

PHOTOPERIOD

Photoperiod effects on growth:

Photoperiod clearly affects growth. Higgins and Talbot (1985) found that food intake during the hours of darkness was limited in salmon parr. There is consistent evidence presented here that fish on longer days (advanced photoperiod cycle in early spring) grow quicker than fish on shorter days (retarded photoperiod cycle in spring), on all temperature regimes. Fish on 12 hours light (12 hours dark) grew less well than all groups on cyclic photoperiod. A daylength of 12 hours represents the photoperiod of the 21st of March and 21st September. Growth of populations on a perceived photoperiod between these dates is likely to be less limited by daylength. In addition, fixed daylength populations received no twilight, this may further extend the feeding possibilities for groups on cyclic photoperiods beyond sunset and before sunrise.

These findings are consistent with Villarreal *et al.* (in press) who showed a relationship between the number of hours daylight and mean length of salmon parr.

Photoperiod as a seasonal timer:

In addition to its effects on growth, photoperiod affects the timing of the split of the two modal groups. Advanced photoperiod regimes (3 months) hastened bimodality by approximately 1 month, and photoperiod regimes retarded 2 and 3 months delayed the appearance of bimodality by about 1 month. This agrees with the findings of Villarreal et al. (in press) who found that delaying photoperiod phase delayed the timing of a reduction in growth rate in LMG fish but that the relationship was not an exact one. Villarreal and his co-workers suggested that photoperiod acted as an exogenous synchroniser (a Zeitgeber) of an internal clock. However when the phase angle of the synchroniser was more than 90 ° (>3 months) from the internal clock then it ceased to act as an effective entrainer of the endogenous rhythm.

The finding that a modal segregation occurs in parr held on fixed constant light regimes, and hence receiving no photoperiod seasonal cues, provides support for the existence of such an endogenous rhythm.

Photoperiod effect on entry to the UMG:

Evidence presented here suggests that both photoperiod and temperature affect growth, photoperiod through daylength constraints on opportunity to feed, and temperature through intake rate and digestion physiology. Thus any photoperiod / temperature pair will represent a particular quantifiable

opportunity for growth. Thus an index of growth opportunity combining these parameters, the thermal-sum, is proposed (Adams and Thorpe in press a). This index has shown to be an effective predictor of growth in the radial flow tank during periods of excess food supply prior to LMG growth arrest.

In addition to its effects on growth it has been shown that photoperiod can act as a timer, providing seasonal information which is probably used to synchronise an endogenous rhythm.

Temperature over the range 0 - 20°C clearly affects the proportion entering the UMG as underyearlings and hence smolting as S1's. Additional +5°C heating greatly increased the proportion entering the UMG on every photoperiod regime. The effects of +2°C additional heating were less marked, however in one group on retarded photoperiod (Experiment 3) there was a slight unexplained reduction in the proportion entering the UMG.

Photoperiod has also been shown to influence the proportion entering the UMG as underyearlings, however to interpret these results fully it was found necessary to separate the growth opportunity and seasonal timing effects of photoperiod by using the thermal sum as an index of growth opportunity.

Advanced photoperiod cycle was found to decrease the proportion entering the UMG as underyearlings. The timing effect of photoperiod in these populations is likely to have accelerated the timing of the smolting decision. This would result in the decision being taken during a period when water temperature and hence growth opportunity is lower than those populations held under ambient conditions (water temperature peaked around July at Almondbank Appendix 2 & 3).

Although retarded photoperiod cycle slightly increased the proportion entering the UMG in Experiment 2, it reduced the proportions in Experiment 3. In both these experiments retarded photoperiod (-3 & -2 months out-of-phase respectively) is likely to have retarded the smolting window (although not necessarily an equivalent phase change). In 1986, (Experiment 3) the water temperature dropped rapidly after mid-July, however 1985 (Experiment 2) water temperatures remained relatively high until into September (Appendix 2 & 3). So for example in 1985 in August the population on retarded photoperiod (-2 months) ambient temperature received a thermal sum of 6365 (hrs-°C),cf. 5547 (hrs-°C) for the retarded population (-3 months) on ambient temperature in 1986. Thus the growth opportunities around the smolting window period were higher for the retarded

photoperiod group in 1985.

Data presented here show that thermal sum correlates well with the proportion entering the UMG, over a range of growth opportunity conditions. Stepwise multiple regression was used to indicate the period during which growth opportunity most influences rate of entry to the UMG. Analysis of the 11 groups in Experiment 3 on cyclic and fixed photoperiod / temperature regimes indicated July/August as the most likely period for the smolting window. This estimate of the smolting window correlates well with the findings of others. Metcalfe et al. (1986) found a reduction in appetite of salmon parr which eventually entered the LMG, between July and August. Villarreal (1983) examining muscle RNA/DNA ratios of parr showed that there was a growth disruption around the end of July. Also Thorpe et al. (1980) estimated that growth arrest in LMG parr commenced around June-July, by backward extrapolation of growth curves.

Although growth opportunity during the smolting window is clearly important in determining the outcome of the smolting decision, it was not the only factor determining the outcome in these experiments. Accelerated incubation populations subsequently held on ambient temperature and photoperiod regimes were found to have higher rates of entry to the UMG than sibling populations hatched under ambient water

temperatures, despite identical growth opportunities (same thermal sum) during the smolting window (in fact throughout the whole growth period of the standard hatched group). Clearly the additional early growth advantage afforded by early hatching influenced the outcome of the smolting decision.

CHAPTER 4

PHOTOPERIOD AND TEMPERATURE INFLUENCES ON REPRODUCTIVE INVESTMENT

4.1 INTRODUCTION

4.1.1 Photoperiod:

Photoperiod has repeatedly been shown to be important in the control of breeding in a number of cyclically spawning fish species (Lundqvist 1980, Bromage et al. 1984, Skarphedinsson et al. 1985, Takashima and Yamada 1984). As with smolting, photoperiod is likely to have two distinct effects on sexual maturation.

Firstly photoperiod may act as a seasonal timer. A number of studies indicate that some aspect of photoperiod may be a proximate cue used to initiate breeding in temperate fish species. For example Takashima and Yamada (1984) in a study of the autumn breeding Masu salmon (Oncorhynchus masou) found that long days stimulated the early stages of the reproductive cycle, whereas shorter photoperiods stimulated the latter stage. Likewise Bromage et al. (1984) showed that long days followed by a period of short days stimulated complete ovarian development in Rainbow trout

(Salmo gairdneri) a winter spawner.

As has already been discussed (section 3.1.1), photoperiod is an inherently more reliable timing mechanism than other environmental cues, such as temperature. As with the timing of other developmental events (e.g. smolting) photoperiod is the most likely external stimulus for the initiation of gonadal development in the majority of fish (for review see Lam 1983).

Secondly photoperiod is likely to have an indirect influence on maturation by modifying population maturation rate through growth.

Roff (1983) suggested that there is a direct trade-off between somatic growth and reproduction. Animals that invest in reproduction do so at the expense of somatic growth. Bagenal (1978) showed that there is a direct relationship between fecundity and body size in fish, thus energy directed into gonad detracts from future fecundity (Wootton et al. 1980).

Several workers have commented on a relationship between growth and maturity (Alm 1959, Thorpe 1986, Myers et al. 1985, Section 1.4.1). Bailey et al. (1980) proposed that salmon parr maturation is dependent on attaining a critical threshold size. Daylength is known to influence growth rate (Higgins and Talbot 1985, Villarreal et al. in press and Section 3.4) and thus photoperiod may exert an influence on

maturation rate through its influence on growth potential.

4.1.2 Temperature:

As with daylength, temperature may affect growth (the mechanisms for this have been explored in Section 3.1.3). Thus if some aspect of growth or size is important in determining the outcome of the physiological maturation decision then temperature may also influence maturation through its effects on growth.

The influence of both timing and growth rate on parr maturation rates in any one year class have been combined in Thorpe's (1986) model (Figure 1.2). The model predicts that high growth opportunity during a supposed "maturation window" in early spring would result in high levels of reproductive investment in the population during the breeding season (in the following winter).

It is the aim of this section to examine how reproductive investment may be influenced by photoperiod (which may influence the timing of the breeding cycle and growth opportunity) and temperature (which influences growth opportunity) with respect to the predictions drawn from Thorpe's model. The null hypothesis was that photoperiod and temperature have no effect on reproductive investment in underyearling salmon parr.

4.2 MATERIALS AND METHODS

In an attempt to estimate the reproductive investment of both male and female parr held under differing growth conditions during their first breeding season, a sample of 50 fish were sacrificed from each population in Experiments 1 - 3, for examination of reproductive condition. As Lundqvist (1980) has shown that photoperiod can alter the timing of ripening in male Baltic salmon parr (Salmo salar L.), parr were examined during the subjective month of January, as determined by a "perceived" January photoperiod, a period when sexual maturity is likely to be encountered. The photoperiod and temperature regimes received by populations from experiments 1, 2 and 3 are summarized in Tables 3.1, 3.2 and 3.3 respectively.

Each fish was measured (fork-length), blotted dry and weighed. The fish were sexed by dissection and gonads over 4 mgs blotted dry and weighed (gonads under 4 mgs were not weighed owing to the difficulty in ensuring complete removal).

The Gonado-somatic index (GSI) (Le Cren 1951, Van den Hurk and Peute 1979) was used as a measure of relative gonadal investment. This is defined as:

$$GSI = \frac{\text{Gonad weight}}{\text{Gonad + Soma}} \times 100$$

To stabilise these proportionate values, GSI values were

arcsine transformed (Sokal and Rohlf 1969) before being regressed on fish fork-length to establish the gonad investment relationship for each treatment group.

To determine the effects of tissue water content on GSI, around 50 fish and their respective gonads were oven dried at 100°C for 7 days. It was found that drying reduced the GSI of female fish proportionally to 63.8% ± 1.78 (mean ± S.E.) of its wet weight value, for fish in the range 40 - 120mm in these experiments. Although this was not a linear relationship (ovary is not likely to have the same water content as soma), it was deemed to be a close enough approximation over these limited size ranges to warrant using wet weights (blotted) as an adequate measure of investment in ovary, and hence these were used throughout.

Histology

Gonad for histological examination was fixed immediately after removal, in buffered formal saline, prior to dehydration, clearing in "Histoclear" and embedding in paraffin wax, using standard histological techniques (Drury and Wallington 1973).

Each gonad sample was sectioned at 3 different transects, at 7 μm thickness. Sections were stained with Mayers haematoxylin and counterstained with eosin. Reproductive state was estimated by comparison of sections

with developmental stages as defined by Sutterlin and MacLean (1984) (ovarian stages, adapted from those of Van den Hurk and Peute 1979) and Henderson (1964) (testis stages also adopted by Grier 1981).

Oocyte Diameter Measurement

Oocyte diameter was measured directly from calibrated images projected by a Leitz "Prado" projection microscope. The oocyte diameter was estimated from the mean of 2 measurements made at right angles, of cells whose nuclei were present in the section (Faucher and Beamish 1980). This technique obviously results in an underestimate in oocytes where the plane of sectioning is not through the equator. Using serial sections an estimated mean error of 10% from the true cell diameter was calculated for this technique. This was regarded as acceptable. The fish mean oocyte diameter was calculated from a minimum total of 60 cell diameter measurements, made at 3 positions along each of the 2 ovaries.

The Energetics of Reproductive Effort

The calorific value of ovaries taken from 11 fish over the size range 60 - 85mm, from a number of different populations from Experiment 3, during subjective January (as determined by photoperiod), was determined by bomb calorimetry. Tissue was oven dried at 55°C for 5 days, then

compressed into pellets of less than 20 mgs prior to combustion in a Phillips Micro bomb (Model AH12 EF2). Benzoic acid (calorific value 250 J/mg) was used to produce a calibration curve. The somatic tissue (i.e. all tissue except gonad) of 15 parr of the size range 60 - 85 mm, taken from a variety of populations was homogenised and then treated in a similar manner to ovarian tissue.

4.3 RESULTS

4.3.1 REPRODUCTIVE INVESTMENT - EXPERIMENTS 1 & 2

Gross morphology of testis (Jones and Orton 1940) and ovary (Sutterlin and MacLean 1984) of a sample of around 50 fish from each population suggested that no parr (male or female) was sexually mature during the "perceived" January of their first year. To confirm this a histological examination of gonad was made of approximately 12 fish from each population.

Males

Approximately 25 fish from each population were sacrificed and their testes examined. These appeared as very small thread-like structures and all weighed less than 4 mgs.

Histological examination of 6 males from each population confirmed that these fish had been functionally immature, as the sections corresponded to Stage I - the spermatogonial proliferation phase of Henderson (1964). The internal structure of the testis appeared ill-defined, lacking clear lumina with primordial germ cells and spermatogonia predominating. The latter were recognised by their relatively large size with acidophilic nuclei densely staining with haematoxylin.

Females

Gross morphology of ovary indicated that females were similarly immature. Histological examination of approximately 6 fish from each population subsequently confirmed this. The majority of oocytes corresponded with Stage II (primary oocyte stage) of Sutterlin and MacLean (1984) (Plate 4.1). This stage of development is typified by oocytes with a large nucleus with numerous small nucleoli on the periphery. Balbiani bodies can be seen around the nucleus at this stage. Stage III oocytes were not observed. In this stage of development, Balbiani bodies disperse and yolk vesicles form in the cytoplasm. The absence of yolk vesicles in the cytoplasm was confirmed by staining some sections with Periodic-acid-Schiff (PAS). This stain has a strong reaction with the mucopolysaccharides found in yolk vesicles (Yamamoto and Yamazaki 1961).

Differential Investment in Ovary

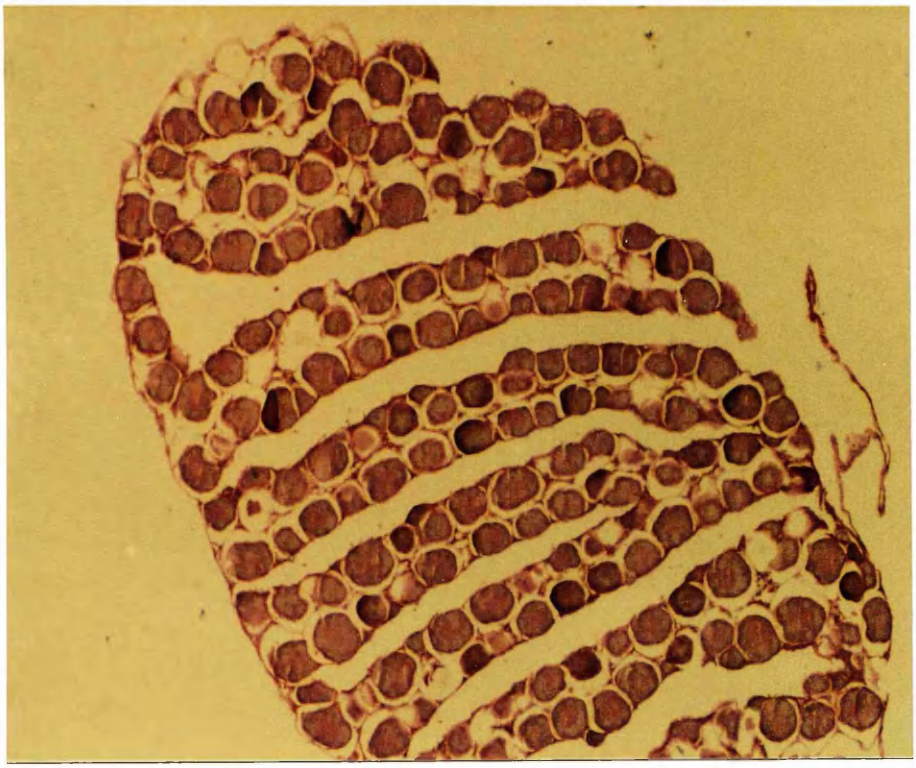
Although no females were found to be sexually mature there was differential investment in ovarian tissue.

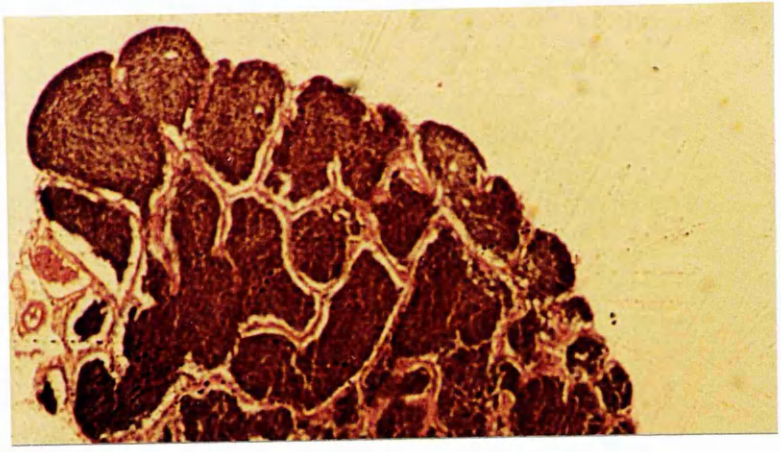
Within Populations:

The relative investment in ovary (as measured by GSI) decreased proportionally with increasing fork-length in all groups ($r = -0.839$ to -0.910 $p<0.001$).

Plate 4.1 Longitudinal section through immature ovary of underyearling salmon parr, showing stage II, primary oocytes (Sutterlin & MacLean 1984) (stained with Haematoxylin and Eosin, mag. c. x60).

Plate 4.2 Transverse section through maturing testis, cellular stage IV (Henderson 1964) of underyearling salmon parr. Lumina filled with densely staining spermatozoa. (Stained with Haematoxylin and Eosin, mag. c. x60).





Between Populations:

The relative investment in ovary also differed between populations held under different environmental conditions.

Experiment 1 - Accelerated Incubation

The Effects of Elevated (+5°C) temperature:

Experimental Population - +3P/+5T

Control Population - +3P/AT

Comparing the regression of GSI on fork-length for population +3P/+5T with that of +3P/AT (Fig. 4.1) shows that both populations have a similar rate of decline in GSI with increasing fork-length (Analysis of Covariance - slopes, no significant difference). However the relative investment in ovarian tissue was greater, at any given length, on a +5°C elevated temperature regime than on an ambient temperature regime (Analysis of Covariance - elevations $p<0.001$).

The Effects of Advanced (+3 months) photoperiod regime:

Experimental Population - +3P/AT

Control Population - AP/AT

Comparing the regressions of GSI on fish fork-length for these two populations (Fig. 4.2) shows that the rate of decline in GSI with length is the same (Analysis of

Figure 4.1 Regressions of GSI (arcsine transformed) (see text for definition) on parr fork-length (mm) in October (= "perceived" January), populations +3P/+5T (o) and +3P/AT (Δ), Experiment 1.

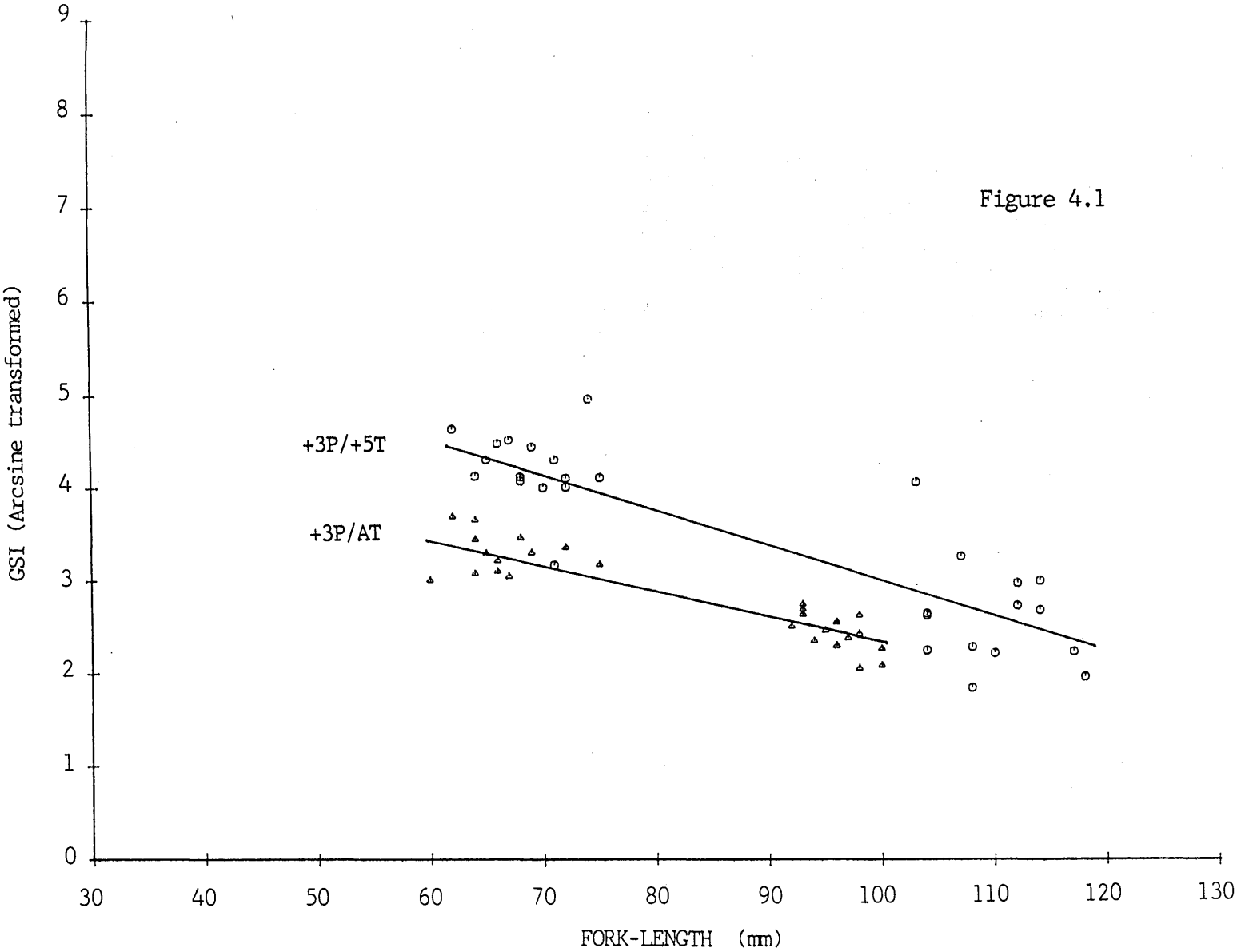

GSI (Arcsine transformed)
9
8
7
6
5
4
3
2
1
0
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)
+3P/+5T
+3P/AT

Figure 4.1

Figure 4.2 Regression of GSI (arcsine transformed) (see text for definition) on parr fork-length (mm) in January, population AP/AT (Δ solid line) and population +3P/AT (broken line), Experiment 1.

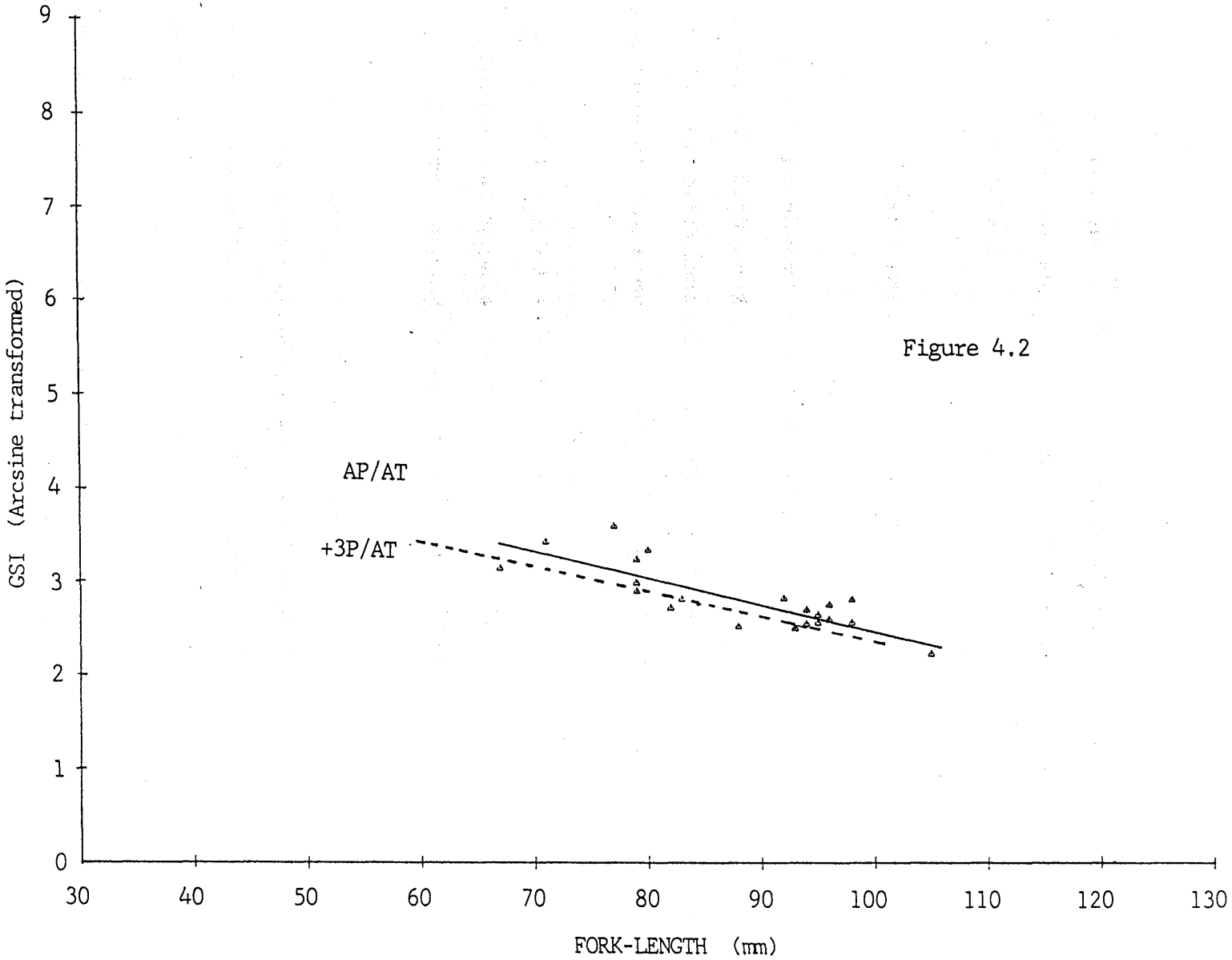

9
8
7
6
5
4
3
2
1
0
GSI (Arcsine transformed)
AP/AT
+3P/AT
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)

Figure 4.2

Covariance - slopes no significant difference). However the group on advanced photoperiod, experiencing a shorter growth period up to "perceived January" invested relatively less in ovary for any given length (Analysis of Covariance - elevation $p<0.05$).

Experiment 2 - Standard Incubation

All groups showed a similar rate of decline in GSI with increasing fork-length (Analysis of Covariance - slopes no significant differences). However there were differences in magnitude of investment in ovary for any given fork-length.

Effects of Elevated +2°C Temperature:

Experimental populations: AP/+2T & -2P/+2T

Control populations: AP/AT & -2P/AT

On an ambient photoperiod regime, additional heating to +2°C increased the relative investment in ovary for a given length (comparing experimental population AP/+2T with the control AP/AT Fig. 4.3 Analysis of Covariance - elevation $p<0.01$).

This finding is borne out by examination of the retarded photoperiod pair. Ovarian investment of the population on elevated temperature (-2P/+2T) was higher than that of the population on ambient temperature (-2P/AT) (Fig. 4.4)

Figure 4.3 Regressions of GSI (arcsine transformed) (see text for definition) on parr fork-length (mm) in January, populations AP/+2T (o, broken line) and AP/AT (Δ, solid line), Experiment 2.

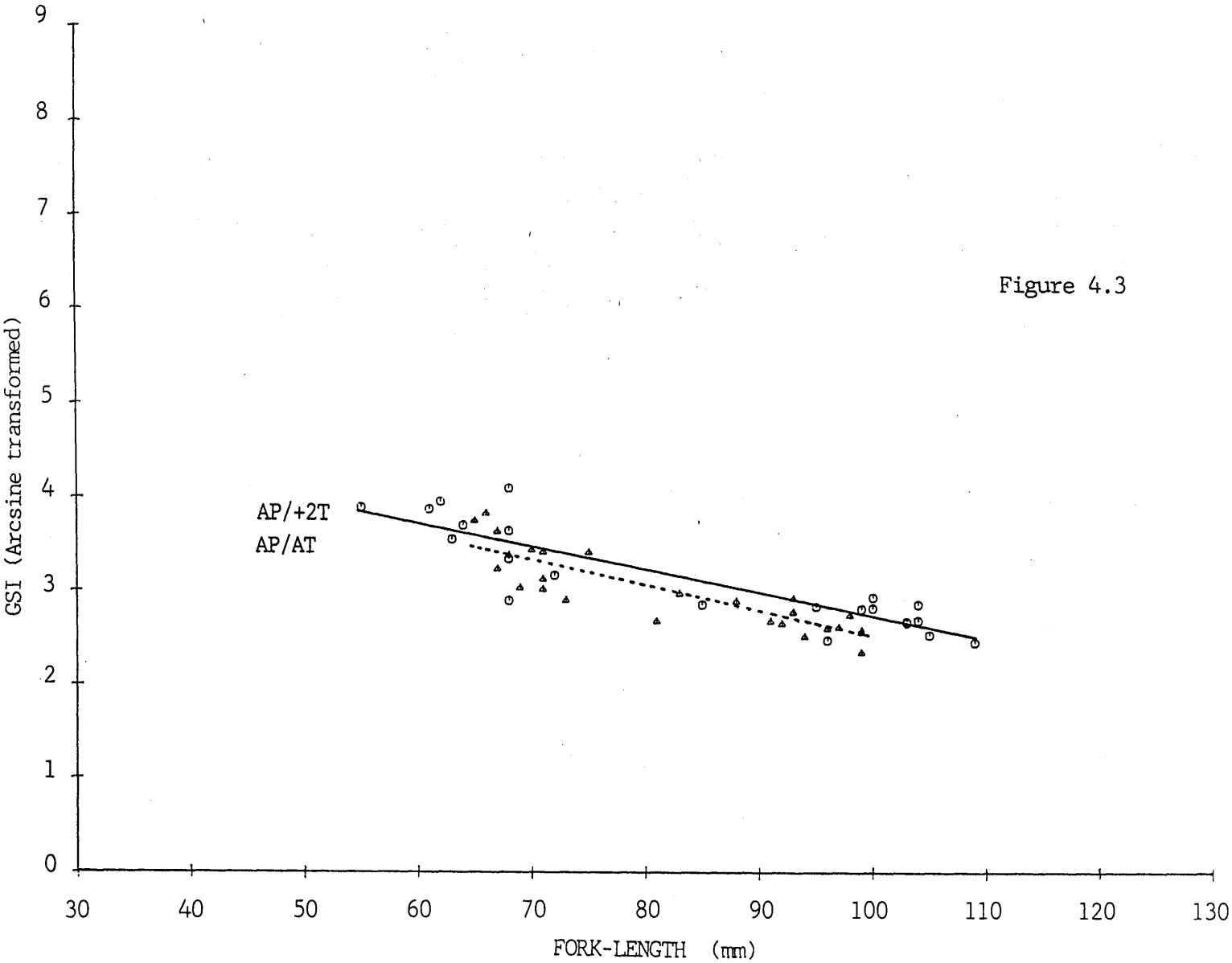

GSI (Arcsine transformed)
9
8
7
6
5
4
3
2
1
0
AP/+2T
AP/AT
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)

Figure 4.3

Figure 4.4 Regressions of GSI (arcsine transformed) (see text for definition) on parr fork-length (mm) in March (= "perceived" January), populations -2P/+2T (o, solid line) and -2P/AT (Δ , broken line), Experiment 2.

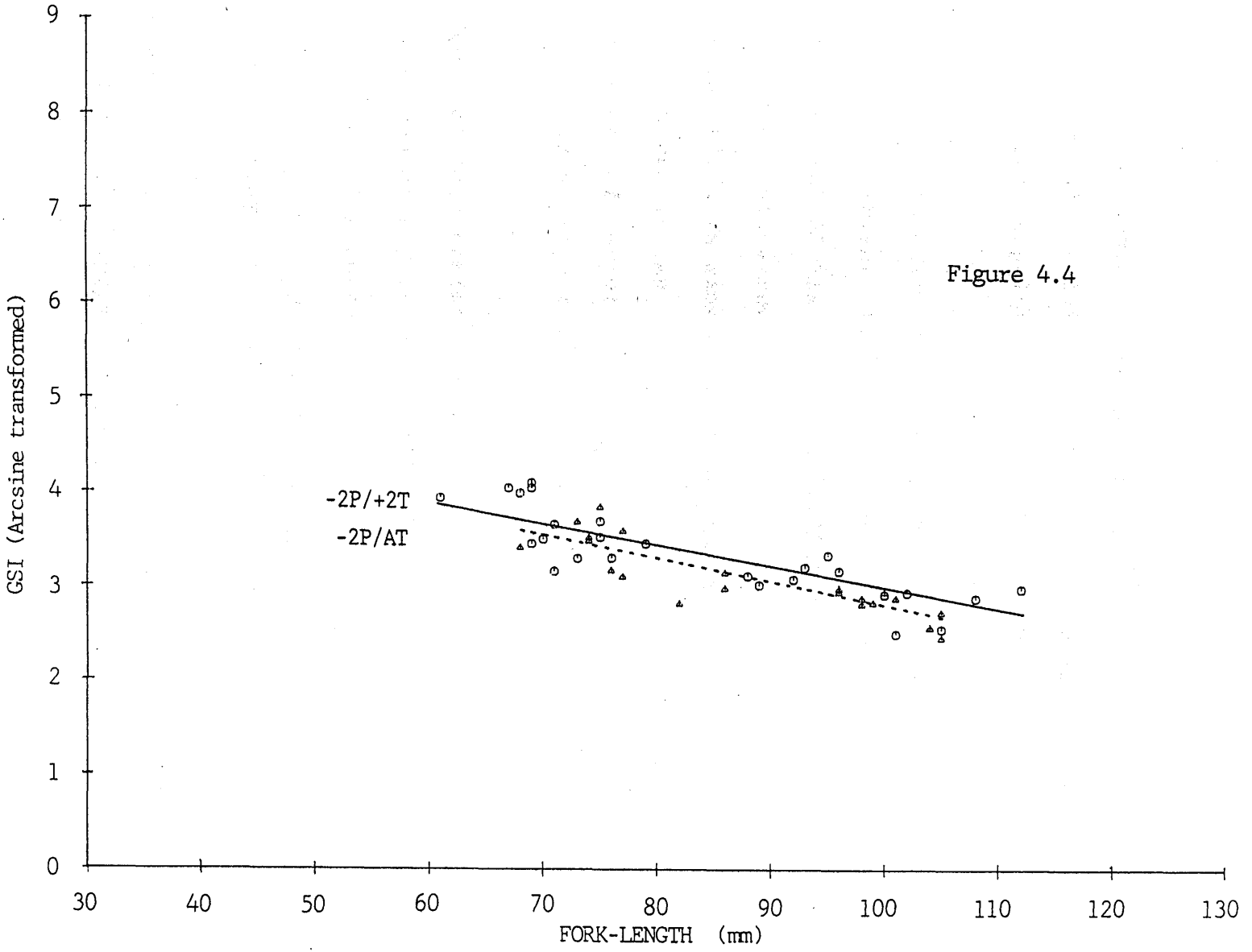

GSI (Arcsine transformed)
FORK-LENGTH (mm)
-2P/+2T
-2P/AT
0
1
2
3
4
5
6
7
8
9
30
40
50
60
70
80
90
100
110
120
130

Figure 4.4

(Analysis of Covariance - elevation $p<0.05$).

Effects of Retarded (-2 months) Photoperiod:

Experimental populations: -2P/AT & -2P/+2T

Control populations: AP/AT & AP/+2T

Retarded photoperiod increased the relative investment in ovary at ambient temperature by "perceived" January (comparing -2P/AT Fig. 4.4 with AP/AT, Fig. 4.3, Analysis of Covariance - elevation $p<0.001$).

This effect is also seen at elevated (+2°C) temperature, where there is an increase in the elevation of the regression of population -2P/+2T (Fig 4.4) compared with that of AP/+2T (Fig 4.3), ($p<0.01$ Analysis of Covariance).

Effects of Accelerated Incubation:

Experimental population: AP/AT - Exper. 1

Control population: AP/AT - Exper. 2

Accelerating the incubation period appears to have had no effect on the relationship between GSI and fork-length by January of the first year (comparing AP/AT - Experiment 1 (Fig. 4.2) with AP/AT - Experiment 2 (Fig. 4.3) Analysis of Covariance shows no significant difference in slopes or elevation).

Fork-length Weight Relationship:

The simplest explanation of these observed differences in relative investment in ovary is that the length weight relationship differs between groups. Figure 4.5 shows accumulated fork-length - total body, wet weight data for all populations of experiments 1 and 2.

Fork-length correlated closely with total body weight and there were no differences between sexes or populations, thus it seems likely that differences in ovarian investment between populations under differing conditions of photoperiod and temperature is not the result of differences in the length weight relationship between groups.

Experiment 3

The extent of gonadal investment was investigated in all populations on cyclic temperature regimes over the breeding season 1986 - 87 during "perceived" January (those on constant water temperature having been lost prior to this period).

Male Reproductive Investment:

Examination of the gross testes characters indicated that the majority of parr were not sexually mature. In these fish the testis consisted of small thread-like structures (Jones 1940) often barely visible and less than 4 mgs in weight.

Figure 4.5 Fork-length (mm), parr weight (g) (wet) curve. Cumulated data from all populations in Experiments 1 & 2, during "perceived" January.

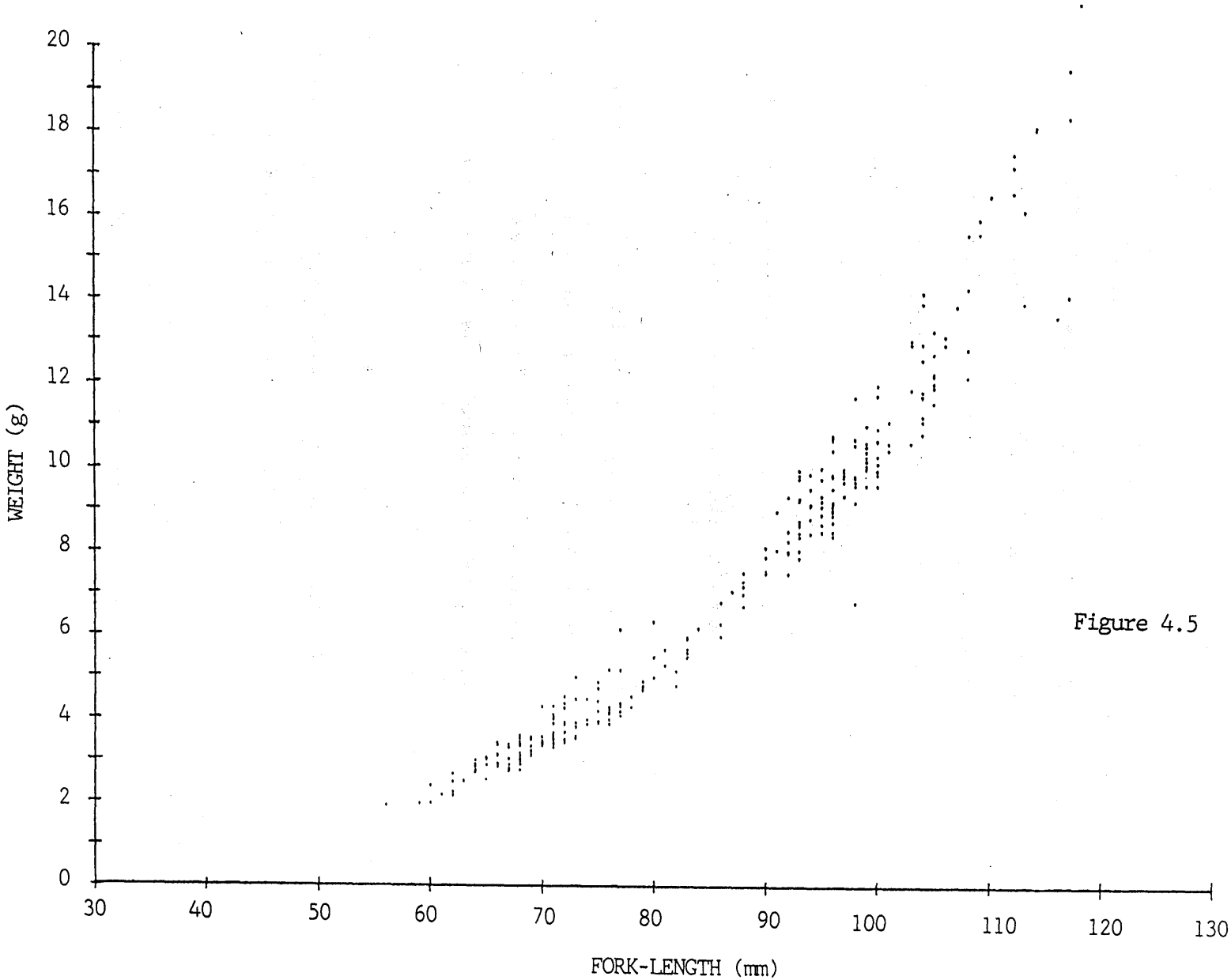

20
18
16
14
12
10
8
6
4
2
0
WEIGHT (g)
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)

Figure 4.5

Difficulty in ensuring complete removal of such testes prevented comparison of gonad investment between and within populations. Immaturity in these fish was confirmed histologically in a sample of at least 6 male parr from each population. As with experiments 1 and 2 all parr classified as "immature" on gross characters were found to correspond to Stage I - spermatagonial proliferation stage of spermatogenesis.

Eight parr from population AP/+5T/A were found to have noticeably larger testes than those characterised as immature.

Histological examination showed that 3 of these parr were at an advanced stage of maturity, Stage IV (Table 4.1, Plate 4.2). In this stage of spermatogenesis, the lumina are filled with spermatozoa (Henderson 1964). These parr did not seem to be "running ripe" as light flank pressure did not result in the shedding of milt, thus the criteria of Henderson's (1964) Stage V - functional maturity did not seem to have been met. However histological examination indicated that functional maturity would be very likely to occur in these fish in the breeding season 1986 - 87.

Functional maturity in another 3 remaining parr from this population with enlarged testis is less certain. Histological examination of the testis indicated late Stage II/early Stage III (rapid phase of spermatogenesis)

Fish Fork-length mm	Developmental Stage	Testis wt. mgs	GSI %
96	early IV	113.3	1.2
107	late IV	27.6	0.2
104	IV	125.9	1.0
95	II	10.1	0.1
96	II	16.3	0.2
87	II	7.6	0.1
88	I	8.3	0.1
112	I	9.3	0.1
Others	I	<4	<0.04

Table 4.1 Stages of spermatogenesis of 8 parr from population AP/+5T/A, showing high investment in testis during their first winter.

(Henderson 1964) with moderate quantities of spermatids/spermatozoa in the testis lumina. Despite the fact that the testis of these fish were at a relatively advanced stage of spermatogenesis, compared with the majority of parr from these experiments, it is questionable if these parr would be able to attain functional maturity during the breeding season of 1986 - 87.

A further 2 parr from this population were found to have enlarged testis, more than twice the weight (wet) of other parr of a similar size. Histological examination showed these two fish to be immature, spermatogenesis being at the early stage I of development, as found in the majority of parr characterised as immature.

In addition to these fish from population AP/+5T/A, one male from population AP/+2T/S was found to have enlarged testis. Histological examination found that this fish would not become functionally mature during the breeding season 1986 - 87. Only one testis showed any signs of spermatogenesis beyond the (stage I) spermatagonial stage of development of the majority of immature parr, and only in a very small number (estimated 3 - 4%) of cysts.

Fish Size at Maturity:

All fish showing marked spermatogenesis were found in the UMG of population AP/+5T/A (Table 4.1). However all were

within the lower 64% of the size range covered by the UMG at the time of monitoring.

4.3.2 Female Reproductive Investment

Of the approximately 25 female parr examined from each population none was estimated as having reached sexual maturity, on the basis of gross ovarian morphology. The ovaries from 213 females, taken from all populations on cyclic temperature regimes were examined histologically, and immaturity subsequently confirmed. No fish were found to have oocytes beyond Stage II of oogenesis (Sutterlin and MacLean 1984, Plate 4.1). The absence of stage III oocytes was confirmed by staining with PAS. However as with Experiments 1 and 2 differential investment in ovary was apparent.

Within Populations:

As with Experiments 1 and 2 relative investment in ovary (as measured by GSI) decreased linearly with fork-length in all populations ($r = -0.661$ to -0.962: $p<0.01$ in population 12P/AT/S, $p<0.001$ - all other groups). In addition there were differences in relative investment in ovary between populations experiencing differing environmental conditions.

Between Populations: Standard Incubation populations:

Analysis of Covariance showed that there was no

significant difference in the slopes of the regressions of GSI on fork-length, between populations in the standard incubation group.

Effect of Elevated (+2°C) Temperature:

Experimental populations: -3P/+2T/S & AP/+2T/S

Control populations: -3P/AT/S & AP/AT/S

There was a slight increase in the relative investment in ovary with elevated temperature at ambient photoperiod (comparing population AP/+2T/S with AP/AT/S Fig. 4.6) (Analysis of Covariance - elevation $p<0.05$). However the other like photoperiod pair (-3P/+2T/S and -3P/AT/S Fig. 4.7) did not show a similar significant difference.

Effect of Retarded (- 3 months) Photoperiod:

Experimental populations: -3P/AT/S & -3P/+2T/S

Control populations: AP/AT/S & AP/+2T/S

Retarded photoperiod increased the relative investment in ovary at ambient temperature (comparing population -3P/AT/S Fig. 4.7 with AP/AT/S Fig. 4.6, Analysis of Covariance - elevation $p<0.01$). However the difference is not significant at elevated temperature (comparing -3P/+2T/S Fig. 4.7 with AP/+2T/S Fig. 4.6).

Figure 4.6 Regressions of GSI (arcsine transformed) (see text for definition) on fork-length (mm) of parr from populations AP/+2T/S (o, solid line) and AP/AT/S (Δ , broken line), Experiment 3 during January.

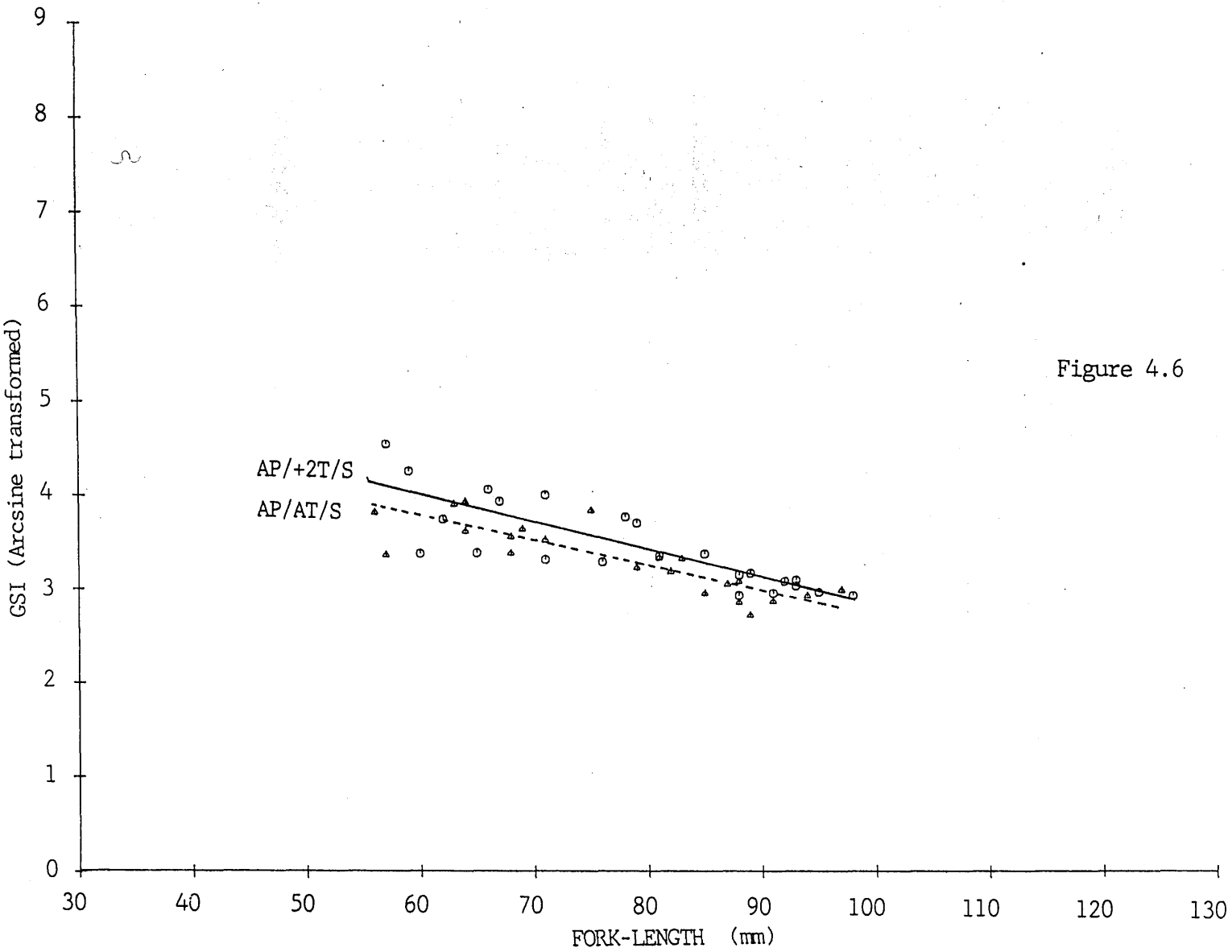

9
8
7
6
5
4
3
2
1
0
GSI (Arcsine transformed)
AP/+2T/S
AP/AT/S
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)

Figure 4.6

Figure 4.7 Regressions of GSI (arcsine transformed)(see text for definition) on fork-length (mm) of parr from populations -3P/+2T/S (o, solid line) and -3P/AT/S (Δ , broken line), Experiment 3 during April (= "perceived" January).

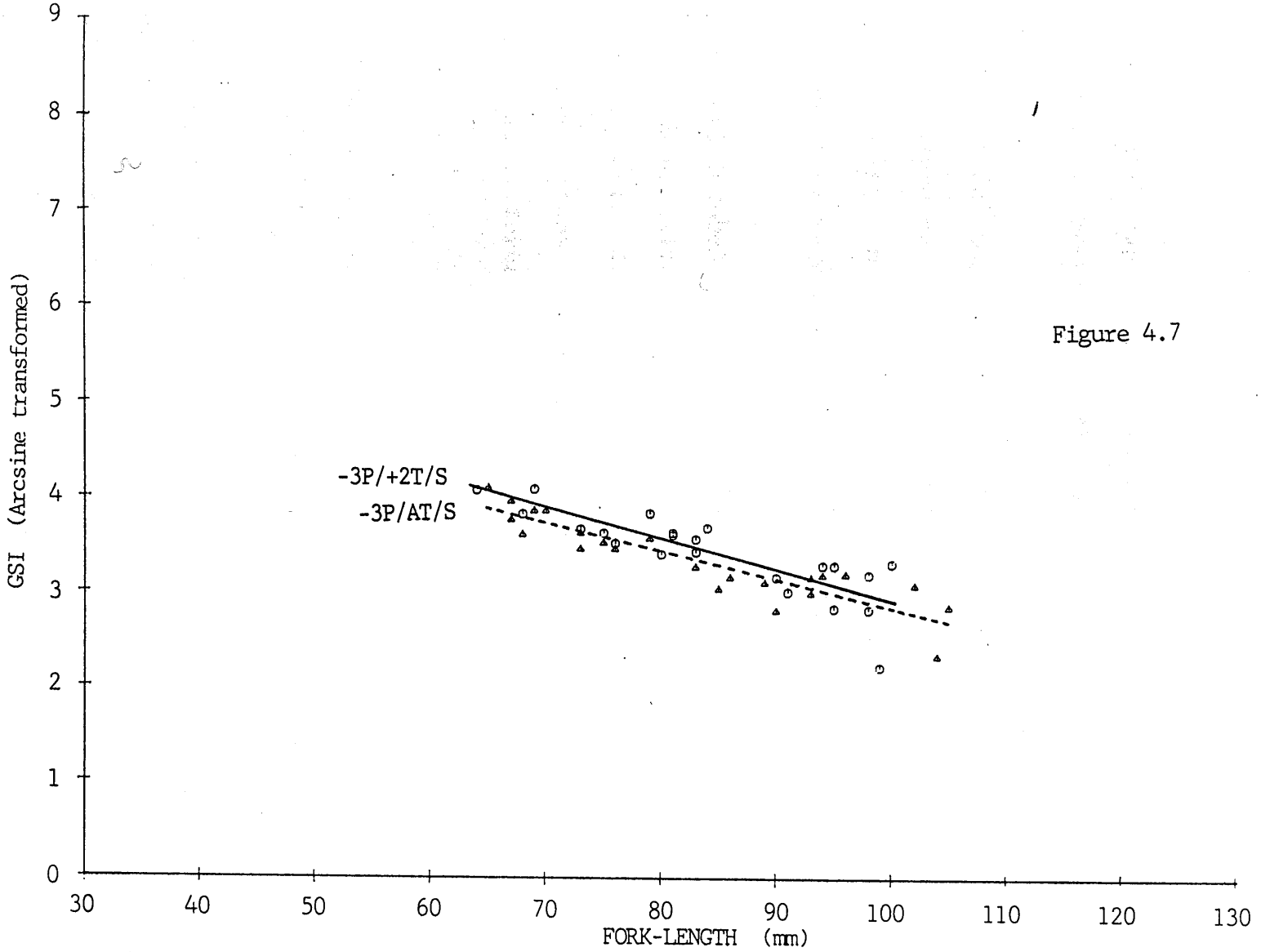

GSI (Arcsine transformed)
FORK-LENGTH (mm)
-3P/+2T/S
-3P/AT/S
0
1
2
3
4
5
6
7
8
9
30
40
50
60
70
80
90
100
110
120
130

Figure 4.7

Effect of Constant (12 hrs) Light Regime:

Experimental population: 12P/AT/S

Control population: AP/AT/S

Females from population 12P/AT/S Fig. 4.8 showed a slightly depressed relative investment in ovary compared with the control on a cyclic photoperiod regime (Analysis of Covariance - elevation $p<0.05$).

Accelerated Incubation Group:

There was no difference in the rate of decline in ovarian investment with increasing fork-length between accelerated incubation groups (Analysis of Covariance - slopes no significant differences).

Effect of Elevated (+5°C) Temperature:

Experimental populations: AP/+5T/A & +3P/+5T/A

Control populations: AP/AT/A & +3P/AT/A

Accelerated populations on elevated temperature showed consistently higher relative investment in ovary over both photoperiod regimes (comparing between populations AP/+5T/A & AP/AT/A Fig. 4.9 and +3P/+5T/A & +3P/AT/A Fig 4.10 Analysis of Covariance $p<0.001$).

Figure 4.8 Regressions of GSI (arcsine transformed) (see text for definition) on fork-length (mm) of parr from populations 12P/AT/S (o, solid line) and AP/AT/S (broken line), Experiment 3, during January.

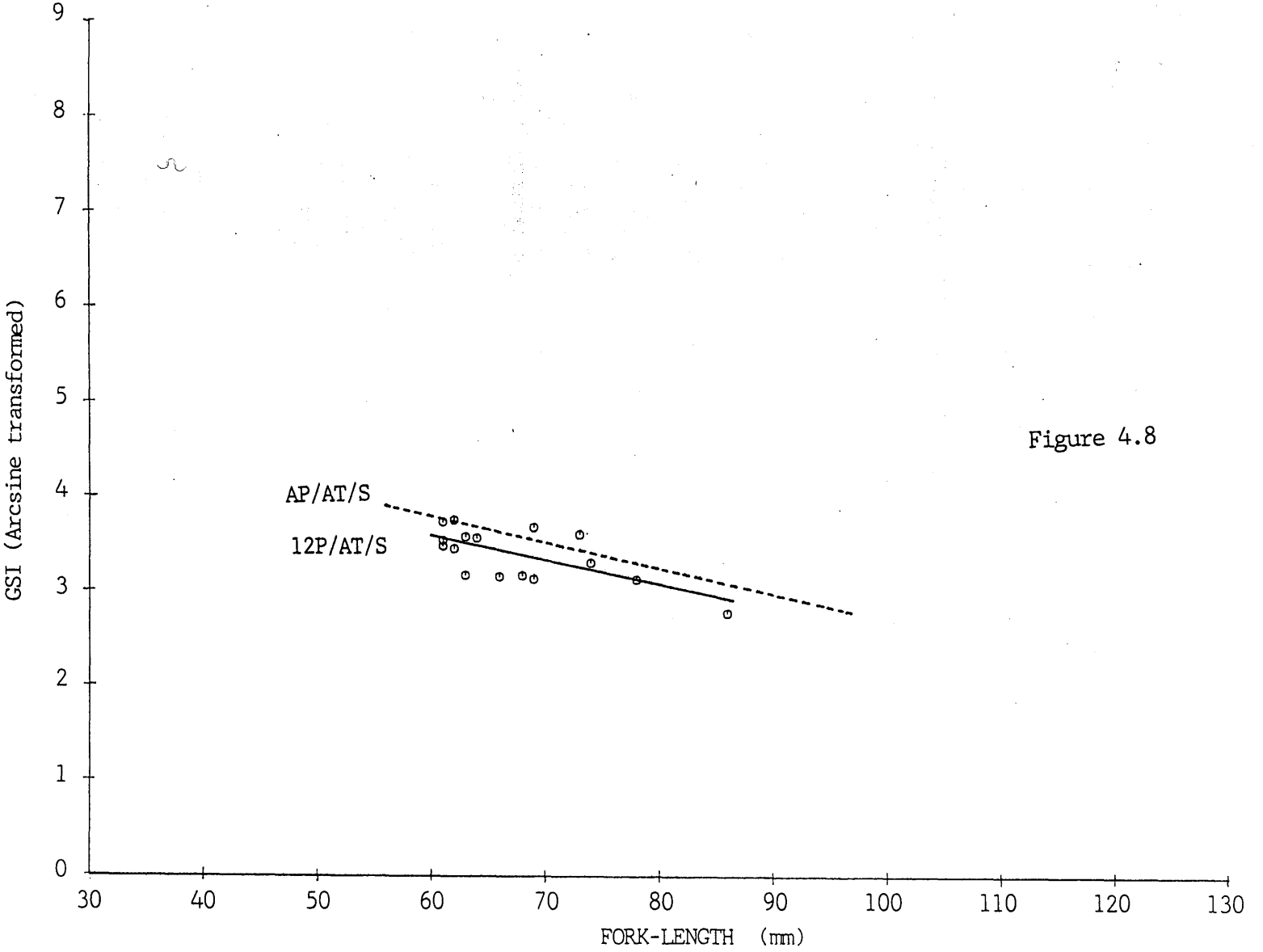

GSI (Arcsine transformed)
FORK-LENGTH (mm)
AP/AT/S
12P/AT/S
0
1
2
3
4
5
6
7
8
9
30
40
50
60
70
80
90
100
110
120
130

Figure 4.8

Figure 4.9 Regressions of GSI (arcsine transformed) (see text for definition) on fork-length (mm) of parr in populations AP/+5T/A (o) and AP/AT/A (Δ), Experiment 3, during January.

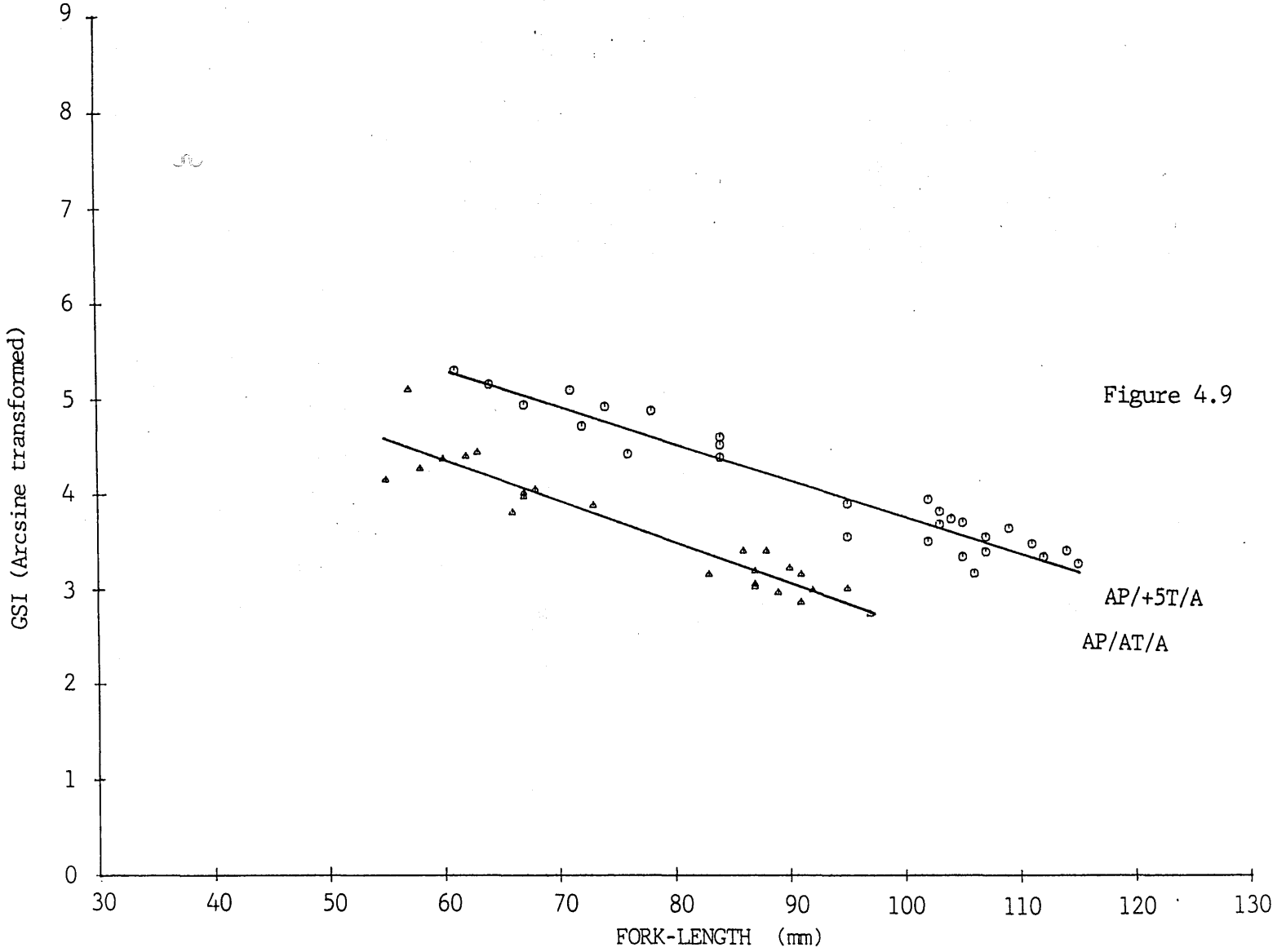

GSI (Arcsine transformed)
9
8
7
6
5
4
3
2
1
0
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)
AP/+5T/A
AP/AT/A

Figure 4.9

Figure 4.10 Regressions of GSI (arcsine transformed) (see text for definition) on fork-length (mm) of parr from populations +3P/+5T/A (o) and +3P/AT/A (Δ), Experiment 3, during November (= "perceived" January).

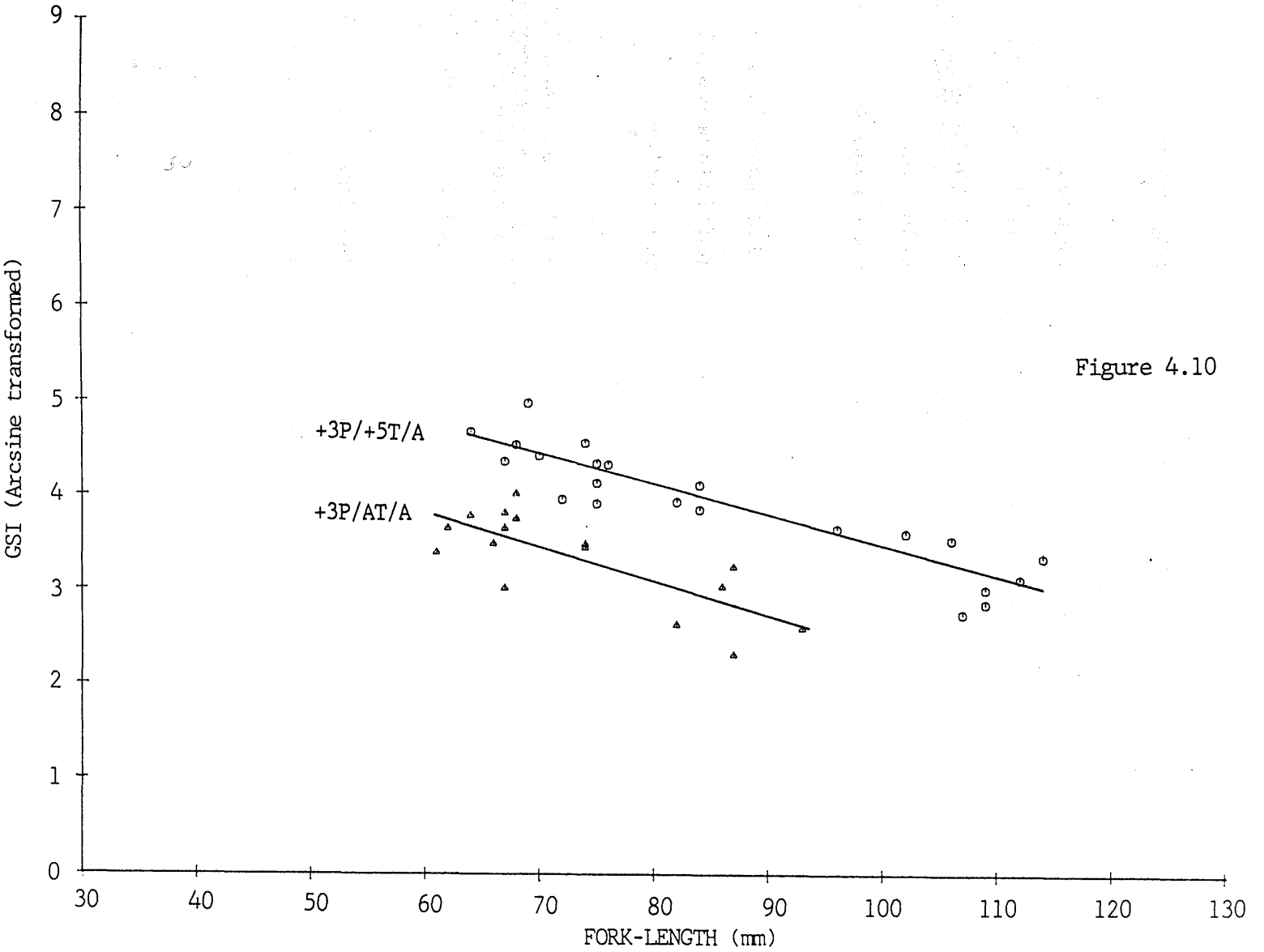

GSI (Arcsine transformed)
FORK-LENGTH (mm)
+3P/+5T/A
+3P/AT/A
0
1
2
3
4
5
6
7
8
9
30
40
50
60
70
80
90
100
110
120
130

Figure 4.10

Effect of Advanced (+3 months) Photoperiod:

Experimental populations: +3P/+5T/A & +3P/AT/A

Control populations: AP/+5T/A & AP/AT/A

Advanced photoperiod decreased the relative investment in ovary over both temperature regimes of the accelerated incubation group (comparing AP/+5T/A Fig. 4.9 with +3P/+5T/A Fig. 4.10 and AP/AT/A Fig 4.9 with +3P/AT/A Fig. 4.10 Analysis of Covariance $p<0.001$).

Effect of Constant (12 hrs) Light Regime:

Experimental population: 12P/AT/A

Control population: AP/AT/A

Relative investment in ovary in population 12P/AT/A (Fig. 4.11) did not differ significantly from that of the control on an ambient photoperiod regime.

Effect of Accelerated Incubation:

Experimental population: AP/AT/A

Control population: AP/AT/S

Comparing accelerated and standard incubation groups on ambient light and temperature regimes shows that the relationship between GSI and fork-length differs between these two groups (Fig. 4.6 & 4.9). The slope of the

Figure 4.11 Regressions of GSI (arcsine transformed) (see text for definition) on fork-length (mm) of parr from populations 12P/AT/A (o, solid line) and AP/AT/A (broken line), Experiment 3, during January.

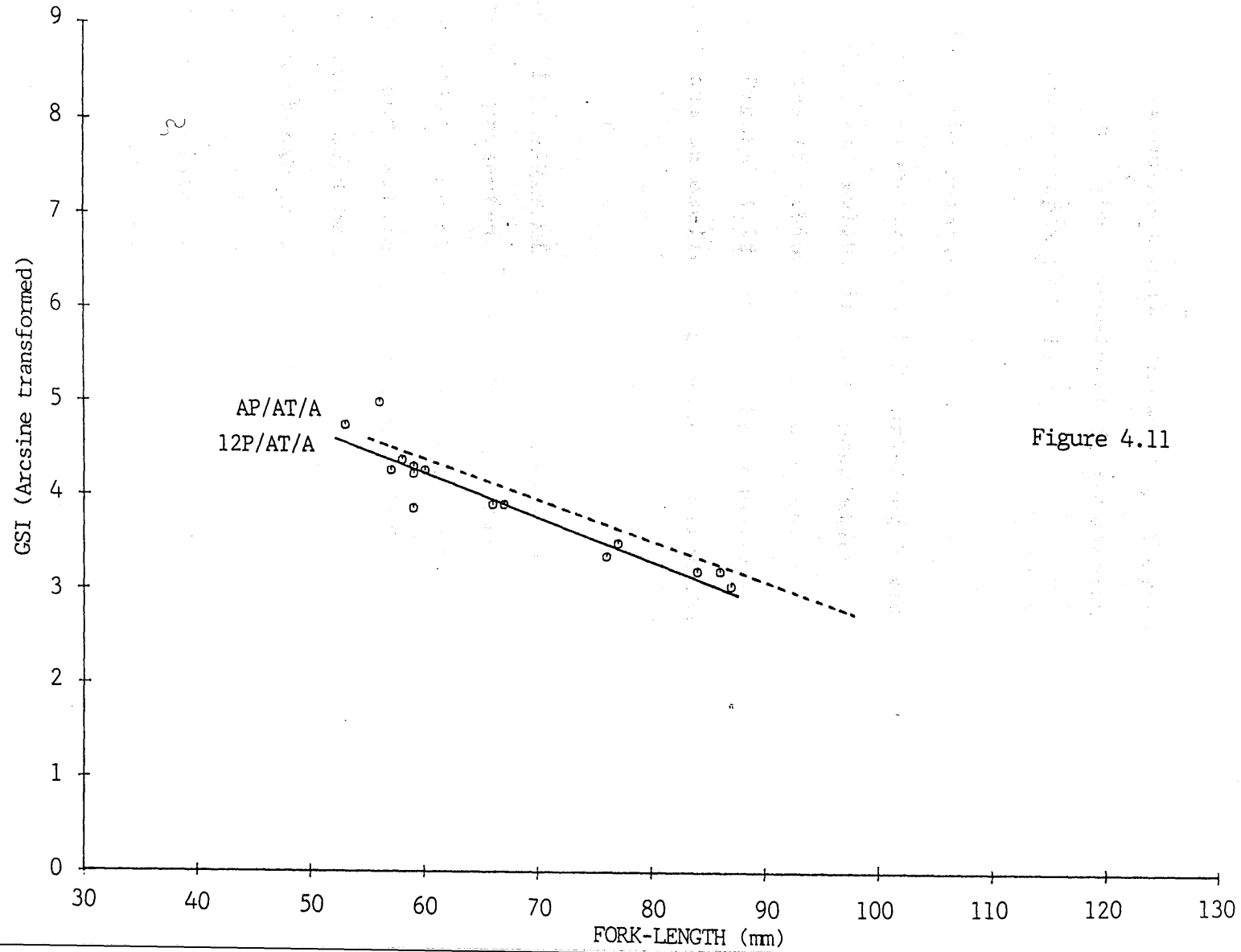

GSI (Arcsine transformed)
FORK-LENGTH (mm)
AP/AT/A
12P/AT/A
0
1
2
3
4
5
6
7
8
9
30
40
50
60
70
80
90
100
110
120
130

Figure 4.11

regression being relatively steeper in the accelerated incubation group indicating a greater reduction in relative ovarian investment with increasing length in "perceived January" in the accelerated population. (Analysis of Covariance - slopes $p<0.01$).

Length weight relationship:

As with experiments 1 & 2 fork-length was closely related to body (wet) weight in all populations. There were no differences in this relationship between populations or sexes. Figure 4.12 shows the cumulated data from all populations on cyclic temperature regimes from experiment 3.

4.3.3 The Nature of Differential Ovarian Investment

Data presented here clearly show that there is differential investment in ovary at any given size both between and within populations. Differences in ovarian investment could be achieved by:

1) a change in oocyte size
2) a change in oocyte number
3) a change in the proportion of non-reproductive ovarian tissue

Initial examination of histological sections showed that ovary consisted primarily of oocytes, with only relatively small proportions of non-reproductive tissue. There were no

Figure 4.12 Fork-length (mm), parr weight (g) (wet) curve. Cumulated data from all populations in Experiment 3 during "perceived" January.

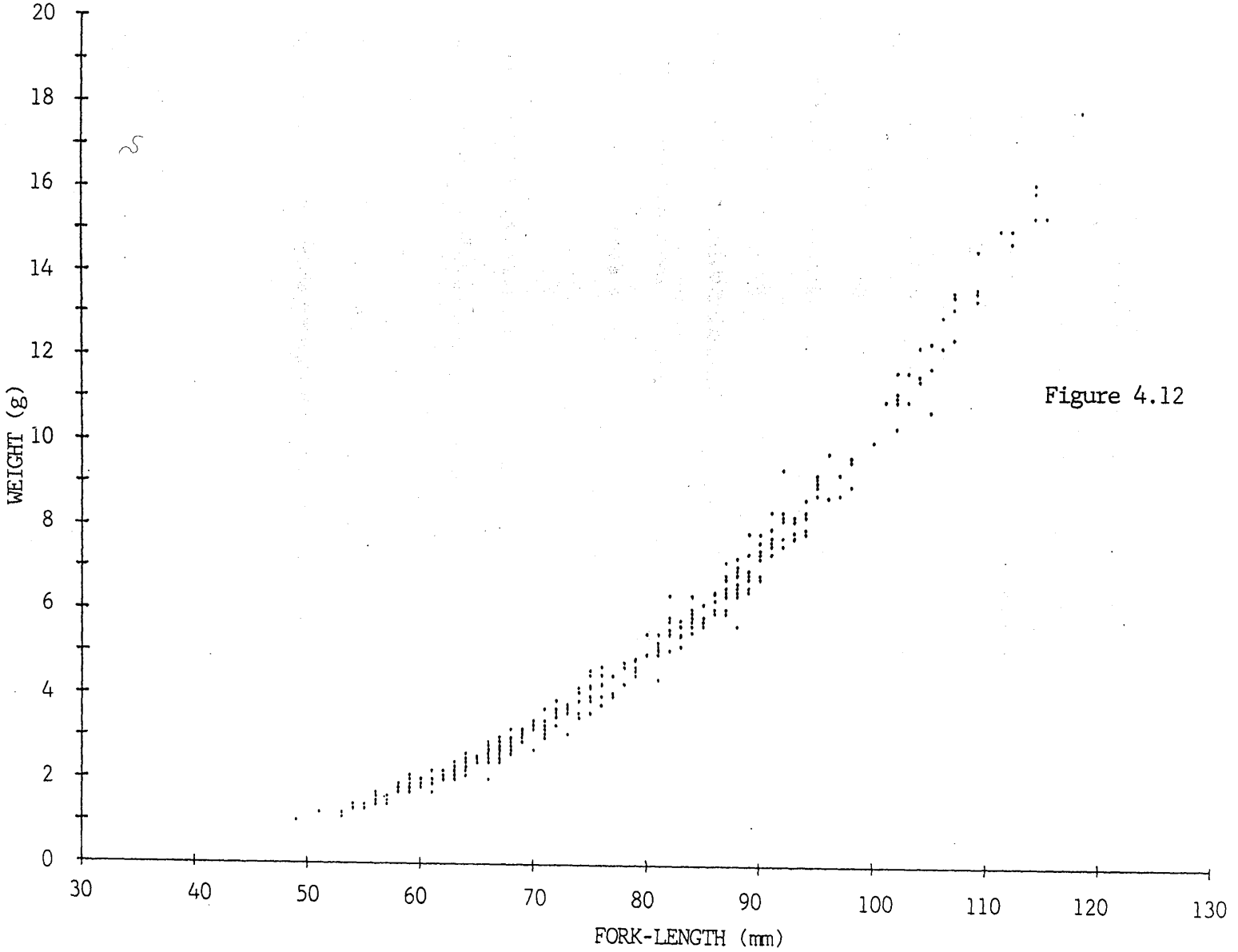

20
18
16
14
12
10
8
6
4
2
0
WEIGHT (g)
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)

Figure 4.12

obvious observed differences between populations in the proportion of non-reproductive tissue.

Thus it was assumed that a change in the proportion of non-reproductive tissue was not the primary cause of differential ovarian investment. To investigate this further the mean oocyte diameter of those fish sacrificed for histological examination in Experiments 1 & 2 was estimated.

Correlation analysis of oocyte diameter against fork-length suggested that there may be a relationship between fork-length and oocyte diameter within populations ($r = 0.06 - 0.997$). However owing to very small sample sizes these correlations were not significant, except in populations +3P/AT Experiment 1 and -2P/+2T Experiment 2 ($p < 0.05$).

Initial examination also suggested that the relationship between oocyte diameter and fish length may differ between populations, however small sample sizes made any firm conclusions impossible. Hence a more extensive examination of oocyte diameters was made during the course of Experiment 3.

EXPERIMENT 3

All populations showed a linear increase in total ovary (wet) weight with fish fork-length ($r = 0.770 - 0.959$ $p < 0.001$) Fig 4.13.

Similarly all groups showed a linear relationship

Figure 4.13 Regression of gross ovary weight on fork-length of parr from population AP/+5T/A, Experiment 3, during January, ($r = 0.950$, $p<0.001$).

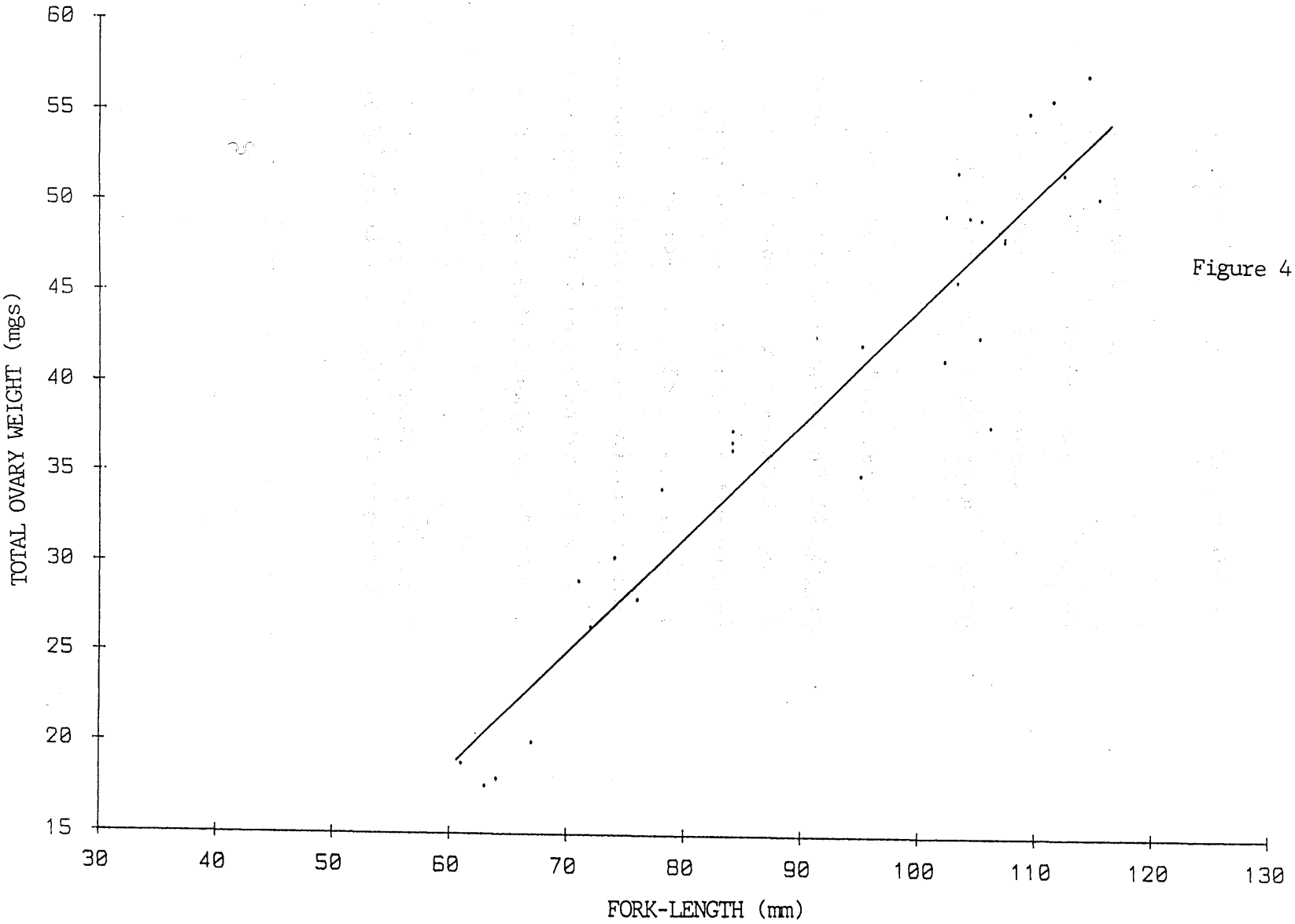

TOTAL OVARY WEIGHT (mgs)
60
55
50
45
40
35
30
25
20
15
30
40
50
60
70
80
90
100
110
120
130
FORK-LENGTH (mm)

Figure 4.13

between fork-length and oocyte diameter (r= 0.878 - 0.960 $p<0.05$ population -3P/+2T/S, $p<0.001$ all other populations) (except the UMGs of populations AP/+5T/A and AP/AT/A).

Standard Incubation Groups

The rate of oocyte diameter increase with fish fork-length was found to be similar in all groups (Analysis of Covariance slopes - no significant differences).

Effect of Elevated (+2°C) Temperature:

Experimental populations: AP/+2T/S & -3P/+2T/S

Control populations: AP/AT/S & -3P/AT/S

Both populations on elevated temperature had a larger mean oocyte diameter for any given length than the corresponding populations on ambient temperature (comparing population AP/+2T/S with AP/AT/S (Fig. 4.14) and -3P/+2T/S with -3P/AT/S (Fig. 4.15) Analysis of Covariance - elevation $p<0.001$)

Effect of Retarded (-3 months) Photoperiod:

Experimental populations: -3P/+2T/S & -3P/AT/S

Control populations: AP/+2T/S & AP/AT/S

Both populations on retarded photoperiod regimes had relatively larger mean oocyte diameters for a given fork-length than the equivalent groups on ambient

Figure 4.14 Regressions of oocyte diameter (μm) on fork-length (mm) of parr from populations AP/+2T/S (o) and AP/AT/S (Δ), Experiment 3, during January.

Figure 4.15 Regressions of oocyte diameter (μm) on fork-length (mm) of parr from populations -3P/+2T/S (o) and -3P/AT/S (Δ), Experiment 3, during April (= "perceived" January.

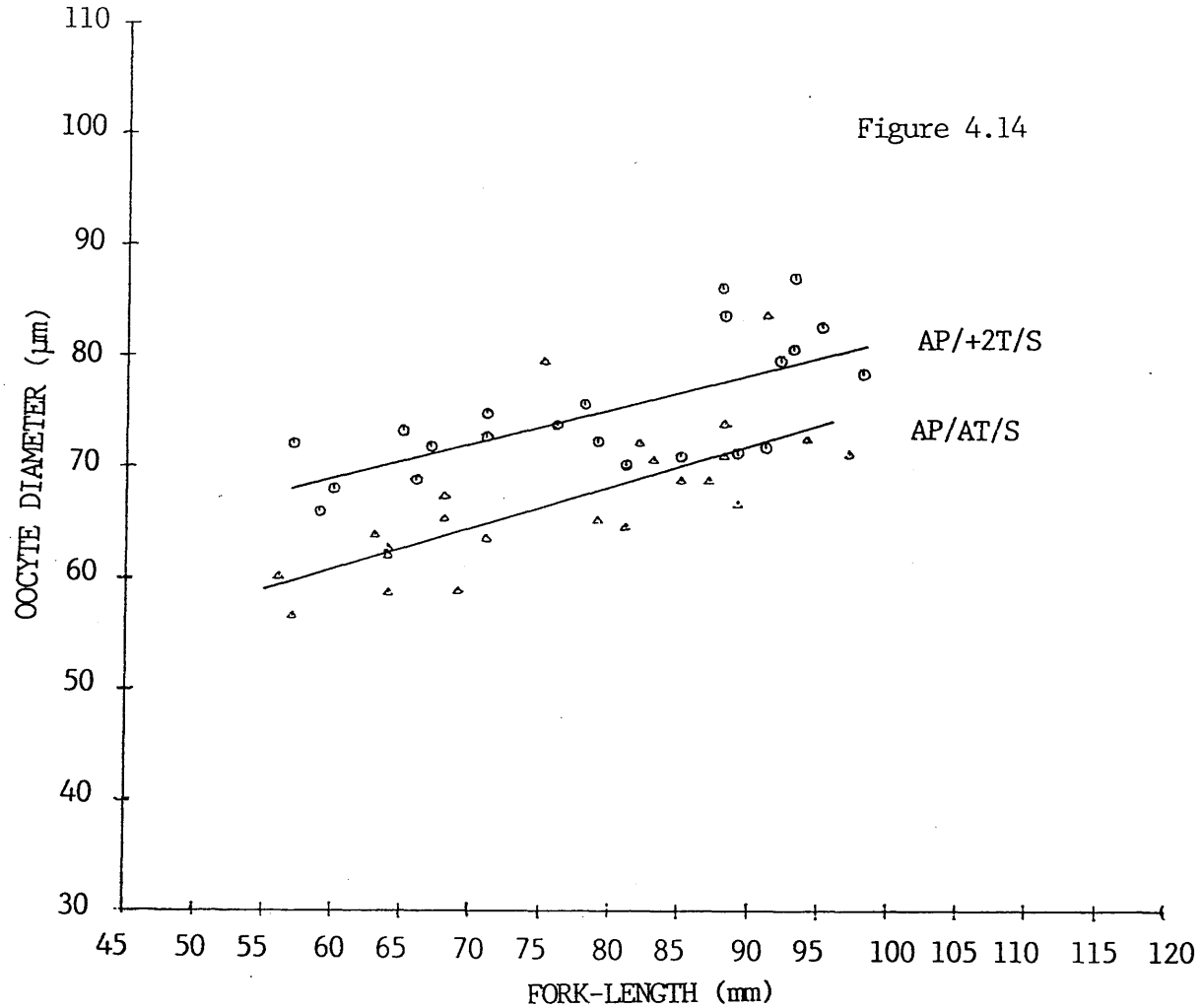


Figure 4.14

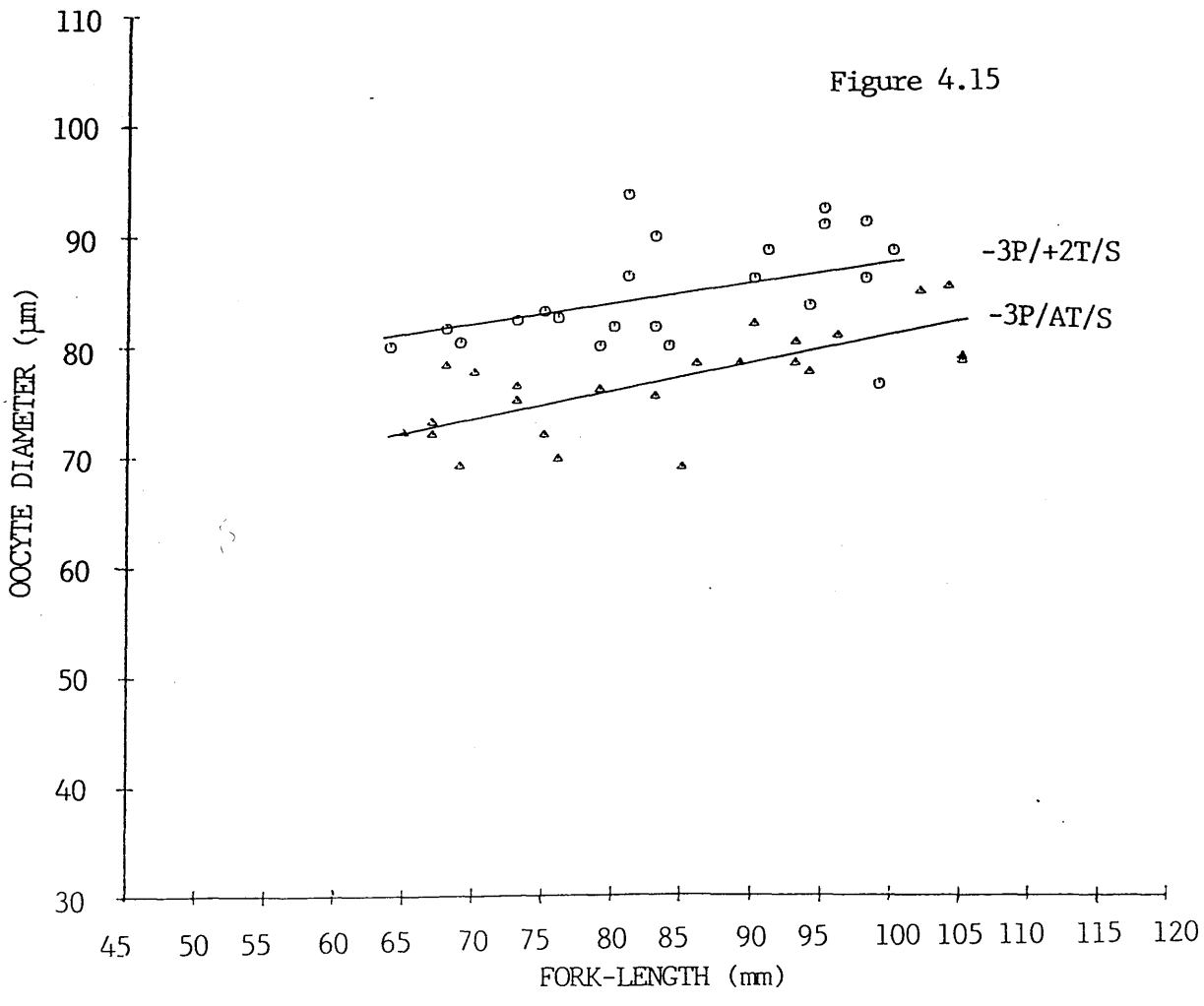


Figure 4.15

photoperiod (comparing population -3P/+2T/S (Fig 4.15) with AP/+2T/S (Fig. 4.14) and -3P/AT/S (Fig. 4.15) with AP/AT/S (Fig. 4.14) Analysis of Covariance - elevation $p < 0.001$).

Effect of Constant (12 hrs) Daylength:

Experimental population: 12P/AT/S

Control population: AP/AT/S

The population on constant 12 hours daylength had significantly larger oocytes for a given length than the equivalent group on ambient photoperiod regime (comparing 12P/AT/S with AP/AT/S (Fig. 4.16) Analysis of Covariance - elevation $p<0.001$)

Accelerated Incubation Group

Effect of Elevated (+5°C) Temperature:

Experimental populations: AP/+5T/A & +3P/+5T/A

Control populations: AP/AT/A & +3P/AT/A

Additional heating increased the mean oocyte diameter for a given fork-length over both photoperiod regimes (comparing populations AP/+5T/A with AP/AT/A LMG (Fig. 4.17) Analysis of Covariance - elevation $p < 0.001$, UMG t-test $p < 0.001$ and +3P/+5T/A and +3P/AT/A (Fig. 4.18) Analysis of Covariance - elevation $p<0.001$).

Figure 4.16 Regression of oocyte diameter (μm) on fork-length (mm) of parr from populations 12P/AT/S (o, solid line) and AP/AT/S (broken line), Experiment 3, during January.

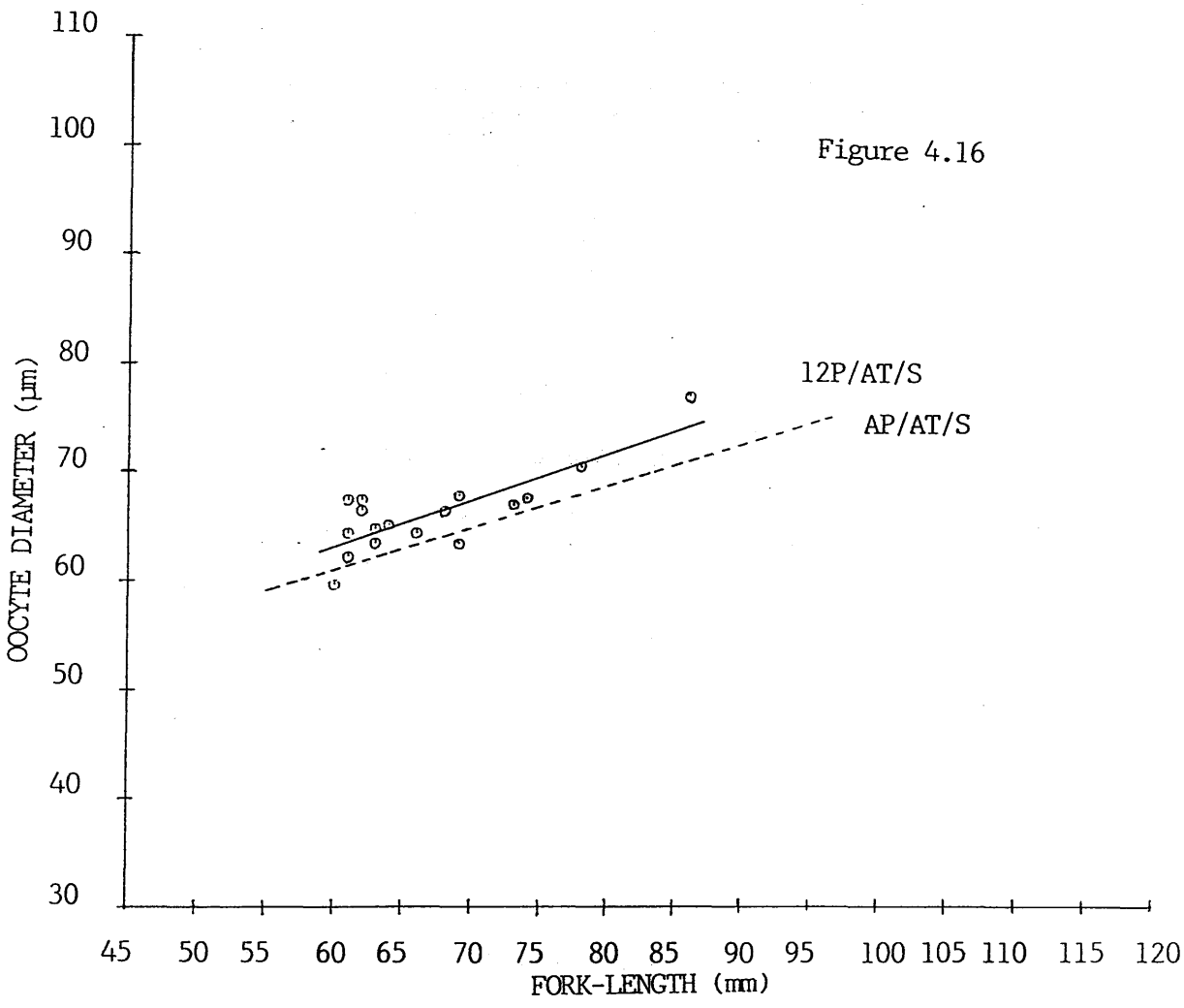

110
100
90
80
70
60
50
40
30
OOCYTE DIAMETER (µm)
12P/AT/S
AP/AT/S
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
FORK-LENGTH (mm)

Figure 4.16

Figure 4.17 Oocyte diameter (μm) vs. parr fork-length (mm) in populations AP/+5T/A (o) and AP/AT/A (Δ), Experiment 3, during January, (regression LMG fish only, see text for explanation).

Figure 4.18 Regressions of oocyte diameter (μm) on fork-length (mm) of parr from populations +3P/+5T/A (o) and +3P/AT/A (Δ), Experiment 3, during November (= "perceived" January).

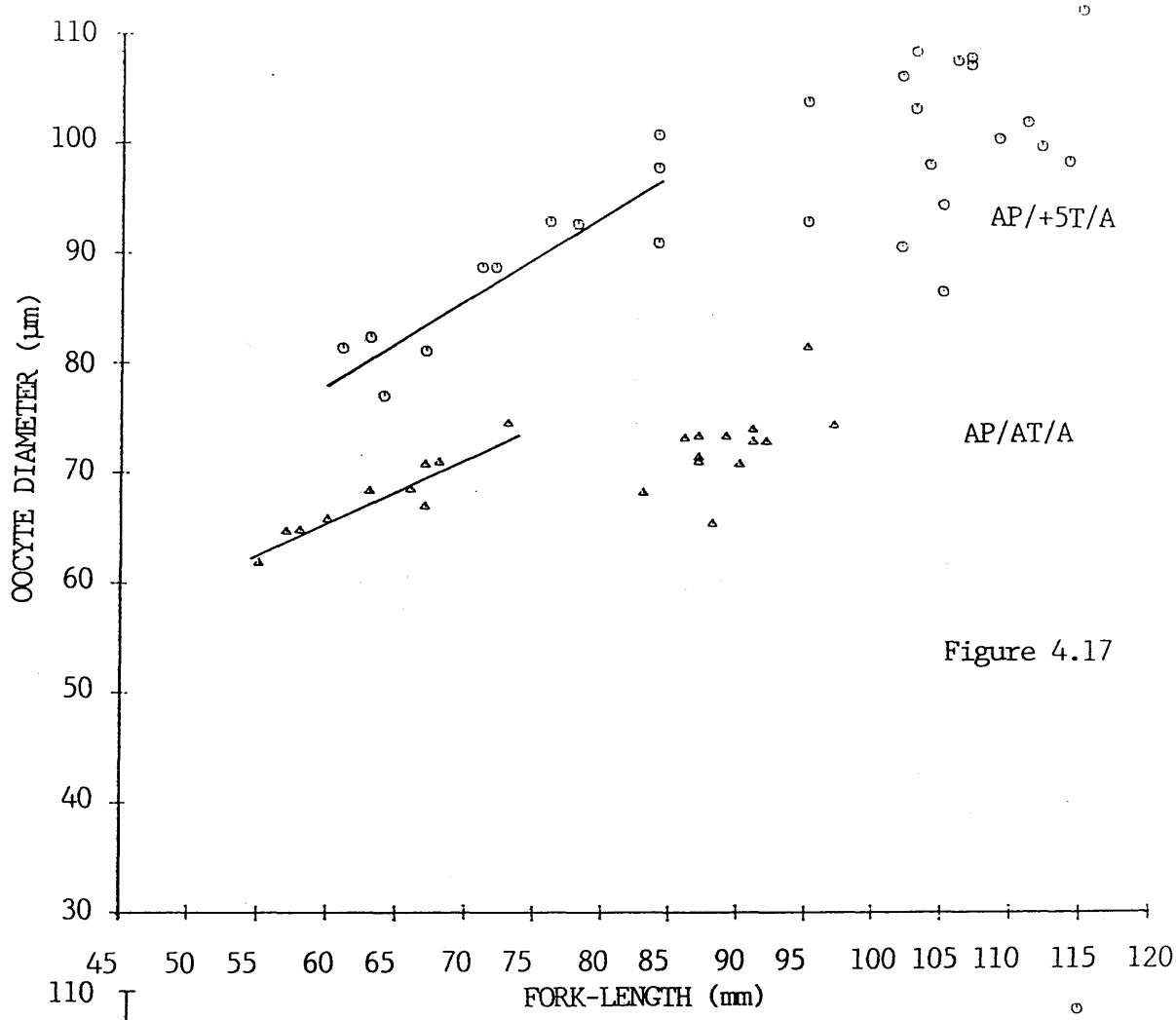


Figure 4.17

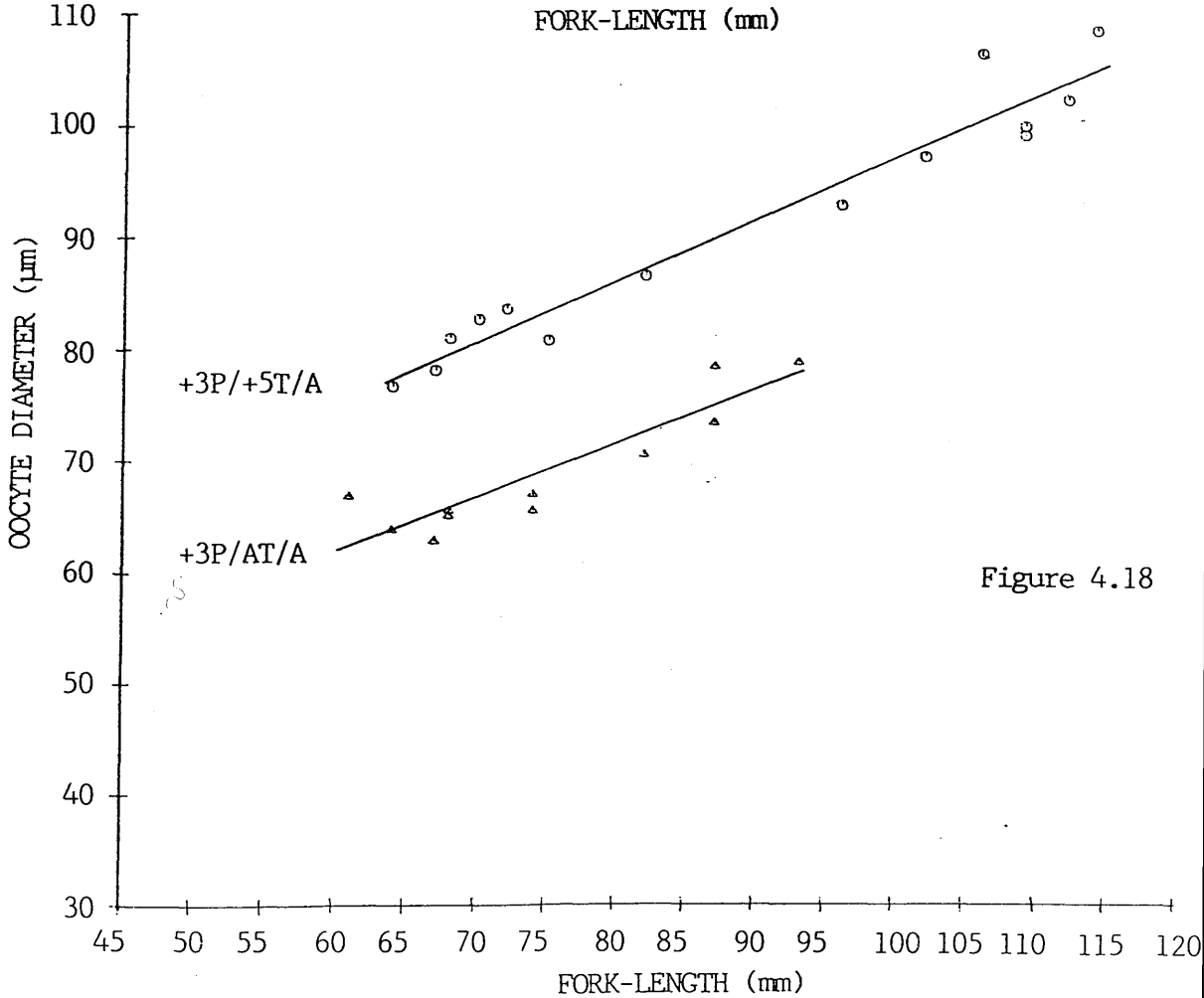


Figure 4.18

Effect of Advanced (+3 months) Photoperiod regime:

Experimental populations: +3P/+5T/A & +3P/AT/A

Control populations: AP/+5T/A & AP/AT/A

Advanced photoperiod decreased the mean oocyte diameter for any given fork-length (comparing populations +3P/+5T/A (Fig. 4.18) with AP/+5T/A (LMG only) (Fig. 4.17) and +3P/AT/A (Fig. 4.18) with AP/AT/A (LMG only) (Fig. 4.17) Analysis of Covariance $p<0.005$).

Effect of Constant (12 hour) Daylength:

Experimental population: 12P/AT/A

Control population: AP/AT/A

Oocyte diameter was found to be smaller for any given fork-length in the population on fixed 12 hours daylength, than in the population on ambient photoperiod (at ambient temperature) (Fig. 4.19 Analysis of Covariance $p < 0.01$ - elevation).

Effect of Accelerated Incubation:

Experimental population: AP/AT/A

Control population: AP/AT/S

Comparing the accelerated incubation group on ambient

Figure 4.19 Regressions of oocyte diameter (μm) on fork-length (mm) of parr from populations 12P/AT/A (o, solid line) and AP/AT/A (broken line), Experiment 3, during January.

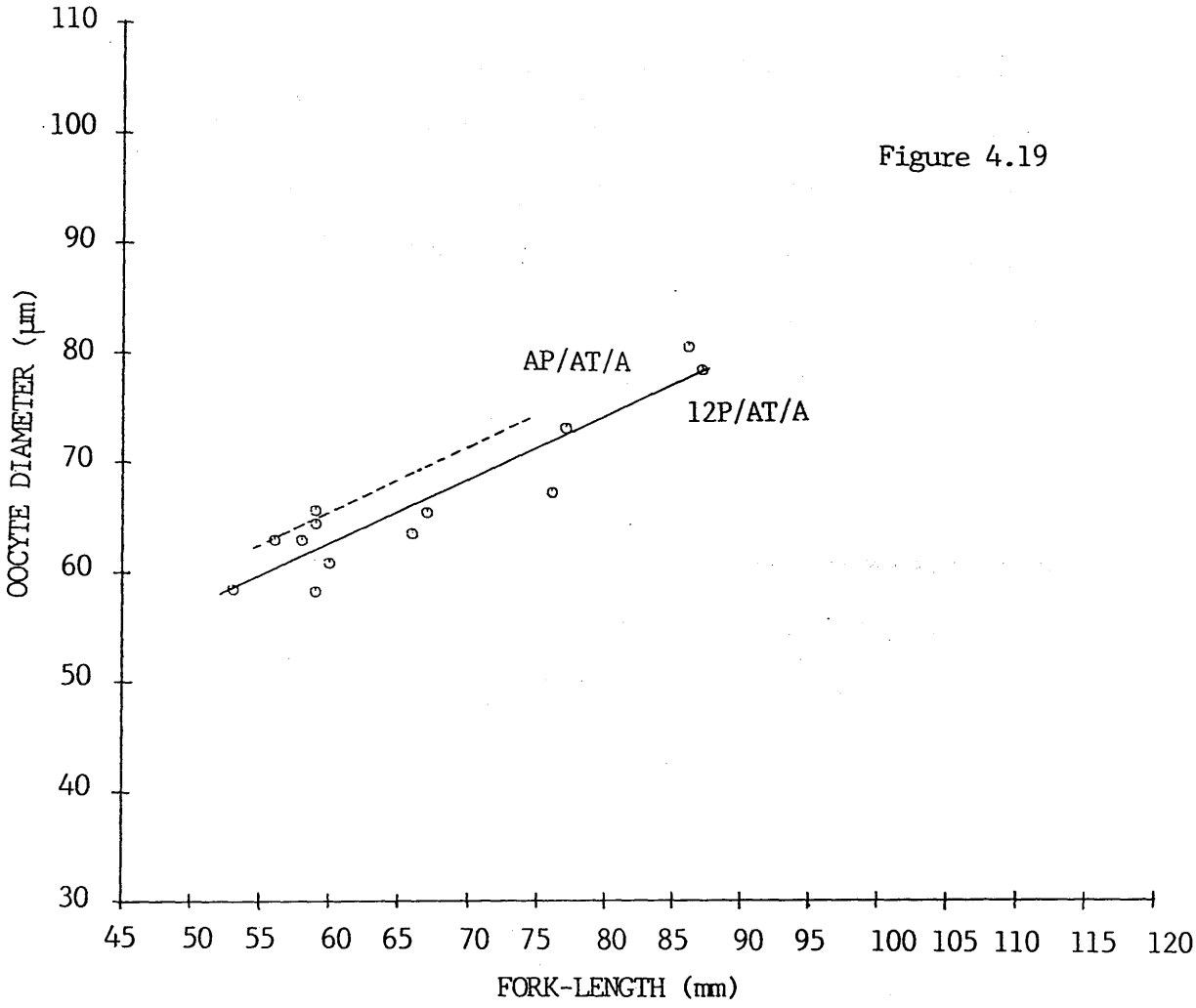

110
100
90
80
70
60
50
40
30
OOCYTE DIAMETER (µm)
AP/AT/A
12P/AT/A
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
FORK-LENGTH (mm)

Figure 4.19

temperature and photoperiod regimes (Fig. 4.17) with the equivalent group hatched under standard conditions (AP/AT/S Fig. 4.14) shows that there is no difference between the rate of oocyte diameter increase with fork-length (Analysis of Covariance - slopes no significant difference). However for any given fork-length the oocyte diameter was relatively larger in the accelerated incubation population, than the standard incubation group (Analysis of Covariance $p < 0.01$).

Energetic Investment in Ovary

The mean calorific value of the immature ovary found in these fish was found to be 22.0 $\pm$ 1.5 J/mg (mean (dry wt.) $\pm$ standard error). This value was slightly (7.3%), but not significantly higher than the energetic value of parr somatic tissue (20.5 $\pm$ 1.0 J/mg).

Results presented here indicate that the observed differential investment in ovary with fish length, within populations can be, at least partly, explained by an increase in oocyte size. However in populations AP/+5T/A and AP/AT/A the observed correlation between gross ovary weight and fork-length ($r = 0.937$ & 0.948 $p < 0.001$) is mirrored by a similar linear relationship between fork-length and oocyte diameter only in the LMG of these two populations (Fig. 4.17).

Ovary consists mainly of oocytes and oocyte weight is

proportional to its volume. To examine this apparent disjunction in the oocyte diameter, fish length relationship in these two groups, oocyte volume was calculated for the 4 populations on cyclic photoperiod and temperature regimes in the accelerated incubation group of experiment 3.

Correlation analysis of oocyte volume with parr fork-length showed a strong relationship in populations +3P/+5T/A and +3P/AT/A ($r = 0.957$ and 0.933 $p < 0.001$ Fig. 4.20) and a highly significant correlation in the LMGs of populations AP/+5T/A and AP/AT/A ($r = 0.904$ & 0.941 respectively $p < 0.001$ Fig. 4.21). However there was no correlation between oocyte volume and fish length in the UMG of population AP/+5T/A ($r=0.266$ not significant) and only slight correlation ($r=0.670$ $p<0.02$) in population AP/AT/A UMG. Fig. 4.21 shows that there is considerable disjunction in the fork-length, oocyte diameter relationship between these two populations. The majority of fish in the UMG showing a lower oocyte volume than would be suggested by extrapolation of the LMG fork-length oocyte volume regression.

In addition an estimate was made of the magnitude of the change in oocyte size (volume) resulting from increased water temperature and advanced photoperiod cycle for a specific size. This was achieved by comparing the ovary weight (obtained from the regression equation) of the experimental group (+5T or +3P) with the control, to obtain

Figure 4.20 Regressions of oocyte volume ($\mu m^3 x 10^5$) on fork length (mm) of parr from populations +3P/+5T/A (o) and +3P/AT/A (Δ), Experiment 3, in November (= "perceived" January).

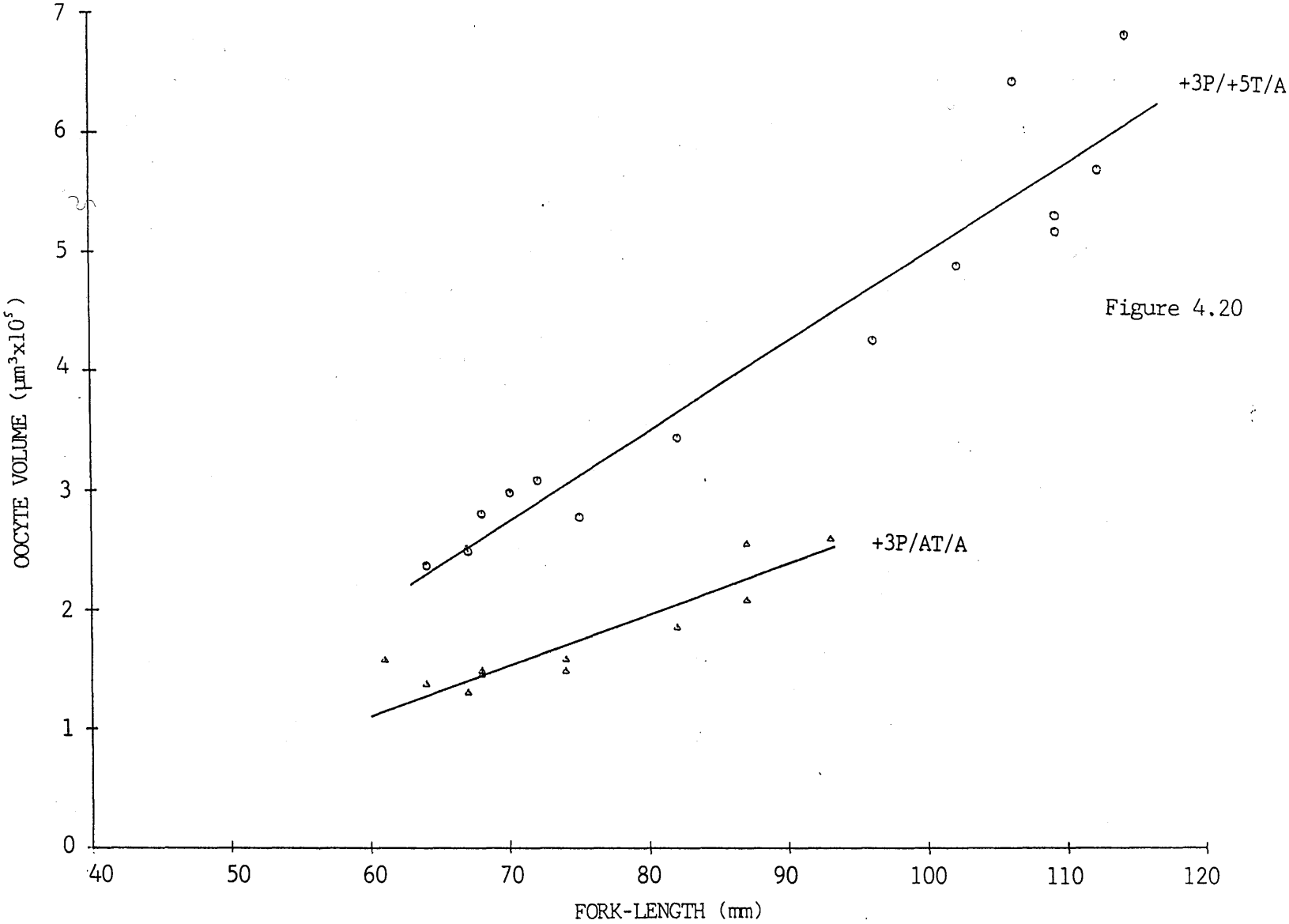

7
6
5
4
3
2
1
0
OOCYTE VOLUME ($\mu m^3 x 10^5$)
40
50
60
70
80
90
100
110
120
FORK-LENGTH (mm)
+3P/+5T/A
+3P/AT/A

Figure 4.20

Figure 4.21 Oocyte volume ($\mu m^3 x 10^5$) vs. fork-length (mm) of parr from populations AP/+5T/A (o) and AP/AT/A (Δ), Experiment 3, in January.

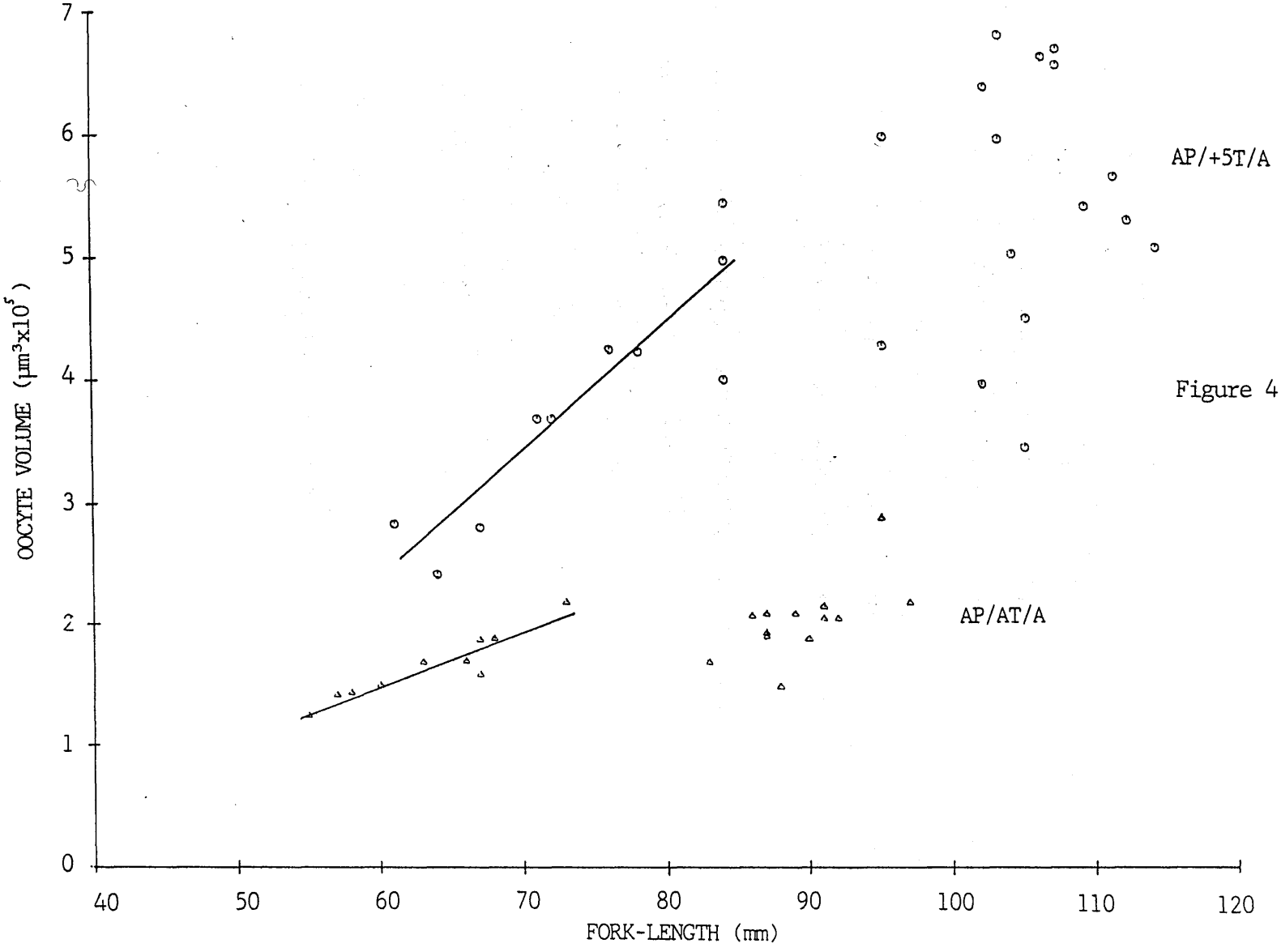

OOCYTE VOLUME ($\mu m^3 x 10^5$)
0
1
2
3
4
5
6
7
40
50
60
70
80
90
100
110
120
FORK-LENGTH (mm)
AP/+5T/A
AP/AT/A

Figure 4.21

a percentage increase change attributable to the particular treatment, for a specific fork-length (70mm was chosen). An identical calculation was performed using the regression equations for cell volume in these 4 populations (UMGs of AP/+5T/A and AP/AT/A were not included in this analysis).

Table 4.2 shows the percentage change in oocyte volume and in total ovary weight attributable to the effects of elevated temperature and advanced photoperiod. In all LMG groups total ovary weight and oocyte volume increases are very similar, indicating that the environmentally induced changes in ovary weight are likely to be the result of increased oocyte size alone. However the disjunction in the regression of cell volume on fork-length in populations AP/+5T/A and AP/AT/A suggests that in the UMGs of these two populations increasing ovary weight with fish length cannot be completely accounted for by an increase in the size of the oocytes, thus implying an increase also in the number of developing oocytes at this stage.

Treatment	Population	Total Ovary Wt. (mgs)	Oocyte Volume $\mu m \times 10^3$	Ovary Wt. Change (%)	Oocyte Size Change (%)
+5T	+5T/AP/A	25.1	3.44	173	178
	AT/AP/A	14.5	1.93		
+5T	+5T/+3P/A	20.6	2.75	187	179
	AT/+3P/A	11.0	1.54		
+3P	+5T/+3P/A	20.6	2.75	82	80
	+5T/AP/A	25.1	3.44		
+3P	AT/+3P/A	11.0	1.54	76	80
	AT/AP/A	14.5	1.93		

TABLE 4.2 Proportional changes in the total ovary weight and oocyte size (calculated from regression equations at 70mm fork-length) attributable to elevated +5°C temperature and +3 months out-of-phase photoperiod.

4.4 DISCUSSION

Given relatively good growth opportunity prior to a supposed maturation window in early spring it is possible to induce sexual maturation in underyearling male Atlantic salmon in Scotland (Adams and Thorpe in press b). The rate of male maturation in these experiments was very low with around 7.5% of males (8 individuals) in the population receiving the best growth opportunity prior to the maturation window showing increased investment in testis, of these 2 were found to have increased gonad investment (by at least 100 - 230% Table 4.1) (over the majority of immature parr in the same population) however cellular development had not occurred. A further 3 had invested approximately 200 - 400% more in testis than immature male parr. These parr also showed some spermatogenesis, however they may not have been capable of functional maturity as 0+ parr. The final 3 showed the largest testis investment 700 - 3000% larger than immature parr. This group had testis in a late stage of spermatogenesis and would be likely to be capable of breeding as 0+ parr. This rate of maturity is very low but would be expected to differ between genetically distinct families (Thorpe et al. 1983).

No mature parr were found in the control population given good growth conditions but not exposed to the photoperiod

conditions found during the supposed maturation window. However this population did produce high proportions in the UMG in their first winter (Adams and Thorpe in press a, Chapter 3). This suggests that the physiological decision to initiate maturation is taken prior to that initiating smolting, and complies with predictions of the model of Thorpe (1986). This also confirms the importance of the annual photoperiod cycle as a timing mechanism for initiating maturation (Scott 1979, Lam 1983).

Eriksson and Lundqvist (1982) demonstrated a strong endogenous circannual rhythm in male parr maturation and suggested that photoperiod acted as an exogenous synchroniser of this rhythm. It has already been shown that attempts to synchronize this endogenous rhythm with photoperiod regimes outwith the range of entrainment leads to asynchrony between photoperiod and the endogenous rhythm (Chapter 3 and Villarreal et al. in press). It is likely that this is occurring in population -3P/+2T/S, where no mature males were found despite the good growth opportunity afforded by relatively high water temperatures, prior to the maturation window. Parr showing increased testicular investment were all found in the lower portion of the UMG. This supports the finding of Saunders et al. (1982) that maturing parr were originally amongst the fastest growers but subsequent to a physiological decision to mature they

grew more slowly.

It has been shown here that the effects of growth opportunity are not restricted solely to sexual maturation, but that gonadal investment in non-maturing females also varies with growth opportunity. Decreasing GSI with increasing fork-length within populations of the same age shown here, has also been reported in salmon parr by Villarreal and Thorpe (1985). This relative reduction in investment in ovarian tissue with fish size implies that ovarian and somatic tissue is not laid down synchronously in the prematuration stages.

A comparison of the energetic cost of such investment showed that immature ovary (perinuclear/primary oocyte, stage II Sutterlin and MacLean 1984) does not represent a significantly greater investment than somatic tissue, weight for weight.

The increased growth opportunity afforded by elevated water temperature was found to increase the relative investment in ovary, for a given fish size. The long growth periods prior to "perceived" January (as determined by photoperiod) conferred by retarded photoperiod also increased ovarian investment for a given size.

Advanced photoperiod had the reciprocal effect of

reducing the relative ovarian investment.

Examination of the nature of this increased investment in ovary showed that the increasing ovary weight with fork-length found in all populations corresponded with an increasing oocyte diameter with fork-length in most groups. Changes in the relative investment between groups on differing environmental treatments was shown to be linked to similar changes in oocyte size in most groups. Indicating that changes in oocyte size were responsible for differential ovarian investment between treatments in most groups. However in two of the fastest growing groups (the UMGs of populations AP/+5T/A and AP/AT/A of experiment 3) increasing gross ovary weight with fish size could not be accounted for wholly by increasing cell size (volume). This implies that increased ovarian investment in these fish is also the result of increased number of developing oocytes. A similar tissue growth pattern as been reported in Atlantic salmon parr muscle. Brooks (1987) has found that parr less than 6 cms long achieve muscle growth by both fibre hyperplasia and hypertrophy, however above 6 cms long muscle growth is predominantly by fibre hyperplasia.

Growth opportunity prior to the first breeding season has been shown to influence ovarian investment in immature female parr. The greater growth opportunity conferred by

higher water temperatures or extended growth period prior to the breeding season increases the percentage of the total body weight that comprises of ovary. Larger ovary in fish exposed to good growth opportunity conditions was found to be mainly the result of larger oocytes. However the UMG of accelerated incubation parr exposed to ambient photoperiod (independently of temperature) showed evidence of an increase in oocyte numbers.

Most published literature on environmental regulation of fecundity, has tended to concentrate on such effects during the latter stages of oocyte development. Nikolskii (review 1969) concluded that rapid growth conditions led to increased fecundity. Thorpe et al. (1984) found that sea-run salmon that had developed rapidly produced a greater number of smaller eggs than slower developing fish.

Results presented here indicate that differential resource allocation into ovary can be detected at a very early stage of cellular development (prior to vitellogenesis) and can be related to environmental manipulation of photoperiod and temperature, good growth potential conditions leading to elevated ovarian investment. However much of the increased investment appears to be in the form of increased oocyte size. The adaptive value of investment in larger oocytes at this very early

developmental stage remains open to question for 2 reasons.

Firstly there is no evidence that larger (Stage II) oocytes will give rise to larger eggs.

Secondly, even if they do, there is evidence that larger egg size is not correlated with better fry survival or growth at least in Rainbow trout (Springate and Bromage 1985).

However evidence has been presented here of ovarian investment in more oocytes, in the UMG's of accelerated incubation groups on ambient photoperiod. In these fish it is more likely that the general level of number of developing oocytes may be higher. This may increase potential fecundity (Nikolskii 1969) however this background fecundity level is liable to constant modification in the light of subsequent growth conditions.

CHAPTER 5

PHOTOPERIOD EFFECTS ON PARR FEEDING RATES

5.1 INTRODUCTION

It has been argued in Chapters 3 and 4, that the effects of both photoperiod and temperature on smolting and maturation rates may be mediated mainly through their influence on growth opportunity. Photoperiod is likely to affect opportunity to feed and temperature may affect both opportunity to feed and assimilation rate (absorption and conversion efficiency).

In addition, it has been argued that a change in food intake is the first indication of the outcome of the developmental course which will be followed in the months following the smolting window in June/July. Around this time those which lose appetite and reduce intake fail to smolt in the following spring, whilst those that continue to feed heavily do smolt then (Metcalfe et al. 1986).

This chapter examines the changes in population ingestion rate around the critical period immediately following the smolting window and the effects of photoperiod on food ingestion rate at constant temperature at this time.

5.2 MATERIALS AND METHODS

To characterise the basis of growth changes occurring around the smolting window period, the food intake of parr from populations AP/15T/S and -3P/15T/S from Experiment 3, held at Rowardennan, was monitored from July until October 1987.

The monitoring technique used has been explained in detail elsewhere (Talbot and Higgins 1983), however the basis of the procedure was as follows. An X-ray opaque iron powder (particle c. 170 um) was incorporated at a concentration of c. 1 - 3% by weight into ground down EWOS salmon food pellets. The feed was dampened and the iron powder thoroughly mixed into it in the required ratio. The pellets were then reconstituted by squeezing the mixture through a 1.2mm sieve, drying them slightly and then resieving to obtain the correct pellet size.

The standard feed hopper was replaced with a hopper of labelled feed at the required time. Subsequently, after measured intervals, samples of c. 40 fish were anaesthetised in "Benzocane", and X-rayed using a Todd Research Model TR80 120 portable X-ray generator and Kodak Industrex MX plates.

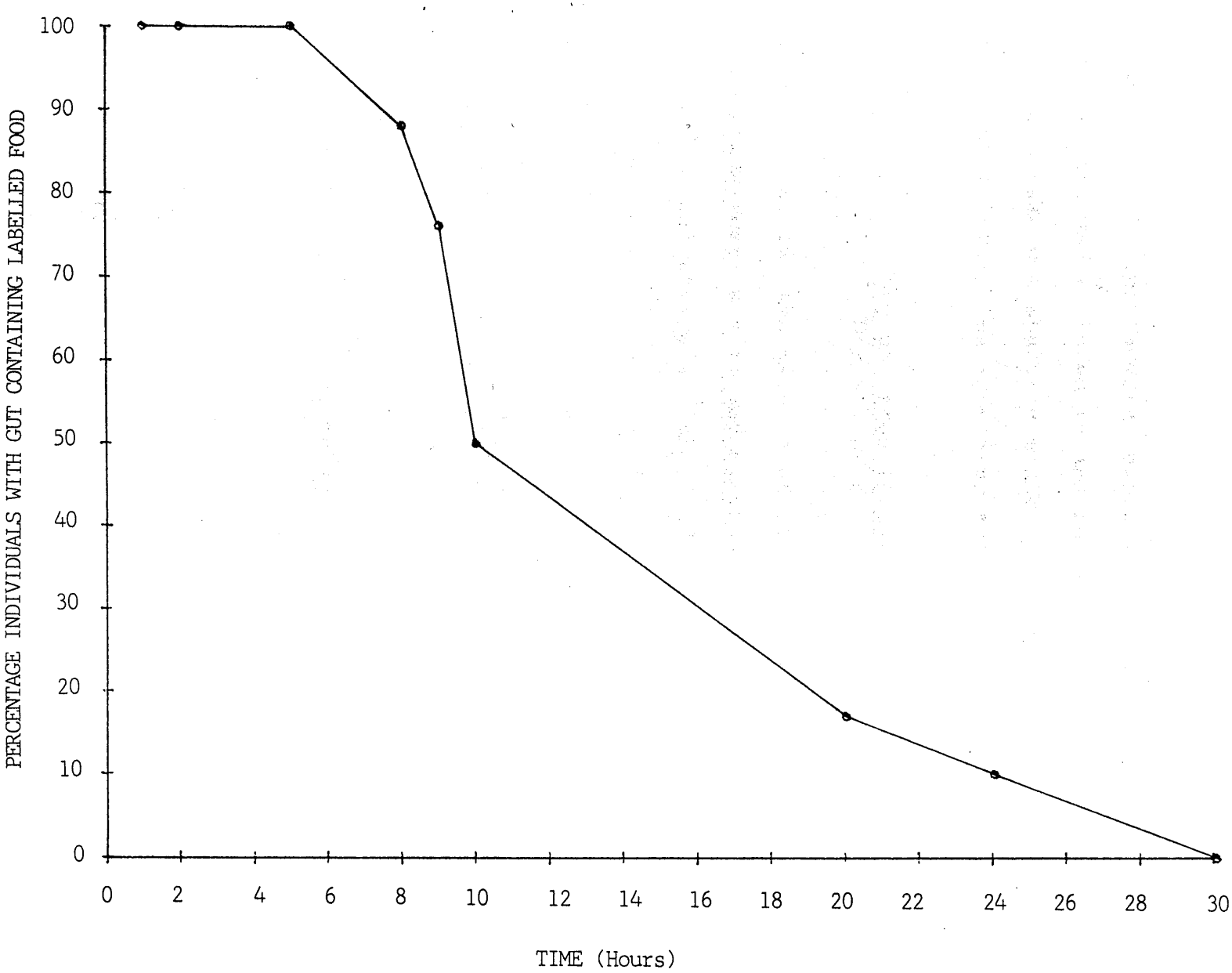
The number of iron particles in the gut of individual fish was counted from the radiograph with a hand lens on a light-table, and the fish fork-length measured.

The ingested food equivalent that these particle counts represented was then determined from a calibration curve constructed from particle counts from X-ray plates of known weights of labelled food.

To determine the total daily intake of fish in these populations it was necessary to divide the 24 hour day into artificial feeding periods. To estimate the best length of each feeding period and the time required to clear iron particles from the gut, the fish were fed labelled food for 5 hours and then put back on the standard diet. Evacuation rate was estimated at the holding temperature of 15°C.

Figure 5.1 shows the change in the proportion of fish in a sample of c. 30 whose gut contained any iron particles with time since labelled feeding. There was no reduction in the number of fish with labelled food in their gut for the first 5 hours after labelled feeding had ceased. Thus at 15°C it was deemed safe to feed the labelled diet for 4 hours before X-ray sampling, without risk of the intake estimation being confounded by evacuation of food over this period. To avoid the over estimation of intake by counting iron particles consumed during a previous labelled feeding bout, it was necessary to allow an evacuation time of at least 24 hours between labelled feeding bouts. After 24

Figure 5.1 Evacuation rate of parr held at 15°C. Percentage of individuals with labelled food in their gut against time (hours) since feeding labelled food.


PERCENTAGE INDIVIDUALS WITH GUT CONTAINING LABELLED FOOD
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (Hours)

hours only 12% of fish retained some iron particles (Fig.5.1) and these were confined to the posterior intestine. During feeding experiments particles in this region were not included in any results.

Food intake was measured monthly during the last week in the month. Intake was expressed as:

$$\frac{\text{Dry wt. food ingested}}{\text{Dry wt. of fish}} \times 100$$

this minimized the effects of fish size on meal size. All proportions were stabilized by arcsine transformations prior to statistical analysis (Sokal and Rohlf 1969).

5.3 RESULTS

Owing to the early demise (section 3.3) of populations AP/15T/S in early September and -3P/15T/S in November, food intake data were obtained only for the months July - October for population -3P/15T/S and for July alone in population AP/15T/S.

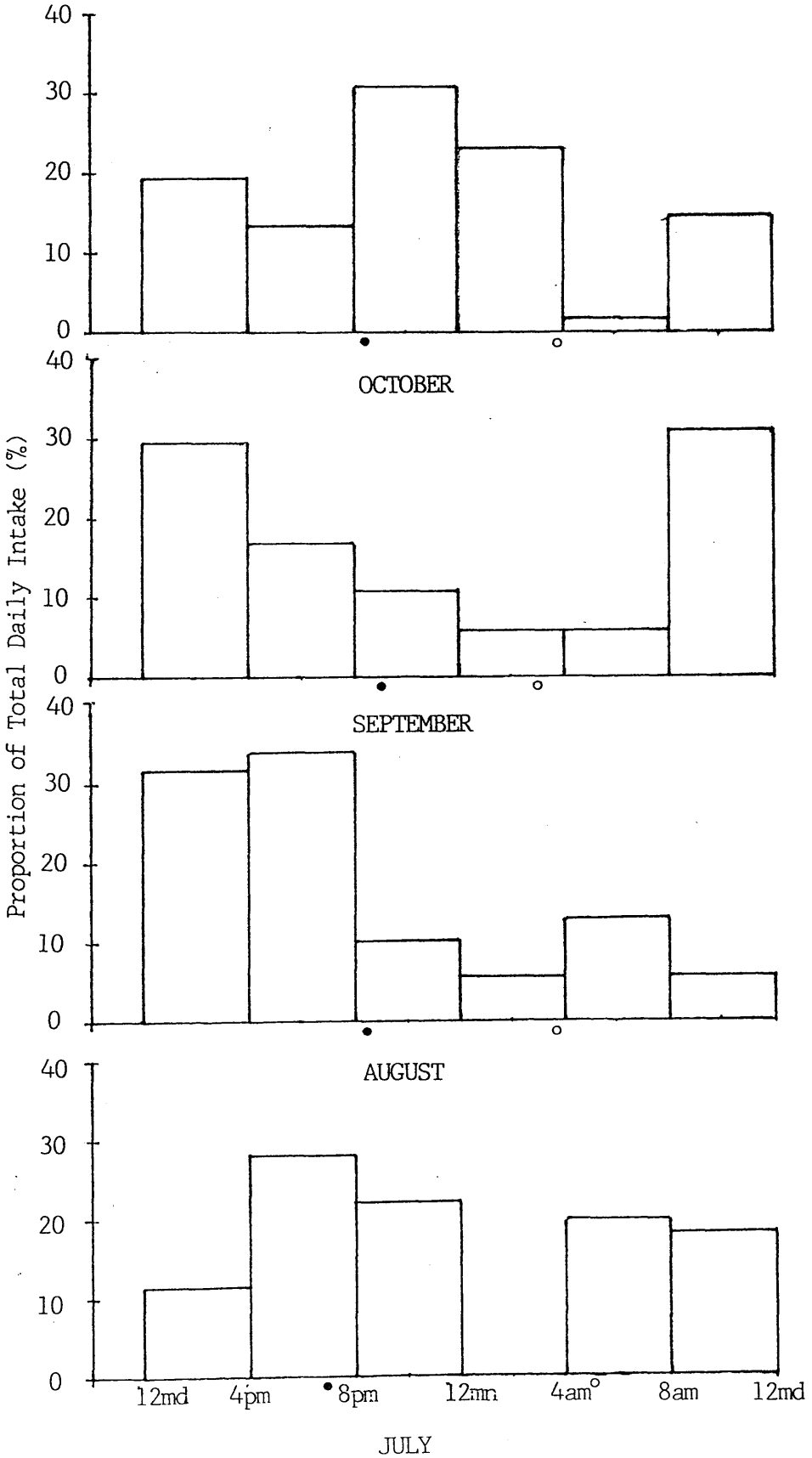
Figure 5.2 shows the percentage of the total 24 hour food intake, during each of the 6 feeding periods over the months July - October 1986, in population -3P/15T/S. Food intake patterns over the 24 hour period changed over the experimental period. During the 12 - 4am period in July (a feeding period with over 2 hours total darkness) no evidence of any food intake could be found. Fish were found to feed after sunset during periods of twilight (twilight extended throughout the night during the months of August, September and October under this 3 months retarded photoperiod regime). During August and September twilight feeding was at a lower rate than during daylight hours. There was some slight evidence for this pattern changing in October where the major proportion of the daily food intake appeared to be after sunset and before sunrise.

Food ingestion rates in July differed between populations. Population -3P/15T/S was exposed to a

Figure 5.2 Percentage of total daily intake taken during 6, 4 hour feeding periods in population -3P/15T/S over the months July - October 1986.

" o " = time of sunrise

" ● " = time of sunset


Proportion of Total Daily Intake (%)
40
30
20
10
0
OCTOBER
SEPTEMBER
AUGUST
JULY
12md
4pm
8pm
12mn
4am
8am
12md

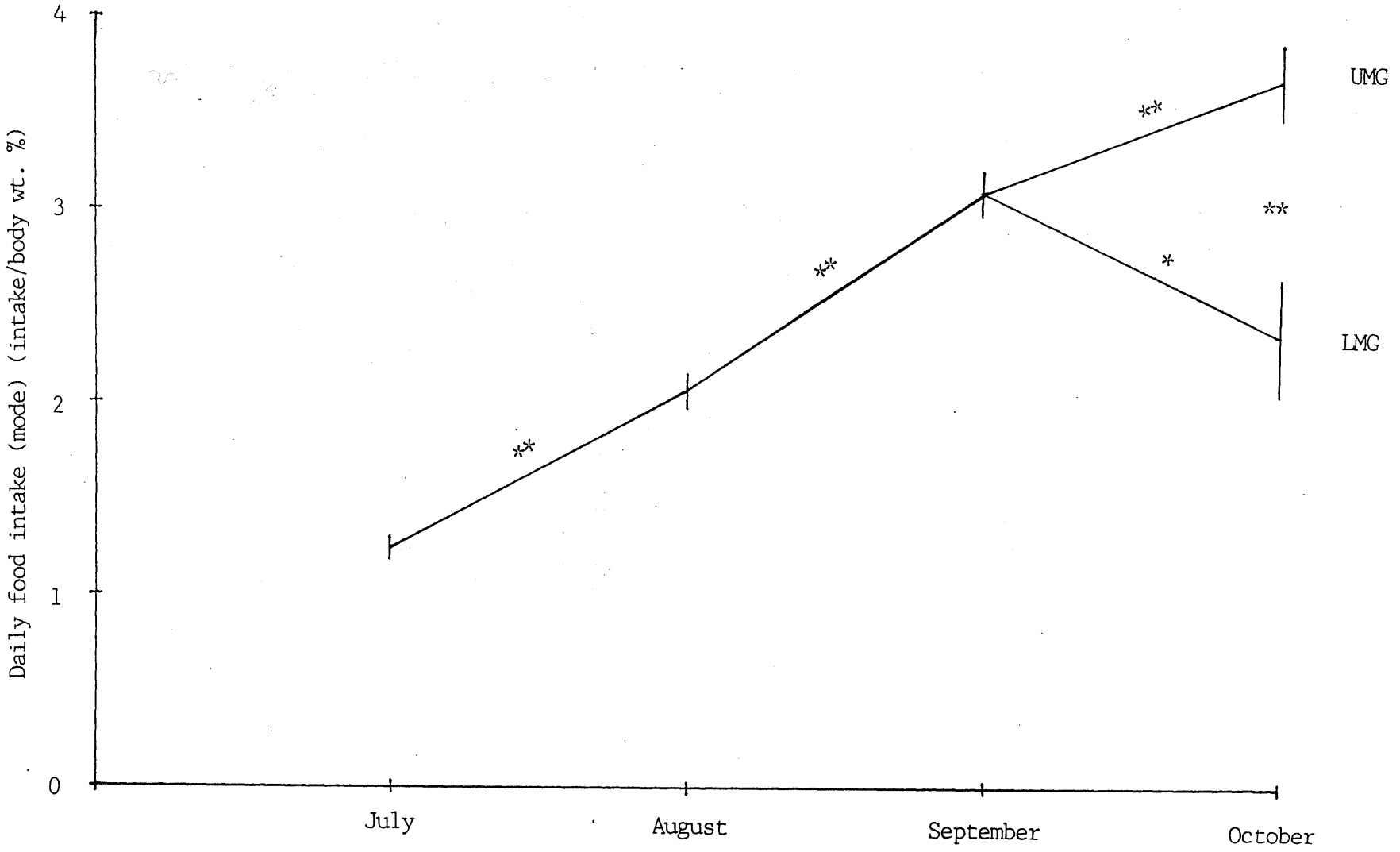
relatively short day (14.5 hours sunrise - sunset, with total darkness i.e. no twilight between c.10 pm and 2 am) during food intake monitoring. In contrast population AP/15T/S was exposed to a relatively longer day, (16 hours between sunrise and sunset, with some twilight all night). The short day population had a daily mean intake of 1.29% $\pm$ 0.07 (mean mgs intake / mgs body wt. $\pm$ S.E.) at this time, compared with 1.87% $\pm$ 0.07 in the long day population ($p<0.001$, t-test, arcsine transformed data).

Over the period 8 pm - 4 am (roughly corresponding to the period of twilight in the long day population) the population mean food intake was significantly lower in the short day population at 0.28% $\pm$ 0.06 compared with 0.44% $\pm$ 0.05 in the longer day population over the same period this difference was significant ($p<0.01$, t-test, arcsine transformed data). In contrast there were no significant differences in mean food intake between long day and short day population in feeding bouts after sunrise and before sunset.

As might be expected population mean daily intake (expressed as a proportion of body weight) was found to increase significantly between each month from July to September (Fig. 5.3, p 0.001, Mann-Whitney U test), as the fish grew. However although fish in the UMG continued to increase food intake significantly ($p<0.001$) over the period

Figure 5.3 Daily food intake (mode) (% food intake/body weight) over the study period July - October 1986 of population -3P/15T/S, (bars indicate standard error).

** = $p<0.001$, * = $p<0.02$ (Mann-Whitney U test)


Daily food intake (mode) (intake/body wt. %)
4
3
2
1
0
July
August
September
October
**
**
**
*
**
UMG
LMG

September - October, LMG parr decreased intake ($p<0.02$) over the same period. This resulted in a difference in proportional intake between the LMG and UMG parr in October ($p<0.001$).

5.4 DISCUSSION

Data from populations -3P/15T/S and AP/15T/S on short days and long days respectively in July shows that mean daily food ingestion rate was significantly lower in the population exposed to a shorter day than the sibling population exposed to a longer day despite identical constant 15°C temperature.

This corresponded to a significantly lower intake rate during the hours of reduced light intensity. Reduced intake during darkness hours has also been recorded by Higgins and Talbot (1985) in 1+ parr in May, on ambient photoperiod and fluctuating temperature. Results presented here for populations on constant temperature confirm the suggestion of these authors that reduced feeding results from photoperiod and not temperature effects. These results support the suggested photoperiod mechanism for the finding of Villarreal *et al.* (in press) and Chapter 3, that daylength influences growth rate.

Proportional food intake increased significantly as fish increased in size (Fig. 5.3) over the period July - September. After September LMG fish reduced intake whereas the UMG parr continued to increase consumption. This resulted in a significant difference in food intake between LMG and UMG parr in October at constant temperature.

Metcalfe et al. (1986) have shown that modal separation results from an appetite loss and reduction in intake in LMG parr, on fluctuating water temperatures. Results presented here have shown that at high constant temperature (15°C) food intake is reduced in LMG parr and continues to increase in UMG parr around the time of modal segregation, and that this occurs independently of temperature.

CHAPTER 6

GENERAL DISCUSSION

6.1 THE PLASTICITY OF SALMON EARLY LIFE HISTORY

It is assumed that natural selection favours the reproductive strategy of an animal that maximizes the number of offspring produced over its lifetime, reaching reproductive age. It has been noted by many authors (see Stearns 1976 for review) that the best reproductive strategy may change as the environment changes. Fish exploiting rapidly changing environments must have the physiological mechanisms to detect environmental changes and to make short-term adjustments to reproductive behaviour (termed tactics by Wootton 1984) to be successful. Such a fish is the Atlantic salmon.

It has been shown that developmental rates (growth, smolting and sexual maturation) in salmon vary greatly not only between populations (Orton et al. 1938, Jones 1959, Bailey et al. 1980 & review Thorpe 1986) but also within sibling groups (Thorpe et al. 1980, Bailey et al. 1980).

Thorpe in 1986 published a model relating three aspects

of development (growth, smolting and maturation) postulating how these may be interrelated. Results presented here have shown that Atlantic salmon parr have the ability to detect environmental cues and have the physiological capability to make considerable changes to their reproductive strategy (Wootton 1984), i.e. reproductive strategy is not genetically fixed.

Thorpe (1986) has argued that the criteria for adoption of a particular reproductive strategy are related to other aspects of development, especially growth rate. There is some evidence to suggest that sexual maturity occurs at an earlier age in fish with a higher growth rate (Alm 1959, Leyzerovich 1973, Bailey et al. 1980, Bagliniere & Maisse 1985). However as the breeding period of salmon is fixed to a relatively short season in winter, a time constraint is placed on the period when growth rate may affect the maturity option. After the lapse of this period termed the "maturation window" by Thorpe, the model predicts that growth opportunity has no effect on initiating maturation.

A similar argument may be applied to the smolt emigration in spring. Growth rates are known to be related to this developmental decision also (Thorpe & Wankowski 1979, Thorpe et al. 1980, Kuzmin & Smirnov 1982). However, as the emigration period is relatively fixed and as smolting involves numerous complex physiological changes, the choice

of this developmental pathway must be made some months prior to emigration. Bailey et al. (1980) have suggested that, as the same criterion, namely growth opportunity, determines the outcome of the developmental decisions of maturation and smolting, then it is likely that these decisions are taken at different times. This poses the question: Why should some aspect of growth determine the outcome of a maturation and smolting decision?

A number of authors (e.g. Stearns 1976, Wootton 1979, Roff 1983 & Myers et al. 1985) have examined the relationship between growth and reproduction. These authors argue that energy channelled into gonad development detracts from somatic growth. As fecundity is thought to be related to body size, investment in gonad will be a trade-off against future fecundity. Any reproductive strategy must balance the benefits of early maturation against the costs of possible loss of somatic growth and hence future fecundity. Thus some measure of growth potential must be essential. This may be expected to be more acute in animals, such as salmon parr, exploiting a rapidly changing environment and showing highly plastic life-history strategies.

The adaptive significance of growth rate as a criterion for determining age of smolt emigration is much less clear. However evidence presented by Peterson (1973) suggests that

mortality on emigration is size related, larger smolts faring better than smaller. While Peterson's conclusion is questionable (his data were based on recoveries from Carlin tagged fish,and could be interpreted as showing that larger fish survive such tagging better that smaller fish) it is reasonable to argue that the larger the fish is on entry to the sea the fewer are the predators capable of capturing it and the faster it can swim to avoid them. Thus there may be a selection pressure delaying smolting in parr that would be small by the time of emigration.

6.2 PARR DEVELOPMENTAL DECISIONS

Atlantic salmon parr in any one year class may be twice faced with developmental decisions (smolting and maturation) of which there are 3 possible outcomes (smolts, sexually mature parr and immature parr). From arguments presented here it appears that there are 3 factors of prime importance influencing these decisions:

1. genetic factors
2. timing - of developmental decisions and events
3. growth opportunity - prior to and during the decision making periods

Item 1 is outwith the scope of this thesis, however several authors have found strong genetic influences on

smolting rate (Thorpe 1977, Thorpe & Morgan 1978, Bailey et al. 1980) and maturation rate (Thorpe et al. 1983, Gjerde 1984) in salmon parr. In these experiments genetic effects were reduced by using sibling groups.

Items 2 and 3 represent environmental effects on smolting and parr maturation and have been combined in Thorpe's (1986) unified model of growth, smolting and maturation (Figure 1.2).

The essential features of this model are that there are two distinct periods during which developmental pathways are fixed in any one year. The first (the maturation window) occurs during spring, when the maturation decision is made. The second (the smolting window) occurs in late summer/early autumn when the outcome of the smolting decision is determined. Growth opportunity prior to and during these periods affect the developmental pathway choice of parr made during these critical periods.

Clearly there are a number of factors, both biological and physical that may affect growth rate and hence may influence smolting and maturation. Some of the biological factors (food availability and competition) have been considered by Randall et al. (1986). However it is principally with the physical factors that this thesis is concerned.

6.3 THE REGULATORY ROLE OF PHOTOPERIOD

It has been argued that photoperiod may have two separate effects with respect to maturation and smolting.

Timing

Previous studies on photoperiod timing effects have often resulted in confusing and complex results, when animals previously exposed to one light regime are abruptly transferred to another, without regard to the endogenous rhythm argued for by Aschoff (1960). These studies have generally arisen from a desire to manipulate cyclic events such as breeding. This study represents a departure from this approach and is an attempt to understand the underlying mechanisms governing photoperiod timing responses. Thus the natural photoperiod cycles (daylength and twilight) were simulated as closely as possible, prior to first-feeding fish were allowed no exposure to light, and cycles were moved out-of-phase from the natural cycle by relatively small amounts.

The results show that photoperiod can act as a timer of life-history events such as smolting and sexual maturation. However the relationship between changes in timing of the photoperiod cycle and the winter growth arrest of LMG parr (indicative of a decision to remain as parr) was not an

exact one. A 3 month advanced or retarded photoperiod cycle only shifted the timing of modal separation by 1 - 2 months in these experiments. These results support the findings of Villarreal et al. (in press) who also found lack of synchrony between delayed photoperiod cycle and timing of modal segregation in 0+ parr. These authors suggest that photoperiod is acting as a Zeitgeber (exogenous synchroniser) of an endogenous timing rhythm. These results plus the fact that the the timing of bimodality under constant light conditions was similar to that under ambient cycled light, support this hypothesis. It is suggested that asynchrony between photoperiod cycles and the timing of modal segregation has resulted because the external Zeitgeber cues are outwith the range of entrainment of the internal rhythm.

Growth

Results presented here consistently showed that groups exposed to longer days showed greater mean size increase, than sibling groups exposed to shorter days. This finding is in accord with Villarreal et al. (in press) who found that parr size was directly proportional to the number of hours of daylight received.

Evidence for the mechanisms of light effects on growth are given by by Higgins and Talbot (1985) who found that food intake was very much reduced during the hours of

darkness. The results of feeding trials presented here indicate that there is little feeding reduction, while twilight persists but that during the hours of complete darkness food intake drops substantially at constant temperature.

6.4 THE REGULATORY ROLE OF TEMPERATURE:

Not surprisingly temperature was found to have a marked effect on growth. Elevation of water temperature by +5°C (and to a lesser extent by +2°C) increased population mean size gain. Temperatures used throughout these experiments were unlikely to be above those which may inhibit growth (Bardach et al. 1972). Increasing temperature (below the optimum for growth) is known to increase stomach evacuation rate, appetite, food intake, absorption and conversion efficiency in a number of fish species (Windell 1978).

In addition temperature was found to delay the onset of total winter growth arrest in LMG parr and to hasten its recommencement in spring.

6.5 FACTORS INFLUENCING THE SMOLTING DECISION

It has been shown clearly that elevated water temperature (at least over the range 0 - 20°C) during the first year of growth increases the proportion of the population smolting

as S1's. In addition it has been shown that water temperature affects growth rate.

Evidence presented here is consistent with the hypothesis that photoperiod has a dual effect on smolting, acting on feeding opportunity and on the timing of a smolting window.

Advanced photoperiod cycle was found to decrease the proportion smolting as S1's. This can be best explained by an examination of the timing of the smolting window and growth opportunity at this time. Photoperiod has been shown to influence the timing of segregation of the UMG and LMG subpopulations. It has been suggested that the smolting decision is taken in mid-summer at a time when the conditions for growth offered by long days and high water temperatures are at their best at the Almondbank hatchery (Appendix 1, 2 & 3). Advanced photoperiod in these experiments accelerated the timing of the smolting decision to a period when water temperatures and hence opportunity for growth is reduced.

Likewise retarded photoperiod was found to have a similar effect. Exaggerated asynchrony between the photoperiod and temperature annual cycles which normally both peak within about 4 - 6 weeks of one another resulted in lower temperatures during the period of maximum daylength (when the smolting decision is taken) when compared with the ambient cycle. This resulted in reduced proportions of S1's in populations on retarded photoperiod in Experiment 3. In

Experiment 2, retarded photoperiod slightly increased the proportions of S1's, this may be because of relatively high water temperatures throughout the summer at Almondbank in 1985.

It has been argued that it is necessary to consider both water temperature and daylength to gauge the opportunity for growth adequately, and so an index combining both of these factors is proposed (Adams and Thorpe in press a) The thermal-sum is defined as the number of hours of daylight X median daily temperature. In practice thermal-sum was found to correlate closely with growth rates and to be a useful tool for predicting growth opportunity from environmental conditions.

6.6 IDENTIFICATION OF THE SMOLTING WINDOW

An attempt was made to identify the smolting window, the period when the smolting developmental option is available. Stepwise-multiple regression was used to determine the period of the year when growth opportunity (defined by thermal-sum) has the greatest effect on the proportion of the population entering the UMG. The evidence points to July/August as the most likely period. This finding agrees well with the finding of Metcalfe et al. (1986) that a reduction in appetite occurs in potential LMG parr between

the months of July and August and with the results of Villarreal (1983) who showed a significant growth disruption around the end of July.

6.7 THE SEXUAL MATURATION OPTION

Sexual maturation can be induced in male salmon parr during their first winter by abiotic manipulation of growth opportunity in spring in Scotland. This has not been achieved before deliberately and confirms the predictions of Thorpe's (1986) model, suggesting that high growth opportunity prior to a maturation window in early spring would induce parr maturation.

The rate of parr maturation remained very low in these experiments, but this would be expected to vary considerably between sibling groups (Thorpe et al. 1983, Gjerde 1984).

Functional maturity was found when the population was allowed to experience early February daylengths under favourable temperatures for feeding and growth - condition AP/+5T/A - and under no other combination of light and temperature conditions. For example, accelerated incubation populations on advanced photoperiod, (i.e. not experiencing February photoperiod) and elevated (+5°C) water temperatures

showed no signs of male sexual maturity. This indicated that, as with smolting, the timing of the maturation decision can be influenced by the photoperiod cycle but the direction of that decision depended on the temperature effects on feeding opportunity. In addition, high smolting rates under both (AP/+5T/A & +3P/+5T/A) photoperiod/temperature regimes confirmed that the smolting decision is taken after the 1st May, and after the maturation decision.

This supports the suggestion of Bailey et al. (1980) that the maturation decision is taken prior to the smolting decision and also remains consistent with the dual critical window proposed in Thorpe's model.

Females did not mature, even under the best conditions for growth. However growth opportunity was found to affect sub-maturational investment in ovary. In general, in non-maturing parr the relative investment in ovary (cf. soma) decreases with increasing size (this has also been found by Villarreal and Thorpe 1985) despite increasing ovary weight with size, indicating allometric growth of ovary with respect to soma. However the relative investment in ovary (at cellular Stage II of development) was greater in parr receiving good growth opportunity for a given size. This is at least partly due to an increased investment in larger oocytes, however in the fastest growing fish this may have resulted partly from a greater number of oocytes, at

that stage of development.

There is relatively little published information on the effects of size and growth on immature ovary, most work concentrating on the relationship of these parameters with egg number, size and quality. A number of studies (see Nikolskii 1969 for review) have concluded that rapid growth conditions also led to increased fecundity. Thorpe et al. (1984) found that sea-run salmon that had developed most rapidly in freshwater produced more (but smaller) eggs than slower developing fish. Results presented here show that ovarian investment differences can be detected even in immature 0+ parr in the form of increased allocation of energetic resources towards ovary under good growth conditions. However as much of this investment took the form of larger oocytes, the adaptive value of this increased investment may be questioned on two counts. Firstly there is no evidence that larger immature (Stage II) oocytes give rise to larger eggs. Secondly, even if they do there is evidence that greater egg size is not correlated with better early fry growth or survival at least in Rainbow trout (Springate and Bromage 1985). Only in the fastest growing fish was there evidence of larger ovaries resulting from increased numbers of developing oocytes. In these fish increased growth opportunity at this early stage of development is more likely to be affecting the general level

of fecundity. However this level will be subject to substantial modification in the light of subsequent growth conditions (Nikolskii 1969).

6.8 BIOLOGICAL REGULATION OF DEVELOPMENTAL RATES

In addition to the environmental effects on smolting and maturation an attempt was made to examine some possible biological effects on growth and smolting, (see Appendix 4).

Small groups of parr held in relatively crowded conditions, where access to food was limited showed high levels of aggression. Aggressive individuals maintained a position in the observation tank current with good access to the food supply. In the wild this behaviour would result in the maintenance of a drift feeding station (Keenlyside and Yamamoto 1962, Wankowski and Thorpe 1979). This suggests a possible mechanism for a biotic influence on food intake. This would directly influence growth opportunity and hence the outcome of smolting and maturation decisions.

Parr used throughout these experiments were held in radial flow tanks designed to reduce social effects on food intake (Thorpe and Wankowski 1979). Despite this there was a trend (not statistically significant) for more aggressive fish to be over-represented in the upper mode of the

separating subpopulations in autumn. Loss of key populations at crucial periods prevented the collection of more complete data. These data provide an interesting indicator of a possible biotic influence on smolting and maturation decisions, which may be increasingly important under poor feeding conditions. These experiments are now being repeated (Metcalfe in prep.).

6.9 CONSEQUENCES OF DEVELOPMENTAL RATE ON SALMON PRODUCTION

It is the aim of salmon fishery managers and farmers alike to maximize production of sea-run (or sea-caged) salmon. In the past this has taken the form of maximizing growth rates through the normal parr - smolt - sea-run adult sequence, through food availability, water temperature, selective breeding, etc. Evidence presented here shows that abiotic factors such as as daylength and water temperatures can indeed be manipulated to increase developmental rates through this route.

However, as Thorpe's (1986) model suggests, the fish may adopt an alternative strategy under very good conditions for development and mature as parr. They then achieve their biological goal without reaching large size, contrary to the salmon industry's requirement. Although some mature parr will subsequently smolt and gain weight at sea, the delay may represent an unacceptable loss to the industry. This

situation appears to have occurred in the Matamek river, Quebec, where male parr maturity increased 31 - 75% between 1967 and 1982, this was linked to increased growth rate over this time (Myers et al. 1985).

This work offers two alternative solutions to this problem suitable for use in the controlled conditions of a fish farm.

As maturation and smolting developmental pathways are initiated during discrete sensitive periods, then manipulation of growth opportunity during these periods should influence the developmental pathway. Ideally low growth opportunity during the maturation window will minimize the proportion of the population becoming mature as parr, whereas high growth opportunity during the smolting window will maximize the proportion opting for the smolt - mature sea-run salmon life history strategy. Two abiotic factors suggested by these experiments capable of manipulating growth opportunity would be water temperature and daylength. Clearly however other factors such as food availability will be important.

Alternatively, it may be possible to manipulate the phase of daylength cycle and so influence the timing of the maturation and smolting windows. Parr populations given continuously good growth opportunity but denied exposure to

a maturation window trigger would be expected to yield few (if any) mature parr, however if subject to a smolting window trigger, this population would yield a high proportion of smolts. This technique would only be expected to work under modest photoperiod phase changes. Under extreme phase change, endogenous timing rhythms would predominate.

6.10 CONCLUSIONS

Results presented here tend to vindicate Thorpe's (1986) unified model of growth, smolting and sexual maturation in salmon parr.

Two separate critical periods (maturation and smolting) were found. The most likely period for the smolting window was around July; the maturation window was not so precisely identified but seems to occur within the months of February, March or April.

Growth opportunity during these critical periods affects the subsequent life history strategy of juvenile salmon.

Photoperiod and temperature clearly act as abiotic regulators of development in juvenile salmon; photoperiod through its effects on the timing of life history events and through its direct action on feeding opportunity; temperature through its effects on feeding opportunity, assimilation efficiency and appetite.

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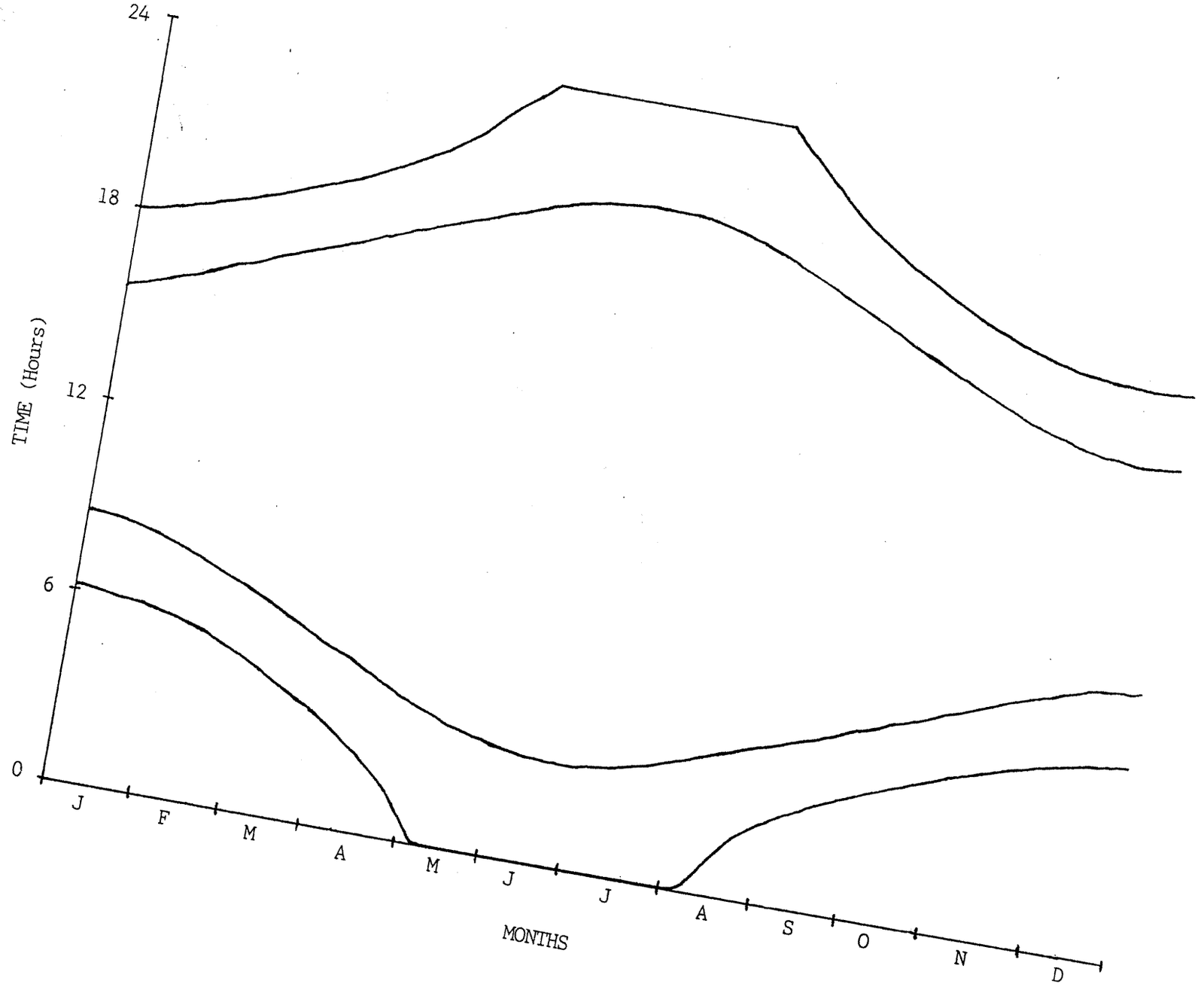
Appendix 1 Annual astronomical twilight and daylength changes, on the 56° N. latitude (data extracted from Whitakers Almanack 1985).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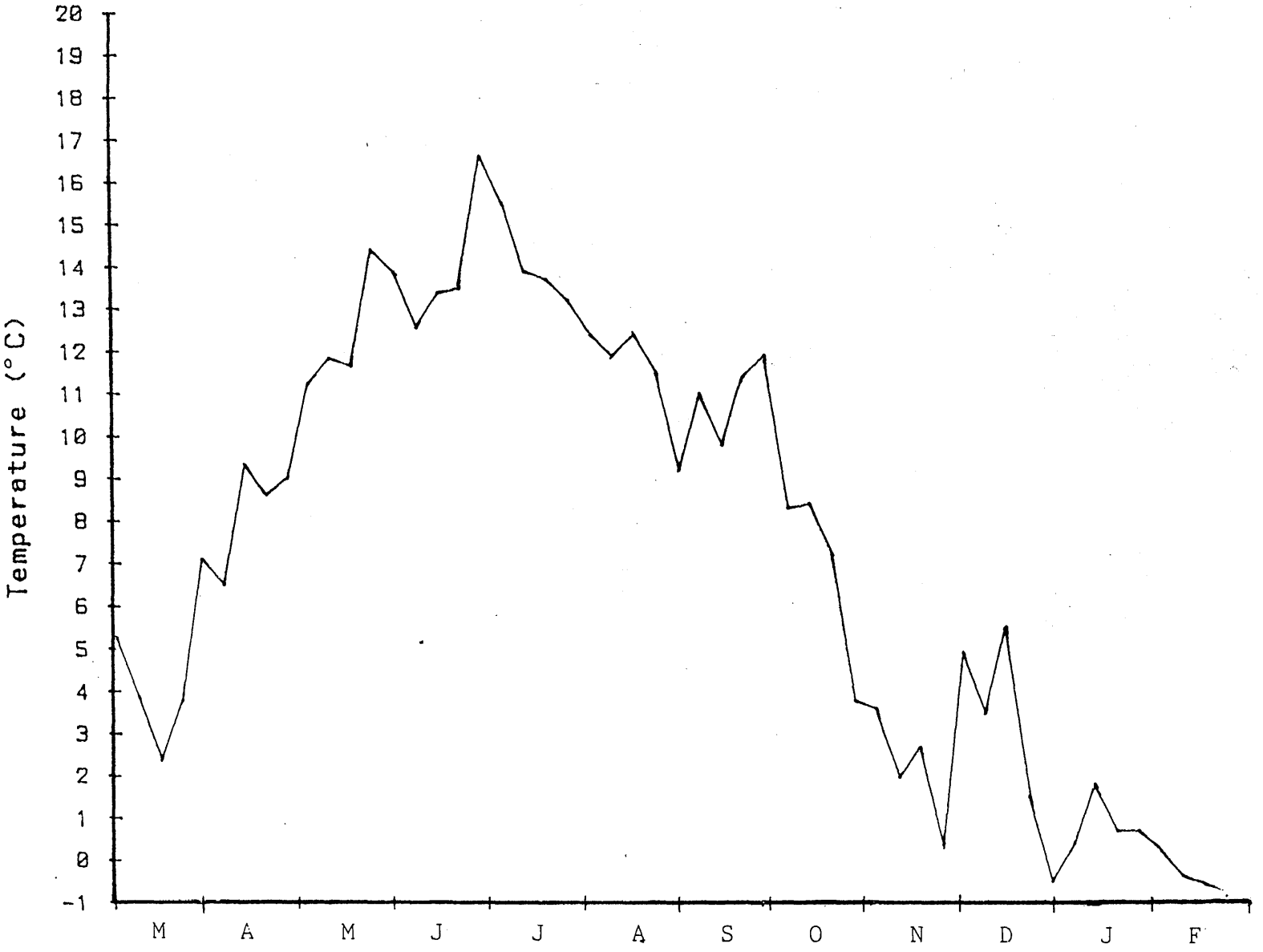
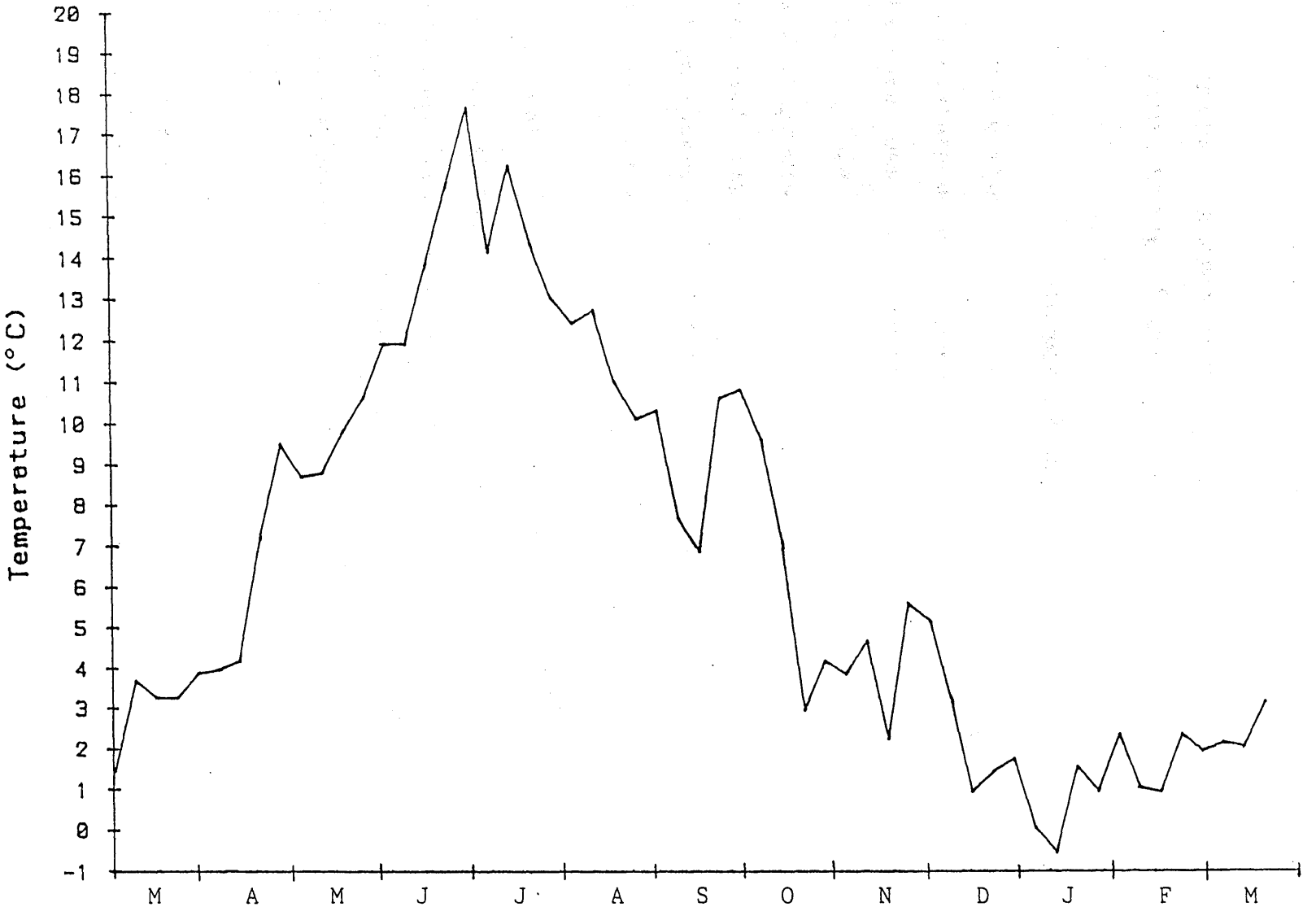


Appendix 2. Mean ambient temperature River Almond water, over the study period March 1985 to March 1986.



Appendix 3. Mean ambient temperature River Almond water, over the study period March 1986 to March 1987.

APPENDIX 4

THE EFFECTS OF AGONISTIC ACTIVITY ON SMOLTING RATE

INTRODUCTION

In addition to the abiotic influences of photoperiod and temperature on developmental rate, it is likely that biotic factors may regulate developmental rate. Competition for a limited food resource is one possible such factor that may affect growth opportunity and hence developmental rate. In parallel experiments to those examining the abiotic effects of photoperiod and temperature, the effects of agonistic activity on smolting rate were examined.

Atlantic salmon parr are known to adopt a "sit and wait" feeding strategy. In the wild they maintain a feeding station, normally on or near to the bottom of a stream or river in a fast flowing current (Keenleyside and Yamamoto 1962, Wankowski and Thorpe 1979). They are territorial (Kalleberg 1958), defending an area around their chosen station, both in a natural situation (Keenleyside 1962, Wankowski and Thorpe 1979) and under artificial observation conditions (Keenleyside and Yamamoto 1962, Symons 1968). Wankowski and Thorpe (1979) showed that the frequency of aggressive encounters was negatively correlated to the

abundance of profitable feeding stations (channels) in salmon parr. The implication was that if food needs were being met, then the fish's motivation to defend an exclusive opportunity to feed was lowered and fighting occurred less frequently. The radial flow tank (Thorpe and Wankowski 1979, Thorpe 1987a) was designed to provide abundant feeding channels and thus to limit the expression of agonistic behaviour. In a contrasting situation Metcalfe (1986) has shown that, in Rainbow trout (Salmo gairdneri) held in tangential flow tanks at high densities the more aggressive, dominant individuals obtained more food and grew faster than subordinates.

In the experiments described here, pilot observations on small groups of juvenile 0+ Atlantic salmon parr showed that in confined conditions, less favourable than the radial flow tanks, overt aggression was commonplace. Most aggression was expressed by relatively few individuals and was directed towards other individuals in a non-random manner (also see Fenderson and Carpenter 1971, and Symons 1968). Also the fish were not randomly distributed, suggesting that some individuals were holding territories.

The reduction in food intake that results in bimodality of length frequency distribution in 0+ Atlantic salmon parr is not primarily the consequence of agonistic activity

(Thorpe 1977, Metcalfe et al. 1986). Although populations throughout this study were maintained in radial flow tanks where agonistic behaviour is minimized (Thorpe and Wankowski 1979) it was hypothesised that parr strongly motivated to defend territories, where such behaviour was essential to maintain food intake, may be disposed to high rates of entry to the UMG as underyearlings.

To examine the possibility that social rank is linked to the developmental outcome of the smolting decision, it was necessary to establish the social status of a number of individuals and then to test the null hypothesis that social rank is not linked with the choice of developmental pathway during the smolting window in late summer.

MATERIALS AND METHODS

Establishing an order of social rank through frequency of aggressive encounters between fish, required observation trials in tanks in which the availability of food was more restricted than in the radial flow holding tanks.

Observations were made on small groups of 6, 0+ salmon parr in tanks (20 X 30 X 14 cms) each with its own supply of continuous running water. The fish used were taken from (and subsequently returned to) populations AP/15T/S and -3P/15T/S of Experiment 3, whose holding conditions are summarized in Table 3.3. They were maintained at 15°C in constant temperature rooms at the University Field Station and at the correct photoperiod for the stock conditions from which they came.

Individual Recognition

To allow recognition of individuals during these experiments a marking technique was developed over several months. The requirements considered for such recognition marks were:

1. effects on behaviour

2. suitability for fish of 25 - 40mm fork-length

3. ease of administration

4. ease of identification (i.e. without handling)

5. durability

6. the number of unique identification combinations

The method finally adopted was as follows. A very fine nylon fishing line (2 LB breaking strain) was threaded through a puncture hole made with a (size 0) entomological mounting pin, in the musculature immediately anterior to the dorsal fin, of fish anaesthetised with Benzocane. One or two pieces of coloured plastic (<1mm length, <1mm width) obtained by stripping the insulation from telephone handset wires, were threaded on to the nylon. The ends were tied in a "thumb knot" and sealed with a small drop of "Superglue". This was allowed to air dry for c. 10 seconds before returning the fish to water. With 8 different colours of plastic available, 36 colour combinations were possible, without using any 2 identical colours on the same tag or any reversed codes. The tags proved clear and easy to read in these experiments.

Experimental Set-up:

The observation tanks were isolated by blinds from each other and from the observer except for a small observation slit (Fig. A4.1). Differential lighting helped to cast shade on the observer, further reducing the likelihood of observer interference. If a current of water was jetted across the floor of the observation tank, then most fish would orientate to the current, spending most of their time on the floor of the tank. Such an arrangement not only simulated a more natural situation but also increased the competition for preferred position in the observation tank.

Pilot observations for these trials confirmed the findings of Keenleyside and Yamamoto (1962) that at least 12 hours settling down period was necessary before consistent behavioural data could be obtained. For this reason throughout the main trials, fish were given a minimum settling down period of 17 hours. During this time they were hand fed, the food being incorporated with the water supply jetted into the tank. Feeding was terminated one hour before commencement of the trial.

A series of successive half hour observations on one group of fish showed that little extra information could be gained by using observation periods of 60 or 90 minutes. Thus 30 minute trials were used throughout, (cf. Fenderson

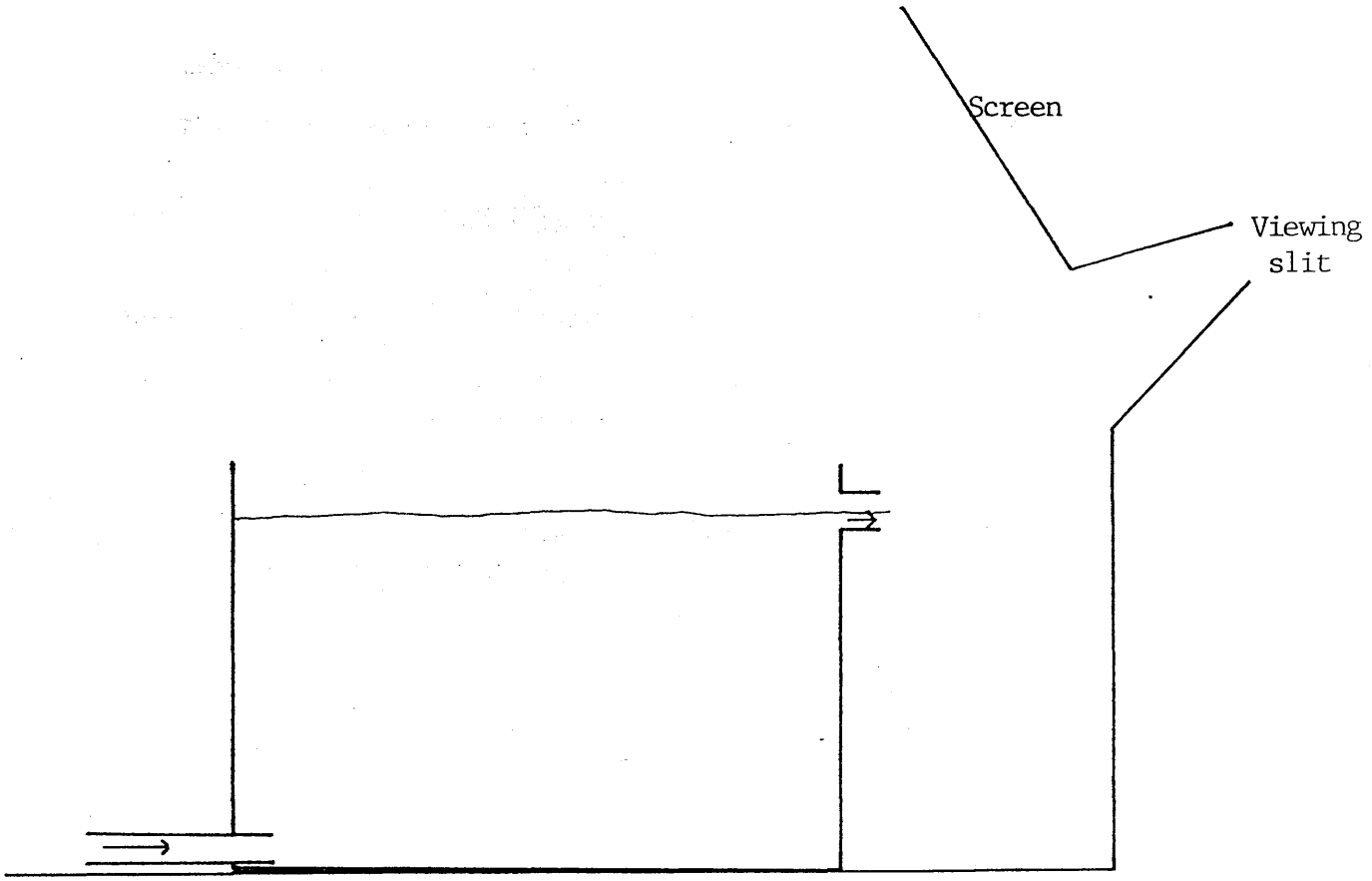


Figure A4.1 The aggression trials observation tank.

and Carpenter 1971). In a few trials a second 30 minute trial was necessary to identify the "principal aggressor" of the group.

Tank Position:

The location of a fish at any time was estimated using 3 coordinates in 3 planes. The base of the tank was divided into a grid of 3 by 5 squares (6 cms long by 6.7 cms broad). To the x,y (width, length) coordinates was added a third, to indicate vertical position in the tank (1 - on the bottom, 2 - anywhere midwater, 3 - on the surface).

Aggressive Behaviour:

Aggressive encounters during the 30 minute trial period were classified into one of 6 agonistic behaviour categories, described below. In only one of approximately 300 overtly aggressive encounters was the outcome of the encounter unclear. In almost all cases the attacking fish was the clear winner. For this reason the attacked fish shall be referred to as the "submissive" fish in any given encounter, and the attacking fish as the "aggressor".

Initial observations on a group of 6 fish without colour tags showed that aggression levels and orientation of fish without tags were comparable to groups of tagged fish. Thus it was assumed that tags themselves did not influence

aggression.

The six agonistic behaviour patterns were defined as follows.

Overt Aggression

Chase and Nip - the aggressor swam very rapidly towards the submissive fish, which moved from its station, the aggressor followed through the attack past the original position of the submissive fish, eventually making contact.

Chase - (also called "chase" by Keenleyside and Yamamoto 1962) similar to the "chase and nip", however the aggressor broke off the attack before contact was made.

Lunge and Nip - (similar to the "charging" of Keenleyside and Yamamoto 1962) the aggressor darted forward and made contact while the submissive fish was still at its station. Subsequently the aggressor might take over the submissive's station or return to another station.

Lunge - similar to the "Lunge and Nip" but without any physical contact.

Subtle Aggression

Approach - one fish moved slowly towards another at a station and the submissive fish was displaced for no obvious reason other than the presence of the aggressor. Normally no contact was made (also described by Symons 1968 and Chiszar et al. 1975). The aggressor did not continue to advance beyond the original station of the submissive.

Backward Displacement - similar to the above but the aggressor reversed from upcurrent, downstream on to the submissive fish which moved off station. This behaviour normally did involve physical contact (also found by Chiszar et al. 1975).

Experimental Protocol

Eleven groups of 6, 0+ salmon parr siblings from each of populations AP/15T/S and -3P/15T/S were tested in observation tanks between the 1st July and 20th July 1986. Five trials from each population used fish which had no prior experience of observation tanks. The remaining six trials were composed mainly of fish that had been involved in an earlier trial. As identification of individuals showing extremes of aggressive behaviour was the main aim of these trials, once a "dominant" (defined as the fish involved in the largest number of attacks in each

population) had been identified from each trial it was excluded from all subsequent trials. Each trial lasted 30 minutes. If after this period no principal aggressor (i.e. the assumed dominant fish) could be identified, then a second trial was completed on the same group within 2 hours of the first. Normally 2 trials were sufficient to identify the dominant fish.

During the trial the frequency of all aggressive encounters between specific participants was recorded and categorised into one of the five described behaviour patterns. In addition, the positions of the individuals in the tank were noted at time intervals 0, 5, 10, 15, 20, 25 & 30 minutes into the trial.

Three degrees of current flow in the observation tank were distinguished:

a) the main current - directly in front of the water inlet on the tank floor where the velocity was around 60 cms/sec.

b) the periphery current - around the margins of the main current where the water flow was unidirectional but not as strong as the main current.

c) slack water - in the corners and above the floor of the tank, where the current direction was variable.

The 3 dimensional coordinates covering each of these areas were noted and the relative amount of time spent in each current category by each fish calculated.

RESULTS

Aggression

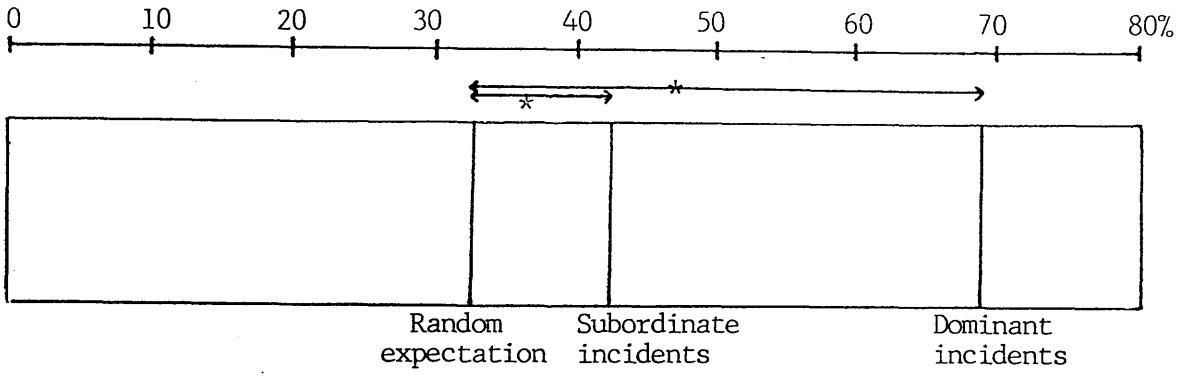
Aggression rates averaged 24.1 incidents per hour, in all trials. In 20 out of the 22 groups tested 80% of all aggressive encounters originated from one individual in each group (nominally the dominant). This was greater than would be expected if aggressive acts originated from random individuals ($p<0.001$ Chi squared test Fig. A4.2).

Aggression was directed towards individuals in a non-random manner: 43% of all aggressive encounters were directed towards one individual (nominally the subordinate) in each trial ($p<0.001$ Chi-squared test). Only 22% of all individuals were not involved in any aggressive encounters.

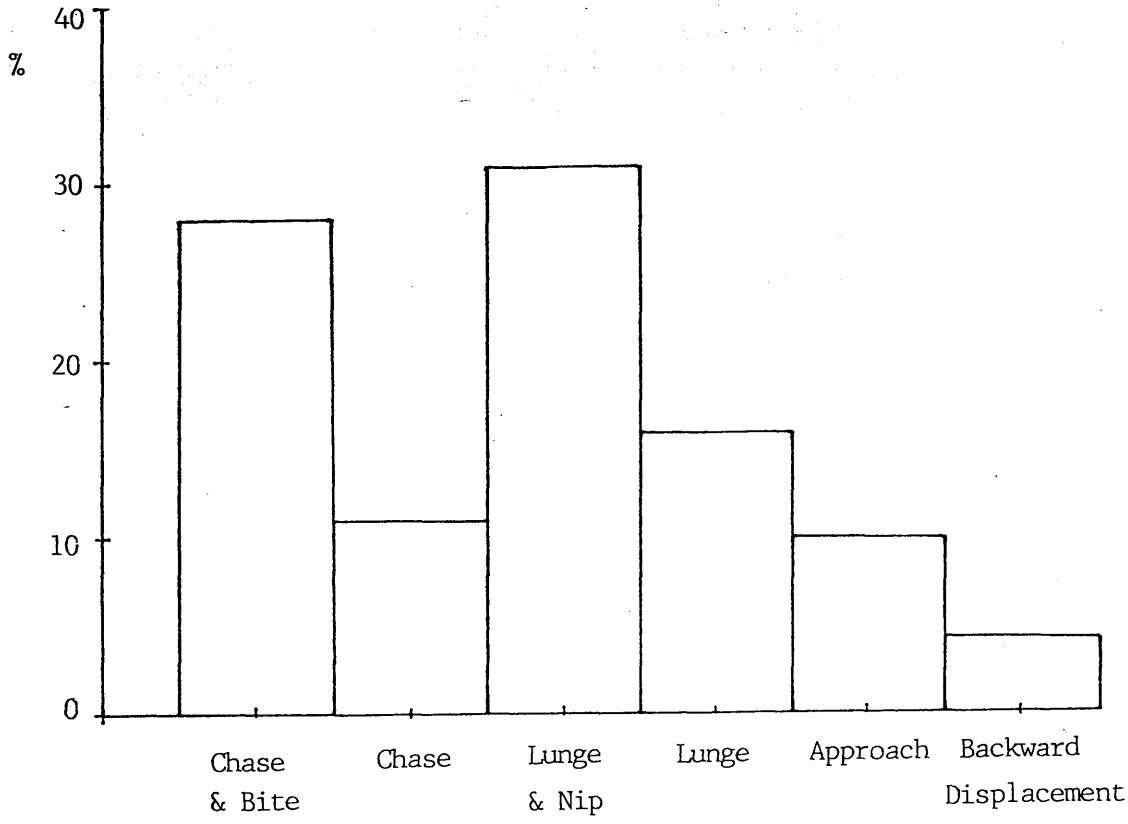
Fish classified as subordinate were not necessarily precluded from being dominant during subsequent trials, and four fish initially identified as subordinates in their first trial, were found to be dominant in subsequent trials.

Figure A4.2 Proportion of agonistic incidents in trials where a dominant fish was identifiable (expressed as a percentage of the total number of observed incidents), percentage of incidents involving the dominant and subordinate individuals and the random expectation of individual involvement.

Figure A4.3 Percentage incidence of individual agonistic behaviour patterns.



* = p<0.001 Chi squared test



Dominance was not determined solely by size. The dominant fish was found to be one of the two largest fish in only 35% of trials (random expectation = 33.3%). The fork-lengths of the dominants were not significantly different from the median fork-lengths of other fish in the trials (paired t-test not significant - Dominant fork-length 40.mm $\pm$ 0.42 (mean $\pm$ S.E.; others fork-length 39.7mm $\pm$ 0.21).

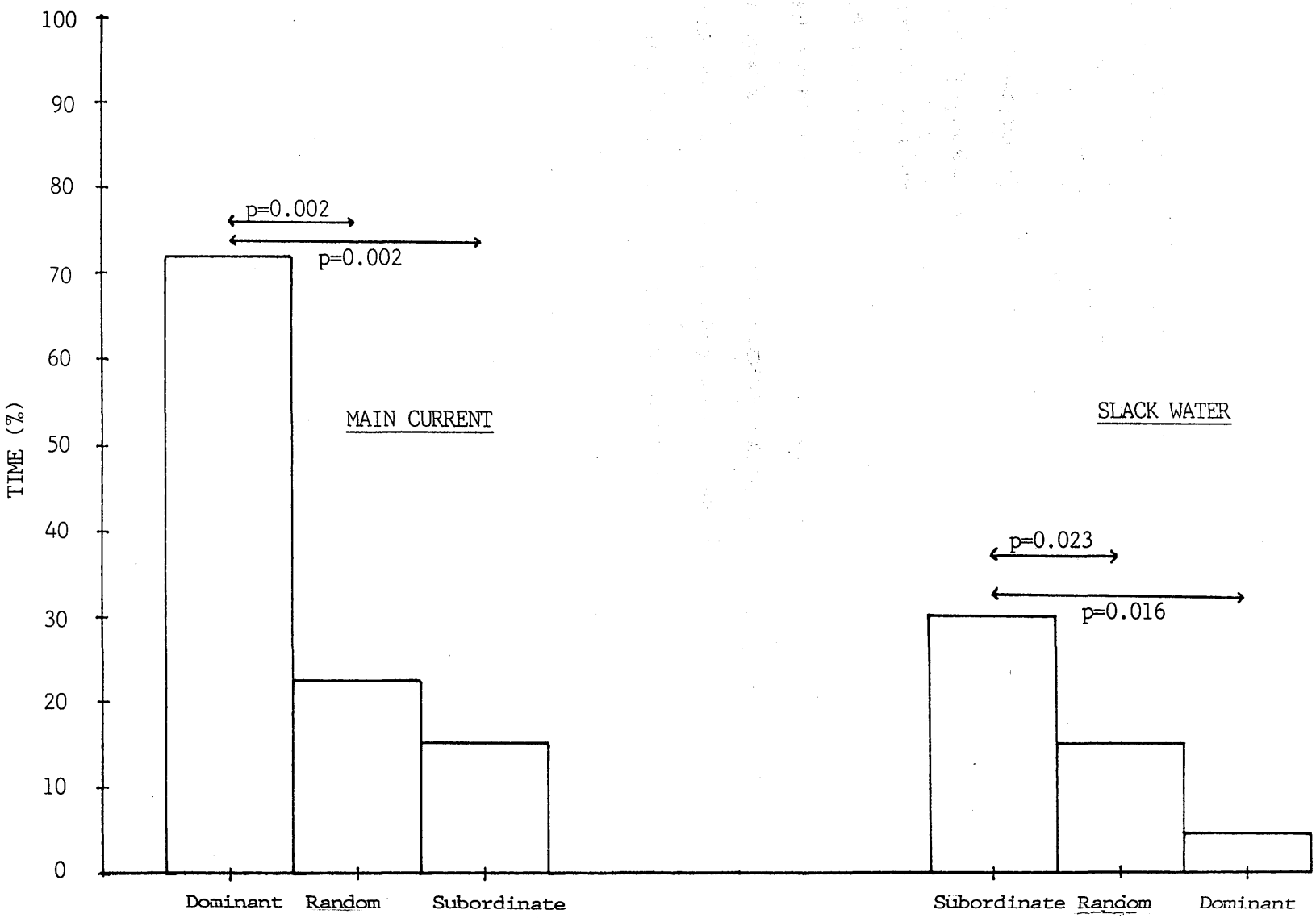
The breakdown of aggressive behaviour patterns observed, (Fig. A4.3) suggests that overt aggression was much more prevalent than the more subtle displacement behaviour patterns and that aggressive behaviour involving contact was more likely than overt action without contact. However the difficulty of observing subtle aggression and establishing contact in overt aggression almost certainly affected the frequency of these observations.

Tank Position.

Fig. A4.4 shows that dominant individuals spent significantly more time in the main current than either subordinates or other parr (neither subordinates nor dominants) ($p=0.002$ Mann-Whitney U test). Likewise subordinates spent more time in slack water than either dominants ($p=0.0016$) or other parr ($p=0.023$ Mann-Whitney U test).

Figure A4.4 Left - Proportion of time spent in the "main current" of the observation tank, by dominant, subordinate and a randomly chosen fish (excluding dominant and subordinate).

Right - Proportion of time spent in "slack water" by subordinate, dominant and a randomly chosen fish.


100
90
80
70
60
50
40
30
20
10
0
TIME (%)
p=0.002
p=0.002
MAIN CURRENT
SLACK WATER
p=0.023
p=0.016
Dominant
Random
Subordinate
Subordinate
Random
Dominant

Movement

The amount of movement around the observation tank was assessed by calculating the distance moved between 5 minute intervals by different categories of fish. Dominants moved on average 5.66 $\pm$ 0.72 cms (mean $\pm$ S.E.) over a period of 5 minutes, significantly more than the 2.96 $\pm$ 0.66 cms of other parr (i.e. not dominant or subordinate) ($p<0.01$ Mann-Whitney U test).

Dominance and Size.

Owing to the loss of populations -3P/15T/S and AP/15T/S it was not possible to determine the fate of all dominant individuals into the bimodal growth phase. However there is some suggestive evidence that dominant individuals may enter the UMG at a higher rate than the LMG. By the end of August 11 out of the 17 (65%) surviving dominant marked individuals were in the upper 50% of the length frequency distribution at this time. This proportion is not statistically greater than might be expected by chance, so the principal null hypothesis of this experiment, that social rank is not linked to choice of developmental pathway during the smolting window, is upheld.

DISCUSSION

Evaluation of the tagging system:

This unique tagging system met the requirements well. The tags were easily identifiable during observations, they did not appear to impair swimming ability, or other obvious behaviour. Tagged fish were randomly distributed in the population when returned to radial flow holding tanks, and they consumed as much food as untagged ones (Chapter 5). The main drawback was tag movement. As a result of the single suture securing it, it could move during swimming, causing the wound to remain open and posing an infection risk. Thus an improvement to this technique has been proposed, where a double suture of nylon is inserted into the muscle anterior to the dorsal fin. The plastic tags are trapped between the two sutures and tag movement is kept to a minimum. This technique is currently being evaluated (D. Rowe pers. comm.).

Given the limited access to food experienced under these experimental conditions parr aggression levels were found to be high. Parr showing high levels of aggression spent significantly more time at stations in the main current with good access to food, than other parr. Parr receiving most attacks spent more time in slack water with no unidirectional current (Fig. A4.4). In addition aggressive

parr spent significantly more time moving around the tank than others. It is suggested that this situation can be likened to parr in the wild where they are generally faced with a limited feeding resource and defence of feeding territories is normal (Keenleyside and Yamamoto 1962, Wankowski and Thorpe 1979). A possible mechanism is suggested where a parr's ability to hold a profitable feeding territory may influence the developmental pathway adopted during the smolting window, through food intake at this time.

Although a high social rank is likely to influence that individual's food availability, dominance may not ensure entry to the UM growth group. If, as has been suggested by Thorpe (1986), the developmental outcome of the smolting decision is based on each individual fish's current growth performance in relation to a genetically predetermined performance threshold, and that individual's thresholds differ, then although maintenance of a profitable feeding territory may raise performance above this threshold, in individuals with a high genetically fixed threshold, it may not. The effects of dominance on smolting rate is likely to be greatly influenced by food availability. In situations with high food availability the relative advantages from maintaining a feeding station are likely to be less than when food is in short supply.

Classification of parr as dominant (principal aggressor) or subordinate (principal submissive) in trial of only 6 fish, is clearly a gross simplification of the situation in the holding tanks. In addition it was found that 4 parr originally classified as subordinate were found to be dominant in later trials. Thus it may be that the recipient of the most attacks from the dominant is the dominants closest competitor and not the fish of lowest social rank in the tank as originally suspected.

Data obtained from these trials do not provide any evidence of a significant relationship between social rank and smolting, therefore the null hypothesis remains upheld.