# Seasonal Ecology and Biochemistry of Juvenile Atlantic Salmon

by
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Pictish carving of an Atlantic salmon, circa 7th century A.D.

This thesis is submitted for the degree of
Doctor of Philosophy,
Department of Zoology, University of Glasgow,
March 1994.



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## Abstract

The ability to hold station against a water current, the efficiency of enzyme catalysis, and various aspects of body morphology were studied in juvenile Atlantic salmon during their period of freshwater residence. This was done in order to assess the effect of varying seasonal temperatures on juvenile salmon and to compare the relative physiological performance of fish following differing life-history strategies, which are manifested by the upper (UMG) and lower (LMG) modes of a bimodal size distribution within a sibling population.

The relative ability to oppose water current (critical holding velocity: CHV) varied with water temperature and was similar for both $0^+$ modal groups until the UMG fish became smolts, at which time they could withstand lower velocities than the LMG fish. $1^+$ LMG fish during their final year in fresh water demonstrated a seasonal pattern of CHV similar to that of UMG fish in their first year. Within the samples of $0^+$UMG and $1^+$LMG fish at smolting CHV was significantly correlated with the degree of silvering seen in smolting fish.

The kinetic characteristics of lactate dehydrogenase (LDH) from juvenile salmon white muscle changed throughout the sampling period, so that the efficiency of this enzyme (as measured by the Michaelis constant) tended to be greatest at the temperature the fish were experiencing when sampled. There was no apparent difference in the seasonal efficiency of LDH between

the $0^+$ modal groups. The efficiency of LDH at the different environmental temperatures also varied significantly with seasonal temperature, being most efficient at the lowest environmental temperatures.

Glucose-6-phosphate dehydrogenase (G6PDH) from the liver also demonstrated a tendency to be most efficient at the environmental temperatures. However, LMG fish appeared to show greater adaptation of this enzyme to seasonal water temperatures than UMG fish. There was no seasonal variation in the efficiency of G6PDH at the environmental temperatures, except for the sample taken during May (the time when UMG fish were smolting) when the apparent efficiency of both modal groups decreased. This enzyme also demonstrated a seasonal variation in the free energy of activation' which tended to increase throughout the first year of fresh water residence.

Truss measurements were taken over the whole body of juvenile salmon from both modal groups throughout the period of freshwater residence and standardized for the size of fish to allow comparisons of body shape. Principal components analysis was used to identify the measurements that differed between modal groups, and the standardized measurements were then analysed directly. With the appearance of the bimodal size distribution relative differences in body morphology became evident, with the UMG fish having relatively smaller jaws and longer heads; longer, deeper trunks; similar post-anal regions; shorter anal fins and longer dorsal and tail fins than the LMG throughout most of

the first year in freshwater. However, by the time that $0^+$ UMG fish were smolting these differences in shape had mostly disappeared, although smolts tended to have slightly deeper heads and trunks. These results contrasted with the general view that at smolting salmon show a relative lengthening of their body. The post-anal region, which is generally considered to be the area of the body that lengthens at smolting, showed no difference between the modal groups at any time.

$1^+$ LMG fish (i.e. in their second year of fresh water residence) demonstrated a similar pattern of overall head, posterior trunk, and dorsal fin measurements to that of $0^+$ LMG fish, whereas the anterior and mid-trunk measurements of $1^+$ LMG fish tended to be intermediate between those of the $0^+$ modal groups. However, by May there was no difference between the standardized measurements of both ages of smolting fish.

The mesenteric lipid levels peaked in November in both modal groups of $0^+$ salmon, but UMG fish maintained a relatively higher level than the LMG fish. Both $0^+$ UMG and $1^+$ LMG fish had generally similar levels of mesenteric lipid, but $0^+$ LMG fish showed a different seasonal pattern.

The results are discussed in relation to each other and in relation to the differing behaviour and ecology demonstrated by fish following different life-history strategies. It is suggested that the LMG fish are not physiologically less capable than those of the UMG, and that any differences in performance, metabolism and

body shape are related to the differing ecologies of these fish.

**Acknowledgements**

During the course of my Ph.D. a great many people have gone out of their way to help me with various aspects of this study. There are too many to name in full, but it is because of them all that this thesis has been completed and I extend to them my heartfelt thanks.

I would like to thank my supervisors Felicity Huntingford, Neil Metcalfe and John Thorpe for having the patience to let my study go its own way and then to continue to provide me with advice and encouragement.

The practical work was carried out at the Freshwater Fisheries Laboratory in Pitlochry and the smolt rearing unit at Almondbank. I would like to thank everyone at these establishments for their help and friendship over the years, especially Derek Pretswell for all the laughs and Bob Morgan, both of whom were always there when I needed them.

Dr. Rob Strang provided me with much advice on biochemistry and was always willing to listen to new ideas. I would also like to thank Karen for putting up with the long months of writing up and, like it or not, learning all about *Salmo salar*. This study was funded by a SERC studentship.

Most of all I would like to thank my parents for their constant love and support through my long number of years as a student. This thesis is dedicated to the memory of my father William Graham, a great man who died before its completion.

# SEASONAL ECOLOGY AND BIOCHEMISTRY OF JUVENILE ATLANTIC SALMON

CHAPTER 3

THEORY AND DEVELOPMENT OF BIOCHEMICAL TECHNIQUES



CHAPTER 4

SEASONAL ADAPTATION IN ENZYMES OF JUVENILE ATLANTIC SALMON: I. LACTATE DEHYDROGENASE



CHAPTER 5

SEASONAL ADAPTATION IN ENZYMES OF JUVENILE ATLANTIC SALMON: II. GLUCOSE-6-PHOSPHATE DEHYDROGENASE

CHAPTER 6

BODY SHAPE, MORPHOLOGY AND AGE OF SMOLTING IN JUVENILE ATLANTIC SALMON

CHAPTER 7

GENERAL DISCUSSION

# CHAPTER 1

# GENERAL INTRODUCTION

## Environmental Physiology

The effect of environmental variables on physiological and metabolic processes has been studied in a wide variety of ectothermic aquatic organisms due to the high level of heat conductivity and the diffusion gradient present between water and the cells/tissues of these organisms. Studies of this type are also facilitated by the relative ease of manipulation of the physical and chemical characteristics of water under laboratory conditions.

Temperature is a major influence on the physiological function and distribution patterns of aquatic ectotherms (Hochachka and Somero 1973; Cossins and Bowler 1987) and freshwater fish may experience large fluctuations in both daily and seasonal water temperatures. Studies on various freshwater fish have shown that fluctuations in temperature can have an affect on the nervous system and levels of spontaneous activity (Lemons and Crawshaw 1985), liver structure and composition (Segner and Braunbeck 1990), swimming performance (Brett 1967; Griffiths and Alderdice 1972), respiration (Roberts 1964; Brett 1971), growth rate (Brett *et al.* 1969; Biette and Geen 1980), food intake (Elliot 1976; Higgins and Talbot 1985) and enzyme activity (Behrisch 1969; Moon and Hochachka 1971; Koch *et al.* 1992). One of the major areas for investigation in ectothermic animals (and fish in particular) has been the adaptation of various aspects of metabolism to

compensate for variation in environmental temperature (review in Hazel and Prosser 1974; Evans 1984).

Photoperiod has also been shown to have a marked effect on various aspects of the metabolism of fish, such as the upper and lower lethal temperature limits (Hoar 1956), critical swimming speed (Kolok 1991) and respiration rate (Roberts 1964). With particular reference to salmonids, photoperiod influences growth and smolting (eg. Saunders *et al.* 1985; Adams and Thorpe 1989) since photoperiod acts as a synchronizer of the endogenous rhythms of appetite and growth (Villarreal *et al.* 1988).

Salmonids of both the *Oncorhynchus* and *Salmo* genera have been the subject of many physiological and metabolic studies (Brett, Saunders, Somero, Thorpe and all their co-workers), due to their economic importance and to the complexity of their life-cycles. The latter results in salmonids being exposed to both freshwater and marine environments, with the consequential effects on osmoregulation, metabolism, food intake and growth (McCormick and Saunders 1987).

## General Salmon Biology

The Atlantic salmon (*Salmo salar*) is an anadromous fish that demonstrates great plasticity in the developmental growth processes preceding sexual maturity (Thorpe 1986; 1989). Spawning takes place between November and January, when the eggs are deposited under the gravel on a stream bed. Hatching usually occurs in early

spring although incubation time is primarily dependant upon the cumulative temperature the fish have experienced (Crisp 1988). The alevins remain in the gravel until their yolk-sac is absorbed before emerging into the water column to begin feeding.

The juvenile growth phase in fresh water (during which time the fish are termed "parr") can vary between 1 and 8 years (Randall *et al.* 1987), with the fish at higher latitudes tending to remain in fresh water for longer periods (Metcalfe and Thorpe 1990). Some fish (mainly males) may become mature and spawn without leaving freshwater. However, in the majority of cases the juvenile salmon then undergo a series of physiological and behavioural changes termed "smolting". These serve to adapt them for life in a marine environment, where they spend one or more years before returning to their natal stream to spawn. Such variation is seen not only between populations living in different environmental conditions, but also within populations living under identical environmental conditions.

## Adoption of life-history strategies

The flexibility of Atlantic salmon is demonstrated by the adoption of different life-history strategies within a single sibling population. As water temperature declines so does the overall food intake of juvenile salmon. However, fish that will smolt the following spring after one year in fresh water (the upper modal group, UMG) maintain a relatively high food

intake and metabolic rate resulting in continued growth over winter (Higgins 1985; Higgins and Talbot 1985). In contrast the fish that will remain in freshwater for at least a further year before smolting (the lower modal group, LMG) show a greatly reduced food intake and a lower metabolic rate and, hence, show little or no growth over winter (Higgins and Talbot 1985). The adoption of these differing strategies and the consequent differing growth rates results in a bimodal size distribution which becomes apparent during the first autumn (Thorpe 1977).

The physiological "decision" of which life-history strategy to follow seems to occur shortly after the summer solstice (Adams and Thorpe 1988; Wright *et al.* 1990) and appears to be based on the performance of the individual fish at this time, as measured by energy acquisition (Thorpe 1989). This of course, is not a conscious decision taken by the fish, but is simply the adoption of a specific behavioural, ecological and metabolic strategy as a consequence of the current environmental conditions, and the opportunity for growth that these present for each individual fish. It has been suggested (Thorpe 1986) that the genetic effect on the proportion of fish entering each modal group is the inheritance of a particular threshold level of performance that must be exceeded at the critical time in July for growth to be maintained.

Living in a temperate fresh water habitat exposes juvenile salmon to fluctuations in environmental

variables (such as water temperature and water flow) and to high rates of competition (Garcia de Leaniz 1990). This is obviously a greater disadvantage to smaller fish and LMG fish respond to the harsh winter conditions by following a cost-minimising strategy which reduces their energy expenditure and the associated inherent cost (Metcalfe and Thorpe 1992) as well as reducing the exposure to the threat of predation (Metcalfe *et al.* 1987).

Growth rates in fresh water are much lower than in the marine habitat (McCormick ans Saunders 1987) so LMG fish incur a cost relative to the UMG by delaying smolting. However, this may be partially offset by the fact that LMG fish enter the sea as larger smolts thus increasing their chance of survival (Hansen and Lea 1982; Hansen and Jonsson 1989).

## General Philosophy

These differing life-history strategies exhibited by juvenile salmon within a temperate environment with the consequent seasonal variations in environmental temperature, provided the opportunity to study the biochemical and physiological performance of an ectothermic animal in direct relation to distinct seasonal ecological and behavioural traits. This thesis describes a study that took advantage of this opportunity.

The thesis approaches the link between ecology and behaviour on the one hand with biochemistry and

physiology on the other from the viewpoint that the whole organism is the level to which the biochemical and physiological processes are adapted by means of natural selection. This may seem an obvious philosophy to ecologists, however, biochemists can still tend to view the evolution of individual types of molecules as being paramount and the level to which the animals as a whole then respond.

The majority of biochemical studies have been carried out either on endothermic animals (generally laboratory rats or humans) or using bacteria associated with endotherms and, therefore, have been performed at one of three standardised temperatures (25, 30 or 37°C) to allow comparisons.

Many ectothermic animals have been shown to adapt to large fluctuations in environmental, and therefore body temperature on a seasonal and sometimes daily basis. Studies on the metabolic processes of such ectotherms provide the opportunity to bridge the gap between ecological and biochemical theory that would not necessarily be afforded by studying the metabolic pathways of endotherms living at constant temperatures. "On the basis of studies on gluconeogenesis in rats in cages, one would not predict that an ectothermic vertebrate [i.e. a salmon] could migrate 1000 km upstream in an 8-10°C river without feeding and while fully developing gonads,..." (Suarez and Mommsen 1987). However, there is a paradox contained within this type of study, which is that the increased accuracy of

laboratory-based observations on biochemical and physiological parameters has to be balanced against ensuring that such observations are still applicable to the animal within its natural environment.

## Experimental work

The differing ecologies and behaviours previously demonstrated in juvenile Atlantic salmon under conditions of seasonally varying temperatures (and described in greater detail in the experimental chapters) suggested two general questions about salmon biology that formed the main focal points for this thesis:

i) Does the efficiency of metabolic and physiological processes vary in relation to seasonal temperature in juvenile salmon ?

ii) What differences and similarities exist between upper and lower modal group fish ?

These questions are investigated in chapters 3, 4, 5 and 6. Each chapter is a self-contained description of each experiment and of how the results relate to the seasonal ecology of upper and lower modal group juvenile salmon.

Chapter 3 examines the maximum relative ability of the different sized fish from the modal groups to hold station on the substrate against a water current throughout the period of fresh water residence. In

particular this addressed the question of whether UMG fish are intrinsically more capable in terms of performance than LMG fish. It also examines the holding performance of smolting fish during the period of seaward migration.

Chapters 4 and 5 are concerned with the study of the efficiency of two enzymes that are each involved in metabolic processes directly linked to aspects of seasonal ecology in juvenile salmon. Chapter 4 is concerned with lactate dehydrogenase (LDH), which is of importance during anaerobic respiration, which in turn occurs during food capture in these fish. A difference in the relative amount of total body lipid has already been demonstrated between the two modal groups (Higgins and Talbot 1985), so chapter 5 examined glucose-6-phosphate dehydrogenase (G6PDH), which is part of the pentose phosphate pathway which is involved in lipid synthesis.

Both chapters 4 and 5 also demonstrated how juvenile Atlantic salmon adapt to seasonally varying temperatures in a manner that has been shown or implied in other salmonids, but has not previously been reported in this species.

Chapter 2 provides a straightforward explanation for non-biochemists of the biochemical theory used in chapters 4 and 5, plus a description of the main points that should be considered when developing an assay for either LDH or G6PDH.

Chapter 6 contains the first detailed account of the use of truss measurements to examine how the relative body shape of both modal groups of juvenile salmon changes throughout the period of fresh water residence. It also focuses on the level of mesenteric lipid present in both modal groups, since it has been suggested that this is used as a store of surplus energy acquisition in juvenile salmon. Both of the topics in this chapter are also examined in relation to the holding velocity results described in chapter 3. Discussions of the results from each experiment and the main implications regarding salmon ecology and the adoption of differing life-history strategies are contained within the respective chapters. However, as well as containing a brief synthesis of all the results and their main implications, chapter 7 discusses some of the more theoretical and wide ranging aspects of salmon biology and suggests further studies to address the questions arising from this thesis.

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# CHAPTER 2

# THE EFFECT OF LIFE HISTORY STRATEGY ON THE SEASONAL CURRENT HOLDING PERFORMANCE OF JUVENILE ATLANTIC SALMON

## Introduction

During the summer months juvenile Atlantic salmon (*Salmo salar* L.) tend to hold station against the current at a chosen focal point within a defended territory (Kalleberg 1958, Keenleyside and Yamamoto 1962). Most inhabit riffle-type habitats in areas of moderate to high velocity, and are usually in ventral contact with a home stone (Rimmer *et al.* 1983). They achieve this by using their pectoral fins as hydrofoils, thus providing negative lift (Wankowski 1981; Arnold *et al.* 1991). This gives them the opportunity to dart into adjacent areas of higher water velocity (with consequent higher rates of drifting prey) to capture particles of food (Wankowski and Thorpe 1979), so allowing them to minimise energy expenditure and maximise profits (Fausch 1984).

Many authors (Allen 1940; Stuart 1957; Bjornn 1971; Bustard and Narver 1975; Gibson 1978; Gardiner and Geddes 1980; Rimmer *et al.* 1983; Cunjak and Power 1986) have shown that during the autumn there appears to be a sudden decrease in the numbers of parr visible in feeding areas of streams and that this disappearance is related to temperature. Rimmer *et al.* (1983, 1984) and Heggenes and Saltveit (1990) showed that the fish do not move away from this area of the stream, but move into interstitial spaces in the cobble/boulder substrate.

This sheltering behaviour may be important in keeping the energy expenditure of these fish to a minimum, and

so maintaining the profit margin of the cost/benefit curve that they experience. Also (being ectothermic) general performance is likely to be impaired at low temperatures. However, feeding does occur over the winter (Jones 1959, Riddell and Leggett 1981), so some fish must emerge into the water column.

Higgins and Talbot (1985) and Metcalfe *et al.* (1986, 1988) have shown in laboratory experiments differences in food intake over autumn and winter that lead to the bimodal distribution of size in a single sibling population of juvenile salmon. The larger fish (Upper Modal Group, UMG) smolt the following spring and the smaller fish (Lower Modal Group, LMG) remain in freshwater for at least a further year (Thorpe 1977). Throughout the winter period (from October to March), the UMG fish maintain a significantly higher daily food intake at any given temperature than the LMG fish (Higgins and Talbot 1985). Obviously this divergence in food intake necessitates a difference in the overwintering ecology of these two types of parr, since the UMG fish (still growing) would have to spend more time in open water capturing prey than sheltering. This may lead to a difference between the two modes of fish in their ability to hold station in a current.

Rimmer *et al.* (1985) have shown that holding performance of juvenile Atlantic salmon generally varied with seasonal temperature, but more specifically fell dramatically at temperatures below 8°C - roughly equivalent to the temperature at which the parr move

into the interstitial spaces. This work was carried out on groups of parr of similar size and, therefore, did not address the question of differences in holding ability between UMG and LMG fish. A spring decrease in swimming ability has been recorded in smolting Atlantic salmon (Kutty and Saunders 1973; Thorpe and Morgan 1978) and in smolting coho salmon (*Oncorhynchus kisutch*) (Smith 1982). Therefore, it would also be of interest to continue measuring critical holding velocity (CHV) into the period of seaward migration to see if the ability to oppose current changes over this period of time.

The purpose of the present study then was to investigate further the relationship between CHV and development in response to changes in environmental temperature since biochemical and physiological processes in teleosts have been shown to acclimate quicker to increases in temperature than to decreases (Brett 1956). Specifically the following questions were addressed:

i) Are any seasonal variations in CHV of juvenile Atlantic salmon related to environmental temperature?

ii) Does the seasonal pattern of CHV differ between fish adopting alternative life-history strategies, and between parr and smolts?

iii) For fish following a given life-history strategy, does fish size and seaward migration affect CHV?

## Material and Methods

*Fish*

The fish used were sibling offspring from sea-run Atlantic salmon raised initially at the SOAFD smolt-rearing station at Almondbank, Perthshire and subsequently at the SOAFD Freshwater Fisheries Laboratory in Pitlochry. In both places the fish were held in radial flow tanks (Thorpe and Wankowski 1979) under conditions of natural light and temperature, and were fed dry hatchery pellets to excess.

The salmon used in the experiment were $0^+$ UMG and $0^+$ LMG fish (i.e. during the first year in freshwater), and $1^+$ LMG fish (i.e. during the second and final year in freshwater). Before each test period the population of $0^+$ experimental fish were weighed (to 0.1g) and measured (forklength, to 1mm) to allow classification into UMG or LMG (Cassie 1954, Thorpe 1977). Fish with forklengths close to the mean forklengths of the respective modal groups were selected for use in the experimental procedures.

*Equipment*

In order to increase the time allowed for habituation of the test fish whilst obtaining sufficient measurements within a suitable time period, a tank with six raceways was built (figs. 2.1a). The raceways each had a tetrahedral cross-section (fig. 2.1b) with the ventral surface wide enough for the largest parr to fully extend their pectoral fins. Each raceway had its

Figure 2.1a Diagram of the experimental tank (total length 1.08m), and associated water supply.

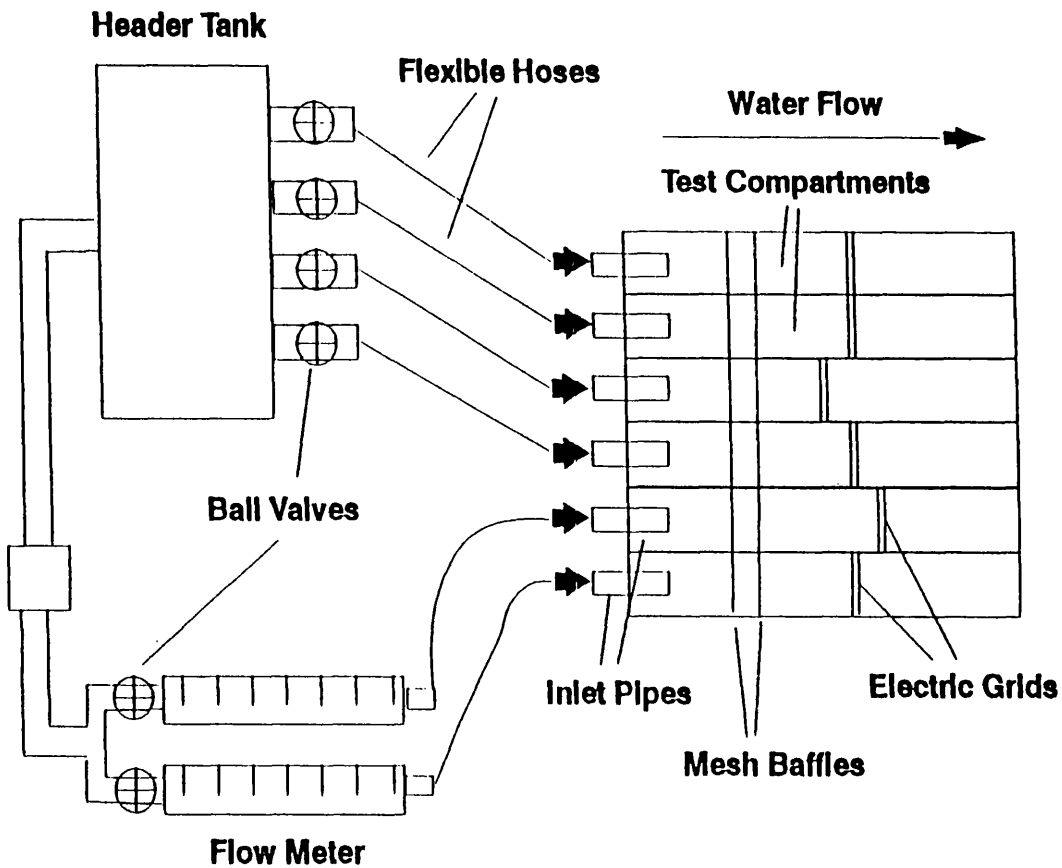
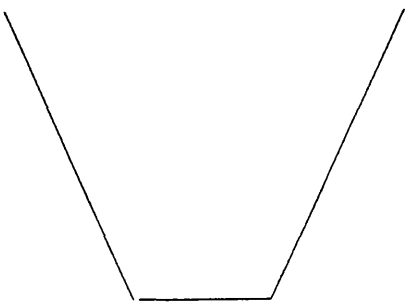
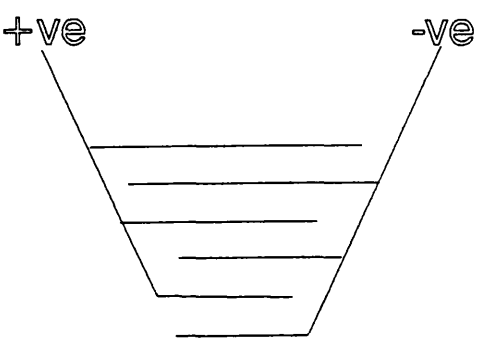

Header Tank
Flexible Hoses
Water Flow
Test Compartments
Ball Valves
Inlet Pipes
Electric Grids
Mesh Baffles
Flow Meter

Figure 2.1b cross section of raceway.

Figure 2.1c diagram of electric grid.

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-ve

own water inlet pipe separated from the test compartment by two plastic meshes to reduce turbulence. The fish were forced to maintain position in the test compartment by an incomplete metal grid placed downstream (Fig. 2.1c), which was connected to the anode and cathode of a DC electrical converter. When a fish touched this grid it closed the circuit and was subjected to an electrical potential of two volts. This was sufficient to keep the fish away from the grid without causing undue shock. The front part of the test compartment was lined with black insulating tape to encourage fish to maintain position in the compartment, and to help reduce any stress during habituation. Rimmer *et al.* (1985) reported that smooth plastic may reduce the measured performance of juvenile Atlantic salmon in velocity tests since they hold station on the substrate. To counteract this the bottom of each test compartment was covered in a layer of silicon sealant which adhered to the plastic and provided the fish with a slightly rougher substrate and, therefore, potentially more grip.

The experimental water supply was introduced through one of two lines, each with taps below a graduated cylinder giving a readout in litres.min$^{-1}$. The water velocity in the test compartment was calibrated with this readout using a Kent-Lea low-speed Miniflo probe (type 265-3) attached to a Nixon Streamflo 422 velocity meter (Nixon Instrumentation, Cheltenham, U.K.).

The output from each of these lines could be attached to the inlet tube of any raceway, as could the other four lines coming from the header tank. This allowed four fish to be acclimated to the raceways while two others were being tested, and eliminated the need to move fish to test areas immediately prior to testing.

*Test Procedure*

Each fish was anaesthetised with benzocaine, measured (forklength), weighed and photographed (for subsequent truss measurements - chapter 6), then allowed to acclimate to the raceway for at least twenty four hours prior to being tested. During acclimation the water flow from the header tank was held below one body length.$sec^{-1}$. When the test started the adjustable experimental flow was attached to the water inlet at a velocity of 1 body length.$sec^{-1}$. The electric grids in the test compartments were movable and were positioned for each fish so that to avoid making contact the fish had to hold station and could not slip backwards to any significant degree. The fish were then continually monitored throughout the experiment.

The critical water velocities that the fish can withstand were calculated by Brett's method (1964) of increasing water velocities by a standard amount every hour until the fish cannot maintain station. This is given by the equation: $CHV = V_i + (t_i/t_{ii} \cdot V_{ii})$

where CHV = critical holding velocity

$V_i$ = highest velocity maintained for prescribed period

$V_{ii}$ = velocity increment

$t_i$ = time (min) fish held at final increment before collapse

$t_{ii}$ = prescribed period at each velocity increment (60 mins)

When the fish could not hold station any longer they collapsed flat against the electrified grid and were immediately removed. This index has been shown to provide a accurate estimate of the maximum sustained speed derived by the fixed velocity technique (Brett 1967).

Because the tests were carried out on parr of varying size, the increment was calculated to be 1 body length.$s^{-1}$ based on the forklength of the fish. The results are referred to as critical holding (rather than swimming) velocities (CHV) (Rimmer *et al.* 1985), since salmon held station on the substrate, rather than swimming. In practice, once the juvenile were introduced into the test compartments they quickly aligned themselves towards the flow of water and on the ventral surface of the race way with their pectoral fins extended - a position they held for the majority of the acclimation and test periods. As the velocity increased so the fish could be seen to press lower on the substrate with occasional flicks of their tails if they started to slide backwards. Actual swimming

behaviour was only ever seen at the highest water velocities when some of the fish would rise off the substrate and show a very brief burst of swimming (usually 1 - 5 seconds) before collapsing onto the electrified grid.

*Sampling protocol*

Experiments on $0^+$ fish were conducted monthly from August 1990 to May 1991 (with the omission of September and April) and on $1^+$ fish during November 1990 and February, April and May 1991. All experiments were carried out under natural temperatures and photoperiods. Sample sizes varied between 6 and 12 fish from each modal group per month due to escapees and predation by feral cats.

During the sampling period in May photographs of the experimental fish were taken immediately prior to introduction into the experimental tank. This allowed the smolting fish to be graded on a scale of 0 to 4 to reflect the extent of silvering.

## Results

*Seasonal changes in critical holding velocity*

Over their first year in freshwater, during the period from August to April (prior to smolting), the absolute CHV (i.e. water velocity measured as $cm.s^{-1}$) of both UMG and $0^+$ LMG juvenile salmon showed a changing relationship with season that was highly significant

(Fig.2.2a, two-way ANOVA, $F_{6,115}=8.283$, $p<0.0001$): CHV declined with temperature over the autumn months and then remained constant during the more stable temperatures over winter. The effect of modal group was also highly significant (two-way ANOVA, $F_{1,115}=50.284$, $p<0.0001$), with the UMG fish (which are larger) being able to withstand higher water velocities than the LMG fish. However, when water velocities were expressed in relative terms (i.e. body lengths.$s^{-1}$) there was no significant difference between the two categories of parr (Fig.2.2b, two-way ANOVA, $F_{1,115}=0.411$, $p=0.565$). In neither case (absolute velocity and relative to body length), was there an interaction between modal group and season, indicating that both types of fish showed the same pattern of change throughout the first year of life prior to the time at which UMG fish smolt.

When the period from May 11 to 31 1991 (the probable final weeks of seaward migration of the UMG fish, during which time most were exhibiting visible signs of silvering) was included, there was a significant difference in relative CHV between the modal groups (two-way ANOVA, $F_{1,130}=4.589$, $p=0.034$), and a significant interaction between season and modal group (two-way ANOVA, $F_{7,130}=3.203$, $p=0.004$). This was due to the relative CHV of the UMG fish remaining unchanged while that of LMG fish increased during a period of rising temperature (Fig. 2.2b), resulting in the performance of UMG fish during May being lower than that of LMG fish. From winter (February) through to and

Figure 2.2a. Seasonal changes in mean absolute critical holding velocity ($cm.s^{-1}$) of $0^+$ UMG (—○—) and $0^+$ LMG (—●—) fish, and water temperature in degrees celcius (————). Vertical bars represent 1 standard error of the mean.

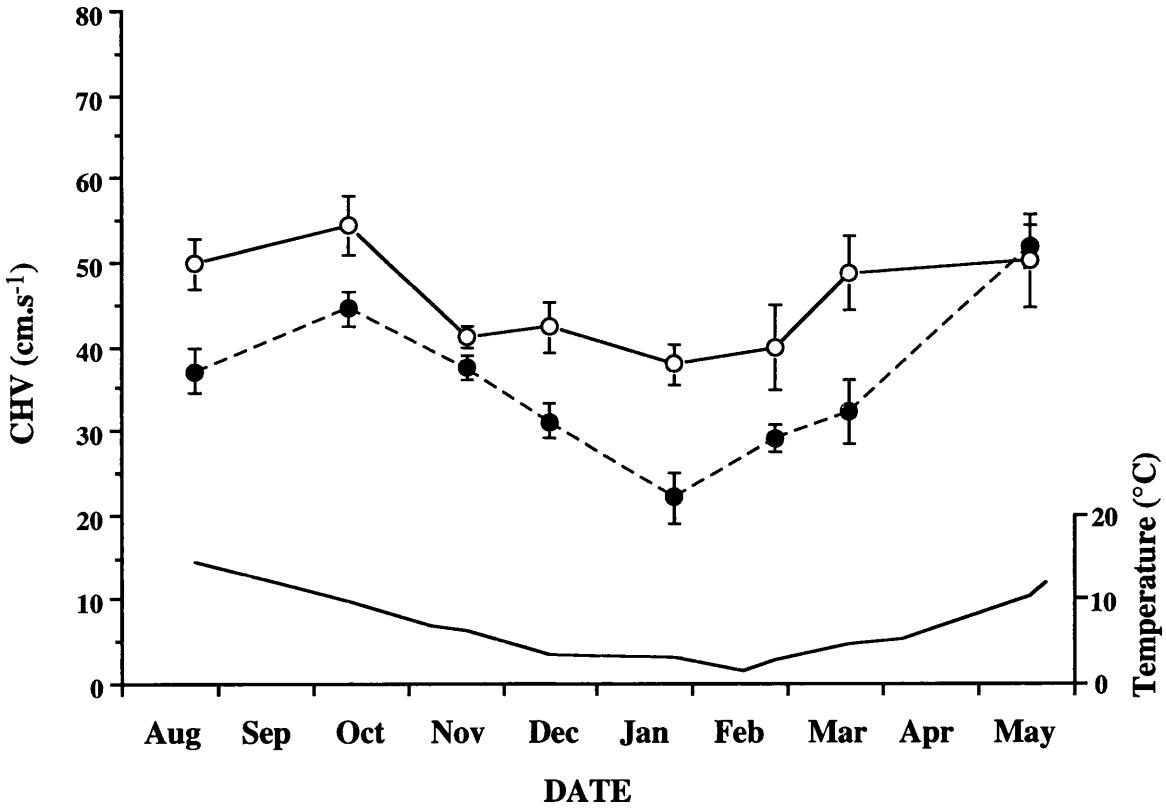
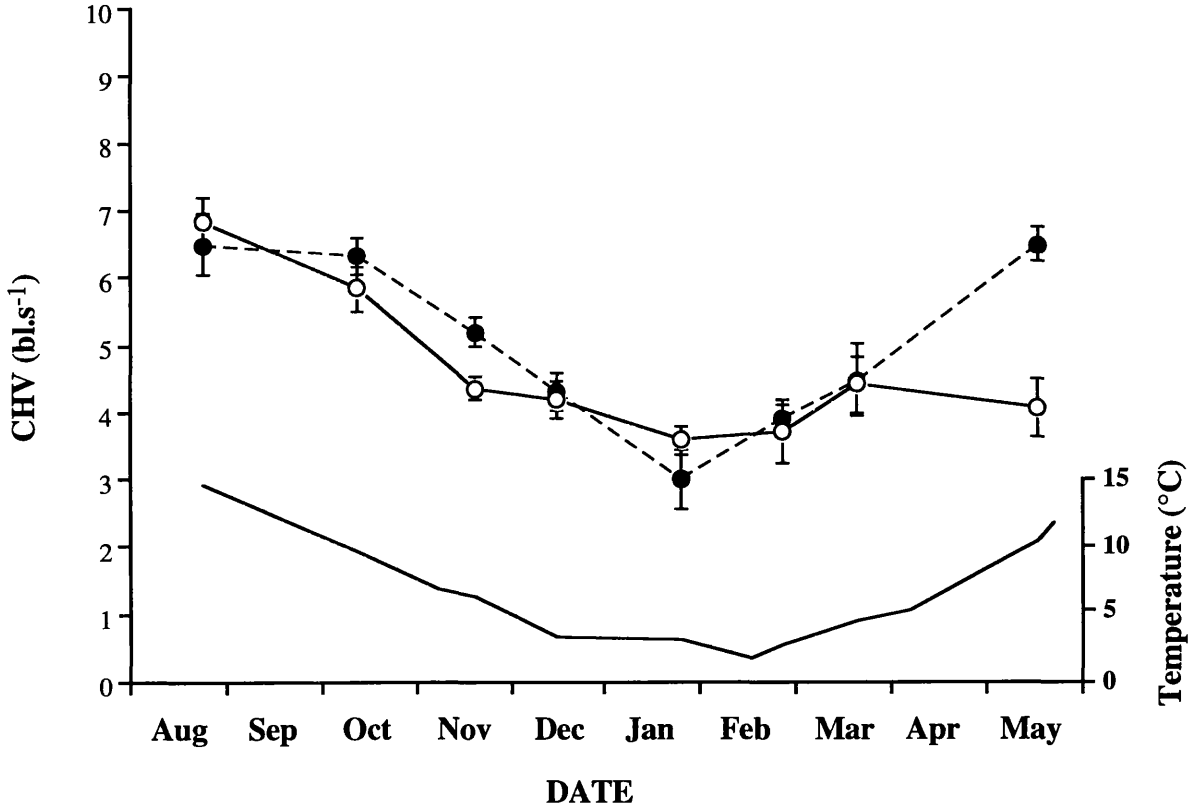

80
70
60
50
40
30
20
10
0
CHV (cm.s-1)
20
10
0
Temperature (°C)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 2.2b. Seasonal changes in mean relative critical holding velocity (bodylengths.$s^{-1}$) of $0^+$ UMG and $0^+$ LMG fish, and temperature (°C). Symbols and vertical bars as in figure 2.2a.


CHV (bl.s-1)
10
9
8
7
6
5
4
3
2
1
0
15
10
5
0
Temperature (°C)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

including the period of seaward migration (May), the absolute CHV of UMG fish showed no significant change (one-way ANOVA, $F_{2,21} = 1.141$, $p=0.339$).

A comparison of the absolute CHV (excluding the period of silvering) of $0^+$ UMG and $1^+$ LMG fish (i.e. during the year prior to seaward migration) shows that the $1^+$ LMG fish have a lower CHV in February and a higher CHV in March/April, causing a significant interaction between modal group and season (Fig. 2.3a. two-way ANOVA, $F_{2,44}=3.798$, $p=0.03$). However, this may be due to the two types of fish being of a significantly different size in March/April (average forklength: $0^+$ UMG=110mm, $1^+$ LMG=120mm), and to their being tested consecutively (rather than concurrently), and so at significantly different temperatures (average temperatures: March=4.6°C, April=5.4°C). When data for absolute CHV in May (the period of silvering) are included, the interaction ceases to be significant because of the greater variation in CHV shown by $1^+$ LMG fish at this time. This may be caused by this sample being taken over a longer than usual period of time, during which the physiological status of the fish may have changed.

However, when CHV is expressed relative to body length there is no statistical difference between $0^+$UMG and $1^+$ LMG fish (Fig. 2.3b, two-way ANOVA, $F_{1,44}=0.145$, $p=0.710$, and $F_{1,57}=0.165$, $p=0.690$ for data excluding (August to April) and including (August to May) the period of silvering respectively). So LMG fish in their

Figure 2.3a. Seasonal changes in mean absolute critical holding velocity ($cm.s^{-1}$)of $0^+$ UMG (—o—) and $1^+$ LMG (----■----) fish, and temperature (°C). Vertical bars represent 1 standard error of the mean.

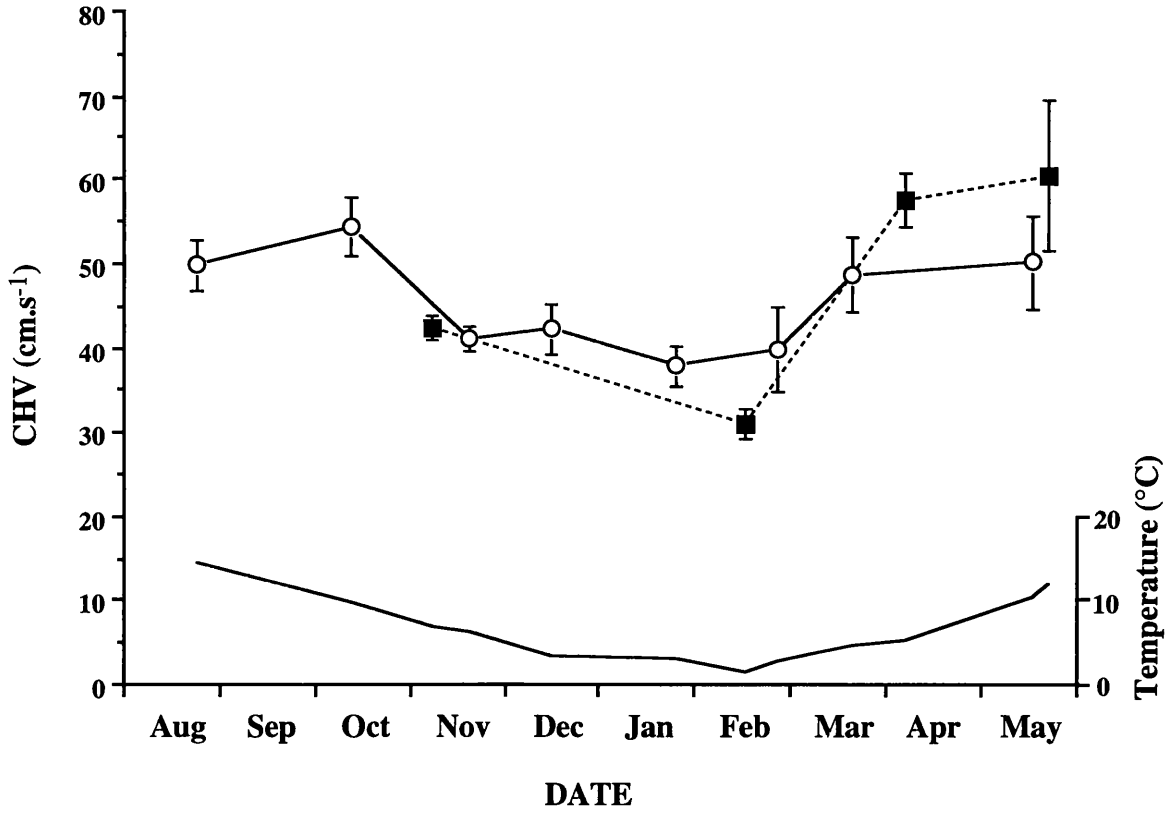
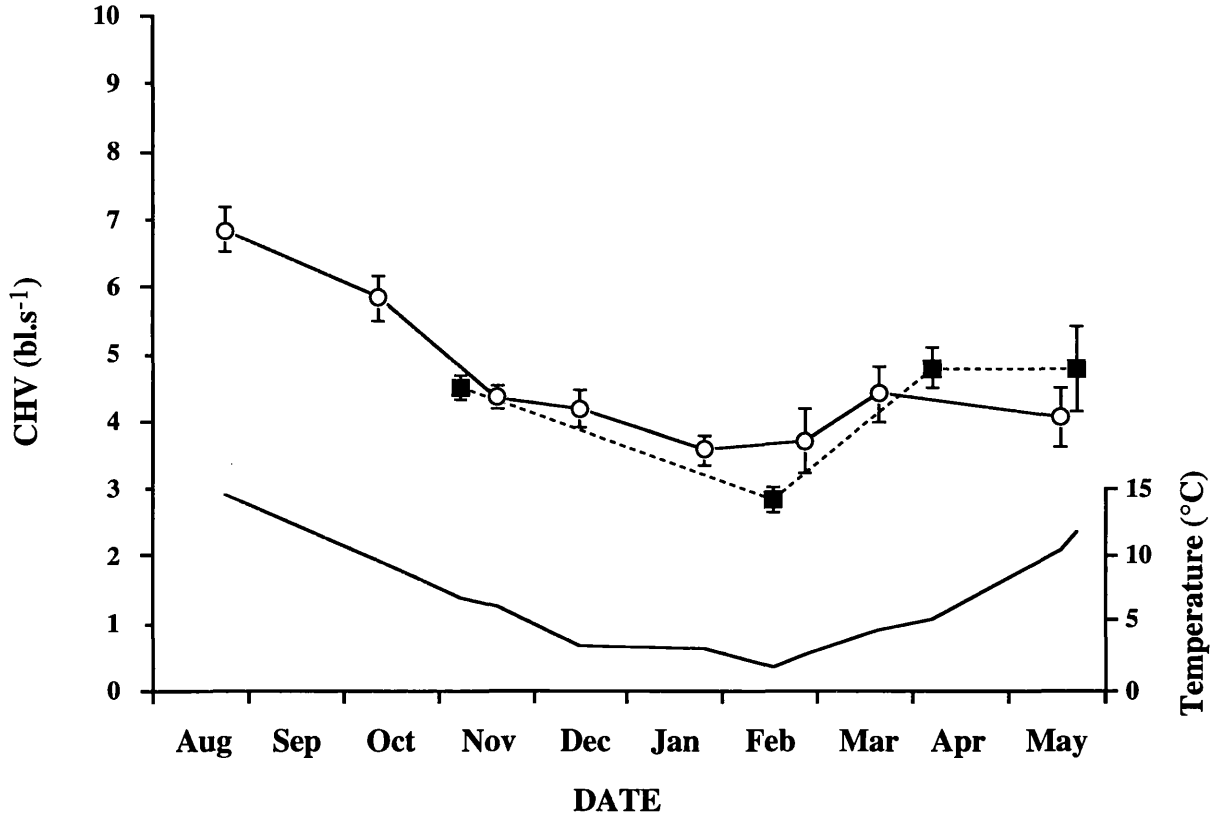

80
70
60
50
40
30
20
10
0
CHV (cm.s-1)
20
10
0
Temperature (°C)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 2.3b. Seasonal changes in mean relative critical holding velocity (bodylengths.$s^{-1}$) of $0^+$ UMG and $1^+$ LMG fish, and temperature (°C). Symbols and vertical bars as in figure 2.3a.


10
9
8
7
6
5
4
3
2
1
0
CHV (bl.s-1)
15
10
5
0
Temperature (°C)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

second year appear to perform as well as UMG fish the previous year.

*CHV in relation to temperature*

Regression analyses performed on relative CHV and the logarithm of relative CHV both showed highly significant effects with both temperature and the logarithm of temperature for $0^+$ UMG, $0^+$ LMG and $1^+$ LMG fish. (Data collected from the last month when the fish were silvering were not used when examining relations with temperature due to the obvious confounding effects.)

Over the whole sampling period all possible combinations of linear regressions between CHV and the logarithm of CHV with temperature and the logarithm of temperature showed highly significant correlations (all $P<0.00001$), however, $0^+$ LMG fish showed a slightly higher $R^2$ value when temperature was expressed as a logarithm of temperature (Table 1). Since this was the largest data set it was decided in retrospect (i.e. after comparing the periods of increasing and decreasing temperatures - see below) to use the correlation between relative CHV and the logarithm of temperature for all modal groups of fish to aid comparison.

The period over which these experiments took place (August 1990 - May 1991) showed temperature reaching its lowest point during early February 1991 (lowest daily mean temperature = 1.68°C). Hence when examining

**Table 1.** Values for linear regressions used to examine the relatonship between relative CHV ($bl.sec^{-1}$) and temperature (°C).

| Modal Group | N | linear regression | corr. coeff. | $R^2$ (%) |
|---|---|---|---|---|
| $0^+$ UMG | 65 | CHV-Temp | 0.750 | 56.24 |
| | | logCHV-logTemp | 0.691 | 47.72 |
| | | CHV-logTemp | 0.746 | 55.63 |
| | | logCHV-Temp | 0.681 | 40.32 |
| $0^+$ LMG | 73 | CHV-Temp | 0.676 | 45.69 |
| | | logCHV-logTemp | 0.682 | 46.49 |
| | | CHV-logTemp | 0.705 | 49.68 |
| | | logCHV-Temp | 0.645 | 41.57 |
| $1^+$LMG | 25 | CHV-Temp | 0.768 | 59.02 |
| | | logCHV-logTemp | 0.795 | 63.15 |
| | | CHV-logTemp | 0.778 | 60.52 |
| | | logCHV-Temp | 0.793 | 62.95 |

the effects of the direction of temperature change, the data set for UMG fish was divided into that collected from August to January (decreasing temperature) and that collected from February to April (increasing temperature), for $0^+$ LMG fish from August to January (decreasing temperature) and February to May (increasing temperature), and for $1^+$ LMG fish from August to February (decreasing temperature) and February to April (increasing temperature).

All types of fish showed highly significant correlations between CHV and logarithm of temperature during periods of increasing and decreasing temperature (minimum correlation coefficient = 0.670, $p<0.001$), except $0^+$ UMG fish with increasing temperature ($r = 0.428$, n.s.) - probably due to only two sampling periods being available during the Spring before downstream migration in May.

There was no significant difference between periods of increasing and decreasing temperature in the slope or elevation of the CHV-log temperature regression lines within fish types (co-variance analysis), so allowing data to be plotted over the whole year (figs. 2.4a, 2.4b and 2.4c). Comparisons between fish types showed similar CHV-temperature (logarithm) slopes for $0^+$ UMG and $0^+$ LMG fish, which were steeper than that for $1^+$ LMG fish ($t=2.28$, $df=86$, $p<0.05$).

Using the equations of these semi-log plots, CHV was then plotted against temperature over the whole year for each modal group (Figs. 2.4d, 2.4e and 2.4f).

Figure 2.4a. Correlation between relative CHV of $0^{+}$UMG fish and the logarithm of seasonal temperature ($y=4.14x + 1.74$, $r=0.746$, $n=65$, $P<0.00001$).

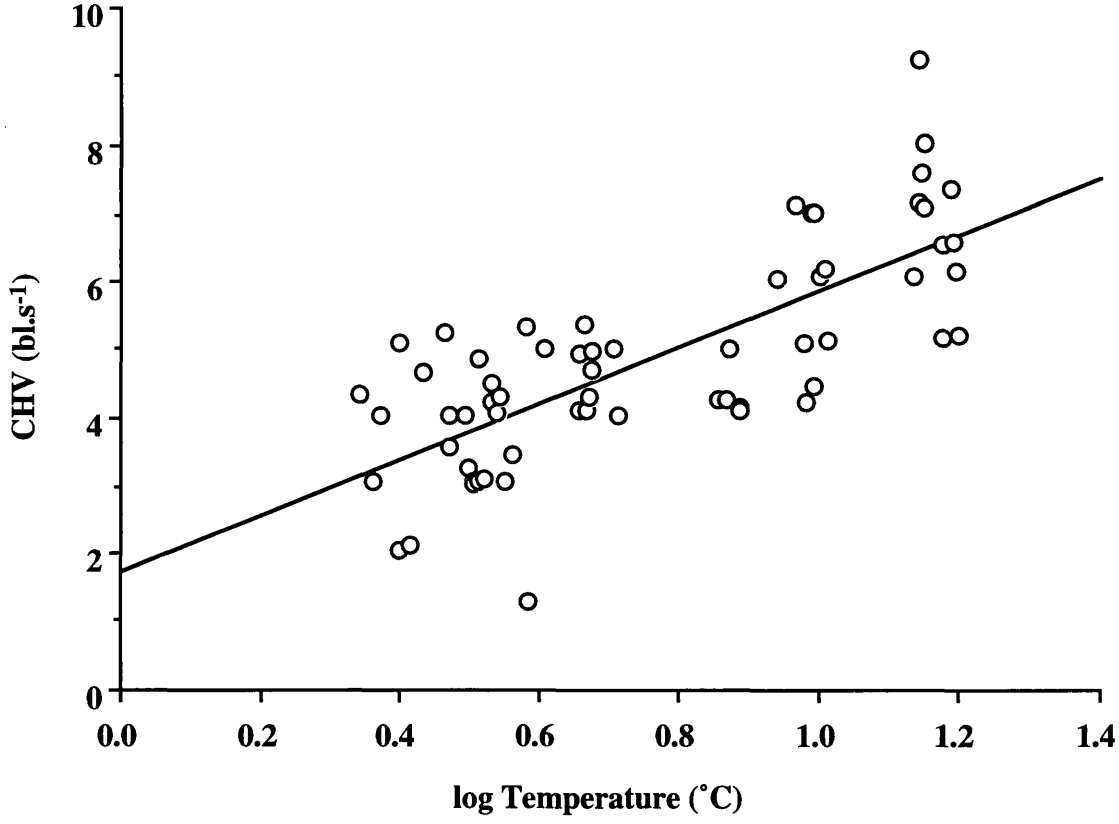

10
8
6
4
2
0
CHV (bl.s-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
log Temperature (°C)

Figure 2.4b. Correlation between relative CHV of $0^{+}$LMG fish and the logarithm of seasonal temperature ($y=4.24x + 1.83$, $r=0.705$, $n=73$, $P<0.00001$).

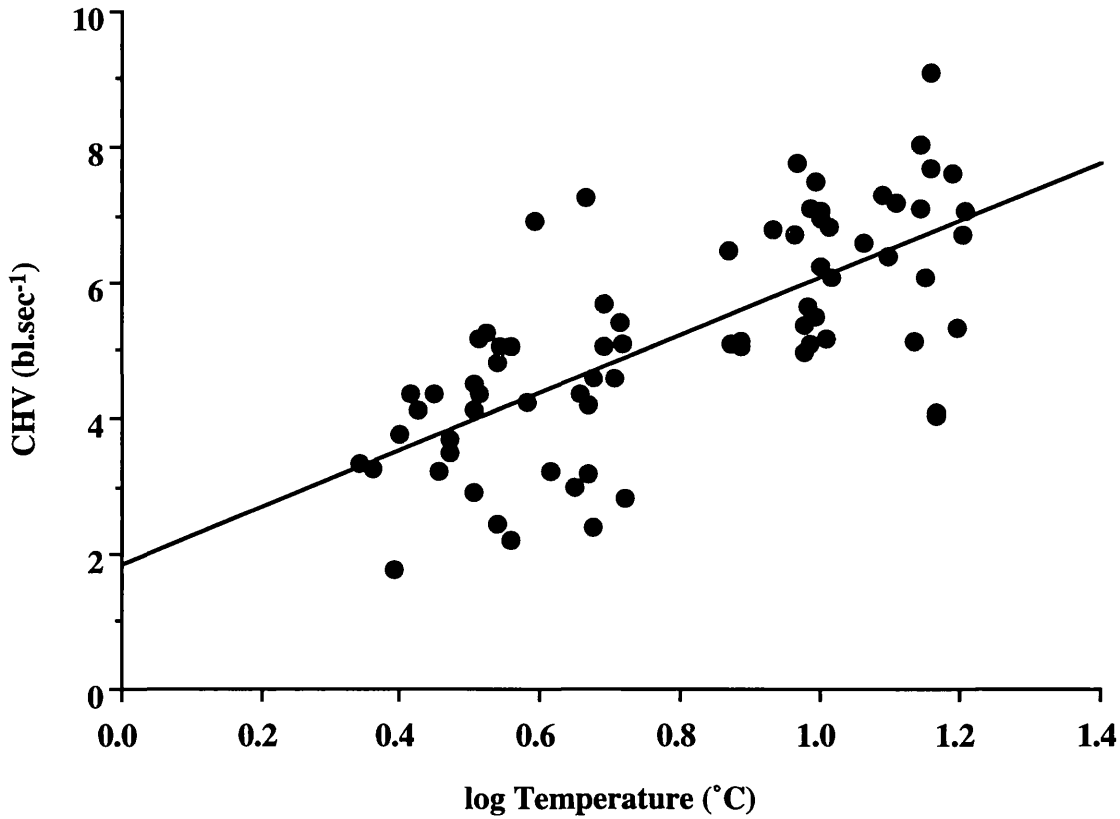

10
8
6
4
2
0
CHV (bl.sec-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
log Temperature (°C)

Figure 2.4c. Correlation between relative CHV of $1^{+}$LMG fish and the logarithm of seasonal temperature ($y=2.67x + 2.41$, $r=0.778$, $n=25$, $P<0.00001$).

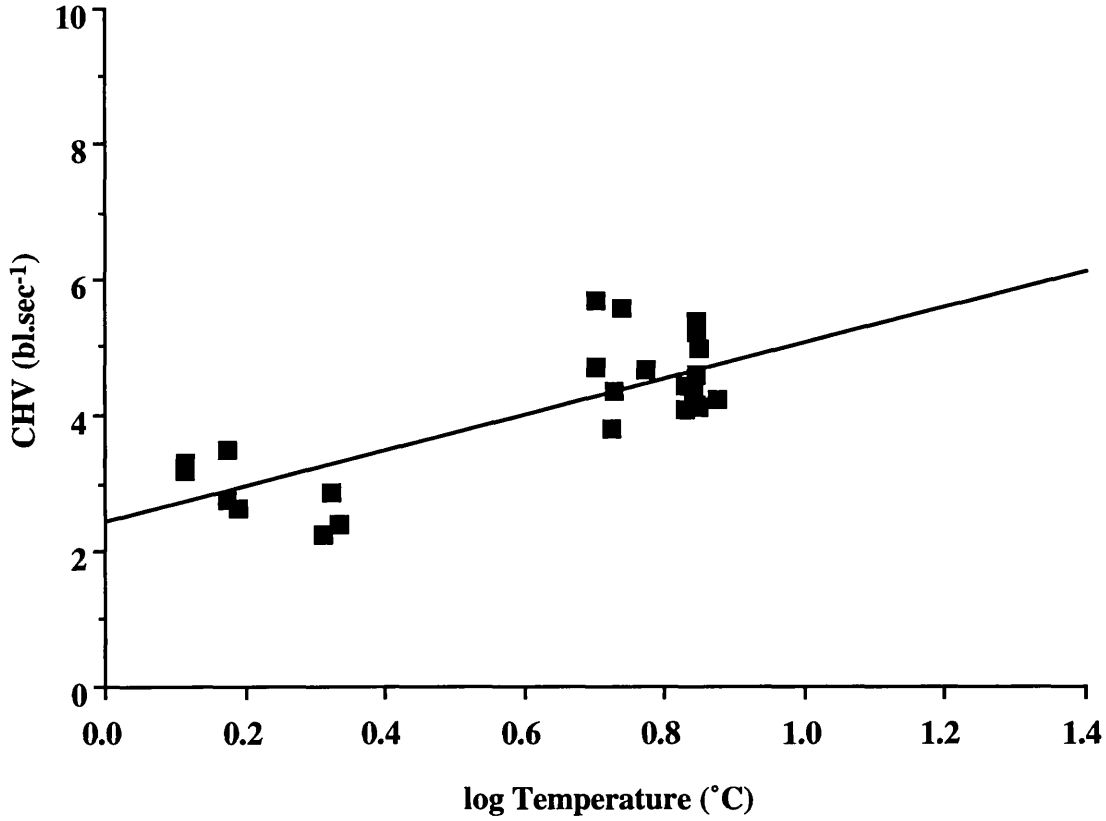

10
8
6
4
2
0
CHV (bl.sec-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
log Temperature (˚C)

Figure 2.4d. Effect of seasonal temperatures on mean relative critical holding velocity of $0^{+}$ UMG fish. The regression line ($y=4.14\ \log X + 1.74$, $r=0.746$, $n=65$, $P<0.00001$) is derived from the semi-log plot of CHV versus temperature (fig 2.4a).

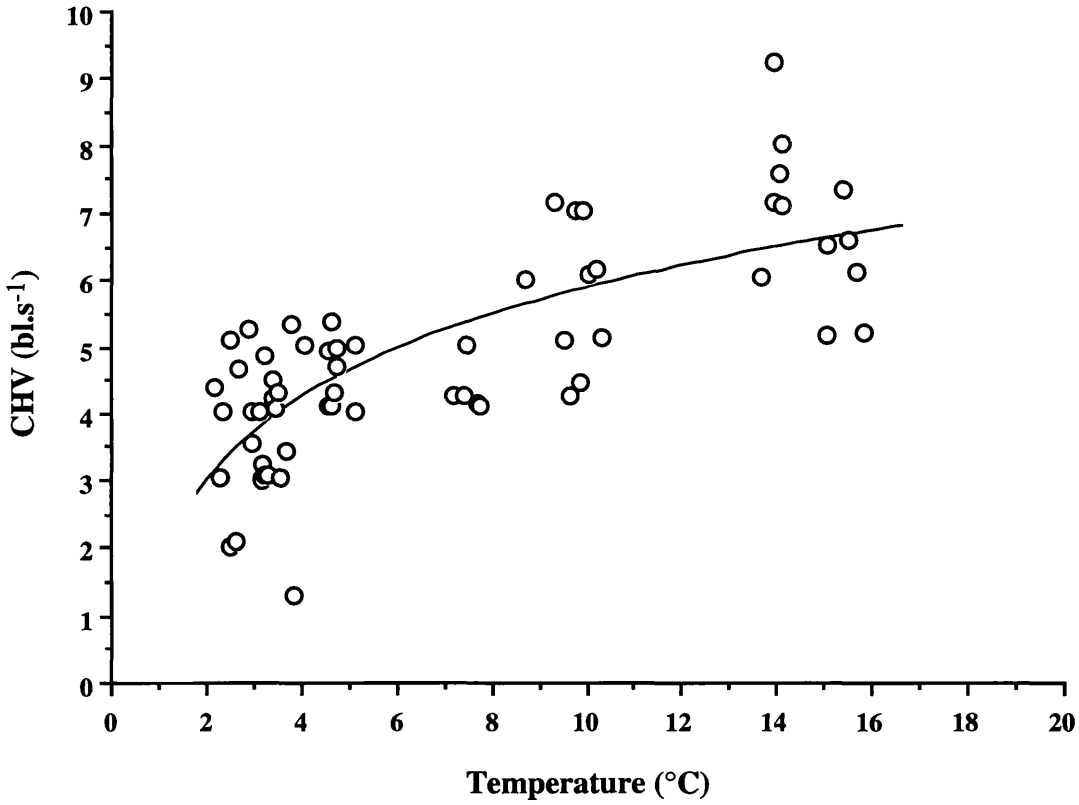

CHV (bl.s-1)
Temperature (°C)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20

Figure 2.4e. Effect of seasonal temperatures on mean relative critical holding velocities of $0^{+}$ LMG fish. The regression line ($y=4.24 \log X + 1.83$, $r=0.680$, $n=73$, $P<0.00001$) is derived from figure 2.4b.

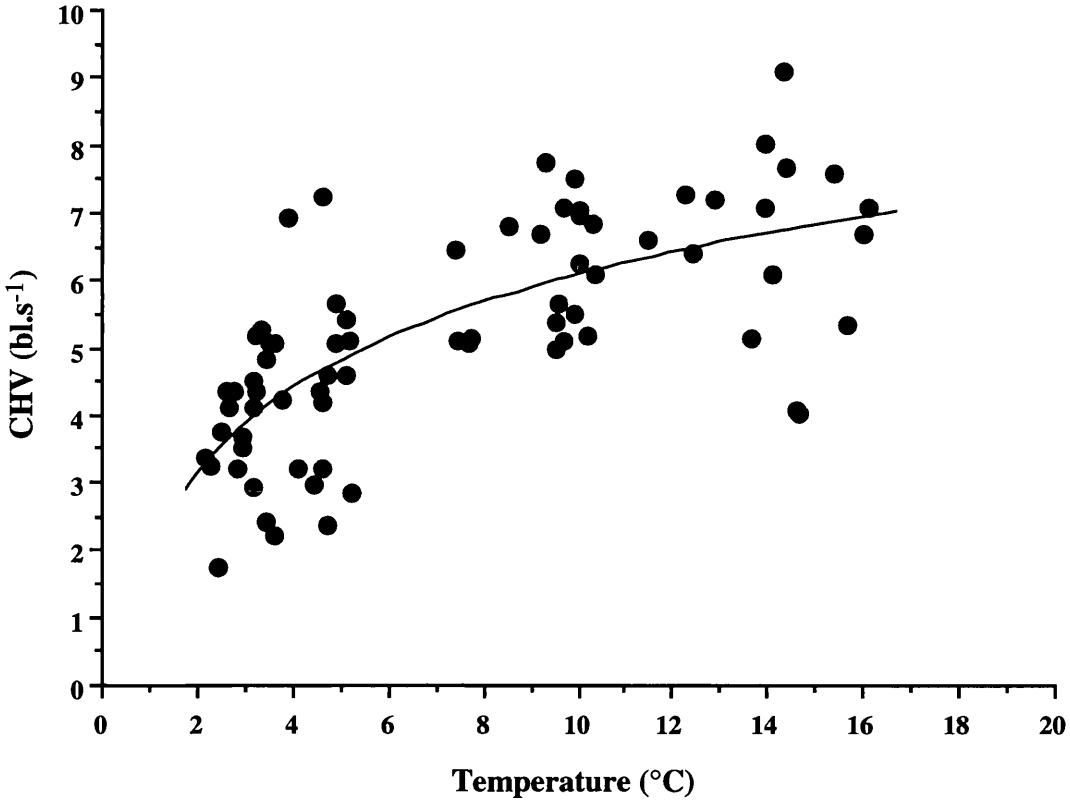
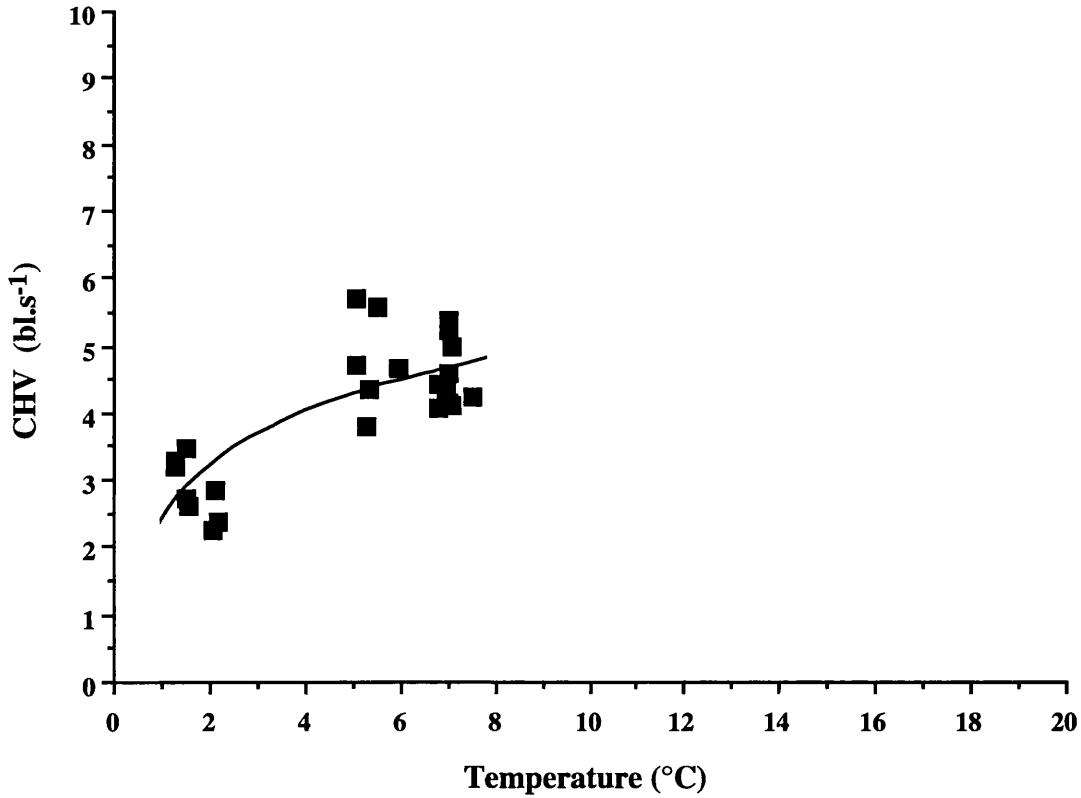

CHV (bl.s-1)
Temperature (°C)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20

Figure 2.4f. Effect of seasonal temperatures on mean relative critical holding velocity of $1^{+}$ fish. The regression line ($y=2.67 \log X + 2.41$, $r=0.778$, $n=25$, $P<0.00001$) is derived from figure 2.4c.


CHV (bl.s-1)
Temperature (°C)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20

*Effect of fish size*

The effect of fish size on relative holding performance was determined for each life-history strategy of fish by regressing relative performance against fork length and temperature over the whole year in a multiple regression.

For $0^+$ UMG and $0^+$ LMG fish temperature was the only variable that had a significant effect on CHV. However, in $1^+$ LMG salmon there was an independent effect of both temperature and forklength, with large fish at a given temperature having a higher relative CHV than small fish (Table 2).

*Effect of degree of silvering*

Figure 2.5 shows that as amount of silvering increases (i.e. the external appearance of the fish become less like a parr and more like a smolt) the relative CHV increases (Spearman rank correlation $r_S=0.779$, N=12, $p<0.02$). There was no correlation between degree of silvering and either water temperature or date.

## Discussion

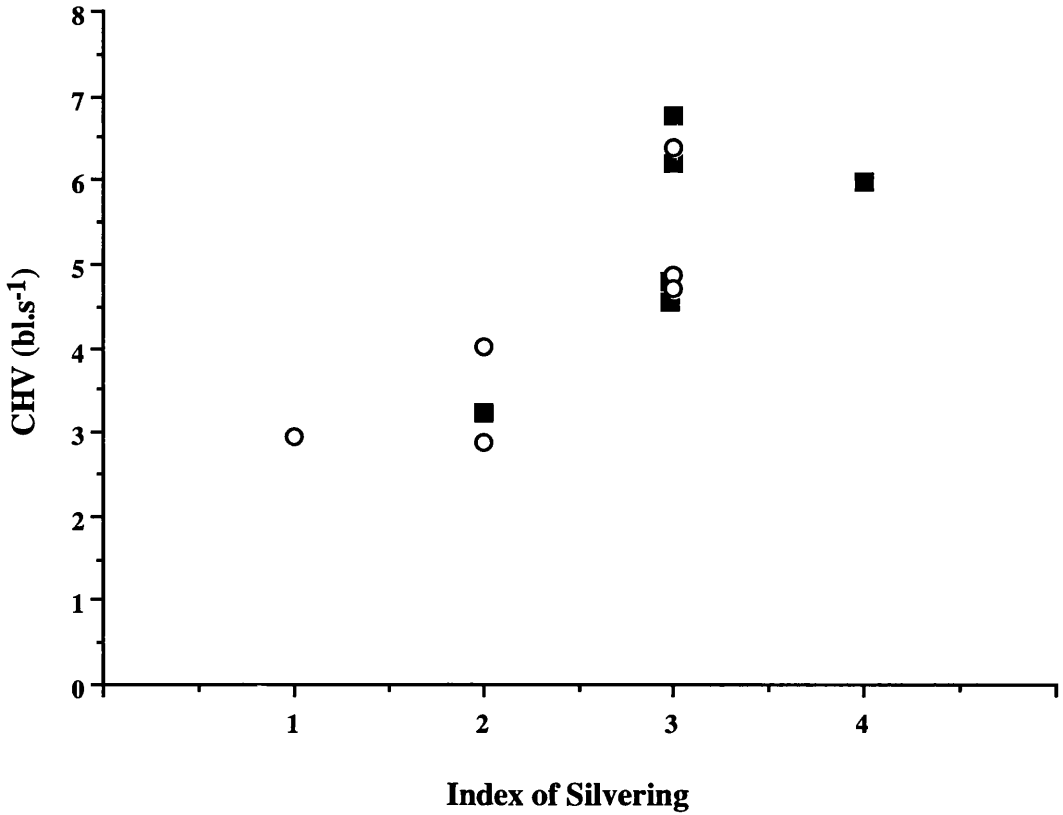
Heggenes (1990) confirmed snout velocity (i.e. the current that the fish actually experiences) to be one of the most important variables in the habitat requirements of juvenile Atlantic salmon. Since feeding stations tend to be in sheltered areas of low velocities close to higher currents (Wankowski and

**Table 2.**

Summary statistics of the multiple regressions relating relative CHV (body lengths.$sec^{-1}$) to temperature (temp) and fish forklength (fl, mm) for 3 categories of fish.

| Variable | regression coefficient | t value | df | $R^2$ | P value |
|---|---|---|---|---|---|
| $0^+$UMG: | | | | | |
| temp | 0.284 | 4.56 | 64 | 0.0550 | <0.0001 |
| fl | 0.010 | 0.50 | 64 | 0.62 | |
| $0^+$LMG: | | | | | |
| temp | 0.292 | 7.68 | 72 | 0.471 | <0.0001 |
| fl | 0.047 | 1.99 | 72 | 0.0502 | |
| $1^+$LMG: | | | | | |
| temp | 0.390 | 6.89 | 24 | 0.657 | <0.0001 |
| fl | 0.031 | 2.59 | 24 | 0.017 | |

Figure 2.5. The effect of silvering on mean relative critical holding velocity (bodylengths.$s^{-1}$) of $0^+$ UMG fish ( O ) and $1^+$ LMG fish ( ■ ) during the period of seaward migration (May).


8
7
6
5
4
3
2
1
0
CHV (bl.s-1)
1
2
3
4
Index of Silvering

Thorpe 1979), this allows the fish to dart out to intercept prey items. This is important for energetic reasons (Bachman 1984; Fausch 1984) and confirms the importance of the availability of low velocity microniches to parr.

Habitat studies have not compared UMG and LMG fish, but have shown slight differences between snout velocities of year classes (Table 3). However, there is a great deal of overlap between year classes of parr, which must be even greater for the different modal groups within a single year class. The present study has shown that LMG fish are unable to withstand such high flows as the UMG fish, when flow is measured in absolute terms ($cm.s^{-1}$). This is a true difference in capability (rather than in preference), since these fish were forced to hold station. The difference in mean absolute CHV between the two modal groups during this study varied between 4 $cm.s^{-1}$ (November) and 17 $cm.s^{-1}$ (January) (Fig. 2.2d), which is a narrower range than those shown previously to be preferred by parr (see above references). Also the lowest absolute velocities that $0^{+}$ LMG fish were shown to be able to withstand (approximately 20 $cm.s^{-1}$ seen in January; Fig. 2.2d) were well within the range of summer velocities seen in $0^{+}$ parr (Table 3). Therefore it seems unlikely that LMG fish would be unable to occupy the same areas of the stream as UMG fish, especially since during the periods of low temperatures LMG fish are likely to spend much

**Table 3.** Recorded snout velocities of juvenile Atlantic salmon of different age classes.

| Reference | Age | Snout velocity | Season |
|---|---|---|---|
| Rimmer *et al.* (1984) | $0^+$ | 10-30 $cm.s^{-1}$ | summer (Jun-Aug) |
| | $1^+$ | 20-40 $cm.s^{-1}$ | |
| | $2^+$ | 30-50 $cm.s^{-1}$ | |
| DeGraaf and Bain (1986) | $0^+$ | 0-35 $cm.s^{-1}$ | summer (Jul-Sep) |
| | Parr | 0-45 $cm.s^{-1}$ | |
| Morantz *et al.* (1987) | $0^+$ | 4-20 $cm.s^{-1}$ | summer (Jun-Sep) |
| | $1^+$ | 8-30 $cm.s^{-1}$ | |
| | $2^+$ | 9-34 $cm.s^{-1}$ | |

more of their time sheltering in crevices or under the substrate.

As long as sufficient suitable substrate is available to create these low-velocity microniches, the decline with temperature in absolute CHV of juvenile salmon may not necessitate large-scale, long distance autumnal migrations of parr to areas of the stream with lower overall velocities, and, therefore, a loss of potentially available habitat. Rimmer *et al.* (1983) showed that there was very little movement to other parts of a river with decreasing temperature, but that juvenile salmon (not distinguished into different modal groups) sheltered beneath the substrate in the areas of summer residence.

However, Cunjak and Randall (1993) have recently shown that the larger $0^+$ juvenile Atlantic salmon tend to stay resident in the same areas of a stream over winter, whereas the smaller $0^+$ salmon are more likely to emigrate. The authors note that this may be due to the severe winter conditions in eastern Canada, since greater site fidelity is seen in juvenile Atlantic salmon in the Girnock Burn, Scotland (Garcia de Leaniz 1989) which has higher winter water temperatures.

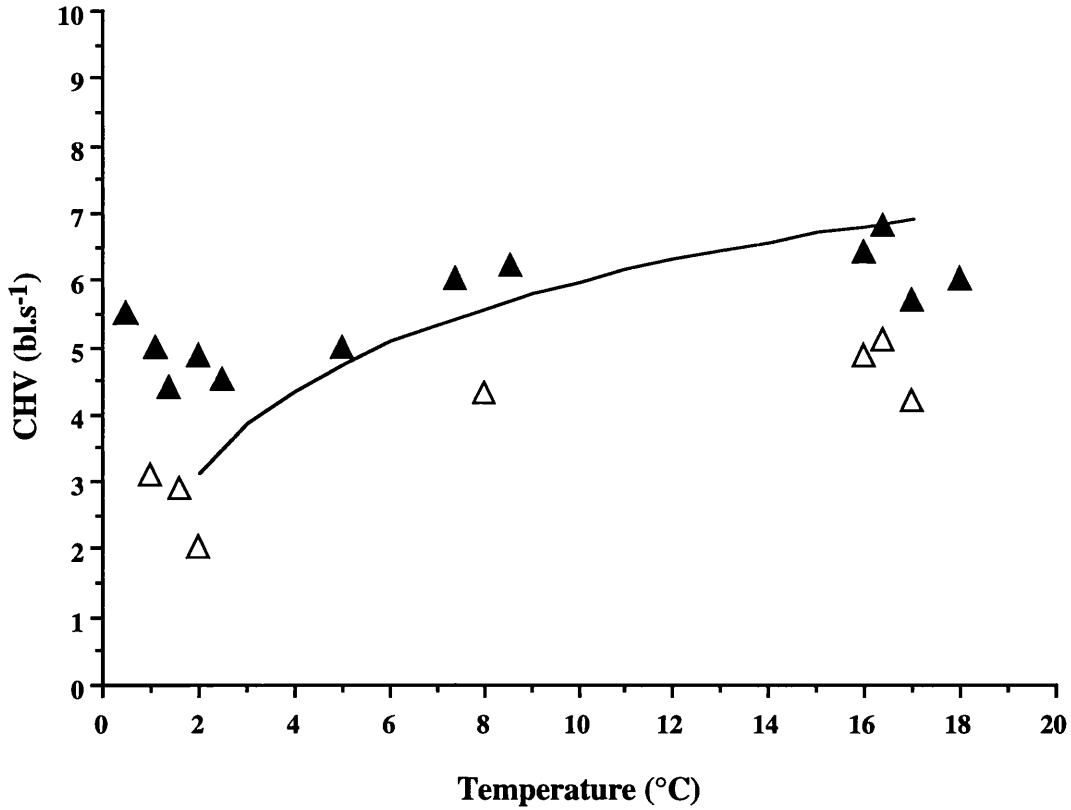
Fraser *et al.* (1993) have recently shown that UMG salmon parr show greater relative nocturnal activity (associated with feeding) as temperature declines, and Heggenes *et al.* (1993) have shown the same nocturnal activity in brown trout (*Salmo trutta* L.) to occur in slow flowing areas of a stream close to the faster

flowing areas used for diurnal sheltering. The strategy of minimising energy expenditure throughout winter adopted by LMG fish, with the concomitant reduction in appetite and food intake (Metcalfe *et al.* 1986, 1988), means that these fish need only emerge into the water column for the amount of time necessary to obtain a maintenance ration. In contrast, since they continue to grow over the winter, UMG fish need to spend more time out of shelters in order to feed. This would lead to a difference in the amount of sheltering behaviour shown by the two modal groups of fish.

The almost exclusive nocturnal foraging seen in UMG fish at relatively high levels of food availability by Fraser *et al.* (1993), may, by necessity, be less pronounced at lower levels of food availability, as observed by Gibson (1978), since the fish would be unable to meet their intake requirements by feeding only at night. This would explain the results of Thorpe *et al.* (1988) (at a lower level of available food), that salmon of both modal groups grew relatively poorly when fed only by night. This in turn may be due to the relatively poor visual acuity of these visual predators at low light levels (Ali 1961). Nocturnal use of slow flowing areas of a stream might therefore allow the fish more time to see their prey, rather than occurring for reasons of energy expenditure.

This study has shown that all fish, regardless of life history strategy, show a decrease in CHV with declining temperature. Figure 2.6 shows the CHV values from non-

Figure 2.6. A comparison of the effect of seasonal temperatures on the relative critical holding velocity (bodylengths.$s^{-1}$) of hatchery $0^{+}$LMG fish (———) from the present study, and wild ( ▲ ) and hatchery ( △ ) fish from Rimmer *et al.* (1985).


CHV (bl.s-1)
Temperature (°C)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
20

smolting wild and hatchery unimodal yearling Atlantic salmon over a whole year (from Rimmer *et al.* 1985), plotted against seasonal temperature, superimposed on the equivalent data for $0^{+}$LMG fish from the present study. The results from the two studies are roughly comparable; the difference in elevation seen between the two sets of values from hatchery fish is probably due to those in the present study having been in contact with a rough substrate, thus allowing greater grip, whereas Rimmer *et al.* (1985) noted that their use of a smooth Plexiglass substrate may have led to an under estimation of CHV values.

Gardiner and Geddes (1980) and Rimmer *et al.* (1985) have postulated the existence of a critical threshold temperature, with sheltering behaviour triggered when water temperatures drop below this point in autumn. Rimmer *et al.* (1985) suggest that this threshold coincides with an abrupt decline in CHV due either to an inability to adapt completely to low temperatures, or to the fact that it becomes more efficient to remain inactive at low temperatures (Gibson 1978). However, there appears to be geographical variation in the temperature at which this switch occurs (Table 4). In the present study there was no abrupt decrease in CHV, but a gradual decline that becomes more severe as the temperature drops below 6°C. This temperature is similar to that at which salmon have been observed to take shelter in the British Isles, whereas the drop in

**Table 4.** Geographical and interspecific variation in the water temperature at which juvenile salmonids commence sheltering (in autumn) in the streambed and emerge again in spring.

| Reference | Temperature (°C) (shelter) | (emerge) | Area | Species |
|---|---|---|---|---|
| Allen (1940) | 7 | 7 | N.W. England | Atlantic salmon |
| Gardiner & Geddes (1980) | 5 | 6-7 | E. Scotland | Atlantic salmon |
| Gibson (1978) | 9-10 | | Quebec | Atlantic salmon |
| Rimmer *et al.* (1983) | 9-10 | | E. Canada | Atlantic salmon |
| Bjornn (1971) | 4-6 | | E. U.S.A | Chinook salmon, Rainbow/ steelhead trout |
| Bustard & Narver (1975) | 4-7* | | W. Canada | Chinook salmon, Steelhead trout |
| Cunjak & Power (1986) | 4.5 | 5 | Quebec | Brook trout |

* dependent upon stream conditions

CHV and sheltering seem to occur at higher temperatures in eastern Canada (Table 4).

This sheltering behaviour may, therefore, be an example of a genetic switch that can respond to different levels (temperatures) depending upon rate of temperature change, as an adaptive response to local conditions (Schaffer and Elson 1975, Taylor 1991). Adult salmon tend to return to spawn in the same parts of the river they inhabited as juveniles, so allowing natural selection acting on local populations to cause at least partial genetic isolation (Berst and Simon 1981; Ståhl 1987).

It has been suggested (Riddell and Leggett 1981) that in juvenile salmon living in high velocity streams, longer pectoral fins are selected for, to reduce energetic costs of maintaining position. However, Rimmer *et al.* (1985) acclimated similar sized parr to different temperatures and showed their CHV to be the same as that of fish experiencing similar temperatures throughout the year, proving that temperature, not variations in relative fin size (due to changes in body size with growth), has the greatest influence on CHV.

An inverse relationship between body size and relative critical swimming speeds has been reported for other salmonids (Brett 1965; Fry and Cox 1970; Glova and McInerney 1977). Explanations for this effect have invoked the increased hydrodynamic drag brought about by an increase in body size outweighing any increase in musculature or metabolism (Brett 1965). Such forces may

not be applicable to the mechanism of opposing the water current adopted by juvenile Atlantic salmon (i.e. in contact with the substrate rather than swimming in the water column). This could explain the trend seen in $1^+$ LMG fish (and also in $0^+$ LMG fish, but not significantly) of CHV being positively correlated with forklength.

There was no difference (prior to smolting) in the relative performance of upper and lower modal groups of parr. Therefore, allowing for differences in body size, LMG fish are capable of working as efficiently as those in the UMG. In physiological terms, the life history strategy adopted by the LMG fish can then be viewed as a developmental option that has been taken, rather than as a strategy the fish have been forced into because they are physiologically less capable than UMG fish.

This view is supported in the present study by the fact that there was an identical seasonal pattern of relative holding velocity for $0^+$ UMG and $1^+$ LMG fish. These were fish of different ages that had followed the two different life history strategies evident in a bimodal split and would both smolt the following spring, but over their first year in freshwater had belonged to dominant and subordinate groups respectively. This also demonstrates that the different behavioural traits exhibited by the LMG parr (Huntingford *et al.* 1988), in particular the seasonal anorexia (Metcalfe and Thorpe 1992) and the subsequent food intake at maintenance ration level (Higgins and

Talbot 1985), do not have long term effects on the subsequent performance of these fish during the year prior to seaward migration.

During the spring, the absolute CHV of LMG fish increased with temperature, but that of the UMG remained constant. This has implications for the causes of seaward migration of smolts. It has been postulated (Tytler *et al.* 1978; Thorpe 1982; 1984 and 1989; Thorpe and Morgan 1978; Thorpe *et al.* 1988) that smolts are flushed out of a river system due to a loss of visual orientation at night. The absolute CHV of these UMG fish shows no significant change from winter through to the migration period in spring which seems to refute any idea that smolts are progressively physically less capable of withstanding water currents. However, the movement of smolts downstream (compared to parr at the same time of year) could be due to an inability to maintain contact with the substrate. The larger size of these fish (reducing availability of shelters), their increased buoyancy (Saunders 1965) and relatively smaller pectoral fins (due to lengthening of the body), along with a possible increase in shoal orientated responses (as seen in pink salmon *Oncorhynchus gorbuscha* (Hoar 1956) and suggested for Atlantic salmon by Thorpe and Morgan (1978) and for salmonids generally by Thorpe (1982)), will all serve to place these fish in mid water where velocities are usually higher than at substrate level. This will tend to increase the likelihood of downstream displacement since holding

station in contact with the substrate has been shown to allow juvenile Atlantic salmon to exceed the maximum sustainable swimming speeds of other salmonids (Beamish 1978).

McCleave and Stred (1975) and Thorpe and Morgan (1978) have shown that, after failure to hold station against increasing water flows, smolts do not show the signs of stress and fatigue shown by parr in a similar situation. Thorpe and Morgan (1978) have interpreted this either as a behavioural refusal or an inability to undergo sustained swimming. From the present study the latter seems most likely, since in all of these tests (including the present study) fish were forced to hold station upstream of an electric grid. This agrees with the findings of Virtanen and Forsman (1987) that smolts of Atlantic salmon have a lower physiological capacity for swimming than parr, possibly due to smolts having higher metabolic rates than parr (Higgins 1985; Maxime *et al.* 1989) which would reduce their capacity for sustained activity (Thorpe and Morgan 1978). However, it should be noted that sustained swimming is not a natural behaviour exhibited by parr.

The results showing that CHV increased with silvering (within the last $0^+$ UMG and $1^+$ LMG samples) seems to contradict this idea of decreased relative holding/swimming performance in smolts compared to freshwater residents. However, the association of degree of silvering with degree of smolting may not be a valid assumption. According to McMahon and Hartman

(1988) silver colouring was not a reliable indicator of migratory behaviour in juvenile coho salmon (*Oncorhynchus kisutch* Walbaum) in Carnation Creek, Canada where only 50 per cent of the migrants entering the estuary were completely silvered. Kato (1972) has shown that silvering can be induced in rainbow trout (*Oncorhynchus mykiss*) by holding them in blue tanks, suggesting that silvering served to camouflage them in a pelagic environment (Denton 1971; Kazakov and Koslov 1985).

The adaptive value of the low CHV values seen in the parr-marked fish may, in conjunction with the aforementioned factors causing them to rise into faster flowing water, act as a trigger for the important initial abandonment of position holding within a stream and the beginning of seaward migration. But since migration occurs primarily at night (Hansen and Jonsson 1985) due to loss of visual cues (Thorpe *et al.* 1988), smolts would have to resist the water current actively during the day (Jonsson 1991). Being unable to withstand mid-water velocities would only be adaptive at the start of the seasonal migration, and could lead to the correlation between CHV and degree of silvering in smolts.

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# CHAPTER 3

# THEORY AND DEVELOPMENT OF BIOCHEMICAL TECHNIQUES

## Theory

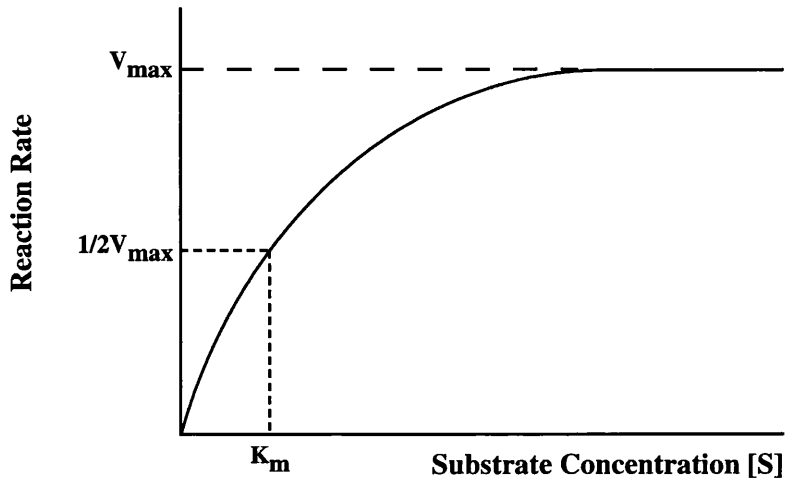
Chapters 4 and 5 deal with the possible adaptation of two areas of the metabolism of juvenile Atlantic salmon to seasonal temperatures. There are several possible mechanisms through which seasonal temperature may affect metabolic pathways: the direction of substrate flow through metabolic branchpoints; regulation of gene expression (and, therefore, enzyme synthesis); enzyme-modulator interactions; conformational changes in enzyme structure; and enzyme-substrate interactions (Hazel and Prosser 1974).

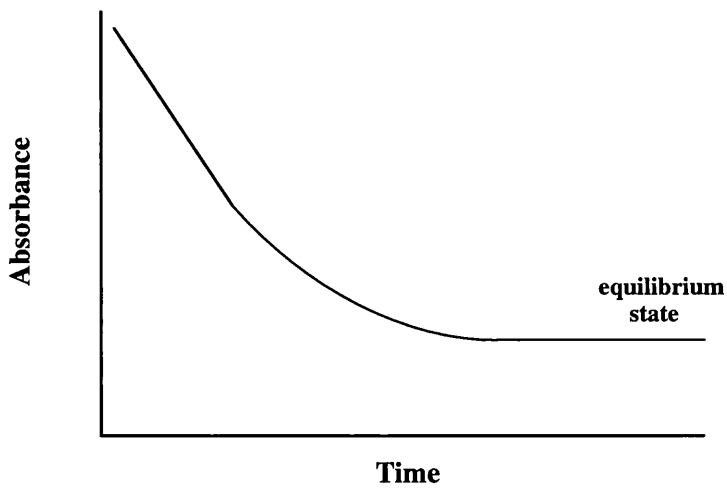
It was decided to concentrate the investigation on the kinetic characteristics of the enzymes involved in enzyme-substrate interactions, and in particular the Michaelis constant ($K_m$) and the maximum rate of reaction ($V_{max}$). Although many biochemical textbooks give complex definitions of these measures and how they may vary under different conditions, the reasoning behind their use is actually fairly straightforward, and they are readily incorporated into ecological studies.

For a simple, single-substrate, enzyme-catalysed reaction, as the substrate concentration ([S]) increases so does the reaction rate ($v$) until it reaches an asymptote (fig. 3.1). This is due to each enzyme molecule having only a finite number of catalytic binding sites for the substrate molecules. Hence, once the enzyme molecules are saturated with

Figure 3.1. Theoretical graph of the effect of increasing substrate concentration ([S]) on the reaction rate of a single substrate equilibrium reaction.

Figure 3.2. Theoretical graph of the decrease in absorbance over time of a single substrate equilibrium reaction as measured by a spectrophotometer.


Reaction Rate
$V_{max}$
$1/2V_{max}$
$K_m$
Substrate Concentration [S]


Absorbance
equilibrium state
Time

substrate molecules, the reaction rate cannot increase any further regardless of increases in substrate concentration, and the reaction has reached its maximum rate ($V_{max}$).

The Michaelis constant ($K_m$) is the [S] that produces a reaction rate equivalent to half of $V_{max}$ (fig. 3.1). The importance of $K_m$ (in terms of relating enzyme catalysis to ecology) is that it is proportional to the reciprocal of enzyme-substrate (E-S) affinity. Since E-S reactions are equilibrium reactions, high E-S affinities in enzymes cause greater binding of the substrate molecules, resulting in a more efficient enzyme. As $K_m$ decreases, E-S affinity increases (and *vice versa*) and the $K_m$ value can thus be regarded as a measure of the efficiency of an enzyme (i.e. the lower the $K_m$ the greater the efficiency).

The reaction rate of enzymes assays performed with varying substrate concentrations can be used in a simple linear regression technique to calculate both the $K_m$ and $V_{max}$ for a given enzyme. The rate of reaction within an enzyme assay is usually followed on a spectrophotometer by recording the change in absorbance within the enzyme-substrate solution caused for instance either by an increase or decrease in the cofactors nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH).

For example the reduction of pyruvate to lactate (see

chapter 4) is given in equation I.

$$\text{pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+ \qquad \text{(I)}$$

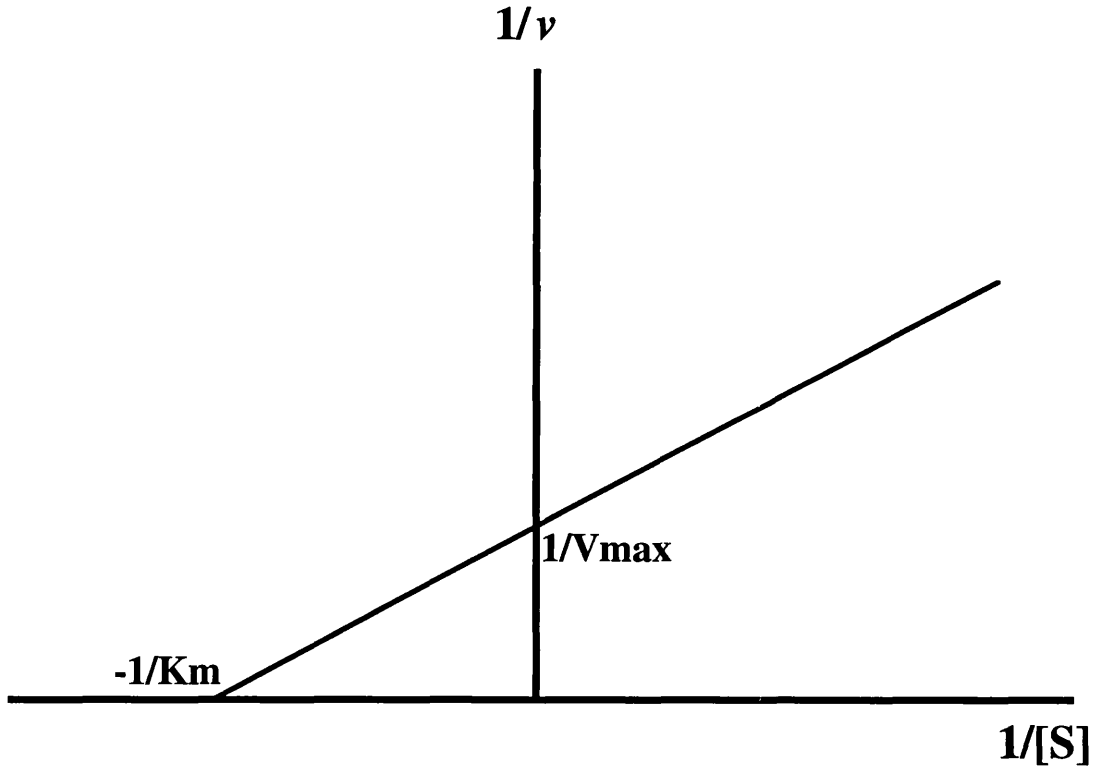
This reaction is followed as a decrease in absorbance due to the conversion of NADH to $NAD^+$.

Over the initial period of an enzyme assay the rate of absorbance increases or decreases at a constant rate as substrate molecules are converted into product molecules with the associated increase or decrease in cofactor, and the graph of change in absorbance remains linear. However, as the reaction approaches equilibrium the change in absorbance decreases (fig. 3.2) and the graph becomes non-linear until it eventually reaches an asymptote. It is this initial change in the level of absorbance when the rate of change over time is constant, that is used to calculate the velocity of a reaction for a given substrate concentration.

As the initial substrate concentration in the assay mixture is increased (as long as it is still below that which would result in $V_{max}$ rates), both the initial rate and the period over which the rate remains constant increases.

To calculate $K_m$ and $V_{max}$ values, the rates of reaction are obtained for different substrate concentrations below saturation and the reciprocal of the reaction rate ($1/v$) is plotted against the reciprocal of [S] ($1/[S]$) in a Lineweaver-Burk reciprocal plot (fig. 3.3).

Figure 3.3. Theoretical graph of a Lineweaver-Burke reciprocal plot based on a rearrangement of the Henri-Michaelis-Menten equation (see text).


1/v
1/Vmax
-1/Km
1/[S]

Simple, single substrate - single product enzyme reactions that are dealt with in this thesis can be described using the Henri-Michaelis-Menten equation II.

$$v/V_{max} = [S]/(K_m + [S]) \qquad \textbf{(II)}$$

When this is rearranged into a linear form (y = mx + b) it becomes equation III:

$$1/v = K_m/V_{max}.1/[S] + 1/V_{max} \qquad \textbf{(III)}$$

and it is this that is used in the Lineweaver-Burk plot. As can be seen both from equation III and figure 3.3 the intercept on the y-axis, when [S] = 0, is $1/V_{max}$. Also when 1/v = 0 (the intercept on the x-axis) then $(K_m/Vmax).(1/[S]) = -1/V_{max}$; therefore $1/[S] = -1/K_m$. Linear regression can then be performed on the data to obtain the equation describing the best fit for a regression line, and this equation can then be used to calculate $K_m$ and $V_{max}$.

An important consideration is the [S] used in the enzyme assays. If the chosen concentrations are too high relative to $K_m$ then the slope will be very shallow and it would be difficult to determine $K_m$ accurately. On the other hand if the [S] are low compared to $K_m$, the intercepts on both the x-axis and the y-axis will both be close to the origin and the accuracy of both $K_m$ and $V_{max}$ estimates will be affected (Segel 1976).

This method then, in theory, provides a fairly simple method for calculating kinetic characteristics of enzyme catalysis that can then form part of an ecological study.

Most studies over the past 25 years that have experimentally determined $K_m$ and $V_{max}$ values for various different enzymes have used this technique. However, one criticism is that use of the reciprocal of initial velocity ($1/v$) magnifies any initial errors in the measurement of the velocity of the reaction, and also that equally spaced increments of $v$ do not produce equally spaced increments of $1/v$, hence biasing the fitting of the line. This latter point was partially addressed in this project by not using equal increments of [S] (see chapters 4 and 5) but this had to be tempered by using substrate concentrations that were suitable for the assay conditions (see below).

There are basically two other methods of using measurements of $v$ and [S] to estimate $K_m$ and $V_{max}$ values: the Hanes-Woolf plot ($[S]/v$ versus [S]) and the Woolf-Augustinsson-Hofstee plot ($v$ versus $v/[S]$), both of which may provide significant advantages over the Lineweaver-Burk plot (Segel 1976). The Hanes-Woolf plot rules out any bias due to the increments of [S], and the Woolf-Augustinsson-Hofstee plot does not use reciprocals and, therefore, would not magnify any errors in the initial measurement of $v$.

All three techniques were used on the initial results from the enzyme experiments, but neither the Hanes-Woolf technique or the Woolf-Augustinsson-Hofstee technique were found to produce any significantly better results with linear regression than were obtained with the Lineweaver-Burk technique. Hence it

was concluded that the errors in measurement of *v* were not significantly magnified by using $1/v$, and that there was no significant bias due to the substrate concentrations used.

With the proliferation of personal computers increased accuracy of determination of $K_m$ and $V_{max}$ values is possible by performing linear regression analysis on the data from the Lineweaver-Burk plot. Also it is now becoming possible to remove the errors caused by using reciprocals of the initial data by using the direct measurements of *v* and [S] themselves to estimate $K_m$ and $V_{max}$ values using specially written computer programs (Brooks and Suelter 1986).

The two enzymes chosen for investigation were each involved in metabolic pathways that are closely related to aspects of the seasonal ecology of juvenile salmon. Lactate dehydrogenase (LDH; 1.1.1.27) is part of the glycolytic pathway associated with anaerobic respiration, which in turn is involved in energy production during the rapid burst-swimming exhibited by juvenile salmon when intercepting prey items. Glucose-6-phosphate dehydrogenase (G6PDH; 1.1.1.49) is the rate-limiting enzyme of the pentose phosphate pathway which has been shown to increase in activity with acclimation to low temperatures (Hochachka and Hayes 1962) which may be related to an increase in lipid synthesis (see chapters 4 and 5 for more detailed descriptions).

## Development of techniques

Assays for lactate dehydrogenase from the white muscle and glucose-6-phosphate dehydrogenase from the liver were both based initially on the diagnostic test kits supplied by Boehringer Mannheim (Catalogue numbers 124885 and 124672 respectively). However, not only were these kits designed to give a measurement of the optimal activity (i.e. $V_{max}$) of these enzymes from samples of blood, but in general, biochemical methods are standardized for temperatures of 25°C, 30°C and 37°C.

Therefore, considerable development of methodology for both enzyme assays had to be undertaken to ensure that these biochemical techniques worked at temperatures related to the seasonal ecology of juvenile salmon. The most important of these developments concerned the identification of the substrate concentrations and the dilutions of tissue supernatant which in conjunction allowed accurate determinations of $K_m$ and $V_{max}$ values.

The periods of development for the enzyme assays were from May to August 1990 for LDH and from February to May 1991 for G6PDH. The fine details of the assays used for each enzyme are given in their respective chapters.

In developing these assays it was found that the period of sampling of the fish or even slight variation in the amount of tissue used to obtain the enzyme, could lead to departure from a linear change in absorbance. This meant that a flexible approach and a periodic re-testing of the assay medium had to be adopted.

Therefore, rather than simply provide an exhaustive list of the different assay mixtures tested, this chapter describes the more general approach taken to developing the biochemical techniques. It is hoped that this will provide a framework and some initial guidance for any biologist wishing to develop similar assays in teleost fishes.

For each enzyme, assays were performed at 3, 7, 12 and 18°C (i.e. covering the range of temperatures annually experienced by salmon in Scotland). Therefore, one of the initial points that had to be addressed was whether to allow pH to vary with temperature or to hold it stable throughout the range of assay temperatures. Yancey and Somero (1978) have suggested that imidazole-HCl buffer (which has a temperature dependence of -0.02 pH units.$^{\circ}C^{-1}$) should be adjusted to a pH of 6.98 at 20°C and then left to vary with temperature. However, recently Butler and Day (1993) have shown that the intracellular pH of red, white and cardiac muscle of brown trout, *Salmo trutta*, is independent of temperature between 5 and 15°C. Based on these observations it was decided to hold the pH stable over all the assay temperatures for both enzymes (see respective chapters).

## Lactate dehydrogenase

LDH activity was assayed from the supernatant of homogenised white muscle from the dorsal trunk of the fish. The assay temperatures (3, 7, 12 and 18°C) were

found to affect the dilution of supernatant that would allow a linear slope of change in absorbance in the assay mixture. As temperature increased so the samples of supernatant had to be diluted to a greater extent. This tended to take the form of a 1:400 dilution of supernatant for assays performed at 3 and 7°C, and a dilution of 1:500 for temperatures 12 and 18°C. However, due to the variation in the amount of tissue excised, this dilution factor had to be changed occasionally in order to maintain a linear initial rate of absorbance, but the changes were relatively minor compared to the overall level of dilution. The concentrations of substrate that were finally found to produce an acceptable Lineweaver-Burk plot (see above) were 0.4, 0.6, 0.8 and 1.2 $mmol.l^{-1}$.

**Glucose-6-phosphate dehydrogenase**

The activity shown by G6PDH in the liver was much less than that seen in the white muscle LDH. Consequently, the amount of dilution of liver supernatant was also much less. In fact, due in part to the low level of activity, and also to the small size of livers from smaller fish, liver supernatant was used either undiluted, or diluted 1:2 with buffer.

This 1:2 dilution of supernatant was used in assays at all assay temperatures in the samples from summer and smolts, whereas in the samples from autumn, winter and spring undiluted supernatant had to be used at the assay temperature of 3°C in order to obtain enough

change in absorbance to allow an accurate calculation of the rate of change.

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# CHAPTER 4

# SEASONAL ADAPTATION IN ENZYMES OF JUVENILE ATLANTIC SALMON. I. LACTATE DEHYDROGENASE

# Introduction

## *Temperature acclimatisation in fish*

Fish such as juvenile Atlantic salmon (*Salmo salar*) that inhabit fresh water in temperate latitudes experience a wide range of seasonal temperatures. Although their body temperature varies with that of the surrounding environment, they are capable of metabolic compensation (Somero and Hochachka 1976), which allows them to adapt to a wide temperature range, the limits of which are genetically determined (Wilson *et al.* 1975). When such a phenomenon is produced under controlled laboratory conditions in response to variation in a single parameter (most commonly temperature) it is termed acclimation. Under natural conditions, where an animal may experience changes in a number of environmental variables (which are not open to control), the process of adaptation is termed acclimatisation (Somero and Hochachka 1971).

Studies on the adaptation of fish enzymes to temperature have highlighted two different strategies, dependent upon genetic structure. Diploid organisms have only two alleles of each gene lying at the same position (locus) on homologous, paired chromosomes (Hood *et al.* 1975). The two possible enzyme variants (allozymes) coded for by this gene must both be produced simultaneously (Somero 1975; Moss 1982), and diploid fish do not exhibit any changes during thermal acclimation in the enzyme variants produced (Somero 1975; Shaklee *et al.* 1977). In this case compensation

for decreased activity at low temperatures must, by necessity, be quantitative; i.e. the enzyme is produced in greater concentrations (Hochachka and Somero 1973; Wilson *et al.* 1975; Sidell 1977).

Polyploid fish, however, have been shown to produce different enzyme variants in response to different temperatures (Baldwin and Hochachka 1970; Moon and Hochachka 1971a). This is made possible by the availability of extra genetic material which has allowed the development of multiple loci (Ferguson 1980) on different parts of the chromosomes to code for a greater number of enzyme variants (isozymes)(Somero 1975). This is the case for lactate dehydrogenase (LDH; 1.1.1.27) in the polyploid salmonids (Moss 1982), which have at least five LDH loci, compared with three in most other teleosts (Bailey *et al.* 1976; Ferguson 1980), with two possible alleles at each loci. The crucial difference between isozymes and allozymes is that since isozymes are coded for by separate genes (Ferguson 1980) they can be synthesized independently from one another. Somero (1975) proposed that these isozymes have evolved functionally adaptive properties, so that only those that are needed are synthesized, thus saving energy (in protein production) and avoiding oversaturating the cells' finite solvent system.

*Smolting strategies in salmon*

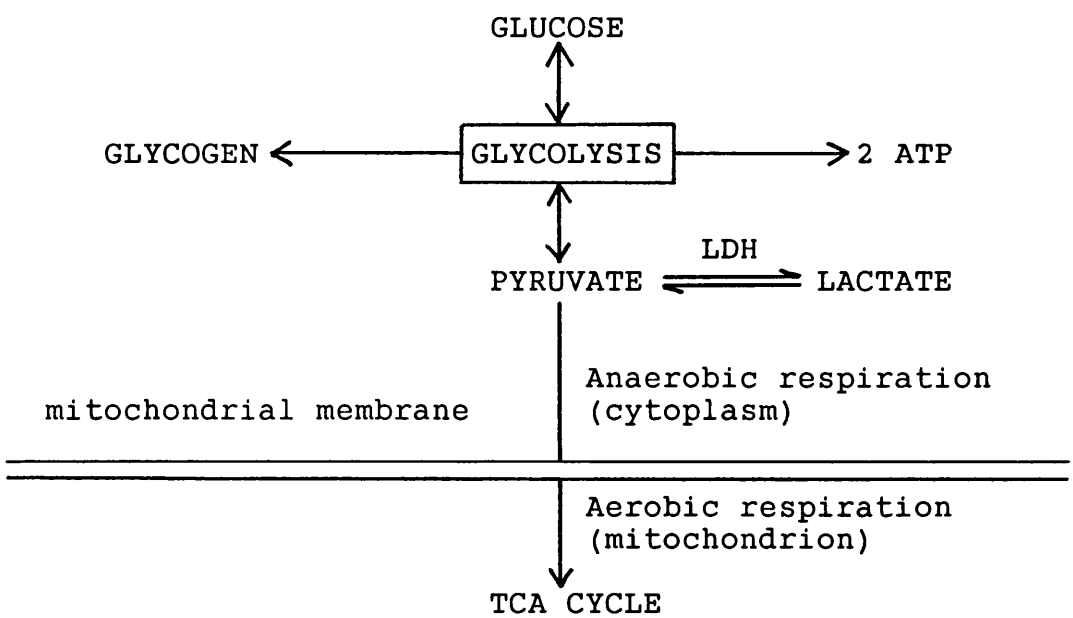
Juvenile Atlantic salmon are polyploid fish (Ohno 1970, Allendorf and Thorgaard 1984) that inhabit streams and

rivers in temperate latitudes, and so experience a wide seasonal variation in temperatures. Under conditions of moderate growth they also exhibit differing life history strategies within a sibling population that lead to different overwintering ecologies (Thorpe *et al.* 1992). This is associated with differences in food intake over autumn and winter leading to a bimodal distribution of size, with the larger fish (Upper Modal Group, UMG) smolting the following spring and the smaller fish (Lower Modal Group, LMG) remaining in fresh water for a further year (Thorpe 1977; Metcalfe *et al.* 1986, 1988). Throughout the winter period (from October to March), the UMG fish maintain a significantly higher daily food intake at any given temperature than the LMG fish (Higgins and Talbot 1985) and, therefore, show a greater level of activity at low temperatures. Specifically, to capture drifting prey, UMG fish must undergo rapid bursts of swimming more often, the energy for which comes from anaerobic respiration in the white muscle. LMG fish tend to adopt a cost-minimising strategy, feeding only at maintenance ration level over the winter (Higgins and Talbot 1985). Juvenile salmon tend to spend some time sheltering beneath the substrate, but LMG fish have been observed to exhibit this behaviour to a greater extent at low temperatures (Huntingford and Thorpe pers. obs.).

*Anaerobic respiration*

During anaerobic respiration in vertebrates the sole method of ATP production is via the glycolytic pathway, the terminal reaction of which is a conversion of pyruvate to the temporary storage product lactate. This is later converted back to pyruvate during the period of recovery, which then enters the TCA cycle to be used in aerobic respiration or is converted back to glycogen by gluconeogenesis. The enzyme that catalyses this reversible reaction is LDH which is also one of the enzymes most extensively examined in studies on thermal adaptation.

**Compartmentalization of glucose metabolism in vertebrates**




Electrophoretic studies have shown that qualitatively different LDH isozymes can be produced in response to thermal acclimation in the polyploid goldfish (*Carassius auratus*)(Tsukuda and Oshawa 1974; Yamawaki and Tsukuda 1979; Tsukuda and Yamawaki 1980), although

some studies have reported unaltered isozyme compositions in the same species (Wilson *et al* 1975). However, there is disagreement as to whether the variation in LDH isozymes present at different acclimation temperatures has any adaptive significance or whether it is due to non-adaptive genetic polymorphism, individual variation or cellular heterogeneity within a tissue (Wilson *et al.* 1975).

Some kinetic studies on fish and other ectotherms have demonstrated an adaptive significance in enzyme-substrate affinity of some enzymes (including LDH), with the Michaelis constant ($K_m$) being lowest (and, therefore, the enzyme being most efficient) at the acclimation temperature experienced by the animal (Aleksiuk 1971; Somero and Hochachka 1971; Narita and Horiuchi 1979; McLeese and Stevens 1986) due to the thermal induction of isoenzymes (Baldwin and Hochachka 1970; Moon and Hochachka 1971). However, other studies demonstrate no such relationship between minimal $K_m$ and acclimation temperature (Wilson *et al.* 1973). Although this adaptation of $K_m$ values to temperature has been shown to occur in rainbow trout (Baldwin and Hochachka 1970; Moon and Hochachka 1971b; Somero and Hochachka 1971), no previous studies have examined whether Atlantic salmon produce isozymes during thermal acclimatisation, and if so whether these are of adaptive significance.

The differing ecological strategies exhibited by juvenile Atlantic salmon in relation to temperature

(Rimmer *et al.* 1983, chapter 3), plus the fact that they show a high incidence of duplicated enzyme loci (Allendorf and Thorgaard 1984), make them ideal subjects on which to study the ecological significance of LDH adaptation to temperature.

This chapter presents the results of a study on the kinetics of LDH from the white muscle of juvenile Atlantic salmon. Specifically this study examined whether this enzyme became adapted to the seasonal temperatures experienced by this species, and whether differing life history strategies exhibited by juvenile salmon had any effect on temperature-related LDH activity over the course of a year.

## Material and Methods

*Experimental Fish*

Sibling juvenile Atlantic salmon of sea-run River Almond stock were reared in radial flow tanks (Thorpe and Wankowski 1979) under natural photoperiods and water temperatures. They were measured for forklength (mm) before each sampling period, to allow classification into upper and lower modal groups. Samples of $0^+$ fish were taken during summer, autumn, winter and spring when the fish were assumed to have acclimatised to the mean water temperatures of the previous two weeks of 18°C, 7°C, 3°C and 7°C respectively. Thus the timing of the sampling periods in autumn and spring were arranged so that both sets of fish had become acclimatised to the same temperature of

7°C to allow a seasonal comparison. Fish used in the holding velocity experiments (chapter 3) provided a May sample of fish termed "smolts" in this paper. This sample consisted of S1 smolts - the one year old UMG fish which by then were ready to migrate to the sea, and one year old LMG fish which would remain in freshwater for a further year before undergoing the same transformation as S2 smolts; S1 and S2 fish thus differ in the age at which they undergo the smolt transformation. The acclimatisation temperature of fish in this sample varied between 7°C and 10°C.

The samples of fish were killed in anaesthetic (benzocaine) and the fish were then stored whole at -80°C until their use in enzyme kinetic studies.

In the statistical analyses, values from summer samples were included in both upper and lower modal groups since $0^+$ fish cannot be differentiated into modal groups by size at this time. Sample sizes at each assay temperature, in each mode and in each season varied between 5 and 9.

*Tissue excision and preparation*

After defrosting the whole fish at room temperature, samples of white muscle (0.01g - 0.1g depending upon fish size) were removed from an area on the left side of the fish above the lateral line and behind the dorsal fin so that tissue samples were consistently obtained from the same area of the body. These samples were homogenised in ice-cold 50 $mmol.l^{-1}$ $KH_2PO_4$ buffer

and the homogenates were centrifuged at 8000g for 10 minutes. The supernatant was diluted with 50 $mmol.l^{-1}$ $KH_2PO_4$ buffer, in amounts determined by assay temperature (see chapter 2). Assays of enzyme activity were performed on the same day as preparation of a sample and the various dilutions of the supernatant were held at ice-water temperatures during this time (i.e. <1°C).

*Enzyme assays and kinetic studies*

Enzyme activity for the reduction of pyruvate to lactate was measured by monitoring the decrease in absorbance over 5 minutes at 340 nm using a Hitachi U-2000 spectrophotometer and a flow-through quartz cuvette. The spectrophotometer was connected to a Grant LTD 6 water bath (Grant Instruments Ltd., Cambridge) which allowed the assay to be performed at a range of temperatures equivalent to the seasonal temperatures experienced throughout the year by the experimental fish and specifically prior to the sampling periods: 3°C (winter), 7°C (autumn and spring), 12°C and 18°C (summer).

The reaction mixture contained 50 $mmol.l^{-1}$ $KH_2PO_4$ buffer with the pH adjusted to 7.5 for each assay temperature, 15 $mmol.l^{-1}$ NADH, and four different concentrations of sodium pyruvate: either 0.2, 0.4, 0.6 and 0.8 $mmol.l^{-1}$ or 0.4, 0.6, 0.8 and 1.2 $mmol.l^{-1}$ depending upon assay temperature and dilution of supernatant. The reaction was started by the addition

of the supernatant and the final volume of the reaction mixture came to 1.5 ml. Protein concentration of the dilution of supernatant used was determined by measuring the absorbance of the mixture after combination with a dye by the method of Bradford (1976).

The initial rates of change in absorbance (when the change over time remained constant) were used to determine the initial velocities of the reactions. Linear regressions were then performed on Lineweaver-Burke double reciprocal plots of these data, to determine the $K_m$ of pyruvate ($K_m$(pyr)) and $V_{max}$ values for the enzymes in each each fish at a given assay temperature. Enzyme activities are expressed in $\mu$mol NAD.min$^{-1}$.mg protein$^{-1}$ and $K_m$ values are expressed in mmol.l$^{-1}$.

## Results

Temperature effects on enzyme-substrate affinity

Figures 4.1 - 4.5 show the $K_m$ values of LDH for both modal groups at each assay temperature in each season. When pooling the data for all fish (i.e. both modes) over the whole year, both assay temperature and season had significant effects on the $K_m$ of LDH (2-way ANOVA, $F_{3,225} = 10.453$, $P < 0.0001$ and $F_{4,225} = 3.018$, $P < 0.02$ respectively). Thus in general, $K_m$(pyr) values were higher at higher assay temperatures and in warmer seasons. However, the effect of assay temperature was strongly dependent on season (2-way ANOVA, assay

Figure 4.1. Temperature profile of mean $K_m$ values of LDH from unimodal juvenile salmon acclimatised to summer temperatures (18°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

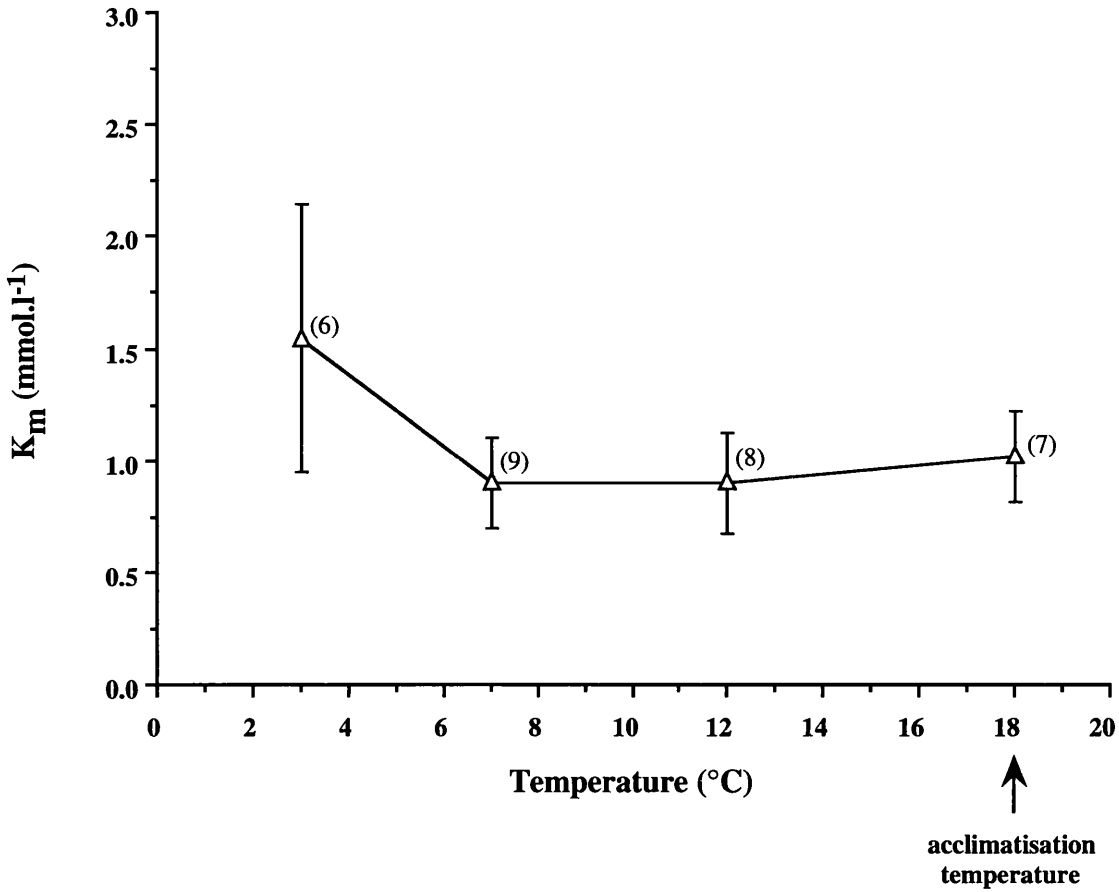

3.0
2.5
2.0
1.5
1.0
0.5
0.0
$K_m$ (mmol.l$^{-1}$)
(6)
(9)
(8)
(7)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation
temperature

Figure 4.2. Temperature profile of mean $K_m$ values of LDH from UMG (——○——) and LMG (–●–) juvenile salmon acclimatised to autumn temperatures (7°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

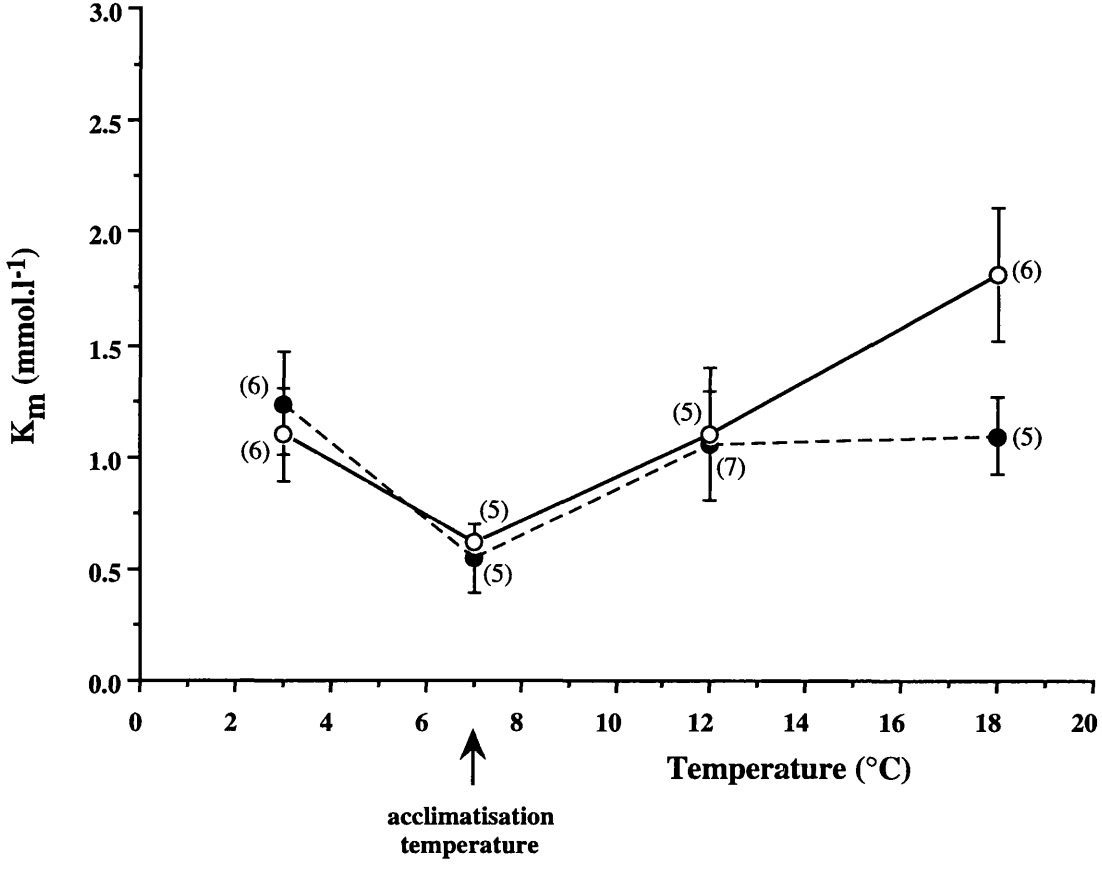

3.0
2.5
2.0
1.5
1.0
0.5
0.0
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation temperature
(6)
(6)
(5)
(5)
(5)
(7)
(6)
(5)

Figure 4.3. Temperature profile of mean $K_m$ values of LDH from UMG (——○——) and LMG (—●—) juvenile salmon acclimatised to winter temperatures (3°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

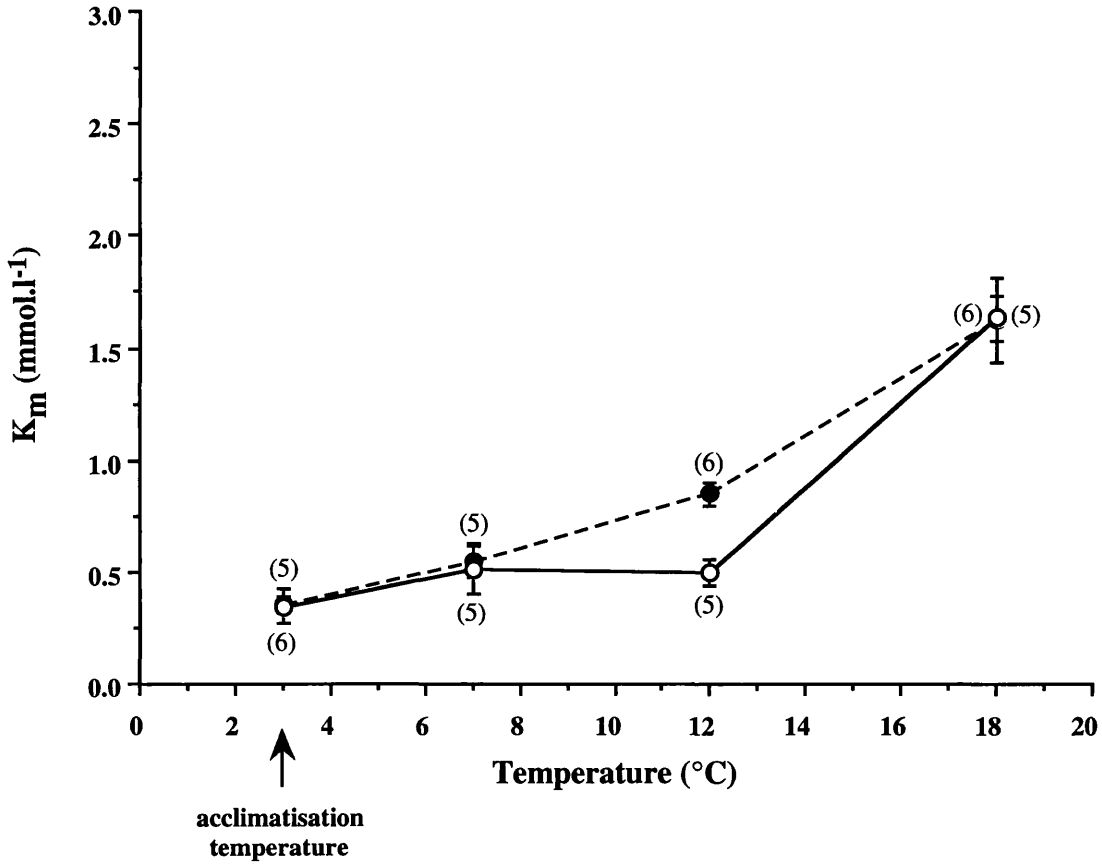

3.0
2.5
2.0
1.5
1.0
0.5
0.0
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation temperature
(6)
(5)
(5)
(5)
(6)
(5)
(5)
(6)

Figure 4.4. Temperature profile of mean $K_m$ values of LDH from UMG (—○—) and LMG (–●–) juvenile salmon acclimatised to spring temperatures (7°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

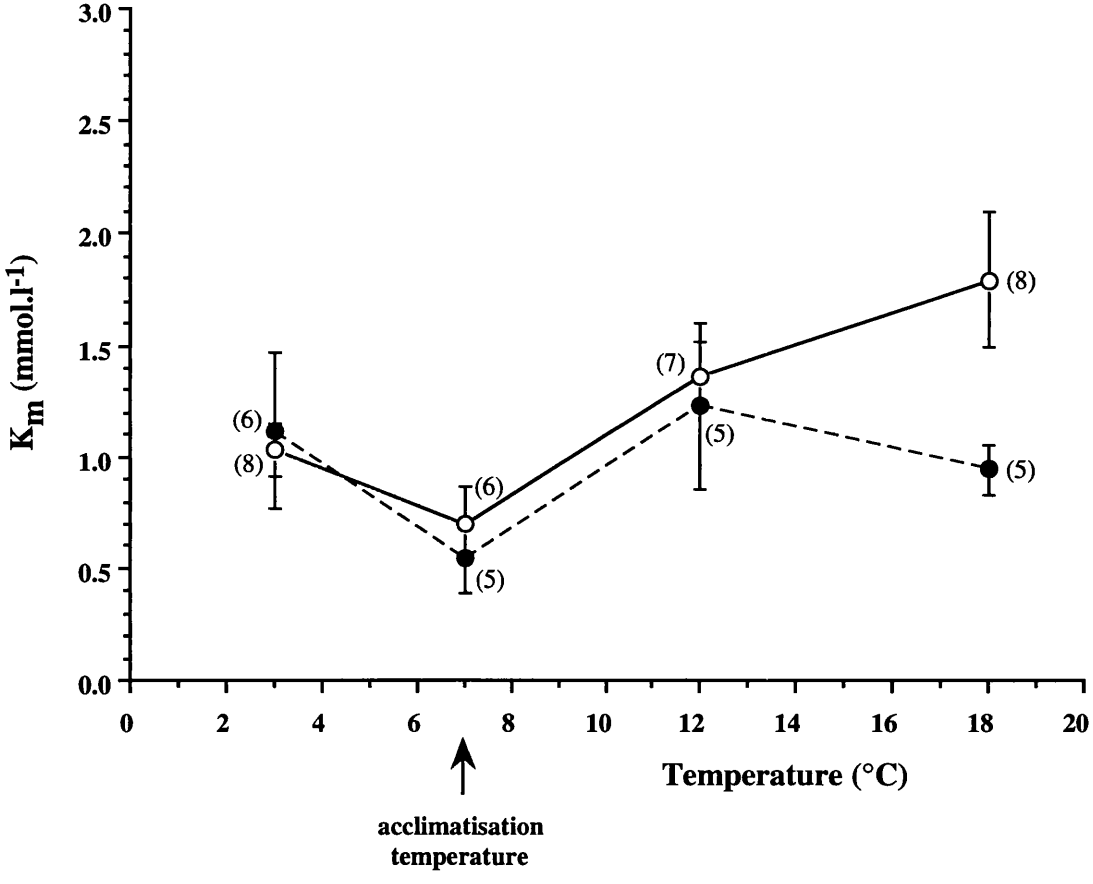
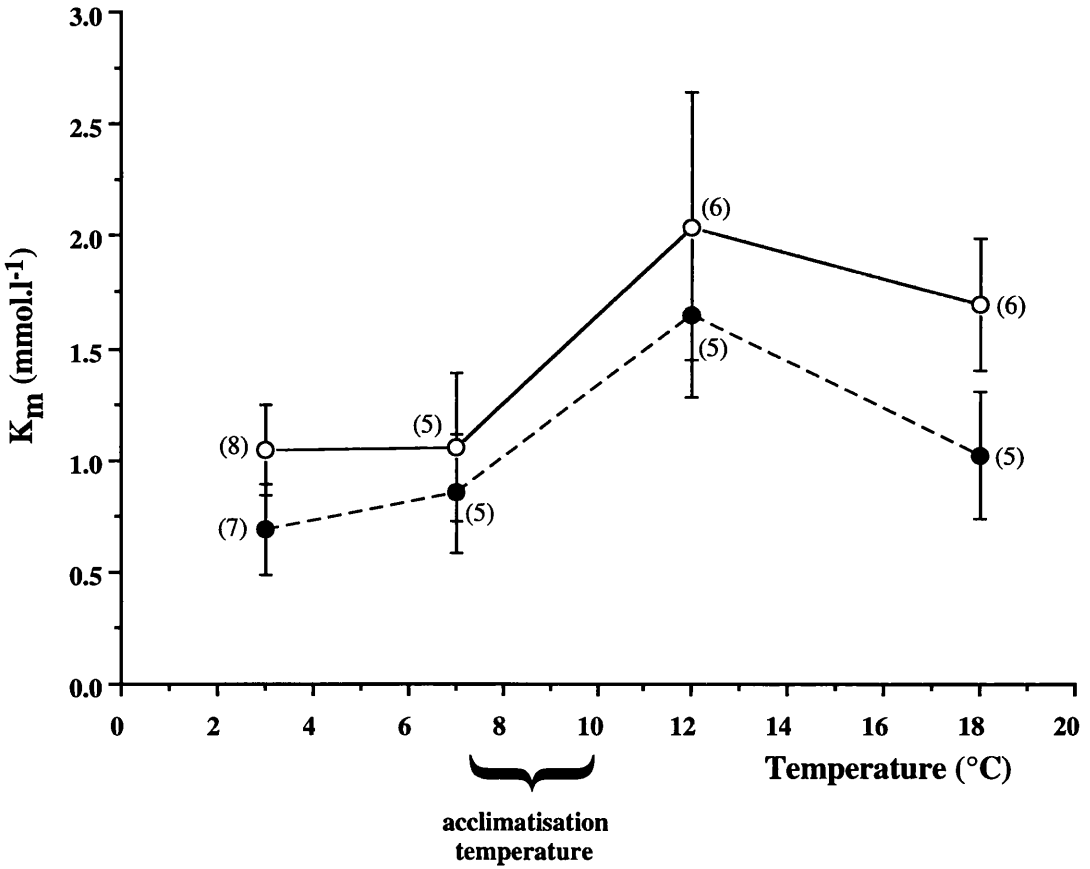

3.0
2.5
2.0
1.5
1.0
0.5
0.0
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation temperature
(6)
(8)
(6)
(5)
(7)
(5)
(8)
(5)

Figure 4.5. Temperature profile of mean $K_m$ values of LDH from smolts (——○——) and the LMG (←—●—→) of juvenile salmon acclimatised to temperatures between 7 and 10°C. Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.


3.0
2.5
2.0
1.5
1.0
0.5
0.0
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation temperature
(8)
(7)
(5)
(5)
(6)
(5)
(6)
(5)

temperature x season interaction $F_{12,225}$ = 3.477, P = 0.0001), although a comparison of $K_m$(pyr) values between autumn and spring samples showed no difference in the temperature profiles between the two seasons for UMG or LMG fish (2-way ANOVA $F_{1,43}$=0.128, n.s. and $F_{1,35}$=0.086, n.s. respectively). Analysing the data from the two modal groups separately showed interesting differences between the two life history strategies; these differences are highlighted below.

*Temperature effects on Km in UMG fish*

For UMG fish, both assay temperature and season had a significant overall effect on $K_m$ (2-way ANOVAs, $F_{3,109}$=8.196, P=0.0001 and $F_{4,109}$=3.453, P=0.01 respectively). The $K_m$-temperature profile of this enzyme also changed with season, as is evident from the significant assay temperature-season interaction (2-way ANOVA, $F_{12,109}$=1.875, P<0.05). When analysing each season separately, assay temperature had a significant effect in autumn, winter and spring for UMG fish (1-way ANOVAs, $F_{3,18}$=4.268, P<0.02; $F_{3,19}$=51.368, P<0.0001; $F_{3,25}$=4.970, P<0.01 respectively).

The lowest $K_m$ values tended to occur at the acclimatisation temperatures that the UMG fish had experienced (figs. 4.1 - 4.5).

*Temperature effects on Km in LMG fish*

For LMG fish the effect of assay temperature on $K_m$ values over the whole year was significant (2-way

ANOVA, $F_{3,96}=2.870$, $P<0.05$), whereas season was not, since the mean $K_m$ level over all assay temperatures remained unchanged throughout the year. Nor was there a significant interaction between season and temperature. When the seasons were examined separately, only in winter did assay temperature have a significant effect on the $K_m$ of LMG fish (1-way ANOVA, $F_{3,17}=28.054$, $P<0.0001$).

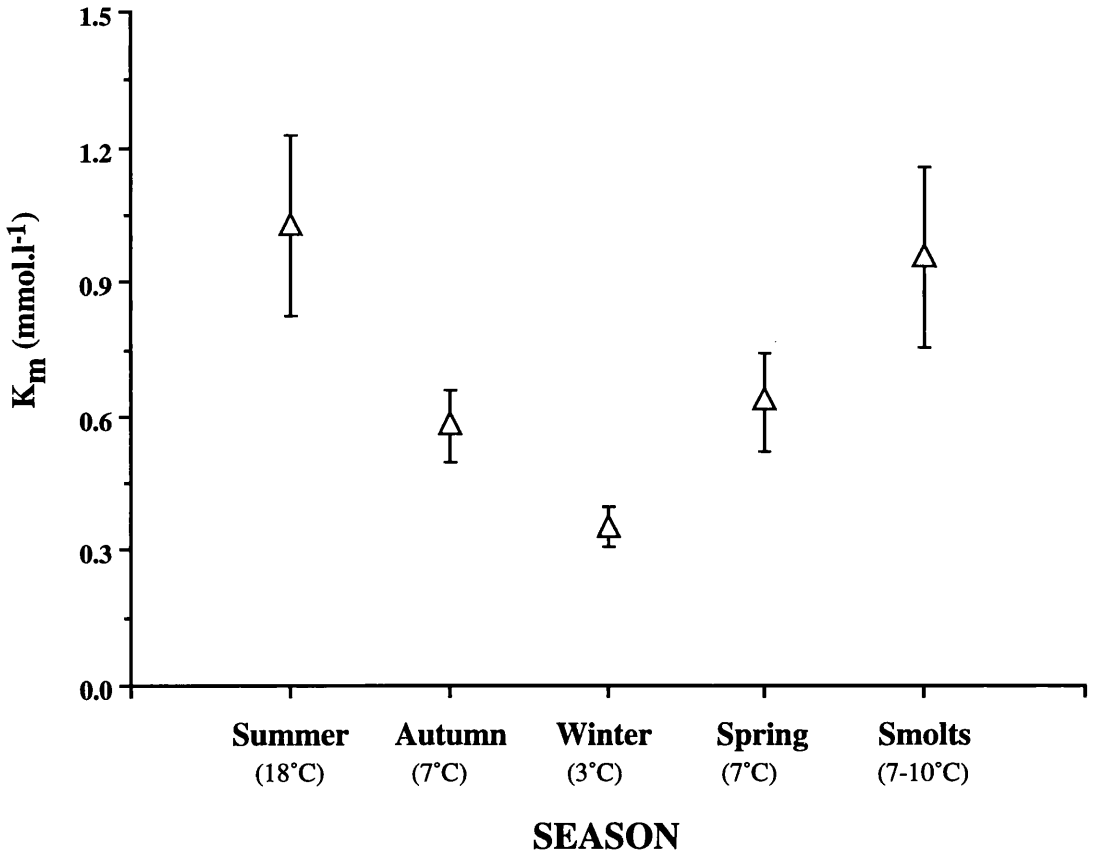
In the sample taken in May (fig. 4.5) there was no difference between the $K_m$-temperature profiles of smolts and LMG fish (2-way ANOVA $F_{1,39}=2.991$, $P=0.09$) and neither modal group showed a significant effect of assay temperature on $K_m$ value (1-way ANOVAs $F_{3,24}=1.789$, $P=0.18$ and $F_{3,21}=2.317$, $P=0.11$ for smolts and LMG fish respectively).

*Seasonal changes in Km at environmental temperatures*

The minimum $K_m$ values tended to occur at the environmental temperatures to which the fish had become acclimatised. A comparison within each season of $K_m$ values at acclimatisation temperatures between the modal groups showed no difference, so the data were pooled for each season in the next analysis.

$K_m$ values at the acclimatisation temperature varied with season (1-way ANOVA, $F_{4,44}=4.199$, $P<0.01$). In the winter, $K_m$ values were lower than those in autumn and spring, and significantly lower than those taken from summer and the period of smolting (fig. 4.6). This

Figure 4.6. Mean $K_m$ values of LDH from both modal groups of juvenile salmon combined at the acclimatisation (environmental) temperatures in each season. Vertical bars represent 1 standard error.


1.5
1.2
0.9
0.6
0.3
0.0
$K_m$ (mmol.l$^{-1}$)
Summer
(18°C)
Autumn
(7°C)
Winter
(3°C)
Spring
(7°C)
Smolts
(7-10°C)
SEASON

indicates that the efficiency of LDH increased at lower acclimatisation temperatures.

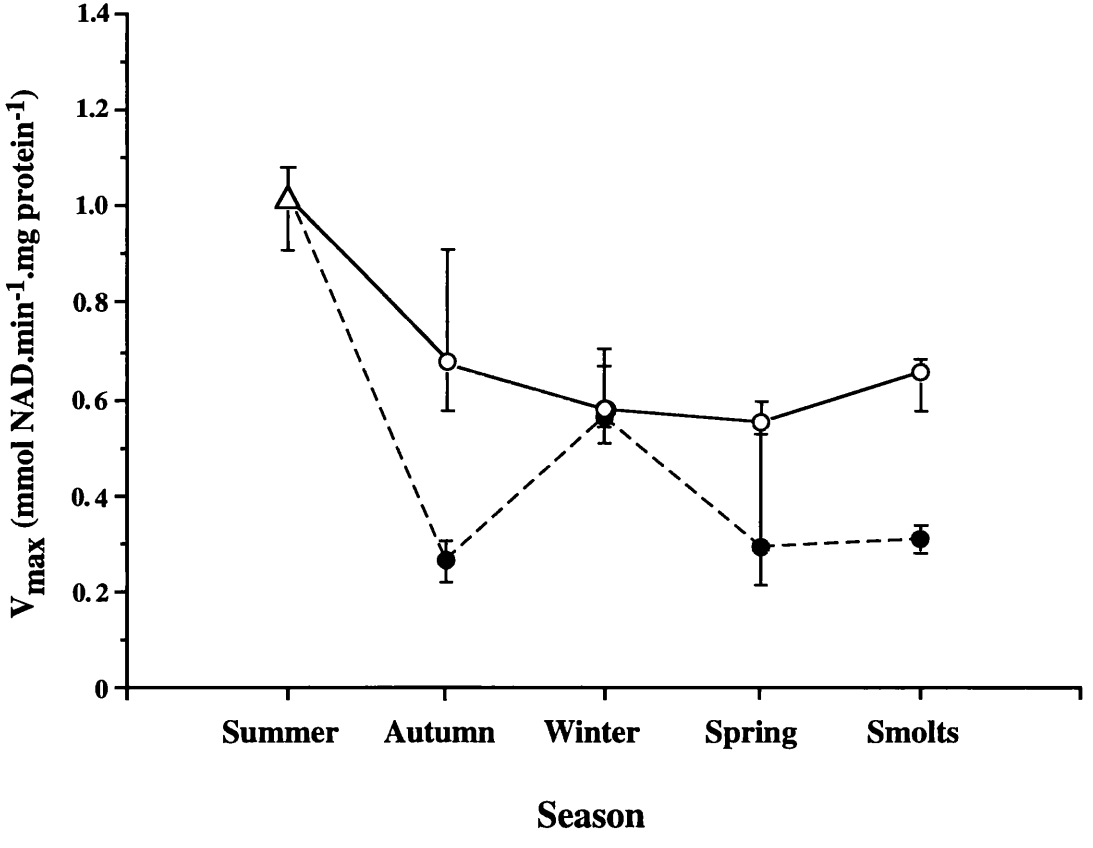
Temperature effects on maximal enzyme activity

A comparison between the maximal enzyme activities ($V_{max}$) from autumn and spring (both sampled at 7°C) for both UMG and LMG fish over all the assay temperatures showed no difference between the two sampling periods for either modal group (2-way ANOVAs $F_{1,40}$ = 1.406, P=0.24 and $F_{1,35}$ = 3.483, P=0.07 for UMG and LMG fish respectively).

There was no significant difference between the seasonal $V_{max}$ values at acclimatisation temperatures of UMG fish (Kruskal-Wallis 1-way ANOVA, H=8.302, N=29, P>0.05). However there was a difference for LMG fish (Kruskal-Wallis 1-way ANOVA, H=12.093, N=27, P<0.05), due to the summer samples being higher than those of the other seasons (fig. 4.7).

When comparing the $V_{max}$ values from the modal groups in each season at acclimatisation temperatures it was found that the variances differed significantly between the two modal groups in some of the sampling periods, hence Mann-Whitney U tests were used for comparisons between the modal groups in each season. The results showed that UMG fish had higher median maximal enzyme activities than the LMG fish in autumn (7°C) and also during the period of smolting in UMG fish (7 - 10°C) (U=0, P<0.05, Mann-Whitney U-test, in both cases), but not in winter or spring (fig. 4.7).

Figure 4.7. Median $V_{max}$ values of LDH from UMG (—○—) and LMG (—●—) juvenile salmon at the acclimatisation (environmental) temperatures in each season. Vertical bars represent semi-interquartile ranges.


1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
$V_{max}$ (mmol NAD.min$^{-1}$.mg protein$^{-1}$)
Summer
Autumn
Winter
Spring
Smolts
Season

Thermodynamic parameters of enzyme catalysis

The slopes of Arrhenius plots of $\log_{10} V_{max}$ against the reciprocal of temperature (°K) can be used to calculate the activation energy (Ea) of an enzyme (equation I).

$$Ea = -b(2.3R) \qquad \text{(I)}$$

where b = slope, and R = gas constant (1.987 $cal.mole^{-1}.°K^{-1}$)

The activation energy is one estimation of how much kinetic energy needs to be present during the collision of enzyme and substrate molecules for the reaction to proceed. All Arrhenius plots were significant (minimum correlation coefficient = -0.504, $P<0.02$), except for UMG and LMG fish during spring (correlation coefficients = -0.369, $P=0.053$ and -0.094, $P=0.68$ respectively), and the activation energies (Ea) are given in Table 1. Comparisons between the two modal groups within each season showed no significant differences and so the data were pooled to compare Ea values between seasons. Comparing the slopes of the Arrhenius plots showed no difference between the Ea values in each season, except that the winter value was lower than the value in smolts ($t=2.110$, 86 d.f. $P<0.05$).

The free energy of activation ($\Delta G^{\neq}$) is considered a more accurate measure of the "energy barrier" that has to be surmounted before a reaction can proceed, since it includes the Ea value in its calculation along with other chemical thermodynamic parameters (equations II

Table 1

Mean values for activation energy (Ea) of LDH at acclimatisation temperatures for upper (UMG) and lower (LMG) modal groups in different seasons.

| Season | Acclimatisation Temp. (°C) | Ea (cal.mole$^{-1}$) UMG | LMG |
|---|---|---|---|
| Summer | 18 | 9344 | |
| Autumn | 7 | 9084 | 8905 |
| Winter | 3 | 6796 | 8706 |
| Spring | 7 | 9900# | 3091# |
| Smolts | 7 - 10 | 15393 | 15841 |

# Arrhenius plot regression line not significant, therefore, less accurate.

and III).

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T.\Delta S^{\ddagger} \quad \text{(II)}$$

$$\Delta H^{\ddagger} = Ea - RT \quad \text{(III)}$$

where $\Delta H^{\ddagger}$ is the energy of activation, and $\Delta S^{\ddagger}$ is the entropy of activation (equation IV) and T is temperature (°K).

$$\Delta S^{\ddagger} = 4.576(\log K - 10.753 - \log T + Ea/4.576T) \quad \text{(IV)}$$

where K (in $sec^{-1}$) = ($V_{max}$/mg of enzyme) x (molecular weight) x ($10^{-3}$ mmol/$\mu$mol) x (1 min./60 sec) (Low *et al.* 1983). Molecular weight of the enzyme is expressed in mg/mmol. Individual $V_{max}$ values were used for each fish, but by necessity the Ea values used were the means for each modal group in each sampling period since they were calculated from collective Arrhenius plots.

$\Delta G^{\ddagger}$ values for the two modal groups were compared within each season using the Mann-Whitney U-test. Results showed that the median $\Delta G^{\ddagger}$ values of the UMG fish were significantly lower than those of LMG fish in autumn and smolt samples ($U=0$, $P<0.05$ in both cases), whereas in winter and spring samples there was no significant difference between modal groups (Table 2). Due to the differences seen between the modal groups, the effect of season on $\Delta G^{\ddagger}$ values was calculated separately. Values for UMG fish showed a significant effect of season (Kruskal-Wallis 1-way AVOVA, $H=13.272$, $N=29$, $P=0.01$), due to the values from the unimodal summer sample being higher than the values from autumn and winter. LMG fish showed no significant effect of

Table 2

Mean values and standard errors for free energy of activation ($\Delta G^{\neq}$) of LDH at acclimatisation temperatures for upper (UMG) and lower (LMG) modal groups in different seasons.

| Season | Acclimatisation Temp. (°C) | $\Delta G^{\neq}$ (cal.mole$^{-1}$) UMG | | LMG |
|---|---|---|---|---|
| Summer | 18 | 12570(±67.56) | | |
| Autumn | 7 | 12123(±85) | * | 12824(±47) |
| Winter | 3 | 12207(±108) | | 12178(±185) |
| Spring | 7 | 12344(±52) | | 12343(±359) |
| Smolts | 7 - 10 | 12230(±107) | * | 12661(±37) |

* $P<0.05$, Mann-Whitney U test comparing modal groups.

season (Kruskal-Wallis 1-way ANOVA, H=7.390, N=27, P=0.12).

*Effect of size and gender*

The effect of size of fish on both enzyme efficiency ($K_m$) and maximal activity ($V_{max}$) was investigated in both modal groups and in both modes combined, at the acclimatisation temperature for each season. The effect of temperature was controlled for by expressing each $K_m$ value as a percentage of the mean $K_m$ value for the appropriate acclimatisation temperature. There was no significant correlation between forklength (mm) and $K_m$(pyr) either in UMG or LMG fish, or in both modes combined (linear regressions: correlation coefficients = 0.08, 0.12 and 0.11 for UMG, LMG and both modal groups combined respectively).

Paired t-tests were used to compare the mean $K_m$ of fish of each sex in each season. This was done using data both from all the assay temperatures and specifically from those coincident with the seasonal acclimatisation temperature. These showed no effect of the gender of fish on $K_m$ values in UMG and LMG fish separately or in both modal groups combined, either over all assay temperatures (t=0.272, 11 d.f., n.s.; t=0.005, 15 d.f., n.s. and t=1.113, 27 d.f., n.s. respectively) or at the acclimatisation temperatures the fish had experienced (t=0.132, 6 d.f., n.s. for both modal groups combined).

## Discussion

Of the relatively few kinetic studies on teleosts that have been carried out on LDH, most have involved acclimation of the fish to two or three arbitrary temperatures within the laboratory (e.g. Wilson *et al.* 1975, Kent and Hart 1976, Shaklee et al. 1977; Almeida-Val *et al.* 1991), and sometimes the enzymes have been assayed at only one temperature (Gaudet *et al.* 1975 and Wilson *et al.* 1975). Therefore, previous studies have not fully assessed whether changes in LDH kinetics seen with differing temperatures have any ecological significance. The present study has demonstrated that the temperature at which $K_m$(pyr) of LDH is lowest does not remain constant throughout the year, but varies seasonally in a predictable manner, suggesting an adaptive response to the environmental temperatures that the fish experience. This is consistent with the results of Javaid and Anderson (1967) who demonstrated that juvenile Atlantic salmon in a temperature gradient selected temperatures similar to those to which they had become acclimated - possibly because their enzymes had become adapted to the acclimation temperature.

Paradoxically, LDH is generally considered to display so much activity that it does not function as a rate-limiting step in glycolysis (Newsholme and Paul 1983; Kleckner and Sidell 1985). However, the large differences in maximal activity seen between LDH and the other enzymes of the glycolytic pathway in birds and mammals are much less pronounced in fishes

(Crabtree and Newsholme 1972). Also the maximal rates of activity of an enzyme may have little physiological significance since they occur at saturating substrate concentrations which rarely, if ever, occur within a cell (Fersht 1977). However, the possibility of there being fluctuations in substrate concentrations is discussed later.

Hochachka (1967) used density of staining in electrophoretic studies of salmonid muscle to show that the relative activity of LDH isozymes depended upon acclimation temperature. Hochachka and Somero (1968) also demonstrated that the thermal optima of different tissue-specific salmonid LDH isozymes (i.e. heart, liver, muscle and eye) fell within the physiological temperature range for these species, and hence appeared to be of adaptive significance.

The present study has shown that LDH in UMG juvenile salmon becomes seasonally adapted, so that the reduction of pyruvate to lactate is most efficient at current environmental temperatures. This has also been shown to occur in LDH of the crayfish *Procambarus clarki* (Narita and Horiuchi 1979).

Different LDH allozymes from diploid fish have also been shown to be of adaptive significance. Merrit (1972) has shown that the north-south cline in white muscle LDH genotypes of the fathead minnow (*Pimephales promelas*), seen over part of North America, corresponded to differences in enzyme-substrate

affinity between the two allozymes at the mean habitat temperature during the hottest part of the year.

Place and Powers (1979) demonstrated the same type of north-south cline in the frequency of the two B-type allozymes of LDH from the liver of the common killifish (*Fundulus heteroclitus*) along the Atlantic coast of North America. These allozymes were shown to have different thermal optima in terms of the efficiency of pyruvate reduction. However, changes in the types of allozymes produced in diploid fish could act as a method of thermal compensation only over evolutionary time periods, not in response to seasonally varying temperatures.

Some studies have proposed a functional significance for different LDH isozymes in salmonids in relation to their ability to convert lactate to pyruvate, which in turn has been linked to the ability to undergo sustained swimming. The isozyme LDH-B' from rainbow trout liver has been shown to have a lower optimum pH for the oxidation of lactate to pyruvate than LDH-B (Tsuyuki and Williscroft 1973), and greater frequencies of LDH-B' (designated LDH-B'') have been shown to be present in the livers of rainbow trout (*Oncorhynchus mykiss*) which inhabit fast flowing-streams (Huzyk and Tsuyuki 1974) compared to those inhabiting slower streams. Davie *et al.* (1986) found a shift in the expression from LDH-B to LDH-B' isozymes in the white muscle of rainbow trout that had undergone long-term swimming. There is still uncertainty as to whether

lactate is converted to pyruvate in the liver (see review in Walton and Cowey 1982) or in the white muscle (Turner *et al.* 1983, Wardle 1978). However, Davie *et al.* (1986) suggested that since lactate causes a decrease in pH, LDH-B' is well suited for preferentially metabolising lactate, and is indicative of white muscle with increased aerobic capacity in addition to its anaerobic function. Unfortunately in the above studies the effect of temperature on the expression of LDH isozymes was not examined.

Juvenile Atlantic salmon tend to oppose water currents by holding station on the substrate rather than actively swimming (Griffiths and Alderdice 1972; Hoar 1976). However, the minimal $K_m$(pyr) values of LDH seen at acclimatisation temperatures in the present study will have a functional significance in relation to the efficiency of the pyruvate to lactate reaction during burst swimming.

In UMG fish the effect of assay temperature on $K_m$ values varies throughout the year so that these fish show a seasonal adaptation in their $K_m$-temperature profile with LDH tending to be most efficient at the acclimatisation temperature. I suggest that this is indicative of differential production of LDH isozymes in relation to temperature.

However, although the $K_m$-temperature profile for LMG fish does not vary significantly with season, the $K_m$(pyr) values of LMG fish are no different at acclimatisation temperatures to those of UMG fish. Thus

the only difference in the kinetic characteristics of LDH between the modal groups occurs at temperatures outwith those currently experienced by the fish. It is not known whether the more accurate "targetting" of minimal $K_m$ values to the acclimatisation temperatures seen in UMG fish has any advantages (e.g. in terms of decreased protein production), but it would appear that LMG fish, despite their cost-minimising strategy, have the potential to reduce pyruvate as efficiently as UMG fish. This potential may reflect the necessity for these fish to feed actively at low temperatures if their energy reserves drop below a predetermined "defended level" (Metcalfe and Thorpe 1992).

This argument is in contrast to the idea of UMG and LMG fish being two distinct physiological populations (Higgins and Talbot 1985) and that LMG fish are metabolically repressed (Higgins 1985), which implies that LMG fish are somehow inferior to UMG fish. The results of this study support the argument that differentiation into modal groups is a developmental "decision", and that, as has been shown for current holding ability (chapter 3), LMG fish are as physiologically capable as UMG fish. However, it does not preclude the possibility that fish enter the lower modal group as a consequence of being subordinate (Metcalfe *et al.* 1989) and, therefore, less efficient at competing for food.

Higgins and Talbot (1985) demonstrated that UMG juvenile Atlantic salmon possess higher gut evacuation

rate in spring (increasing photoperiod) at any given temperature than in autumn (decreasing photoperiod). The present study found no such effect of photoperiod on the $K_m$(pyr) values of juvenile Atlantic salmon of either modal group.

Low *et al.* (1973) have suggested that $\Delta G^{\neq}$ is a more accurate measure of the "energy barrier" of a reaction than Ea since it takes into account more thermodynamic parameters of the reaction. The values for each measurement in this study are in broad agreement with those given for tuna and halibut muscle LDH by Low *et al.* (1973).

The apparent seasonal effect on $\Delta G^{\neq}$ values of UMG fish at acclimatisation temperatures was due to the values from the summer sample being higher than those from the rest of the year. Since it was not possible to distinguish fish that would become UMG or LMG on the basis of size in the summer sample, the possibility that this sample contained a majority of pre-LMG fish with a resulting higher mean value cannot be precluded. The reason for the differences in $\Delta G^{\neq}$ between the modal groups in autumn and during smolting is not clear, but this may be a consequence of alterations in other parameters of the enzymes (i.e. $V_{max}$) relative to differing ecology during the same period (see later).

The present study indicates that the $K_m$ value at the acclimatisation temperature is itself also open to variation and may be of adaptive significance. Previous studies on rainbow trout pyruvate kinase from white

muscle (Somero and Hochachka 1971), rainbow trout brain acetylcholinesterase (Baldwin and Hochachka 1970), and isocitrate dehydrogenase from rainbow trout liver (Moon and Hochachka 1971) have suggested that the minimal $K_m$ values of a particular enzyme at acclimation temperatures are roughly equivalent in fish acclimated to different temperatures. However, $K_m$(pyr) values of LDH in juvenile salmon increased with seasonally increasing acclimatisation temperatures (fig.4.6). This phenomenon may be linked to the mechanism behind lower $K_m$ values and, therefore, higher enzymic rates. Lower $K_m$ values in cold-adapted enzymes are brought about by less rigid secondary and tertiary structures, hence the conformational change that occurs during enzyme activation requires less kinetic energy because fewer weak bonds are broken in the process (Cossins and Bowler 1987). However, this adaptation does incur a cost in terms of thermal stability with the less rigid cold-adapted enzyme being less stable at higher temperatures. This effect has only previously been demonstrated between teleosts living in different geographical regions (Johnston and Walesby 1977) but may also be an explanation for the differing $K_m$ values at the acclimatisation temperatures shown in figure 4.6 and lends further credence to the idea that these are due to the production of isoenzymes with differing thermal optima. This phenomenon is also apparent in rainbow trout LDH (Hochachka and Somero 1968). As acclimation temperature decreased different isozymes of

LDH were expressed that had lower thermal optima and lower $K_m$(pyr) values.

Another possible explanation for this effect being observed in salmonids but not other fish relates to the possible link between the activity of the glycolytic pathway and the behaviour of these animals. Feeding activity has been shown to increase with temperature (Higgins and Talbot 1985) and, therefore, will vary throughout the year with seasonal temperatures. Glycolysis proceeds at higher rates in active fish compared with inactive fish (Malinovskaya 1989), and the glycolytic flux in skeletal muscle has been shown to increase by up to 100-fold with the onset of activity (Newsholme and Start 1973). Diet can also affect the amount of blood glucose present in salmonids (Bergot 1979) and the rate of glycolysis in yeast has been shown to oscillate with respect to the level of substrates present in the environment (Hess 1973). Thus there could well be a direct correlation between the intracellular substrate concentrations and the activity of juvenile salmon. In this scenario, the observed increase in $K_m$ values at higher environmental temperatures would be of adaptive significance with respect to the higher concentrations of substrate caused by the increased levels of activity and feeding at the higher temperatures.

Such possible seasonal and temperature dependant variation in the amount of material passing through the glycolytic pathway may have important consequences for

the interpretation of biochemical parameters and in particular whether $V_{max}$ values are of any physiological significance.

During October $0^+$ UMG fish show a growth spurt (Metcalfe *et al.* 1988) with (presumably) an associated higher level of feeding activity. Data collected from the same period in this study showed $V_{max}$ values for UMG fish significantly higher than those from LMG fish. Smolting fish also displayed significantly higher LDH $V_{max}$ values compared with sibling non-smolting fish. Juvenile Atlantic salmon undergoing seaward migration have higher metabolic rates than their cohorts remaining in fresh water (Virtanen and Forsman 1987; Maxime *et al.* 1989) and although smolts are generally considered to be less active than parr it has been suggested that they may still oppose the water current during daylight hours (chapter 3). Such factors may again lead to increases in substrate concentrations towards levels similar to maximum values. However, whether the cellular substrate concentrations and the amount of substrate passing through a metabolic pathway do vary is a very contentious issue. Although this idea seems to fit in well from a functional viewpoint with changing $K_m$ values of enzymes there is still little substantial evidence on this point, and so this is a major topic that requires investigation.

The general observation that cellular substrate concentrations are maintained at levels approximating that of the $K_m$ is probably true in the majority of

cases. However, such observations are usually made under tightly controlled (and, therefore, unnatural) laboratory conditions. Studies conducted under natural and semi-natural conditions (i.e. seasonally varying photoperiod and temperature) could lead to a greater understanding of the direct links between biochemical and physiological parameters and the more transient aspects of whole animal biology such as behaviour patterns.

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# CHAPTER 5

# SEASONAL ADAPTATION IN ENZYMES OF JUVENILE ATLANTIC SALMON: II. GLUCOSE-6-PHOSPHATE DEHYDROGENASE

## Introduction

### *Temperature dependence of metabolic pathways in fish*

Changes in the environmental temperature (and, therefore, body temperature) of fishes have been shown to affect both the activity and overall organization of metabolic pathways (Hochachka and Somero 1973; Malinovskaya 1989), including an increase in the activity of the pentose phosphate pathway (the hexose monophosphate shunt) during acclimation to low temperatures (Hochachka and Hayes 1962; Fried et al. 1969; Hochachka and Clayton-Hochachka 1973). The pentose phosphate pathway is one of three metabolic pathways in the liver of teleosts which produce NADPH used in lipid synthesis (Henderson and Tocher 1987; Sargent *et al.* 1989). The increased activity in this pathway has been linked with the shift towards greater synthesis of lipid, seen as a general trend in teleosts during acclimation to low temperatures (Hoar and Cottle 1952; Whitmore and Goldberg 1972; Hazel and Prosser 1974). This in turn, has been linked to lipid becoming a greater fuel source in fish at low temperatures (Walton and Cowey 1982).

### *G6PDH in relation to temperature*

Despite being the first, and rate limiting, enzyme in the important pentose phosphate pathway, relatively little work has been done on glucose 6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) in relation to temperature. The molecular form of G6PDH under cellular

conditions and the number of isozymes present has not been studied extensively in fish. Kent and Hart (1976) demonstrated that the liver of a cold acclimated cyprinid, *Semotilus atromaculatus*, possessed three electrophoretically distinct bands, which increased to five on warm acclimation. In a comprehensive study of G6PDH, Hochachka and Clayton-Hochachka (1973) have shown that juvenile mullet, *Mugil cephalus*, produce two tetrameric isozymes of G6PDH that function most efficiently during cold and warm acclimation respectively. Bautista et al. (1984) have purified two forms of G6PDH from the bass *Dicentrarchus labrax*, and Shatton et al. (1971) have shown that rainbow trout, *Oncorhynchus mykiss* possess three distinct forms of G6PDH.

The present study examined how the efficiency and activity of liver G6PDH varied in relation to assay temperature in juvenile Atlantic salmon sampled from various acclimatisation temperatures throughout the year.

It is not known if Atlantic salmon are able to synthesize multiple forms of G6PDH as other salmonids do for LDH (Hochachka 1967; chapter 4). Cederbaum and Yoshida (1976) have suggested that this is not the case and that liver G6PDH in the closely related rainbow trout is determined by two alleles at a single locus with complex electrophoretic patterns being due to post-translational modification.

*Seasonal ecology in salmon*

The effect of differing life-history strategies (with the consequent differences in body lipid) on the efficiency of G6PDH was also investigated. Juvenile Atlantic salmon (*Salmo salar*) exhibit differing life-history strategies which become evident in gross morphology in $0^+$ fish from autumn onwards as a bimodal distribution of sizes. The larger fish (Upper Modal Group, UMG) will migrate to sea in the following spring and the smaller fish (Lower Modal Group, LMG) remain in freshwater for a further year (Thorpe 1977). There are also concomitant differences in levels of food intake, metabolic activity, and general overwintering ecology (Higgins 1985; Higgins and Talbot 1985; Metcalfe *et al.* 1986, 1988; Thorpe *et al.* 1992; chapter 4). Both modal groups show a decrease in total body lipid (relative to weight) between autumn and spring during their first year in freshwater, but the UMG fish always maintain a relatively higher level of lipid compared to LMG fish (Higgins and Talbot 1985). This chapter presented the results of a study that related the activity of G6PDH from the liver of juvenile Atlantic salmon with the seasonal water temperatures experienced by these fish and the expression of different life history strategies within a population.

## Material and Methods

*Experimental Fish*

The sibling juvenile Atlantic salmon used in this study were from the same family as those in chapter 4 and were reared and sampled under the same conditions.

Briefly, samples of $0^{+}$ fish were taken once from each season which, along with associated acclimatisation temperatures, were as follows: summer (18°C); autumn (7°C); winter (3°C); spring (7°C); and smolts (7-10°C). The latter sample consisted of fish undergoing smolting and of fish destined to spend a further year in fresh water (as described in chapter 4). Autumn and spring fish were both sampled at similar acclimatisation temperatures in order to compare the effect of increasing (spring) and decreasing (autumn) photoperiod on enzyme activity while controlling for ambient temperature.

Fish were sampled and stored at -80°C until enzyme analyses as described in chapter 4.

*Tissue excision and preparation*

Adipose tissue is not a synthetically competent site in teleosts, the majority of lipid synthesis taking place in the liver (Fried *et al.* 1969). The liver was removed from each fish and homogenised separately in ice-cold buffer (consisting of 50 $mmol.l^{-1}$ triethanolamine and 5 $mmol.l^{-1}$ EDTA), except for the summer samples in which the livers from two fish had to be combined due to the small size of the fish. Liver weights ranged from

approximately 0.003g (summer samples) to approximately 0.3g (smolts) depending upon size and, therefore, modal group. These were homogenised in volumes of buffer ranging between 0.5 ml and 3.0 ml depending upon size of liver.

The homogenates were centrifuged at 8000g for 10 minutes and the supernatants were then either used undiluted in enzyme assays or diluted (1+1 or 1+3) with TEA/EDTA buffer to create sufficient volume of liquid to be used in the assays.

Assays from each sample were performed on the same day as tissue excision, and supernatants were held in ice-water during this time.

*Enzyme assays and kinetic studies*

Enzyme activity for the oxidation of glucose-6-phosphate to 6-phosphoglucono-$\delta$-lactone was measured by monitoring the increase in absorbance (due to the production of NADP) over 5 minutes at 340 nm using a Hitachi U-2000 spectrophotometer and a flow-through quartz cuvette. The spectrophotometer was connected to a Grant LTD 6 water bath (Grant Instruments Ltd., Cambridge) which allowed the assay to be performed at temperatures representative of the range experienced throughout the year by the experimental fish, namely: 3°C, 7°C, 12°C and 18°C.

The reaction mixture contained a buffer of 50 $mmol.l^{-1}$ triethanolamine and 5 $mmol.l^{-1}$ EDTA with the pH adjusted to 7.6 for each assay temperature, 15 $mmol.l^{-1}$

NADP, and four different concentrations of glucose-6-phosphate: 0.005, 0.01, 0.02 and 0.04 mmol.$l^{-1}$. The reaction was started by the addition of 0.1 ml of the supernatant and the final volume of the reaction mixture came to 1 ml. Protein concentration of the dilution of supernatant used in the enzyme assay was determined by measuring the absorbance of a protein-dye mixture (Bradford 1976).

The initial rates of change in absorbance (when the change in absorbance over time remained constant) were used to determine the initial velocities of the reactions. Linear regressions were then performed on Lineweaver-Burke double reciprocal plots of these data to determine $K_m$ and $V_{max}$ values for each fish at a given assay temperature. Enzyme activities are expressed in $\mu$mol NADP.$min^{-1}$.mg $protein^{-1}$ and $K_m$ values are expressed in mmol.$l^{-1}$.

At the time of taking the summer sample it was not possible to allocate fish to modal groups, since the size separation is not apparent at this age. Therefore, in the statistical analyses data from the summer sample were included with those of both modal groups, unless otherwise stated.

## Results

### Temperature effects on enzyme-substrate affinity

When the $K_m$(g6p) data were analysed over the whole year for juvenile salmon irrespective of modal group, both assay temperature and season had significant effects

(2-way ANOVA $F_{3,240}$ = 2.803, P<0.05 and $F_{4,240}$ = 21.008, P<0.0001 respectively), with $K_m$ values overall tending to be lowest at low temperatures and in the autumn (figs. 5.1 - 5.5). However, this relationship with assay temperature varied greatly throughout the year (2-way ANOVA, assay temperature-season interaction $F_{12,240}$ = 3.257, P=0.0002). In particular UMG fish had higher $K_m$(g6p) values in spring than in autumn (2-way ANOVA, $F_{1,46}$=21.428, P<0.0001), although there was no such difference between LMG fish in the two seasons (2-way ANOVA, $F_{1,41}$=0.002, n.s.). Subsequent analyses were therefore carried out separately on the two categories of fish.

*Temperature effects on Km in UMG fish*

If the unimodal $0^+$ summer sample was included with the UMG data for the rest of the year, $K_m$(g6p) was not significantly affected by assay temperature (2-way ANOVA $F_{3,102}$=1.376, P=0.25). However, if the summer sample was excluded, the effect of assay temperature was significant (2-way ANOVA $F_{3,89}$=4.251, p<0.01). Figures 5.1 - 5.5 indicate that inclusion of the summer sample (which displayed greater variation, and a $K_m$-assay temperature profile that was the opposite of that of the other samples) in the data analysis may have caused the overall mean $K_m$ values in each season to be similar over the period of a year. Regardless of whether summer data were included or excluded, the effect of season (2-way ANOVAs $F_{4,102}$=14.274, P<0.0001

Figure 5.1. Temperature profiles of mean $K_m$ values of G6PDH from unimodal juvenile salmon acclimatised to summer temperatures (18°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

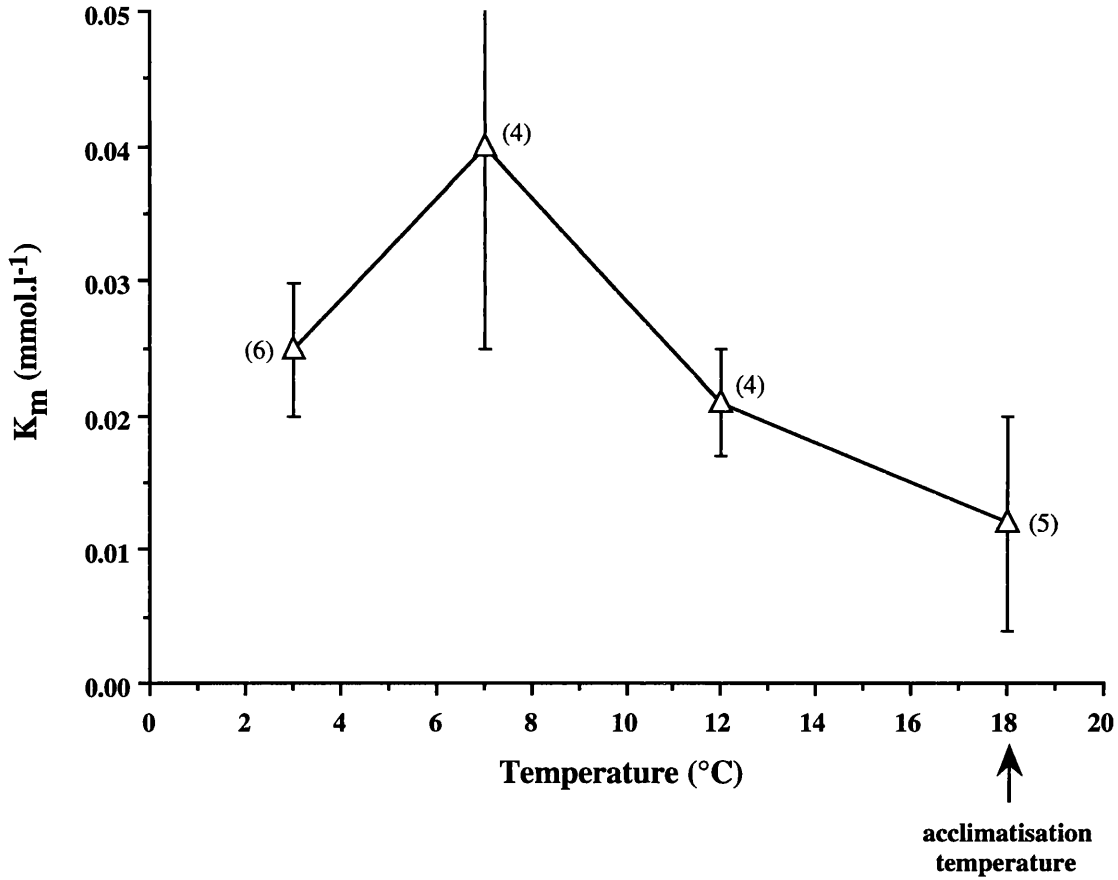

0.05
0.04
0.03
0.02
0.01
0.00
$K_m$ (mmol.l$^{-1}$)
(6)
(4)
(4)
(5)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation
temperature

Figure 5.2. Temperature profiles of mean $K_m$ values of G6PDH from UMG (—○— ) and LMG (— ● —) juvenile salmon acclimatised to autumn temperatures (7°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

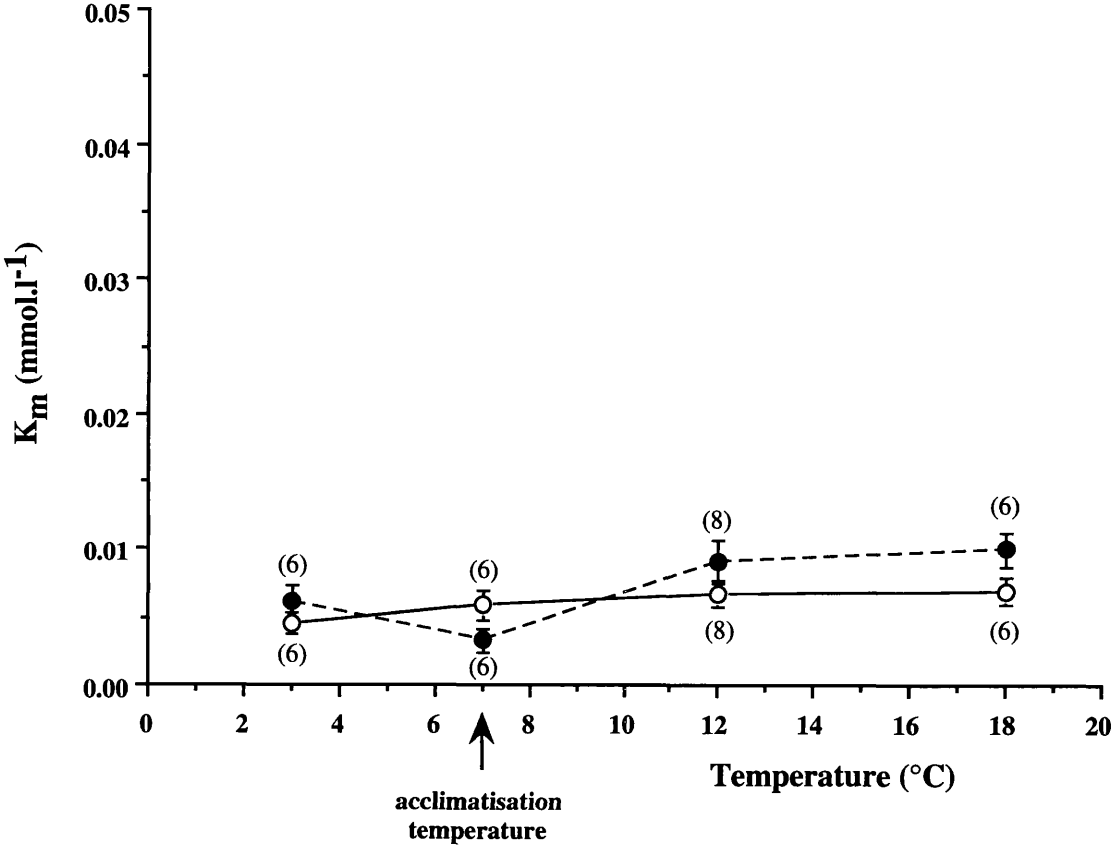

0.05
0.04
0.03
0.02
0.01
0.00
$K_m$ (mmol.l$^{-1}$)
(6)
(6)
(6)
(6)
(8)
(8)
(6)
(6)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation
temperature

Figure 5.3. Temperature profiles of mean $K_m$ values of G6PDH from UMG (——○——) and LMG (– –●– –) juvenile salmon acclimatised to winter temperatures (3°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

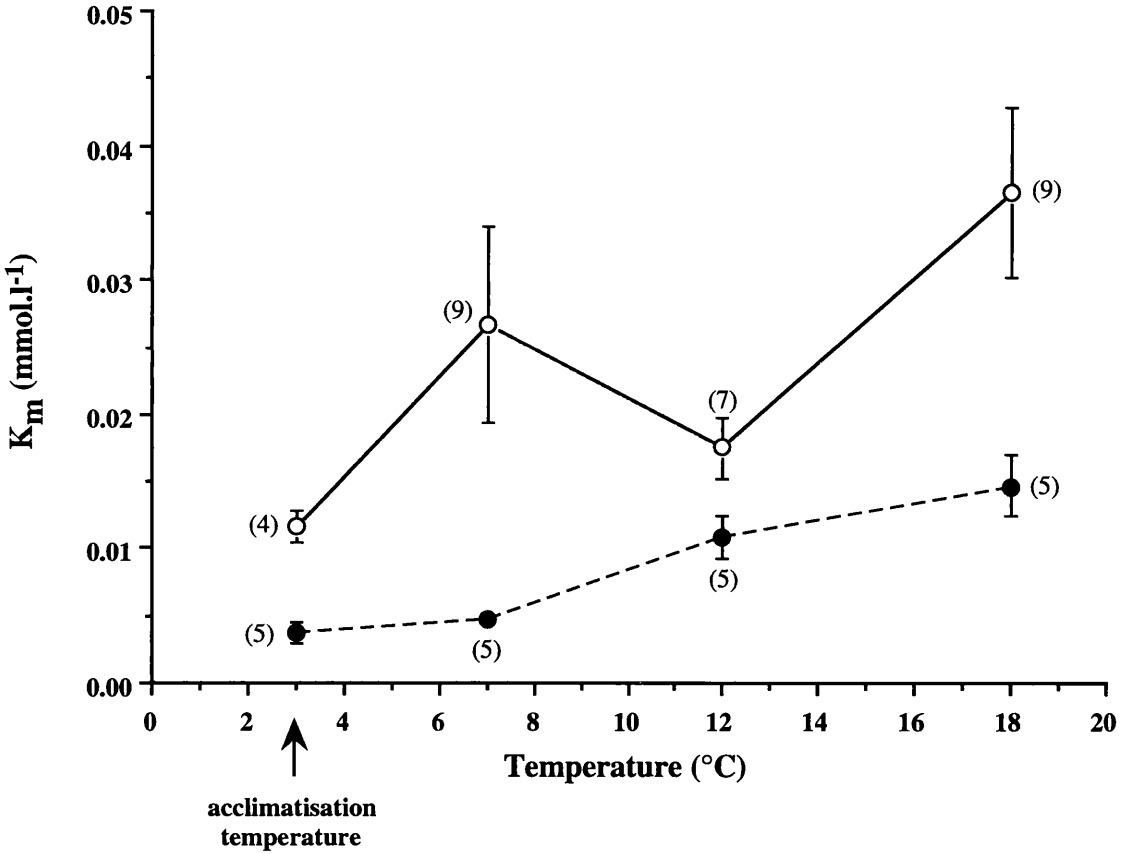

0.05
0.04
0.03
0.02
0.01
0.00
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation temperature
(4)
(9)
(7)
(9)
(5)
(5)
(5)
(5)

Figure 5.4. Temperature profiles of mean $K_m$ values of G6PDH from UMG (——○——) and LMG (– –●– –) juvenile salmon acclimatised to spring temperatures (7°C). Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.

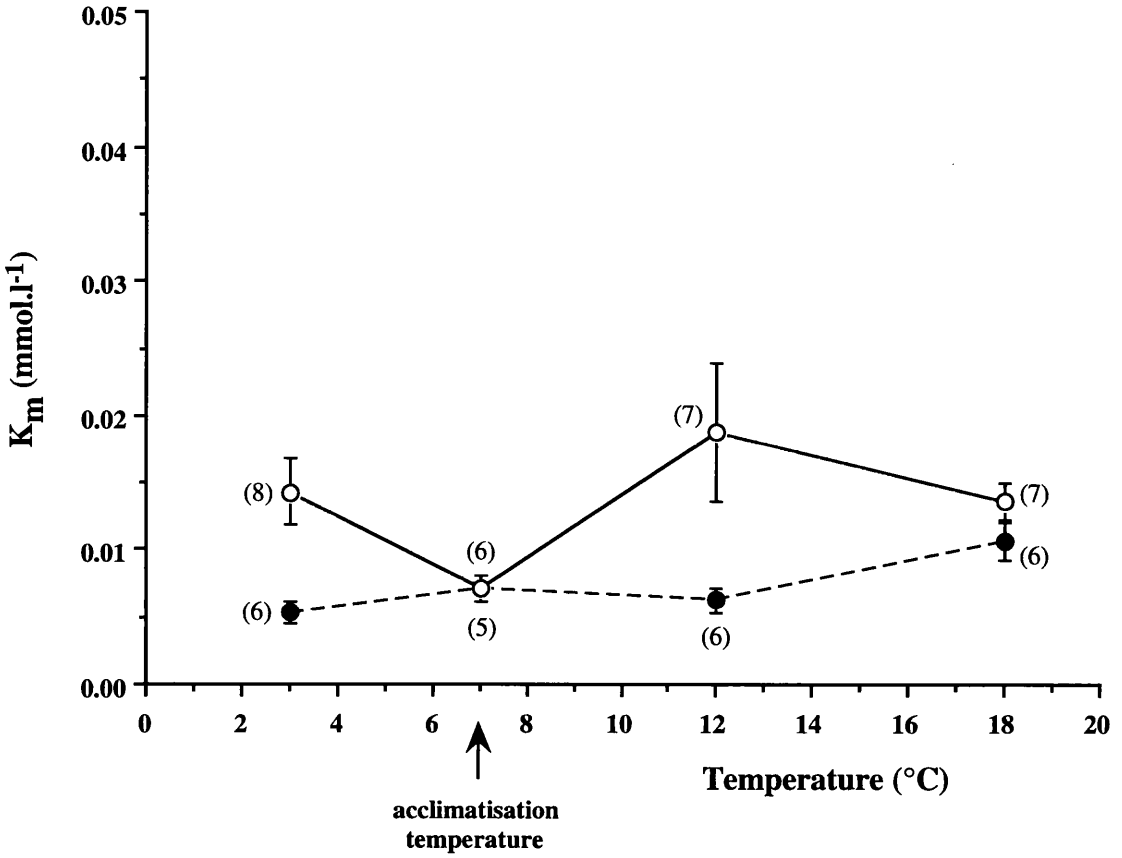
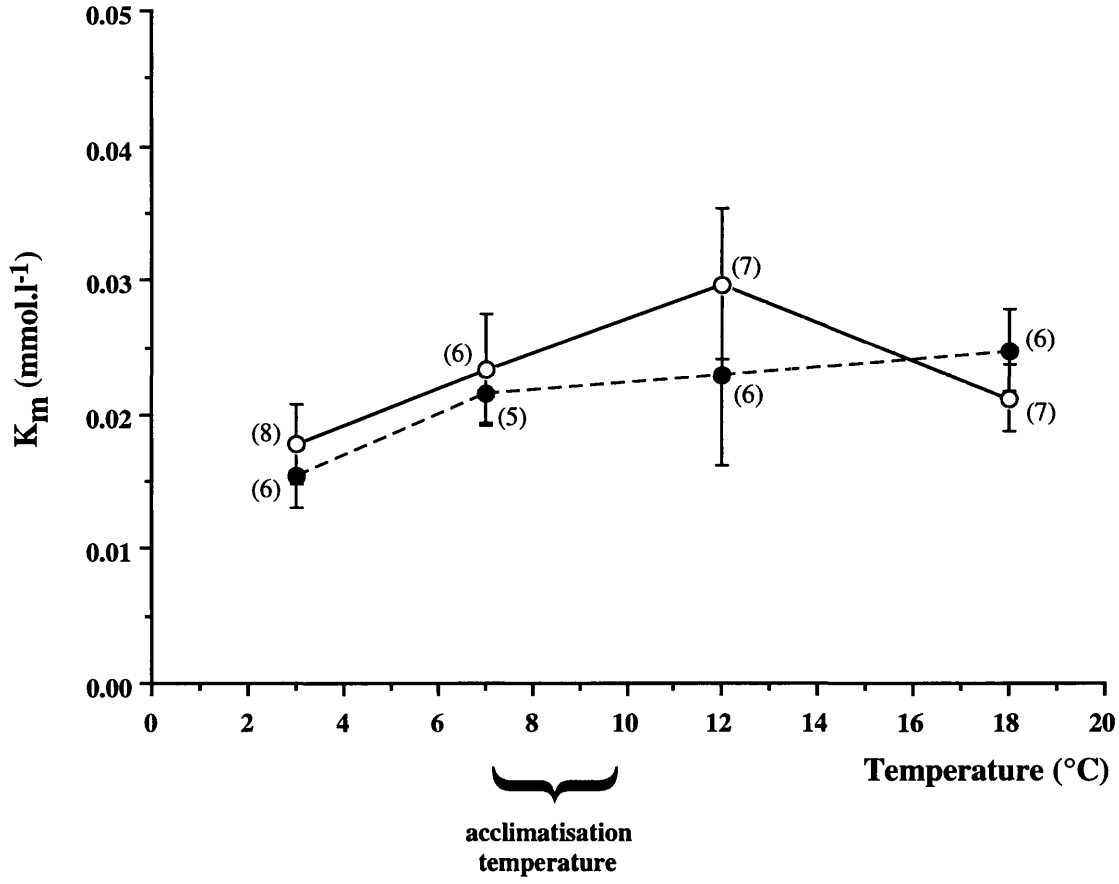

0.05
0.04
0.03
0.02
0.01
0.00
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
acclimatisation temperature
(8)
(6)
(6)
(5)
(7)
(6)
(7)
(6)

Figure 5.5. Temperature profiles of mean $K_m$ values of G6PDH from smolts (—○—) and the LMG (– ●– –) of juvenile salmon acclimatised to temperatures between 7 and 10°C. Vertical bars represent 1 standard error, numbers in parentheses are sample sizes.


0.05
0.04
0.03
0.02
0.01
0.00
$K_m$ (mmol.l$^{-1}$)
0
2
4
6
8
10
12
14
16
18
20
Temperature (°C)
(8)
(6)
(6)
(5)
(7)
(6)
(6)
(7)
acclimatisation
temperature

and $F_{3,89}$=19.370, P<0.0001 respectively) and the assay temperature-season interaction (2-way ANOVAs $F_{12,102}$=3.380, P=0.0004 and $F_{9,89}$=3.200, P<0.005 respectively) were highly significant.

*Temperature effects on Km in LMG fish*

The same general pattern was seen in LMG fish (figs. 5.1 -5.5); with summer values included there were no significant variations with assay temperature (2-way ANOVA $F_{3,82}$=1.374, P=0.26), but when summer was excluded the effect of assay temperature became significant (2-way ANOVA $F_{3,69}$=11.038, P<0.0001). In both cases $K_m$ values varied significantly with season (2-way ANOVAs $F_{4,82}$=23.083, P<0.0001 and $F_{3,69}$=41.603, P<0.0001 for analyses including and excluding summer respectively). The assay temperature-season interaction was significant when the summer sample was included (2-way ANOVA $F_{12,82}$=2.735, P<0.004), but was not significant when it was excluded (2-way ANOVA $F_{9,69}$=1.306, P=0.25). This was due to the response to assay temperature being radically different in summer to the pattern seen at other times of the year (figs. 5.1 - 5.5).

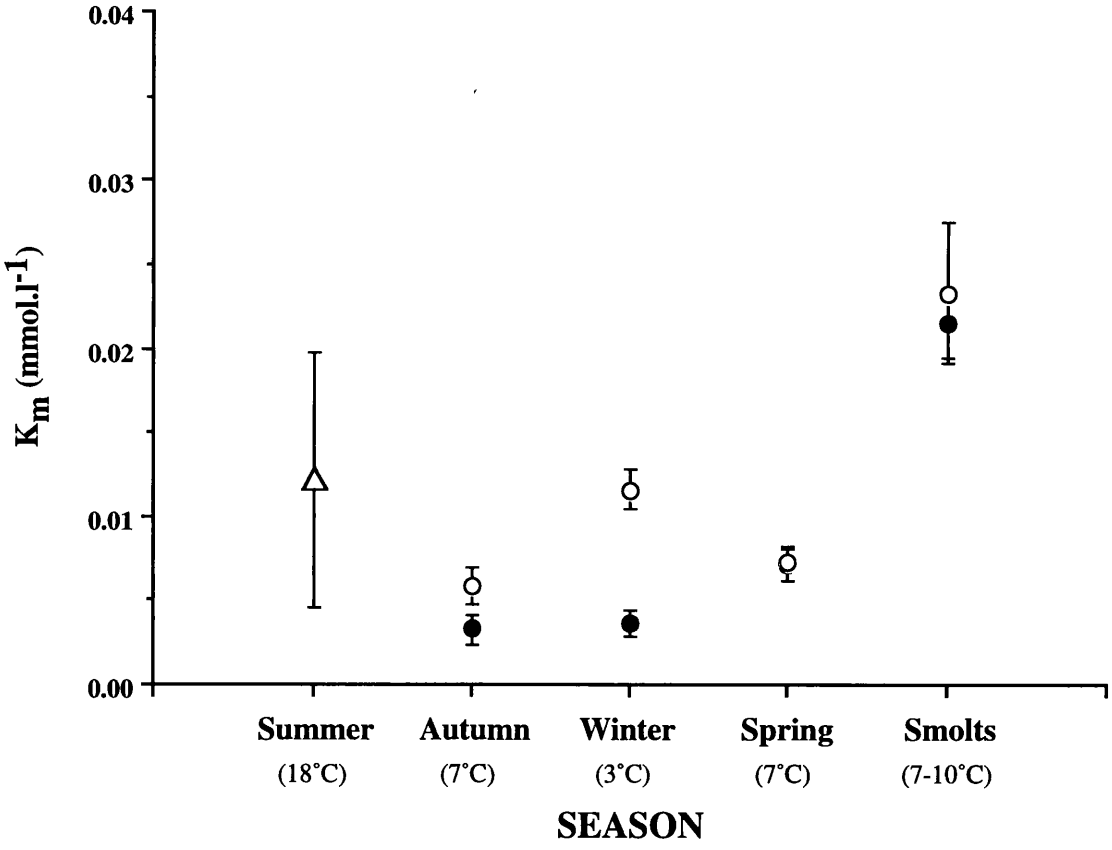
The difference in response to seasonal temperatures between the modal groups is shown by the fact that within a season the $K_m$ for LMG fish was significantly affected by assay temperature in autumn, winter and spring (1-way ANOVAS, maximum P=0.01), whereas in UMG

fish assay temperature only had a significant effect on the $K_m$ in winter (1-way ANOVA $F_{3,23}=5.472$, $P<0.01$).
In the sample taken during May (fig. 5.5) there was no difference in the $K_m$-temperature profiles of smolts and LMG fish (2-way ANOVA $F_{1,39}=0.362$, $P=0.56$) and neither modal group showed a significant effect of assay temperature on $K_m$ value (1-way ANOVAs $F_{3,16}=1.646$, $P=0.23$ and $F_{3,17}=0.183$, $P=0.91$ for UMG and LMG fish respectively).

*Seasonal changes in Km at environmental temperatures*

The $K_m$ values of UMG and LMG fish were similar at acclimatisation temperatures, except during winter when UMG fish showed higher $K_m$ values (fig. 5.6, $t=5.007$, 7 df. $P<0.01$). Generally, the $K_m$(g6p) values were lowest at acclimatisation temperatures which correspond to the environmental temperatures at that time of year.
It was possible to test whether the $K_m$ values of seasonally acclimatised fish varied with season by comparing the $K_m$ of autumn fish at 7°C, winter fish at 3°C, and so on. Season did indeed have a highly significant effect on the $K_m$ values coincident with environmental temperatures for both UMG and LMG fish (1-way ANOVAs, $F_{4,20} = 5.994$, $P<0.003$ and $F_{4,18} = 9.955$, $P=0.0002$ respectively). This seasonal effect was due to the high $K_m$ values at acclimatisation temperatures for both modal groups during the period of seaward smolt migration of the UMG (fig. 5.6).

Figure 5.6. Mean $K_m$ values of G6PDH from UMG (—○—) and LMG (–●–) juvenile salmon at the acclimatisation (environmental) temperatures in each season. Vertical bars represent 1 standard error.


0.04
0.03
0.02
0.01
0.00
$K_m$ (mmol.l$^{-1}$)
Summer
(18°C)
Autumn
(7°C)
Winter
(3°C)
Spring
(7°C)
Smolts
(7-10°C)
SEASON

Temperature effects on maximal enzyme activities

There was no significant difference between the modal groups in maximal activities ($V_{max}$) of G6PDH at acclimatisation temperatures in any of the seasons, and so the data from both modes were pooled within each season. For the combined $V_{max}$ values there was a significant effect of season (1-way ANOVA, $F_{4,43}=137.87$, $P<0.0001$) due to the values from the summer samples being much higher than those at other times of the year (fig. 5.7).

The activation energies (Ea) of G6PDH in different seasons were calculated using Arrhenius plots (log $V_{max}$ versus the reciprocal of temperature in °K) and all were significant (minimum correlation coefficient = -0.816, maximum P = 0.00001). The G6PDH from UMG fish had significantly higher activation energies than did the LMG fish in winter ($t=2.66$, d.f.=43, $P<0.05$), but not in any other seasons (Table 1).

The free energy of activation ($\Delta G^{\neq}$) is another measure of the "energy barrier" that has to be surmounted before a reaction can proceed, and includes Ea in its calculation (see chapter 4).

The mean $\Delta G^{\neq}$ values did not differ between the modal groups in any of the seasons (Table 2) and so the data were pooled in the next analysis. A 1-way ANOVA showed that the $\Delta G^{\neq}$ values tended to vary seasonally, being lowest in summer (fig. 5.8a, $F_{4,47}=2.692$, $P<0.05$). Linear regression of $\Delta G^{\neq}$ values on forklength showed that this was not simply an effect of increasing fish

Figure 5.7. Mean $V_{max}$ values of G6PDH for both modal groups of juvenile salmon combined at the acclimatisation (environmental) temperatures in each season. Vertical bars represent 1 standard error.

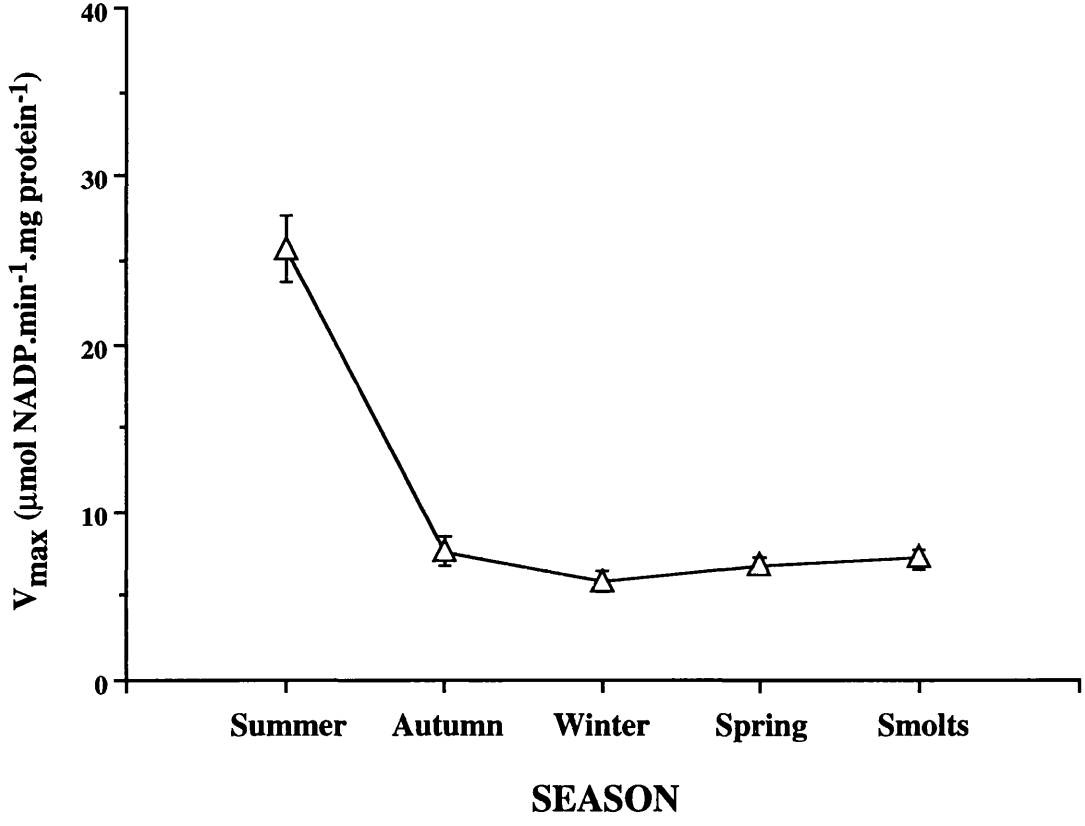

40
30
20
10
0
$V_{max}$ (µmol NADP.min$^{-1}$.mg protein$^{-1}$)
Summer
Autumn
Winter
Spring
Smolts
SEASON

**Table 1**

Mean values for activation energy (Ea) of G6PDH for upper (UMG) and lower (LMG) modal groups in different seasons.

| Season | Acclimatisation Temp. (°C) | Ea (cal.mole$^{-1}$) UMG | | LMG |
|---|---|---|---|---|
| Summer | 18 | 10390 | | |
| Autumn | 7 | 12126 | | 14877 |
| Winter | 3 | 16511 | * | 12128 |
| Spring | 7 | 10426 | | 12545 |
| Smolts | 7 - 10 | 11771 | | 13027 |

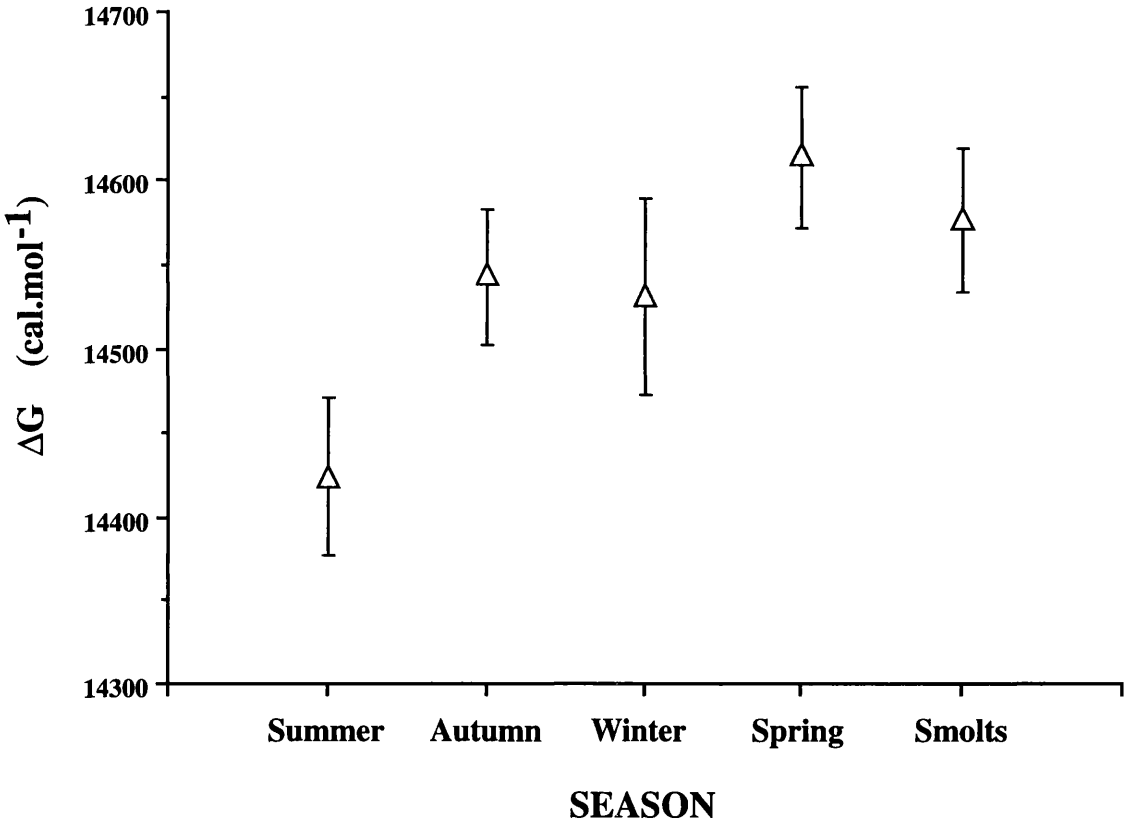
* $P<0.05$, Mann-Whitney U tests comparing modal groups.

Table 2

Mean values and standard errors for free energy of activation ( $\Delta G^{\ddagger}$ ) of G6PDH at acclimatisation temperatures for upper (UMG) and lower (LMG) modal groups in different seasons.

| Season | Acclimatisation Temp.(°C) | $\Delta G^{\ddagger}$ (cal.mole$^{-1}$) UMG | LMG |
|---|---|---|---|
| Summer | 18 | 14424(±38) | |
| Autumn | 7 | 14475(±11) | 14612(±71) |
| Winter | 3 | 14464(±41) | 14494(±69) |
| Spring | 7 | 14599(±48) | 14632(±66) |
| Smolts | 7 - 10 | 14582(±57) | 14567(±54) |

Figure 5.8a. Mean $\Delta G^{\neq}$ values of G6PDH from both modal groups of juvenile salmon combined at the acclimatisation (environmental) temperatures in each season. Vertical bars represent 1 standard error.


14700
14600
14500
14400
14300
ΔG (cal.mol-1)
Summer
Autumn
Winter
Spring
Smolts
SEASON

size throughout the year (r=0.151, P=0.31). Stepwise multiple regression analysis using date (represented in calculations by days since the start of the experiment), date$^2$, forklength, temperature and temperature$^2$ showed that only temperature and temperature$^2$ were significantly correlated with $\Delta G^{\neq}$ values (t=2.176, P,0.05 and t=-2.385, P<0.05 respectively), the resultant quadratic equation (I) being shown in figure 5.8b.

$$\Delta G^{\neq} = 14369.29 + 44.29.x - 2.29.y \qquad (I)$$

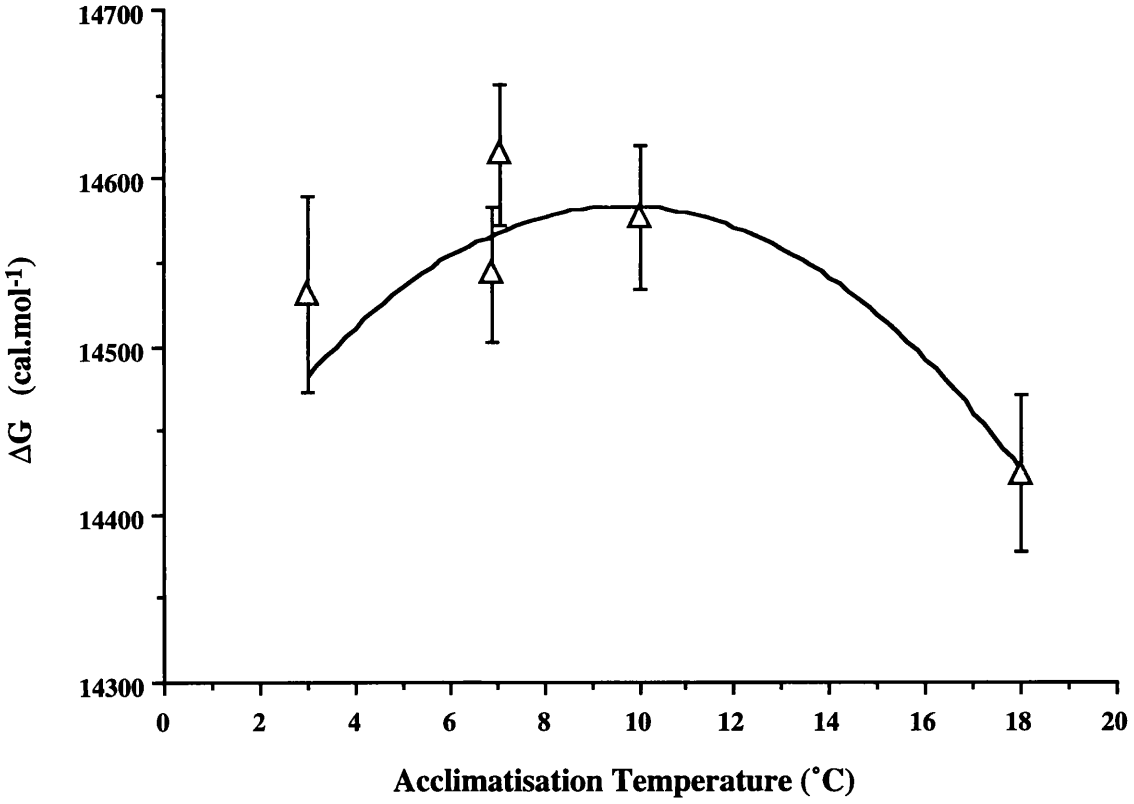
where x = temperature and y = temperature$^2$

*Effect of fish size on enzyme activity*

Size scaling of enzyme activity was investigated (for $K_m$ and $V_{max}$ values, with respect to forklength (mm)) at the assay temperatures concurrent with acclimatisation temperature (i.e. 3°C for winter, 7°C for autumn and spring, etc.). Summer samples were excluded from this analysis since in some instances it had been necessary to combine the livers from two fish from the summer sample to provide sufficient volume of supernatant for the enzyme assays. To control for the different assay temperatures, enzyme activities (either $K_m$ or $V_{max}$) were expressed as a percentage of the mean of all values at that acclimatisation temperature. (N.B. these percentages were not constricted to values of 0-100% and, therefore, did not require arcsin transformation). The logarithms of these percentage values were then regressed against the logarithm of forklength.

Figure 5.8b. The relationship between mean $\Delta G^{\neq}$ values of G6PDH at the acclimatisation (environmental) temperature for both modal groups of juvenile salmon combined and acclimatisation temperature (temp) itself as described by the equation:

$$\Delta G^{\neq} = 14369.29 + 44.29 \text{ (temp)} - 2.29. \text{ (temp)}^2$$


14700
14600
14500
14400
14300
ΔG (cal.mol-1)
0
2
4
6
8
10
12
14
16
18
20
Acclimatisation Temperature (°C)

Both UMG and LMG fish showed significant correlations between $K_m$(g6p) and fish size (fig. 5.9, r=0.819, P<0.00001, y=2.92x -3.89 and r=0.492, P<0.05, y=3.55x-4.65 respectively) and there was no difference between the two slopes (t=0.406, df=37, n.s.). However, covariance analysis did show that LMG fish had higher relative $K_m$ values than did UMG fish of the same size (2-way ANOVA $F_{1,40}$=6.473, P=0.015).

When $V_{max}$ values were compared with forklength in a similar fashion there was no effect either in the 2 modes separately or for both modal groups combined (correlation coefficients = -0.150, -0.153, 0.092 all n.s. for UMG, LMG and both modes combined, respectively).

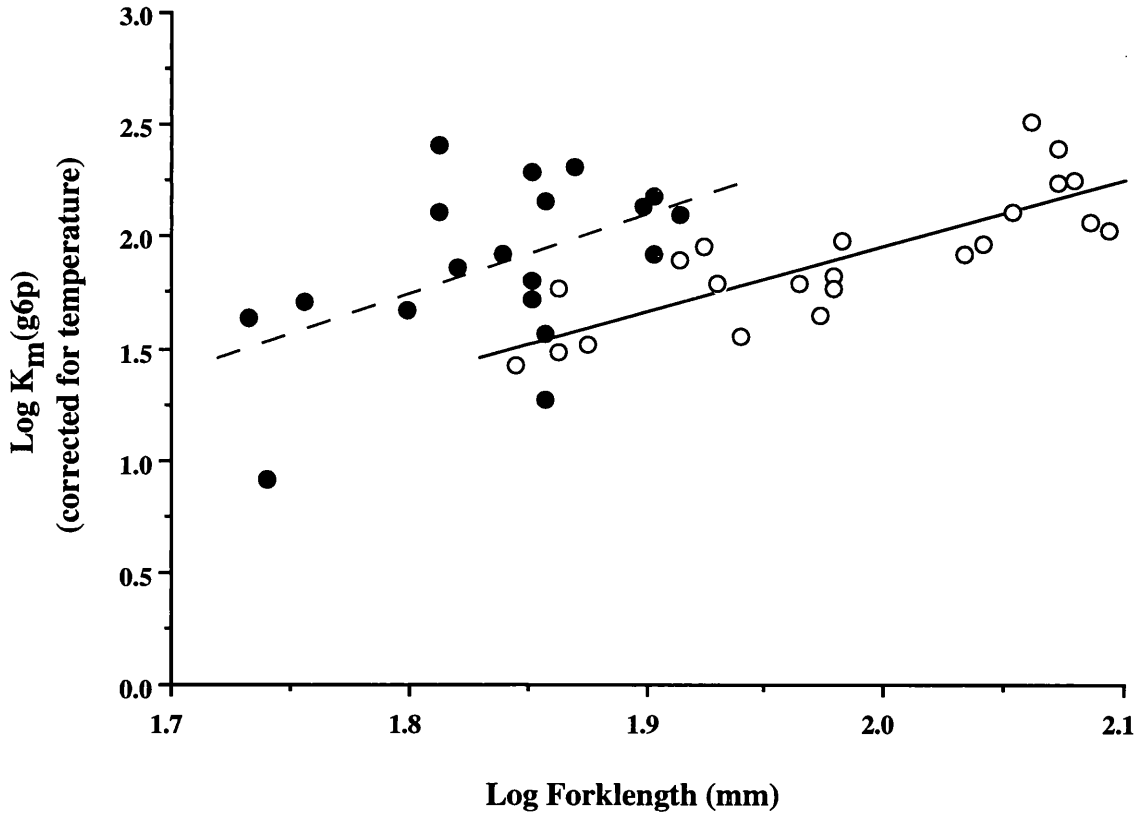
*Effect of gender on Km values*

A paired t-test between the mean $K_m$ values of the male and female fish at each temperature in each season showed no significant difference due to gender for either upper or lower modal groups or for both modal groups combined (t=0.585, 11 d.f., n.s.; t=0.759, 14 d.f., n.s.; t=0.893, 26 d.f., n.s. respectively). Nor was any gender difference apparent when only data from acclimatisation temperatures were used (t=0.961, 5 d.f., n.s. for both modal groups combined).

*Seasonal effects on lipid levels*

NADPH generated by the pentose phosphate pathway provides a reducing agent used in lipogenesis. The data

Figure 5.9. Correlation between log forklength (mm, FL) and the log of the respective $K_m$ values ($\log K_m$%, corrected for temperature - see results) for UMG (—O—) and LMG (— ●— —) juvenile salmon.

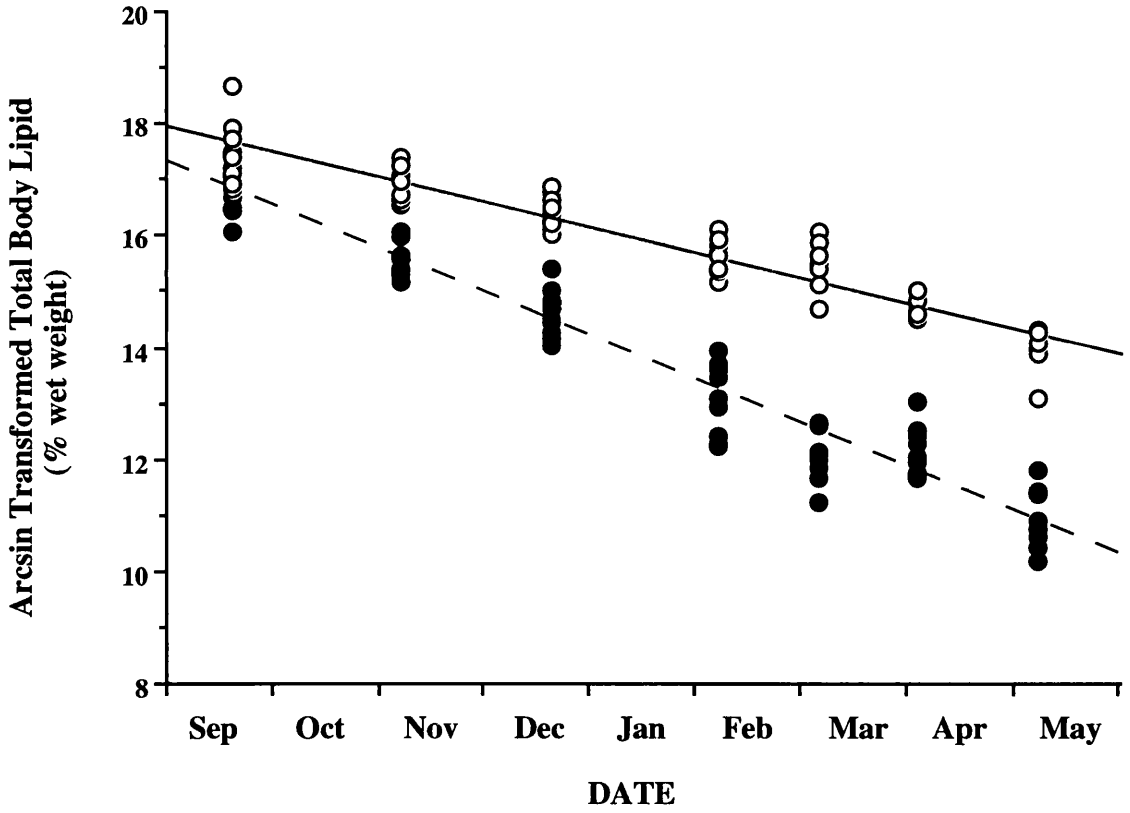

3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.7
1.8
1.9
2.0
2.1
Log $K_m$(g6p)
(corrected for temperature)
Log Forklength (mm)

presented by Higgins and Talbot (1985) showing seasonal changes in whole body lipid levels of UMG and LMG fish were re-analysed to investigate the rate of decline in lipid level from autumn to spring for both modal groups. (These data were used in preference to those of my own (chapter 6) since the former were of total body lipid levels rather than only mesenteric lipid levels). After arcsin transformations of the lipid data (expressed as percentage of wet body weight), both modal groups showed a highly significant linear decline in body lipid from September through to May (fig. 5.10, $r=-0.950$, $df=69$, $P<0.00001$ and $r=-0.967$, $df=69$, $P<0.00001$ respectively). Comparison of these regression lines indicated that the rate of decline in body lipid in UMG fish was less than that for LMG fish ($t=10.625$, $df=136$, $P<0.001$).

## Discussion

Previous studies have demonstrated an effect of environmental temperature on the $K_m$ (i.e. enzyme-substrate affinity) of different enzymes. The adaptive value of this phenomenon of thermal modulation is that it causes reaction rates to remain relatively stable over varying environmental temperatures (Hochachka and Somero 1971). This is seen not only within a given form of an enzyme during immediate adaptation to sudden temperature changes (Somero 1969), but also throughout the year with the production of enzyme variants that display minimal $K_m$ values at differing seasonal

Figure 5.10. Linear regression of arcsin transformed lipid data (as a percentage of wet weight) between september and May for UMG (—○— ) and LMG (– –●– –) juvenile salmon. Data from Higgins and Talbot (1985).


Arcsin Transformed Total Body Lipid
(% wet weight)
20
18
16
14
12
10
8
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

temperatures (Aleksiuk 1971; Baldwin and Hochachka 1970; Somero and Hochachka 1971; Narita and Horiuchi 1979; chapter 4).

The present study has shown that temperature does affect the enzyme-substrate affinity of G6PDH in juvenile Atlantic salmon and that this effect differs between seasons. Figures 5.1 - 5.5 show that this response also differed both qualitatively and quantitatively between modal groups at different times of the year, implying that the activity of the pentose phosphate pathway responds differently in the two modes to changing seasonal temperatures. A closer examination of the $K_m$ values suggests that this may reflect fundamental differences in the overwintering ecology of these animals.

Whereas LMG fish demonstrated a change in $K_m$ values with assay temperature in autumn (7°C), winter (3°C) and spring (7°C), this only occurred in UMG fish during winter, suggesting that LMG fish have forms of the enzyme that are more adapted to seasonal temperatures than those present in UMG fish. Since G6PDH is the rate-limiting "control" enzyme in the pentose phosphate pathway, the reason for this may be tied to differential metabolic reorganization between the modal groups in response to temperature change. Reduced activity levels and concomitant reduction in maintenance metabolism as seen in fish at low temperatures (Brett et al. 1969, Hochachka and Somero 1973) also occur in LMG fish in comparison to UMG fish

(Higgins 1985). This will result in fewer of the metabolites being used in energy producing pathways and may allow the LMG fish to channel a greater percentage of their food into storage compounds such as lipid (Hochachka and Hayes 1962, Hochachka and Somero 1973). The fact that UMG fish have consistently higher levels of body lipid than LMG fish, and that these are lost at slower rates from autumn to spring does not necessarily contradict this argument. UMG fish had up to five times the daily food intake of LMG fish over this period (Higgins and Talbot 1985), so even though the LMG fish may have been converting a greater percentage of their food into lipid, the relative values would still be less than those seen in UMG fish. During the period of anorexia seen in LMG fish over autumn and winter, appetite is maintained to a sufficient extent to keep energy reserves (estimated in terms of body lipid) above a predetermined "defended level" (Metcalfe and Thorpe 1992). In such a case it would make sense for the LMG fish to concentrate their resources on lipogenesis, whereas the energy reserves (body lipid) of UMG fish do not drop to such critical low levels.

Active metabolism, e.g during prey capture and other strenuous activities, may increase the concentration of substrates in pathways over those seen during basal metabolism (Somero 1969, Hochachka and Somero 1973). $K_m$ values lower than the physiological concentrations of substrate would lead to complete saturation of an enzyme and a consequent build up of substrate, whereas

high $K_m$ values would mean low enzyme-substrate affinities and consequently low rates of enzyme catalysis (Hochachka and Somero 1973). Hence for maximum efficiency, $K_m$ values have to be at approximately the same levels as the cellular concentrations of substrate (Williamson et al. 1967, Somero 1969). Therefore, the higher $K_m$ values of G6PDH shown by UMG fish during winter may actually be more efficient in this situation since these more active fish (Fraser 1994) have a larger food intake which will lead to higher cellular concentrations of substrate for the pentose phosphate pathway, and hence the higher $K_m$ values could be viewed as adaptive. This could explain why UMG fish maintain a higher level of body lipid throughout the year than the LMG fish, despite at first sight apparently having less efficient G6PDH.

It would also not be true to say that the lower $K_m$ values of the LMG fish at winter temperatures meant that these enzyme variants were more efficient, since the G6PDH of both modes could be viewed as efficient with respect to differing cellular substrate concentrations. This highlights an important point: during metabolic reorganization, adaptive changes in enzyme efficiency are such that they bring about optimal activity of pathways, both in relation to intracellular concentrations of metabolites/molecules, and the activities of other pathways (Hochachka and Somero 1973). However, it should again be mentioned that the idea of fluctuations in the concentrations of

substrate in a pathway is not yet proven and requires further clarification.

The $V_{max}$ values did not differ between the modal groups, and only the summer sample displayed higher values than those in the rest of the samples. This single difference may be similar to the variation in the expression of LDH isozymes seen during the early ontogeny of juvenile fish (Frankel 1980; Chingjiang and Schroder 1984). Other studies have used maximal enzyme activities to investigate ecological effects on the metabolism of fishes (Shaklee *et al.* 1977, Tranulis *et al.* 1991), but the biological significance of these results must be limited since physiological cellular substrate concentrations are normally well below the saturating levels that cause maximal activity. Atkinson (1966) proposed that the use of physiological substrate concentrations in an experimental system is an absolute necessity if the data are to have any biological meaning. The lack of seasonal and modal variation in $V_{max}$ values from the present study may indicate that maximal enzyme activity is not a suitable indicator of seasonal variation in enzyme kinetics. Also most of the kinetic studies that have been carried out on G6PDH, have used non-physiological saturating concentrations of substrate to compare maximal enzyme activities (Shaklee *et al.* 1977, Soengas *et al.* 1993), sometimes at temperatures well in excess of the lethal limit for the animals that provided the enzyme (Bautista *et al.* 1989). The results from such studies, while

demonstrating differing structures and functions of G6PDH enzymes, must be limited in their applicability to animals living under natural or semi-natural conditions.

Many previous studies have examined the ability of enzymes to lower the amount of energy that the reacting molecules must possess (the "energy barrier") for a reaction to proceed, and how variation of this aspect may provide a means for thermal acclimation. These have tended to use activation energy (Ea) (Hochachka and Somero 1968; Behrisch 1969; Behrisch and Hochachka 1969; Somero 1969; Moon and Hochachka 1971) rather than the free energy of activation ($\Delta G^{\neq}$) (Low *et al.* 1973). Interspecific comparisons of enzymes from fish inhabiting different thermal regimes have shown that some enzymes do exhibit a positive correlation between Ea and acclimatisation temperature (eg. G6PDH in mullet, Hochachka and Clayton-Hochachka 1973). Such a relationship would be advantageous, since it would result in a more efficient enzyme under conditions of low thermal energy and this has been interpreted as being important in evolutionary terms (Hochachka and Somero 1971). Selection for reduction of Ea during acclimation to low temperatures, would be more likely in rate-limiting enzymes especially if the inherent Ea of an enzyme is high (Hochachka and Somero 1971). However, the entropy of activation ($\Delta S^{\neq}$) may also vary both inter- and intraspecifically at different temperatures thus affecting the relative velocity of a

reaction. Hochachka and Somero (1973) and Low *et al.* (1973) suggest that $\Delta G^{\ddagger}$ is a better measure of the "energy barrier" to a reaction since it combines Ea with other thermodynamic parameters of a reaction.

The present study might seem to indicate that UMG fish have less efficient G6PDH during low winter temperatures, since they have higher Ea values. However, $\Delta G^{\ddagger}$ values suggest no modal difference in the efficiency with which G6PDH lowers the "energy barrier" for the reaction.

Figure 5.8a shows that there is a seasonal effect on $\Delta G^{\ddagger}$ values, with a small increase throughout the first year, but no difference between modal groups. Although other studies have compared the $\Delta G^{\ddagger}$ values for various enzymes between fish acclimatised to two different temperatures (review in Low *et al.* 1973), to my knowledge no other work has been done to examine any possible seasonal effect. The reason for the quadratic function (fig. 5.8b) describing the seasonal $\Delta G^{\ddagger}$ values in terms of acclimatisation temperature is not immediately obvious, since there does not seem to be any readily understandable correlation between the level of this "energy barrier" and available thermal energy. However, a more detailed study is needed on this aspect of enzyme kinetics and it is not proposed that the relationship shown in figure 5.8b is definitive.

The tendency for $\Delta G^{\ddagger}$ values to rise throughout the sampling period (fig. 5.8a) may be related to the

increase in $K_m$ with size shown in figure 5.9. $\Delta G^{\ddagger}$ is a measure of how much kinetic energy needs to be present in the reacting molecules for the reaction to proceed. On the other hand, $K_m$(g6p) values were expressed as a percentage of all the values for that given acclimatisation temperature in each season and thus are independent of seasonal effects. Hence, both are measurements of the efficiency of an enzyme, and both show a decrease in efficiency of G6PDH with increasing body size and age throughout their first year in freshwater. This observation is in concordance with the general trend of an inverse correlation of metabolic rate and body size (Brett and Groves 1979).

Although it has been shown that on a seasonal basis the G6PDH of LMG fish is equally, if not better, adapted to the environment than is that of UMG fish, figure 5.9 shows that at any given size LMG fish have less efficient G6PDH. This would seem to indicate a physiological difference between the two modal groups. It also shows that differences in biochemical rates can be detected between fish over a relatively small size range, whereas Brett (1964) was unable to distinguish a difference in metabolic rate (measured as mg $O_2.kg^{-1}.h^{-1}$) between $1^+$ sockeye salmon (*Oncorhynchus nerka*) which varied between 32.9 and 62.6g in weight.

At first sight increasing photoperiod in spring seems to have the effect of increasing the $K_m$(g6p) of UMG fish over the values seen in autumn. However, when analysed at the acclimatisation (i.e. environmental)

temperature, there is no difference between the seasons. This phenomenon of a more specific targetting of the action of enzymes to the acclimatisation temperature, with differences between UMG and LMG fish only being apparent at assay temperatures outwith those of the current environment, was also seen in LDH (chapter 4). Its significance, if any, is unclear.

The effect of temperature on the activity of G6PDH, the rate-limiting enzyme of the pentose phosphate pathway, is important to the seasonal ecology of fish due to the involvement of this pathway in lipid synthesis. It has been suggested that changes in the activity of the pentose phosphate pathway during acclimation are transitory (Hochachka and Somero 1971, Yamauchi *et al.* 1975, Tranulis *et al.* 1991) and are simply associated with the increase in the degree of unsaturation of fatty acids in cell membranes during the process of acclimation to low temperatures (review in Sellner and Hazel 1982) and that once acclimation is complete then the level of activity in the pentose phosphate pathway returns to pre-acclimation levels. In relation to fish under natural conditions this is a moot point because, in contrast to laboratory environments, the water temperature in temperate latitudes is almost always changing, both within and between days. For example, the fish from the present study experienced temperatures that ranged from 23°C in June 1989 to below 1°C in January 1990, with respective diel temperature ranges of 7 and 1 C°. Fish under natural

conditions will very rarely become acclimatised to a single temperature since the levels of saturated fatty acids in cell membranes do not undergo a sudden change at a certain temperature, but gradually increase or decrease with temperature change (Cossins 1983). This is obviously an important point that raises questions as to the biological significance of the results from laboratory-based studies where fish are acclimatised to a stable temperature for a period of weeks.

When studying the biochemistry of an animal, especially an ectotherm, it is important that the enzymic reactions and metabolic pathways are viewed in the context of the whole animal, and in relation to its various activities and changing priorities in a physically variable and seasonally changing environment. The idea that adaptive changes in enzymes do not occur in isolation but as an integral part of metabolic reorganization of the whole animal then becomes all-important.

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# CHAPTER 6

# BODY SHAPE, MORPHOLOGY AND AGE OF SMOLTING IN JUVENILE ATLANTIC SALMON

## Introduction

Juvenile Atlantic salmon (*Salmo salar*) have been shown to develop a bimodal distribution in length during the first year of growth, both in hatchery conditions (Thorpe 1977; Bailey *et al.* 1980) and in some wild populations (Heggenes and Metcalfe 1991; Nicieza *et al.* 1991). The group of larger fish are termed the upper modal group (UMG) and migrate to sea the following spring as S1 smolts, whereas the smaller fish (lower modal group - LMG) remain in fresh water for a further year before smolting as S2 smolts. This divergence in length within a single sibling population first becomes apparent during late summer, but the "physiological decision" about which strategy to follow seems to occur earlier in the summer (Adams and Thorpe 1989), and is influenced by a variety of factors including food supply, energy level, growth rate, dominance status, and temperature (reviewed in Thorpe *et al.* 1992).

As the water temperature drops through autumn the growth rate of juvenile salmon also decreases (Higgins and Talbot 1985; Kristinsson *et al.* 1985; Stewart *et al.* 1990). The UMG are larger, more dominant fish, and maintain a higher feeding rate than those in the LMG; they also show a short burst of intense feeding and growth during October which then declines again to previous levels (Kristinsson *et al.* 1985; Metcalfe *et al.* 1988). A similar short increase in feeding is seen in the group of smaller fish (LMG), but to a much

lesser degree. LMG fish adopt a cost-minimising strategy and by winter are feeding at or about a maintenance ration level, resulting in little or no growth during this period. In contrast, although the food intake of UMG fish is also reduced over winter, they continue to feed relatively actively (Metcalfe *et al.* 1988) and their energy intake remains sufficient to allow growth over the winter (Higgins and Talbot 1985; Kristinsson *et al.* 1985) and this leads to a continuing divergence in the bimodal length distribution.

As well as the obvious difference in lengths and weights seen between the two modal groups, UMG fish also tend to be viewed as having larger, deeper trunks, with greater condition factors, and generally being more "robust-bodied" fish.

When salmonids migrate to the sea they change physiologically, behaviourally and morphologically (Hoar 1976; Folmar and Dickhoff 1980; McCormick and Saunders 1987) and these processes are grouped together under the term "smolting". With respect to changes in morphology, in addition to changes in pigment from darker colours to a silvery appearance, smolting is also typified by a general lengthening of the trunk and/or post anal region (Nikolskii et al. 1947; Vanstone and Markert 1968) to give a more fusiform fish, resulting in a decrease in condition factor (Hoar 1939; Fessler and Wagner 1969).

The analysis of the shape of Pacific salmon has recently been refined from these more general

observations by the use of morphometric characteristics (Strauss and Bookstein 1982). Measurements are taken over the whole body between relevant morphological features and this allows a statistical description of body shape over the whole fish (Winans 1984).

This procedure has been used to discriminate between stocks of chinook salmon *O. tshawytscha* (Winans 1984), and between anadromous and non-anadromous forms of sockeye salmon, *O.nerka* (Taylor and Foote 1991). It has also been used to describe shape changes at smolting in coho salmon *O. kisutch* (Winans and Nishioka 1987), as well as to define the differing shapes of downstream migrant and resident Arctic charr *Salvelinus alpinus* (Damsgård 1991).

Body shape and size are not the only morphological difference seen between modal groups of juvenile Atlantic salmon - the relative levels of body constituents also differ. The difference in growth rates between the modal groups (resulting from differences in feeding behaviour) is also reflected in the relative amount of total body lipid in each modal group, which has been shown to peak in September and then decline through to May (Higgins and Talbot 1985). Not only do UMG fish maintain a higher level of body lipid than LMG fish throughout the whole year, but the rate of decline in body lipid is less in UMG fish than in LMG fish (chapter 5).

Total body lipid can be divided into two main lipid stores: that stored in the muscle and that stored in

and around the mesenteric tissue. This mesenteric lipid is the more labile store in which lipid is preferentially deposited during feeding and lost during periods of starvation (Jezierska *et al.* 1982; Miglavs and Jobling 1989). Consequently it might be expected that the seasonal differences in feeding rates seen in modal groups of juvenile Atlantic salmon would result in differing patterns of deposition and mobilization of mesenteric lipid.

The general aim of the work described in this chapter was, therefore, to investigate how aspects of body morphology varied both seasonally and between modal groups. More specifically, the aspects of morphology chosen were body shape (as described by morphometric analysis) and relative mesenteric lipid level. The following questions were addressed:

1) Does the relative shape of juvenile salmon vary throughout the period of freshwater residence ?
2) Does the relative shape of modal groups differ ?
3) What shape changes occur specifically at smolting?
4) How does the relative shape of fish in their second year of freshwater residence compare to that of fish in their first year ?
5) How does the level of mesenteric lipid vary seasonally and in relation to modal group ?

## Material and Methods

*Experimental Fish*

The fish used in this study were from the same populations as those used in the CHV experiments (chapter 3) and, therefore, were raised under identical conditions.

Prior to their introduction into the experimental raceways for CHV testing, each fish was anaesthetized with benzocaine, weighed (to 0.1g), measured (forklength, to 1 mm) and photographed with the left side uppermost and lying parallel to a ruler.

Fish were measured and photographed in this way in monthly samples from August to May after first feeding, with the omission of September. Photographs from between 6 and 12 fish from each modal group from each monthly sample were used to obtain morphometric measurements for this study. Mesenteric lipid level was also obtained from a subsets of between 8 and 17 fish from each monthly sample.

*Measurements*

Condition factor was calculated using the formula:

$$CF=W/L^3$$

where CF indicates condition factor, W is weight (g) and L is length (cm).

Consideration was given to using Ricker's formula for condition factor (Bolger and Conolly 1989):

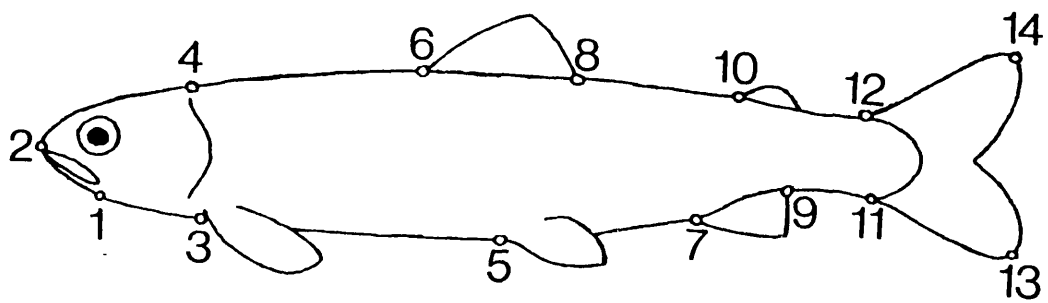
$$CF=W/L^b$$

where b is the slope of the regression of $\log_{10}$ weight on $\log_{10}$ forklength. However, it was found that due to the small size range of the 1+LMG fish over the sampling period the slope of the regression line was relatively shallow. This resulted in the apparent condition factor of $1^{+}$LMG fish being much smaller than those of the other two groups of $0^{+}$ fish - which on examination of the fish was obviously not the case.

Mean condition factors were calculated for each modal group from the fish used in the CHV experiments in chapter 3, subsets of which were used for morphological and mesenteric lipid analysis as described above.

Winans (1984) described a series of truss measurements based on morphological characters of salmonids (fig. 6.1). They then used principal component analysis (PCA) to combine all these measurements into a series of groups (principal components), each of which described a portion of the total variation in body shape. This allowed a numerical description of body shape without the inherent bias towards certain regions of the body seen in more traditional measurements. It also allows a quantitative comparison of various aspects of body shape between different groups of fish and within a group over time. It was initially this system that was used in this study. Measurements (to 0.1mm) were taken from each photograph using vernier callipers. These measurements were then adjusted to the actual lengths of the fish using the ruler contained in the photograph.

Figure 6.1. Diagramatic representation of the location of 14 points and associated morphometric distances for truss network data in a juvenile salmon. These result in the body of the salmon being divided into 6 regions: head, anterior trunk, mid trunk, posterior trunk, post anal, and caudal fin. The different morphometric measurements represent the following features: jaw length (1-2); post-maxilla head length (1-3); ventral head length (2-3); ventral-dorsal head diagonal (1-4); dorsal cranium length (2-4); opercular depth (3-4); ventral anterior trunk length (3-5); dorsal-ventral anterior trunk diagonal (4-5); ventral-dorsal anterior trunk diagonal (3-6); dorsal anterior trunk length (4-6); mid trunk depth (5-6); ventral mid trunk length (5-7); dorsal-ventral mid trunk diagonal (6-7); ventral-dorsal mid trunk diagonal (5-8); length of dorsal fin attachment (6-8); posterior trunk depth (7-8); length of anal fin attachment (7-9); dorsal-ventral posterior trunk diagonal (8-9); ventral-dorsal posterior trunk diagonal (7-10); dorsal posterior trunk length (8-10); post anal depth (9-10); ventral post anal length (9-11); dorsal-ventral post anal diagonal (10-11); ventral-dorsal post anal diagonal (9-12); dorsal post anal length (10-12); caudal fin attachment depth (11-12); ventral caudal fin length (11-13); dorsal-ventral caudal fin diagonal (12-13); ventral-dorsal caudal fin diagonal (11-14); dorsal caudal fin length (12-14); caudal fin depth (13-14).


1
2
3
4
5
6
7
8
9
10
11
12
13
14

In order to eliminate the influence of actual body size on the data each measurement was standardised for length using a variation of Ricker's formula:

$$X'=X/L^b$$

where X' is the standardised truss measurement, X is the actual truss measurement in question, L is the forklength and b is the slope of $\log_{10}(X)$ on $\log_{10}$(forklength) for all modal groups combined.

The mesenteric tissue of each fish used in the CHV experiments was dissected out and dried to a constant weight in an oven. The lipid in this tissue was then extracted using boiling chloroform in a Soxhlet apparatus (Schifferli 1976); the tissue was again dried to a constant weight and the amount of lipid calculated as the difference between the two dry weights. It was then expressed as a percentage of the whole body wet weight.

## Results

### Length and Weight

Over the period of sampling (13/8/90 - 29/5/91) the forklengths (mm) and weights (g) of $0^+$UMG, $0^+$LMG and $1^+$LMG fish varied as shown in figures 6.2 and 6.3 respectively.

$0^+$UMG fish showed a general increase in forklength and weight over the sampling period except, paradoxically, between October and November - the period when Metcalfe *et al.* (1988) demonstrated that these fish showed a brief spurt in growth.

Figure 6.2. Mean forklengths (mm) of $0^{+}$UMG (—○—), $0^{+}$LMG (– –●– –) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

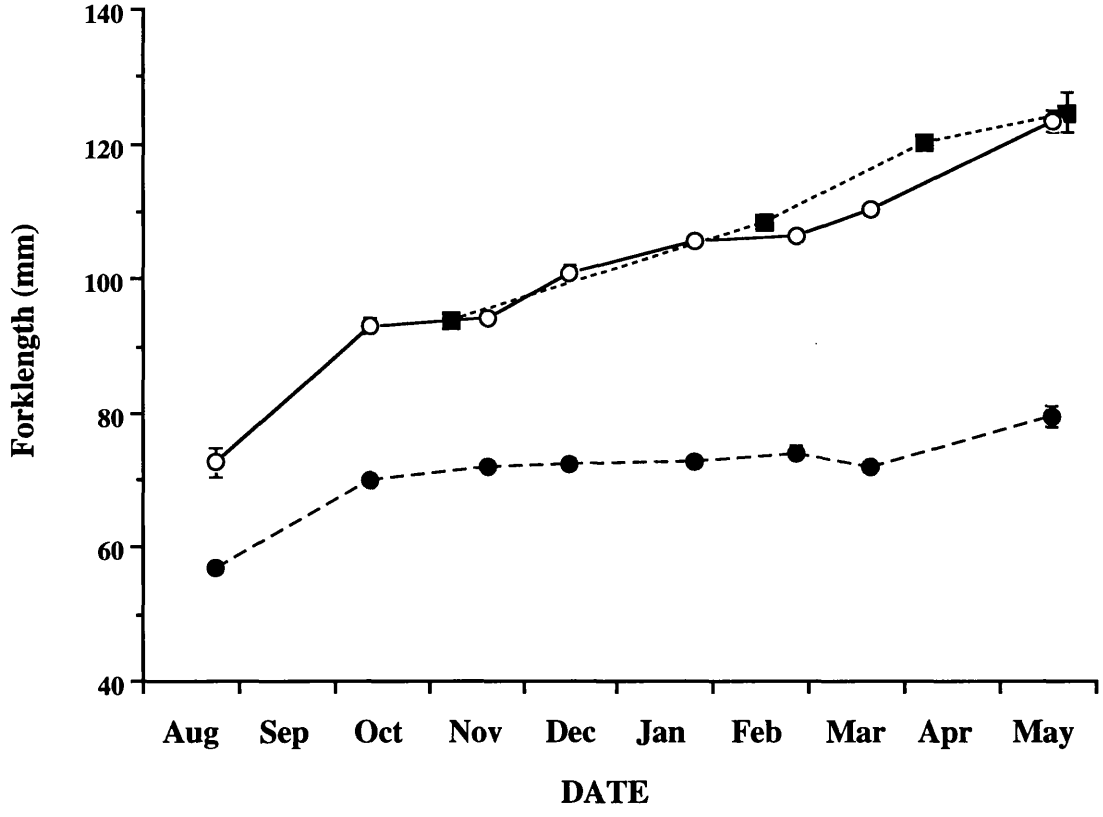
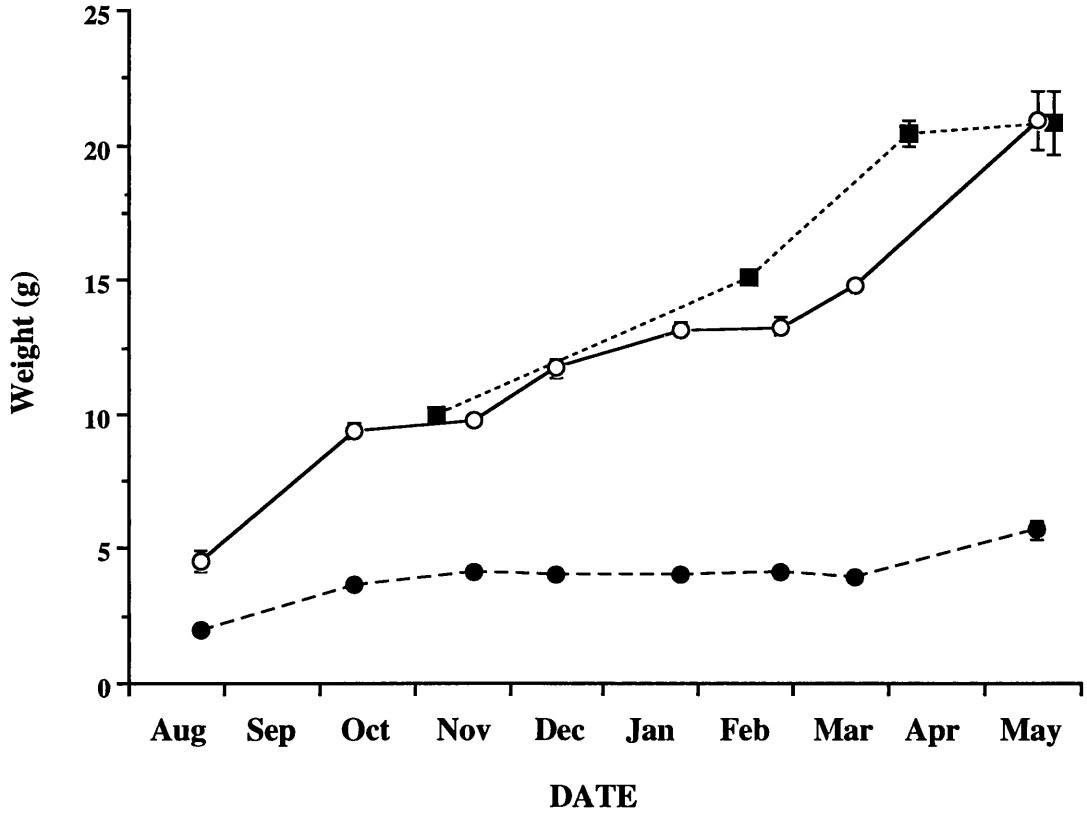

140
120
100
80
60
40
Forklength (mm)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.3. Mean wet weights (g) of $0^{+}$UMG (—○—), $0^{+}$LMG (– –●– –) and $1^{+}$LMG (·---■---·) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


25
20
15
10
5
0
Weight (g)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

The $1^{+}$LMG fish used in this study had longer forklengths and were heavier than $0^{+}$UMG fish, which in turn were longer than $0^{+}$LMG fish (figs. 6.2 and 6.3). However, it should be noted that these fish were those used in the CHV experiments described in chapter 3 and so to some extent had been selected as representative of each modal group in each sampling period, hence the relatively small error bars in figures 6.2 and 6.3. But forklengths from the entire population of fish used in this study demonstrated a divergence throughout the year due to the differing life history strategies and resulted in the development of a bimodal size distribution (shown in figure 6.4).

However, when both $0^{+}$UMG and $1^{+}$LMG fish were exhibiting external signs of smolting (i.e. in May) there was no difference between the forklengths of these fish ($U=25.5$, n.s., Mann-Whitney U test).

Condition Factors

$0^{+}$UMG, $0^{+}$LMG and $1^{+}$LMG fish all showed significant changes in condition factor over the sampling period ($H=52.76$, $P<0.0001$; $H=21.00$, $P<0.005$; $H=33.70$, $P<0.0001$ for $0^{+}$UMG, $0^{+}$LMG and $1^{+}$LMG fish respectively) (fig. 6.5).

Both $0^{+}$UMG and $1^{+}$LMG fish tend to show a decrease in condition factors over the sampling period (i.e. towards the period of smolting), whereas $0^{+}$LMG fish show an increase in condition factor through spring. Over most of the year $1^{+}$LMG fish were heavier for a

Figure 6.4. Development of bimodal size distribution of $0^+$ juvenile salmon over the sampling period.

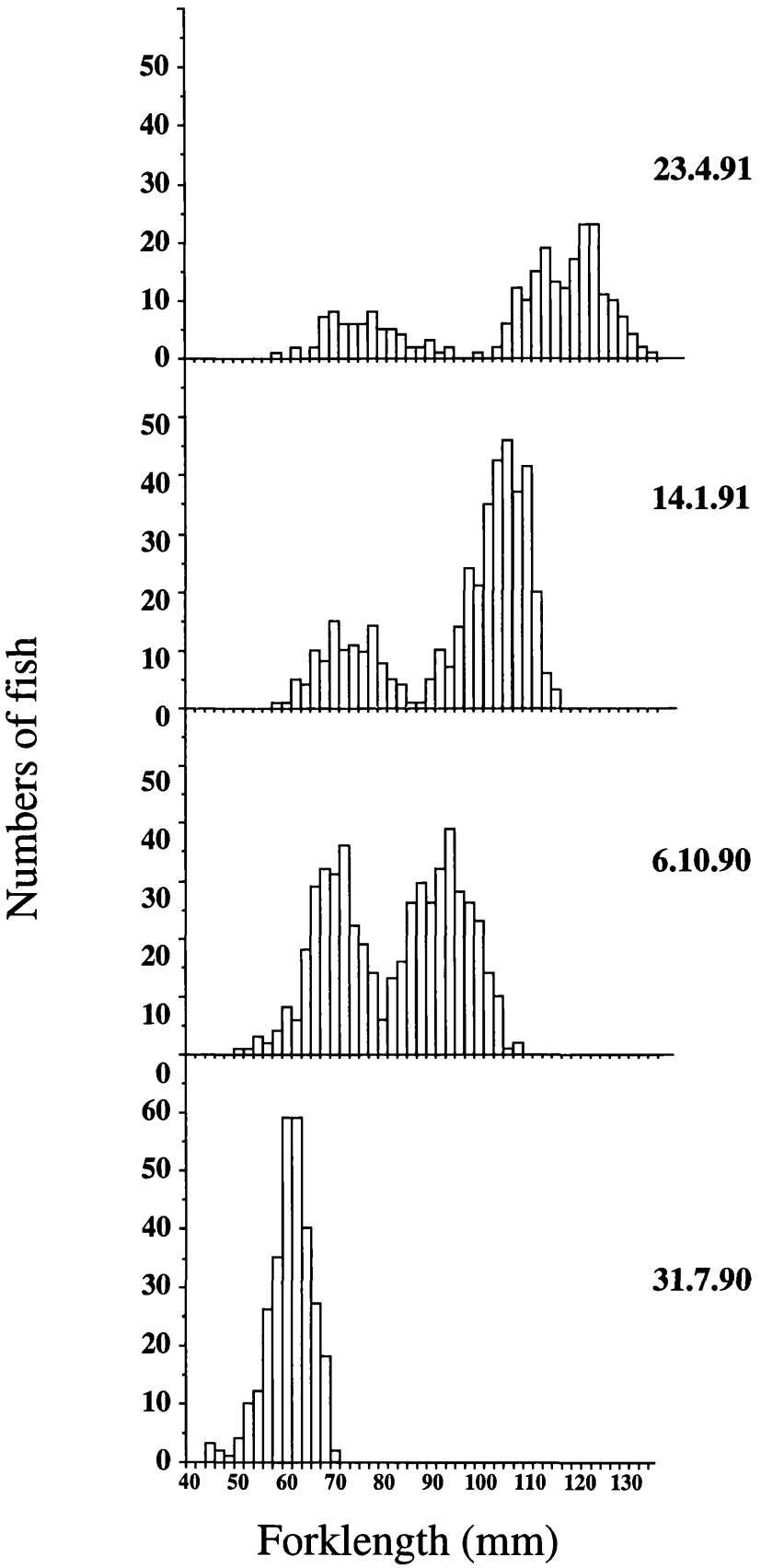
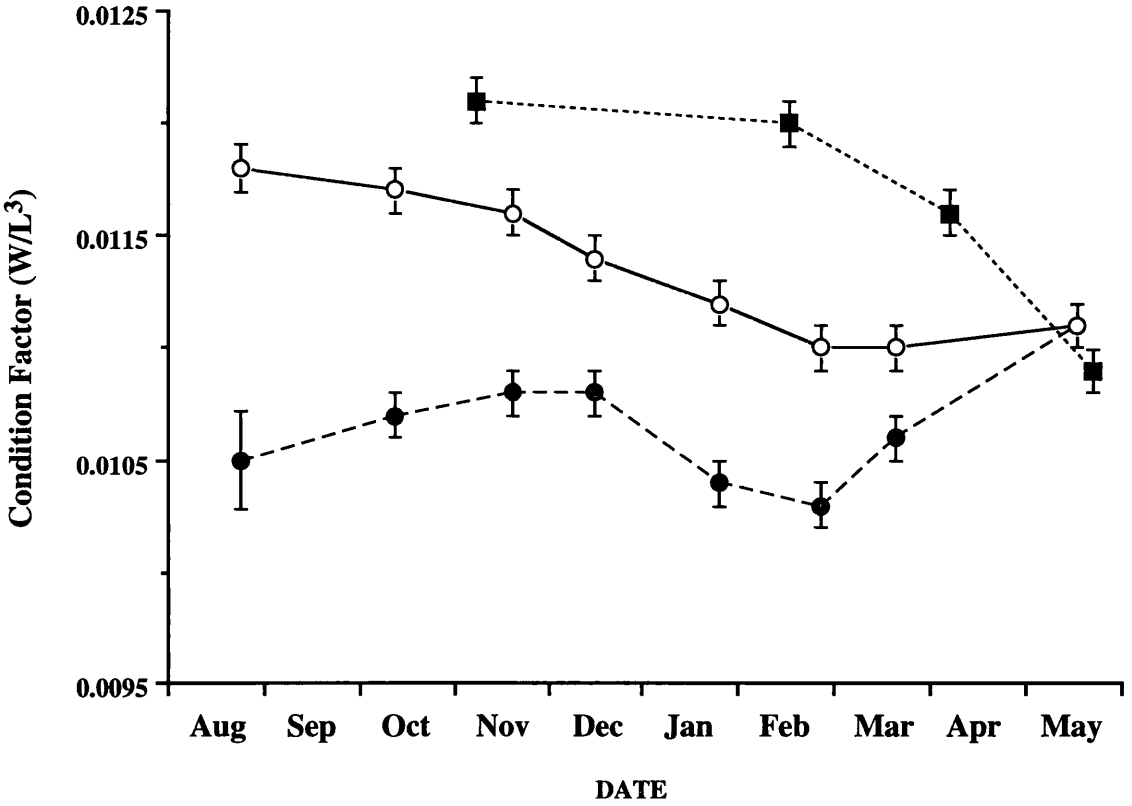

Numbers of fish
23.4.91
14.1.91
6.10.90
31.7.90
0
10
20
30
40
50
60
40 50 60 70 80 90 100 110 120 130
Forklength (mm)

Figure 6.5. Mean condition factor ($W/L^3$) of $0^+$UMG (—○—), $0^+$LMG (– –●– – ) and $1^+$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


0.0125
0.0115
0.0105
0.0095
Condition Factor (W/L3)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

given size than were $0^{+}$UMG fish which in turn were in better condition than $0^{+}$LMG fish ($P<0.0001$ in all cases). However, by the period of smolting in $0^{+}$UMG and $1^{+}$LMG fish, all fish had similar condition factors (fig. 6.5).

Morphometrics

The percentage of the variation in truss measurements (standardised for fish length) explained by each of the first seven principal components is given in Table 1. These principal components were chosen for analysis since they each explained more than 5 per cent of the variation, and cumulatively they accounted for 72.5 per cent of the total variation.

The relatively small amount of variation accounted for by the first principal component, and the relatively large spread of variation accounted for throughout the principal components compared with other similar studies is due to the overall effect of fish size having already been controlled for in the truss measurements; PC1 is, therefore, not just a measurement of absolute fish size as in previous studies (Swain and Holtby 1984; Winans 1984; Winans and Nishioka 1987).

The scores for each PC of each modal group are given in Tables 2-8 in tandem with the respective diagrams (figs. 6.6 - 6.12) of the truss measurements that loaded strongly (>0.2; either positively or negatively) in each PC. Tables 2-8 also indicate whether there was a difference in the scores for each PC over the

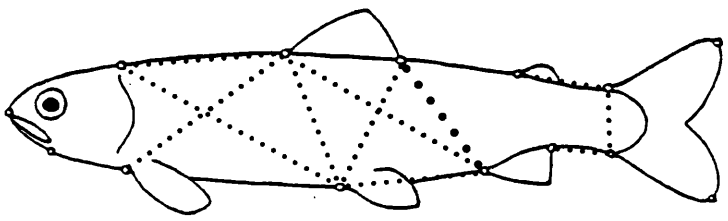
**Table 1.**

Percentage variation in standardised truss measurements explained by each of the first 7 principal components.

| Principal Component | % variation explained |
|---|---|
| 1 | 20.5 |
| 2 | 13.6 |
| 3 | 10.6 |
| 4 | 9.9 |
| 5 | 6.3 |
| 6 | 6.2 |
| 7 | 5.5 |

Figure 6.6. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 1.

Positive Loadings



..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

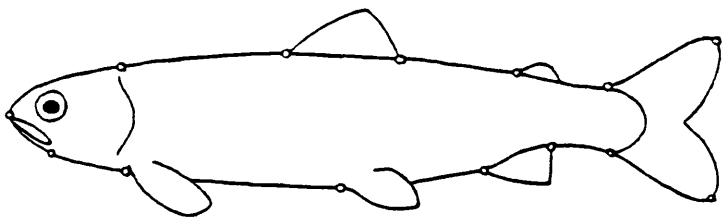


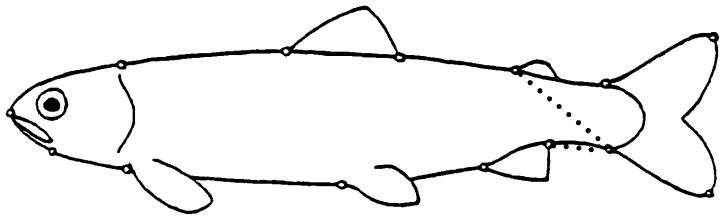
Table 2

Scores for PC 1 in each modal group of juvenile salmon over the period of sampling. The strong loadings (>0.2) desribed variation in some lengths, depths and diagonals of the anterior and mid trunk, as well as some variation of length and depth in the post anal region. All of these loaded positively and thus all varied in the same direction. Less negative values, therefore, indicated longer and deeper anterior- and mid-trunks, and post anal regions.

| Series | | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|---|
| 24.8.90 | | 6.32 | 4.53 | |
| 12.10.90 | | 1.33 | 0.12 | |
| 7.11.90 | | | | 0.86 |
| 19.11.90 | | -0.43 | -1.07 | |
| 15.12.90 | | -0.61 | -2.48 | |
| 24.1.91 | | -1.12 | -2.86 | |
| 15.2.91 | | | | -0.54 |
| 25.2.91 | | -0.93 | -0.11 | |
| 21.3.91 | | -1.41 | -2.16 | |
| 7.4.91 | | | | -0.21 |
| 18.5.91 | | 0.48 | 0.58 | |
| 23.5.91 | | | | -0.27 |
| Effect of date (Kruskal Wallis) | | H=37.61 P<0.00005 | H=39.68 P<0.00005 | H=1.68 P=0.64 |
| Modal effect (2-way ANOVA) | $0^+$UMG: | | $F_{1,109}$=12.873 P=0.0005 | $F_{1,67}$=2.975 P=0.089 |
| | $0^+$LMG: | | | $F_{1,66}$=6.989 P=0.01 |

Figure 6.7. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 2.

Positive Loadings



..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

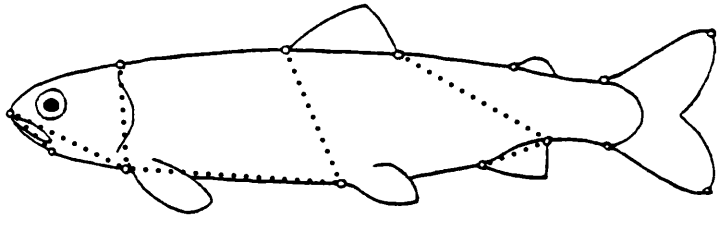


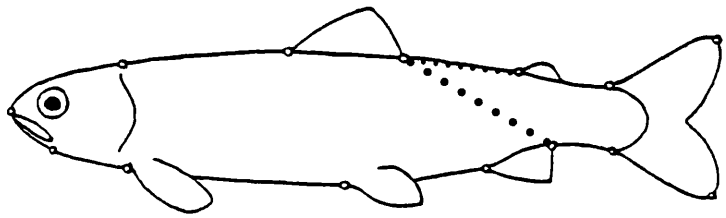
**Table 3**

Scores for PC 2 in each modal group of juvenile salmon over the period of sampling. This PC has components describing variation in length, depth and diagonals of the head and posterior trunk, and length and depth of the anterior trunk - all of which load negatively. Positive loadings are concerned with the length and depth of the post anal region.

| Series | | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|---|
| 24.8.90 | | 0.62 | 4.23 | |
| 12.10.90 | | -0.35 | 0.27 | |
| 7.11.90 | | | | 0.39 |
| 19.11.90 | | 0.21 | -0.36 | |
| 15.12.90 | | -1.03 | -0.15 | |
| 24.1.91 | | -0.43 | -1.18 | |
| 15.2.91 | | | | -1.11 |
| 25.2.91 | | 0.59 | 2.22 | |
| 21.3.91 | | -0.17 | 0.92 | |
| 7.4.91 | | | | 0.32 |
| 18.5.91 | | -0.28 | -1.57 | |
| 23.5.91 | | | | 1.04 |
| Effect of date (Kruskal Wallis) | | H=11.06 P=0.14 | H=41.13 P<0.000001 | H=23.0 P<0.00005 |
| Modal effect (2-way ANOVA) | $0^+$UMG: | | $F_{1,109}=2.198$ P=0.141 | $F_{1,67}=0.956$ P=0.342 |
| | $0^+$LMG: | | | $F_{1,66}=6.989$ P=0.01 |

Figure 6.8. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 3.

Positive Loadings



..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

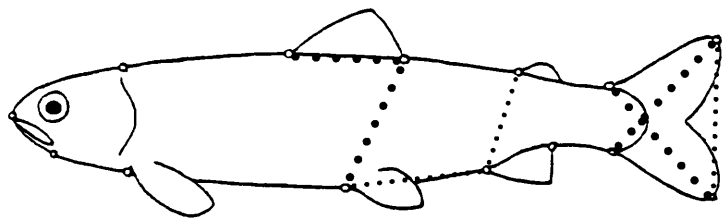


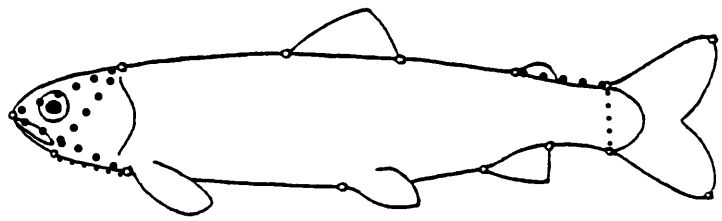
**Table 4**

Scores for PC 3 in each modal group of juvenile salmon over the period of sampling. This loads negatively for length and diagonal measures of the mid- and posterior-trunk, and depth and a diagonal of the posterior trunk. The highest loadings of 0.3-0.39 in the mid trunk length and diagonal measurements, and in the posterior trunk diagonal are negative and positive respectively indicating that these are negatively correlated.
UMG and LMG fish become significantly different in this aspect of body shape over autumn and winter but are similar again at smolting.

| Series | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|
| 24.8.90 | -0.69 | -1.18 | |
| 12.10.90 | -0.92 | 0.09 | |
| 7.11.90 | | | 1.37 |
| 19.11.90 | -1.48 | 0.88 | |
| 15.12.90 | -0.97 | 0.62 | |
| 24.1.91 | -1.27 | 0.84 | |
| 15.2.91 | | | 1.01 |
| 25.2.91 | -1.57 | 1.12 | |
| 21.3.91 | -1.89 | 0.73 | |
| 7.4.91 | | | 1.99 |
| 18.5.91 | -0.65 | -0.12 | |
| 23.5.91 | | | 0.52 |
| Effect of date (Kruskal Wallis) | H=7.42 P=0.39 | H=10.59 P=0.16 | H=5.55 P=0.14 |
| Modal effect (2-way ANOVA) $0^+$UMG: | | $F_{1,109}=29.244$ P<0.00001 | $F_{1,67}=67.578$ P<0.00001 |
| $0^+$LMG: | | | $F_{1,66}=2.835$ P=0.097 |

Figure 6.9. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 4.

Positive Loadings



..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

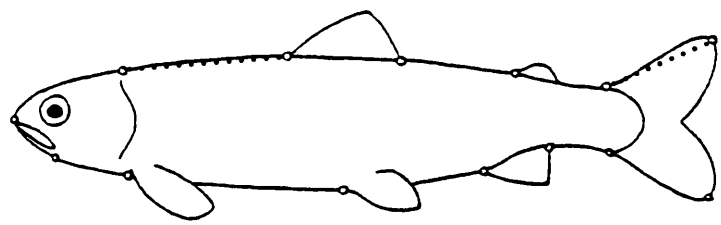


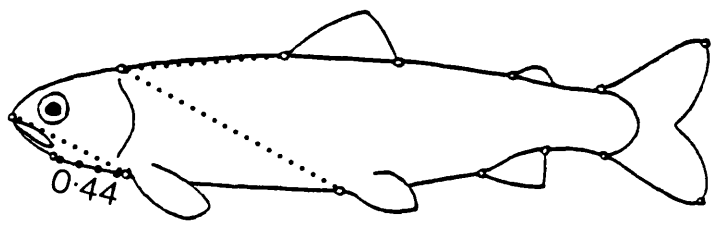
Table 5

The scores for PC 4 in each modal group of juvenile salmon over the period of sampling. PC 4 has negative loadings for anterior trunk length and upper caudal fin length, and very strong positive loadings for the head and post-anal regions.

| Series | | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|---|
| 24.8.90 | | 1.21 | 0.29 | |
| 12.10.90 | | -1.57 | 0.60 | |
| 7.11.90 | | | | -1.39 |
| 19.11.90 | | -2.39 | -0.37 | |
| 15.12.90 | | -0.07 | 0.45 | |
| 24.1.91 | | 0.15 | 1.90 | |
| 15.2.91 | | | | 0.21 |
| 25.2.91 | | -0.07 | -0.66 | |
| 21.3.91 | | -0.29 | 0.10 | |
| 7.4.91 | | | | 0.91 |
| 18.5.91 | | -1.08 | 0.26 | |
| 23.5.91 | | | | 1.00 |
| Effect of date (Kruskal Wallis) | | H=23.91 P<0.005 | H=10.18 P=0.17 | H=14.03 P<0.005 |
| Modal effect (2-way ANOVA) | UMG: | | $F_{1,109}$=15.904 P=0.0001 | $F_{1,67}$=22.384 P<0.0001 |
| | LMG: | | | $F_{1,66}$=0.966 P=0.334 |

Figure 6.10. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 5.

Positive Loadings




..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

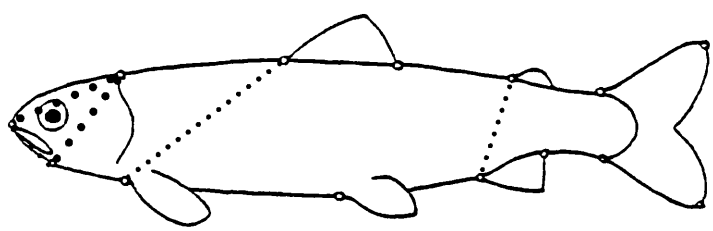


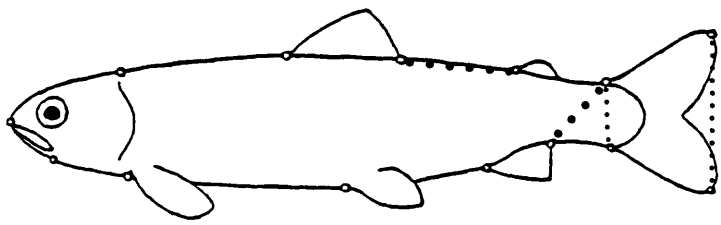
Table 6

The scores for PC 5 in each modal group of juvenile salmon over the period of sampling. Negative loadings represent variation in head length and diagonal, plus anterior and posterior trunk diagonals. Positive loadings show variation in anterior trunk length and diagonal, and head length as well as a very strong loading of the post-maxilla head length (1-3).

| Series | | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|---|
| 24.8.90 | | -0.44 | -0.58 | |
| 12.10.90 | | -0.93 | 0.14 | |
| 7.11.90 | | | | -0.29 |
| 19.11.90 | | -1.12 | -0.33 | |
| 15.12.90 | | -0.45 | 0.05 | |
| 24.1.91 | | -0.23 | -0.46 | |
| 15.2.91 | | | | -0.64 |
| 25.2.91 | | 0.31 | 1.00 | |
| 21.3.91 | | 1.38 | 1.12 | |
| 7.4.91 | | | | 0.61 |
| 18.5.91 | | 0.67 | 0.23 | |
| 23.5.91 | | | | 0.35 |
| Effect of date (Kruskal Wallis) | | H=25.91 P<0.001 | H=12.66 P=0.081 | H=5.59 P=0.13 |
| Modal effect (2-way ANOVA) | UMG: | | $F_{1,109}$=1.193 P=0.28 | $F_{1,67}$=0.466 P=0.13 |
| | LMG: | | | $F_{1,66}$=1.848 P=0.18 |

Figure 6.11. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 6.

Positive Loadings



..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

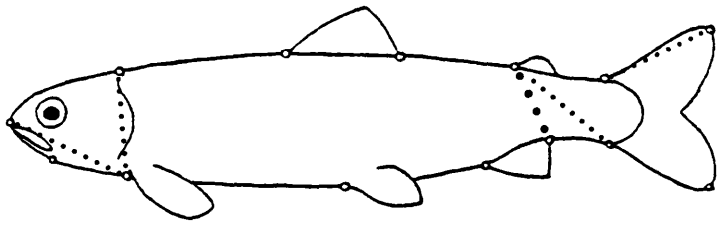


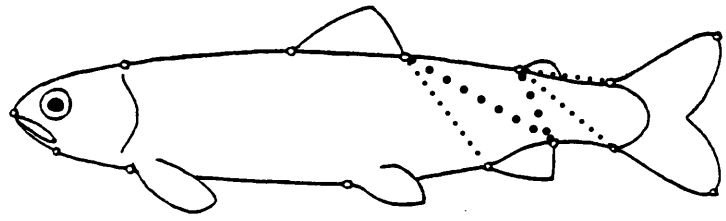
Table 7

Scores for PC 6 in each modal group of juvenile salmon over the period of sampling. PC6 loaded negatively for measures of head length and depth, post-anal depth (very strongly) and a diagonal, and for upper caudal tail length. Positive loadings concern posterior trunk length (very strongly), post anal diagonal (very strongly) and depth, and caudal tail depth.

| Series | | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|---|
| 24.8.90 | | 0.02 | -0.31 | |
| 12.10.90 | | 0.31 | 0.20 | |
| 7.11.90 | | | | -2.44 |
| 19.11.90 | | 0.72 | -0.14 | |
| 15.12.90 | | 1.23 | -0.57 | |
| 24.1.91 | | 1.10 | 0.74 | |
| 15.2.91 | | | | -0.35 |
| 25.2.91 | | -0.88 | -1.44 | |
| 21.3.91 | | 0.21 | -0.01 | |
| 7.4.91 | | | | -0.01 |
| 18.5.91 | | 0.91 | 0.99 | |
| 23.5.91 | | | | -0.14 |
| Effect of date (Kruskal Wallis) | | H=12.61 P=0.082 | H=15.37 P=0.031 | H=21.34 P<0.0001 |
| Modal effect (2-way ANOVA) | $0^+$UMG: | | $F_{1,109}$=6.069 P=0.015 | $F_{1,67}$=31.548 P<0.0001 |
| | $0^+$LMG: | | | $F_{1,66}$=10.579 P<0.002 |

Figure 6.12. Diagrams of the truss measurements that loaded strongly (> 0.2, both positively and negatively) in PC 7.

Positive Loadings



..... 0·2 – 0·29

••••••• 0·3 – 0·39

Negative Loadings

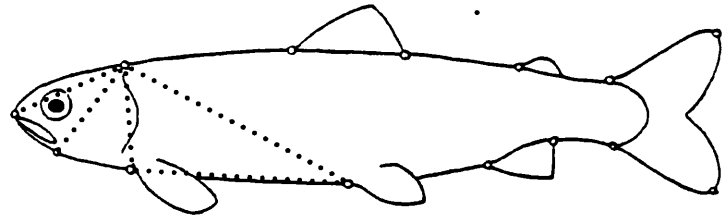


Table 8

The scores for PC 7 in each modal group of juvenile salmon over the period of sampling. PC7 tended to be split positively and negatively into the anterior and posterior part of the fish and the difference between $0^+$UMG and $0^+$LMG fish at smolting would seem to suggest that the migrating fish had relatively longer anterior regions of their bodies whereas non-migrating fish had relatively longer posterior regions of their bodies. Positive loadings were for head length, depth and a diagonal, and anterior trunk length and the dorsal-ventral diagonal. Negative loadings were for posterior trunk depth and dorsal-ventral diagonal (very strongly), post anal depth (very strongly), length and dorsal-ventral diagonal.

| Series | | $0^+$UMG | $0^+$LMG | $1^+$LMG |
|---|---|---|---|---|
| 24.8.90 | | 0.75 | -0.59 | |
| 12.10.90 | | 0.91 | 0.37 | |
| 7.11.90 | | | | -0.20 |
| 19.11.90 | | -0.49 | -0.02 | |
| 15.12.90 | | 0.59 | 1.23 | |
| 24.1.91 | | 0.69 | 0.45 | |
| 15.2.91 | | | | -1.23 |
| 25.2.91 | | 0.53 | 1.62 | |
| 21.3.91 | | 0.45 | -0.99 | |
| 7.4.91 | | | | -1.16 |
| 18.5.91 | | 0.63 | -1.18 | |
| 23.5.91 | | | | -0.45 |
| Effect of date (Kruskal Wallis) | | H=7.44 P=0.38 | H=21.48 P<0.005 | H=13.26 P<0.005 |
| Modal effect (2-way ANOVA) | $0^+$UMG: | | $F_{1,109}=5.26$ P=0.024 | $F_{1,67}=20.13$ P<0.00001 |
| | $0^+$LMG: | | | $F_{1,66}=2.684$ P=0.11 |

sampling period (13/8/90 - 29/5/91) between each of the modal groups.

The PCA revealed marked changes in body shape both over time and between the different categories of fish. However, due to the intrinsic complexity of each PC value these can only give an overall picture of change in body shape. Figures 6.6 - 6.12 show that only in PC1 did the truss measurements all load positively, all the other PC values were made up from truss measurements that loaded both negatively and positively. Hence the seasonal pattern of PCs 2 - 7 were each a combination of changes in size that were in opposition and, therefore, any given PC value may have been the result of a negative loading for a measurement from one area of the body, and a positive loading from another. In this situation it is impossible to relate changes in the value of a PC directly with specific changes in body shape without knowing how each actual truss measurement has changed. So in order to examine changes in body shape at a finer level, the PC values were only used to identify those aspects of body morphology which showed greatest variation, and then the standardised measurements themselves were compared.

Standardised measurements

For each truss measurement that had a loading indicating a relatively high level of variation (i.e. >0.2) Mann-Whitney U-tests were used to compare the actual standardised measurements for the whole sampling

period between each of the three modal groups (Table 9).

This resulted in 87 Mann-Whitney U-tests in order to make all the comparisons between $0^{+}$UMG, $0^{+}$LMG and $1^{+}$LMG fish, so the significance level was set at $P<0.01$. It was mainly the probability values of these results that were used to decide on the measurements that were analysed further, although two measures (mid trunk depth 5-6 and posterior trunk depth 7-8) were included since previous studies had suggested that differences in feeding behaviour might cause these measurements to vary between modal groups (Currens *et al.* 1989).

The resulting measurements were from all areas of the body (fig. 6.13: diagram of salmon showing which truss measures were used). Kruskal-Wallis 1-way analyses of variance were then used to examine the effect of time of year on each modal group individually (since the variances differed between the data for different sampling periods thus precluding the use of parametric one-way analyses of variances). Kruskal-Wallis 1-way ANOVAs were also used to examine any effect of modal group during May - the period of visible "smolting" and seaward migration for $0^{+}$UMG and $1^{+}$LMG fish. For both these sets of tests significance levels were set at $P<0.01$. Two-way ANOVAs were used to detect the combined effects of time of year and modal group of each measurement. Due to the large number of analyses that this involved (57) the significance level was set at $P<0.005$, which precluded "significant" results (at

**Table 9.** Intermodal differences between the standardised measurements that load strongly (>0.2) in PCA. U=$0^{+}$UMG, L=$0^{+}$LMG, S2=$1^{+}$LMG.

| Measurement | Z value | P value | |
|---|---|---|---|
| 1-2 | 3.25 | 0.001 | L>U |
| | 5.20 | $2\times10^{-7}$ | S2>U |
| | 1.81 | 0.07 | |
| 1-3 | 0.28 | 0.78 | |
| | 1.94 | 0.05 | |
| | 1.30 | 0.19 | |
| 2-3 | 4.34 | $2\times10^{-5}$ | L>U |
| | 5.58 | $2\times10^{-8}$ | S2>U |
| | 2.84 | 0.005 | S2>L |
| 1-4 | 2.15 | 0.03 | |
| | 3.83 | 0.0001 | S2>U |
| | 1.78 | 0.08 | |
| 2-4 | 2.55 | 0.01 | L>U |
| | 3.83 | 0.0001 | S2>U |
| | 1.83 | 0.07 | |
| 3-4 | 1.21 | 0.23 | |
| | 5.83 | $5\times10^{-9}$ | S2>U |
| | 4.65 | $3\times10^{-6}$ | S2>L |
| 3-5 | -0.24 | 0.81 | |
| | 2.08 | 0.04 | |
| | 2.08 | 0.04 | |
| 4-5 | -0.55 | 0.58 | |
| | 2.28 | 0.02 | |
| | 2.59 | 0.01 | S2>L |
| 3-6 | -3.29 | 0.001 | U>L |
| | 0.52 | 0.52 | |
| | 3.08 | 0.002 | S2>L |
| 4-6 | -1.39 | 0.16 | |
| | 0.43 | 0.67 | |
| | 1.51 | 0.13 | |
| 5-6 | -2.77 | 0.01 | U>L |
| | -0.05 | 0.96 | |
| | 2.50 | 0.01 | S2>L |
| 5-7 | -4.68 | $3\times10^{-6}$ | U>L |
| | -4.88 | $1\times10^{-6}$ | U>S2 |
| | 0.57 | 0.57 | |

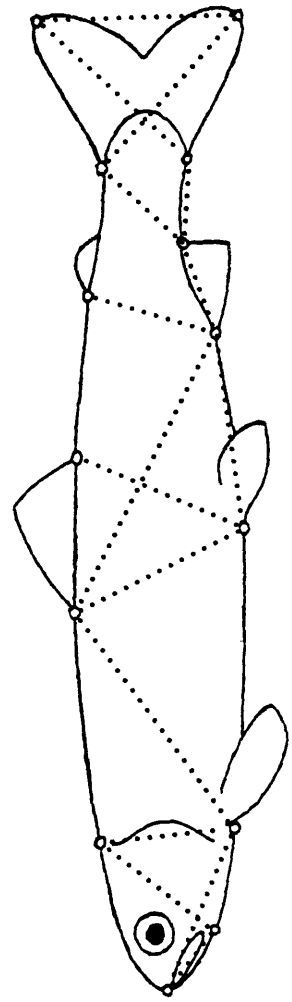
**Table 9** (contd.)

| Measurement | Z value | P value | |
|---|---|---|---|
| 6-7 | -4.47 | $1x10^{-5}$ | U>L |
| | -4.02 | 0.0001 | U>S2 |
| | 1.71 | 0.09 | |
| 5-8 | -4.58 | $5x10^{-6}$ | U>L |
| | -3.28 | 0.001 | U>S2 |
| | 1.78 | 0.08 | |
| 6-8 | -2.94 | 0.003 | U>L |
| | -4.20 | $3x10^{-5}$ | U>S2 |
| | -1.69 | 0.09 | |
| 7-8 | -2.26 | 0.02 | |
| | -0.22 | 0.82 | |
| | 2.21 | 0.03 | |
| 7-9 | 3.04 | 0.002 | L>U |
| | 1.93 | 0.05 | |
| | -1.21 | 0.23 | |
| 8-9 | 1.55 | 0.12 | |
| | 2.07 | 0.04 | |
| | -3.27 | 1.00 | |
| 8-10 | -0.91 | 0.36 | |
| | -2.36 | 0.02 | |
| | -0.92 | 0.36 | |
| 9-10 | 0.61 | 0.54 | |
| | 2.25 | 0.02 | |
| | 1.22 | 0.22 | |
| 9-11 | -0.22 | 0.82 | |
| | -3.01 | 0.003 | U>S2 |
| | -2.08 | 0.04 | |
| 10-11 | 1.51 | 0.13 | |
| | 1.04 | 0.30 | |
| | -0.64 | 0.52 | |
| 9-12 | -1.28 | 0.20 | |
| | -3.49 | 0.0005 | U>S2 |
| | -1.95 | 0.05 | |
| 10-12 | 1.03 | 0.19 | |
| | -0.56 | 0.56 | |
| | -1.59 | 0.06 | |
| 11-12 | -2.20 | 0.03 | |
| | -3.28 | 0.001 | U>S2 |
| | -0.61 | 0.55 | |
| 12-13 | -3.27 | 0.001 | U>L |
| | -4.99 | $6x10^{-7}$ | U>S2 |
| | -2.49 | 0.01 | L>S2 |

**Table 9** (contd.)

| Measurement | Z value | P value | |
|---|---|---|---|
| 11-14 | -4.73 | $2x10^{-6}$ | U>L |
| | -5.34 | $1x10^{-7}$ | U>S2 |
| | -1.94 | 0.05 | |
| 13-14 | -2.40 | 0.02 | |
| | -4.23 | $2x10^{-5}$ | U>S2 |
| | -2.69 | 0.007 | L>S2 |

Figure 6.13. Diagram of the 16 standardised measurements that were analysed further to describe changes in body shape in this study.



$P<0.05$) as a result of chance alone. With so many variables analysed the lower significance level also helped to focus attention on those areas of morphology which displayed greatest variation between sampling periods and/or modal groups.

In order to reduce further the number of variables presented here, 3 measurements (dorsal cranium length 2-4, posterior trunk depth 7-8 and ventral-dorsal post anal diagonal 9-12), which all showed no significant difference between modal groups over the whole year at the $P>0.005$ significance level) are not presented here. Figures 6.14 - 6.30 show the seasonal and modal variation in the standardised measures of truss measurements in the head, anterior, mid and posterior trunks, post-anal and tail regions.

*Head measurements* (figs 6.14 - 6.17)

The relative jaw size (1-2) of $0^{+}$UMG fish did not change throughout the sampling period, but that of the $0^{+}$LMG fish tended to increase as the fish got older ($H=20.9$, $P<0.004$), except for the drop seen in February (fig. 6.14). This resulted in the relative jaw size of LMG fish being larger than that of $0^{+}$UMG fish throughout most of the sampling period (2-way ANOVA $F_{1,109}=14.511$, $P=0.0002$) although by the time of smolting (May) this difference had disappeared ($H=5.14$ $P=0.08$).

$1^{+}$LMG fish were similar in relative jaw size to $0^{+}$LMG fish (2-way ANOVA $F_{1,66}=2.356$, $P=0.13$) and larger than

Figure 6.14. Mean values for the standardised jaw length (1-2) of $0^{+}$UMG (—○— ), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (---■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

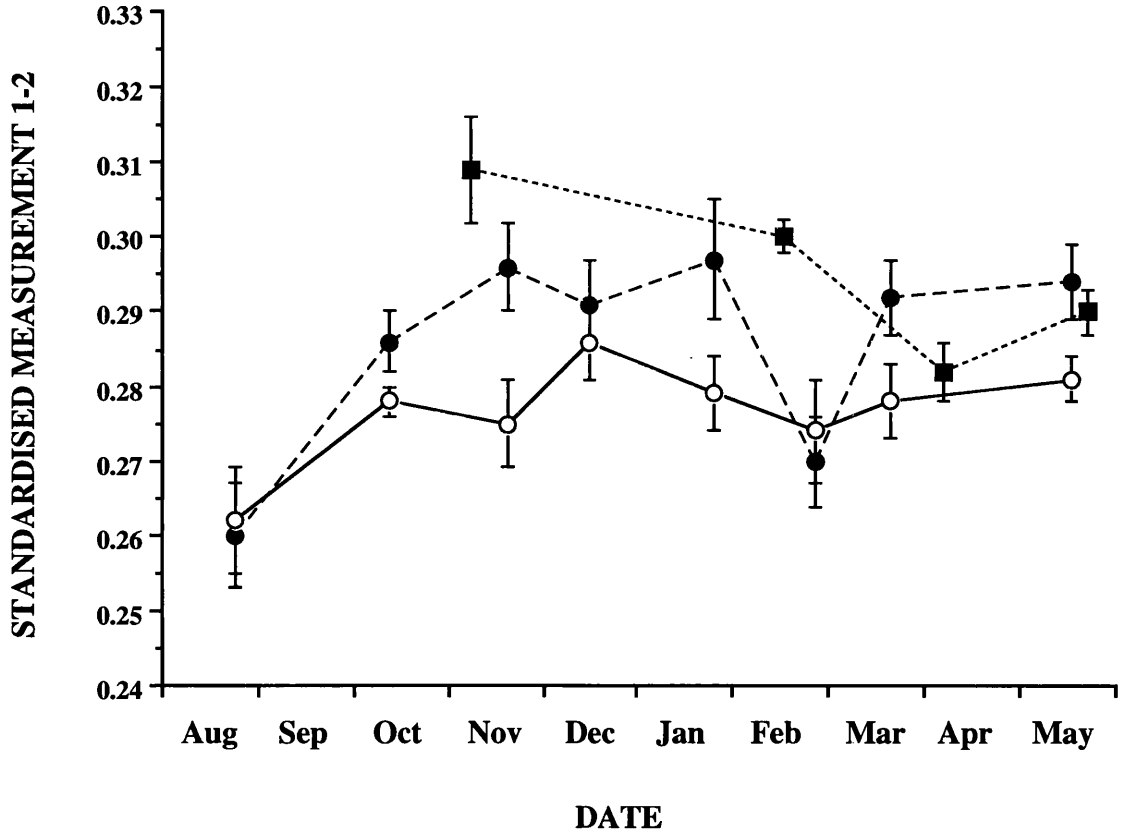

STANDARDISED MEASUREMENT 1-2
0.33
0.32
0.31
0.30
0.29
0.28
0.27
0.26
0.25
0.24
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.15. Mean values for the standardised ventral head length (2-3) of $0^{+}$UMG (—○—), $0^{+}$LMG (—●—) and $1^{+}$LMG (---■---) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

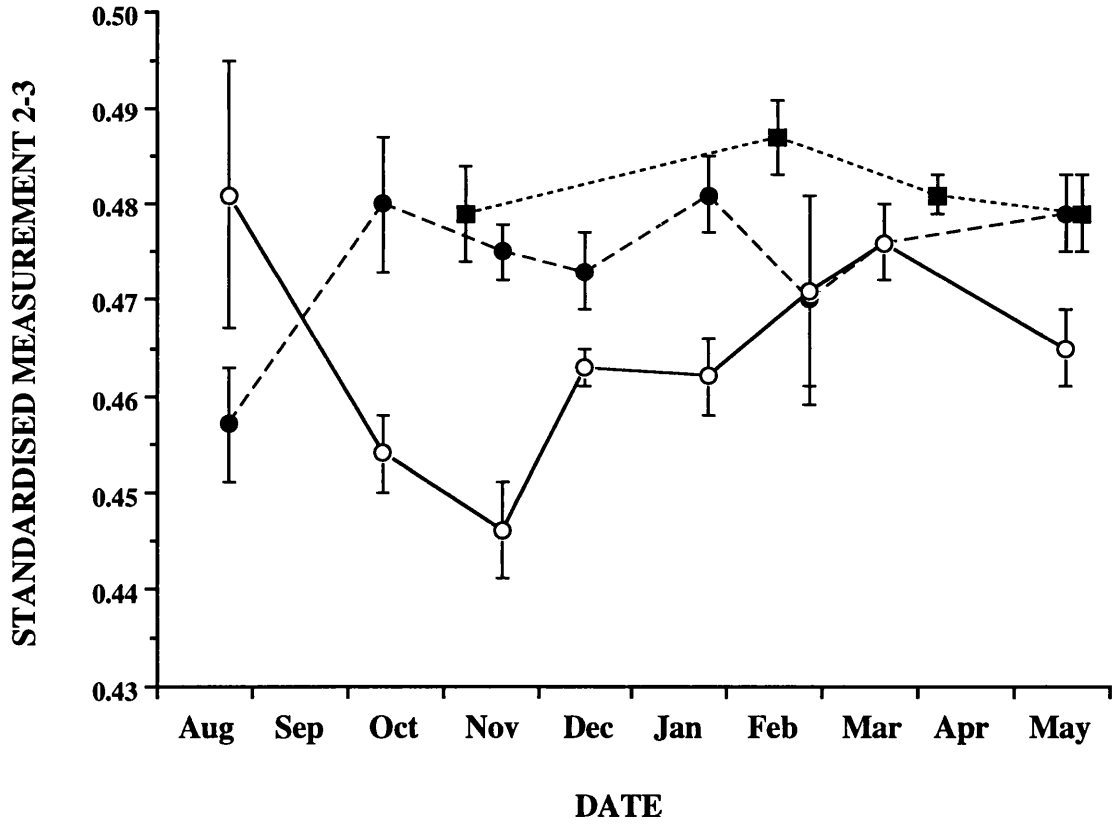

STANDARDISED MEASUREMENT 2-3
0.50
0.49
0.48
0.47
0.46
0.45
0.44
0.43
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.16. Mean values for the standardised lengths of the ventral-dorsal head diagonal (1-4) of $0^{+}$UMG (—○—), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (---■---) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

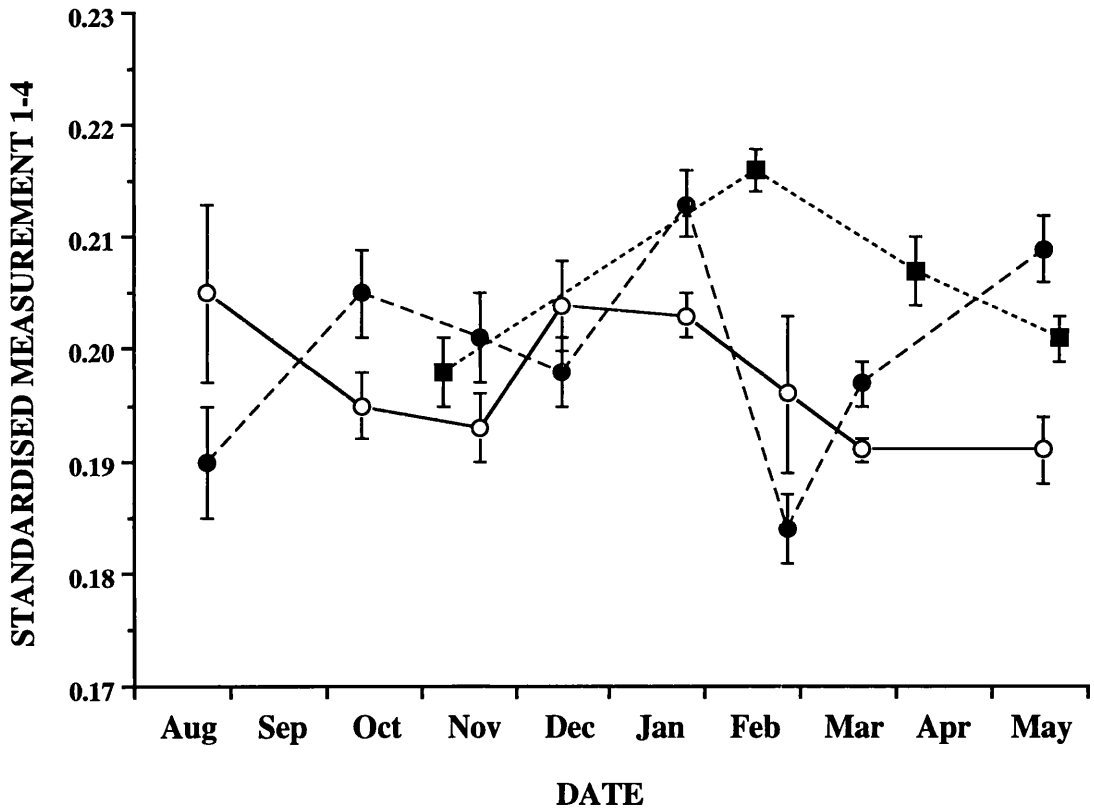
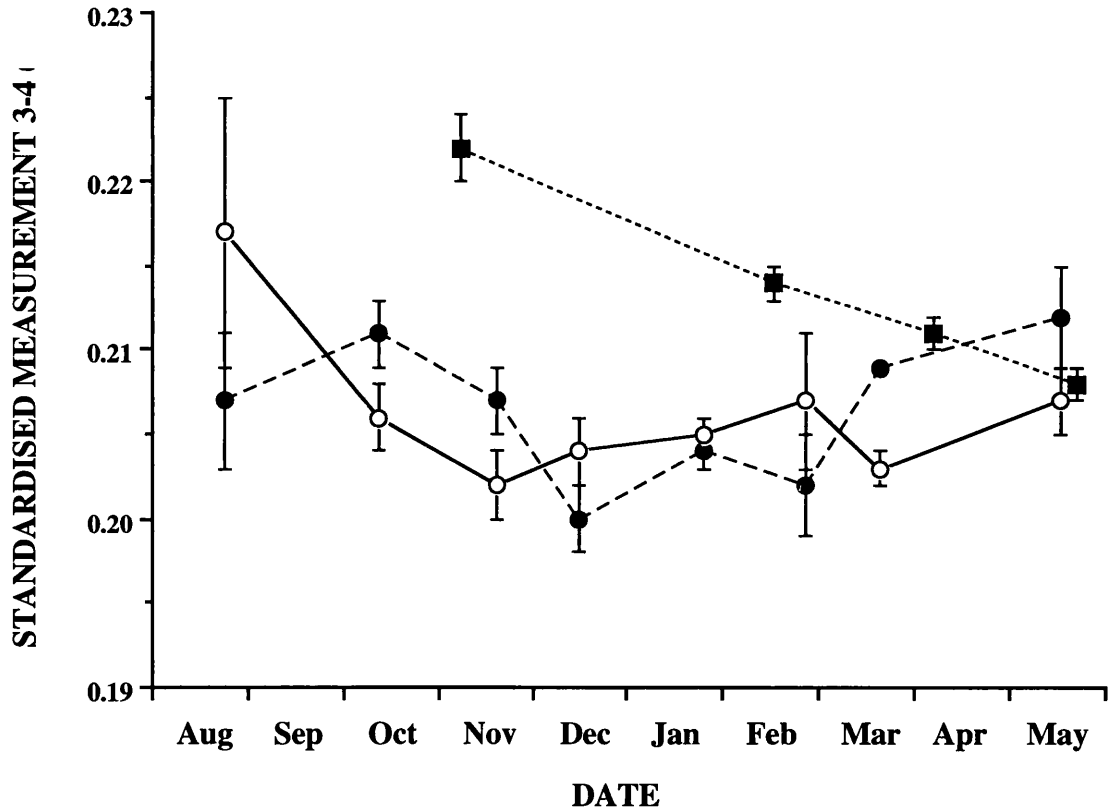

STANDARDISED MEASUREMENT 1-4
0.23
0.22
0.21
0.20
0.19
0.18
0.17
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.17. Mean values for the standardised opercular depth (3-4) of $0^{+}$UMG (—○—), $0^{+}$LMG (—●—) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


STANDARDISED MEASUREMENT 3-4
0.23
0.22
0.21
0.20
0.19
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

$0^+$UMG fish (2-way ANOVA $F_{1,67}=24.259$, $P<0.0001$). However, their relative jaw size tended to decrease throughout the sampling period ($H=13.6$, $P<0.005$), so that by smolting there was no difference in size from those of $0^+$UMG and $0^+$LMG fish.

Another measure of head length (ventral head length 2-3, fig 6.15) in $0^+$UMG fish tended to decrease from summer to winter and then to increase again through spring ($H=20.5$, $P<0.005$), but did not vary with season in $0^+$LMG or $1^+$LMG fish ($H=10.4$, $P=0.17$ and $H=1.4$, $P=0.7$ respectively). This resulted in $0^+$UMG fish having relatively shorter heads than both $0^+$LMG or $1^+$LMG fish (2-way ANOVA $F_{1,109}=13.419$, $P=0.0004$ and $F_{1,67}=29.526$, $P<0.0001$ respectively), which themselves were similar (2-way ANOVA $F_{1,66}=3.079$ $P=0.084$). This difference did not continue into smolting, due to the increase seen in standardised measures of $0^+$UMG fish resulting in no significant differences between $0^+$UMG, $0^+$LMG and $1^+$LMG fish at this time.

$0^+$UMG fish did not differ over the sampling period in either ventral-dorsal head diagonal length (1-4, fig 6.16, $H=16.1$, $P=0.024$) or opercular depth (3-4, fig 6.17, $h=8.0$, $P=0.345$).

The pattern in $0^+$LMG fish however, was more complex. The standardised 1-4 measurement in $0^+$LMG fish increased from the August sample until January then there was a sudden drop, followed by a further increase through spring (Fig. 6.16, $H=26.2$, $P<0.0005$).

Opercular depth (3-4) varied little seasonally in either $0^{+}$UMG or $0^{+}$LMG fish. There was no difference between the head depths or opercular depths of $0^{+}$UMG and $0^{+}$LMG fish over most of the year (2-way ANOVAs F1,109=4.442, P=0.037 and F1,109=0.191, P=0.668 for 1-4 and 3-4 respectively), although by smolting $0^{+}$LMG had relatively deeper heads (1-4) than $0^{+}$UMG fish (H=14.6, P<0.001) but showed no difference in opercular depths.

$1^{+}$LMG fish showed an increase in the ventral-dorsal head diagonal (1-4, fig 6.16) between November and February followed by a decrease through to May (H=16.8, P<0.001), whereas for opercular depth (3-4, fig 6.17) they showed a steady decrease throughout the sampling period (H=22.5, P<0.0001). This resulted in $1^{+}$LMG fish having significantly larger standardised measures than $0^{+}$UMG fish for both ventral-dorsal head diagonal and opercular depth (2-way ANOVAs $F_{1,67}$=34.796, P<0.0001 and $F_{1,67}$=53.585, P<0.0001) throughout most of the year.

No difference was seen between $1^{+}$LMG and $0^{+}$LMG fish in ventral-dorsal head diagonal (1-4, fig 6.16, 2-way ANOVA F1,66=6.352, P<0.014) although $1^{+}$LMG fish did have relatively deeper opercular depths (3-4) (2-way ANOVA $F_{1,66}$=21.445, P<0.0001). By smolting there was no significant difference between $1^{+}$LMG fish and either $0^{+}$UMG or $0^{+}$LMG fish in either measure of depth although $1^{+}$LMG fish again seemed to be intermediate.

In general, prior to May $0^{+}$LMG fish tended to have larger jaws and longer heads than $0^{+}$UMG fish, but show

no difference in depth of head. However, by the time the $0^{+}$UMG fish were smolting, the difference in length had disappeared and the $0^{+}$LMG fish tended to have deeper heads. The lack of difference in length between the modal groups at smolting in $0^{+}$UMG fish seems to have been due to a relative increase in growth on the part of UMG fish, whereas the difference in depth was due to a relative increase by $0^{+}$LMG fish. When the UMG fish became smolts the main difference between $0^{+}$ fish seemed to be a relative lengthening of the head.

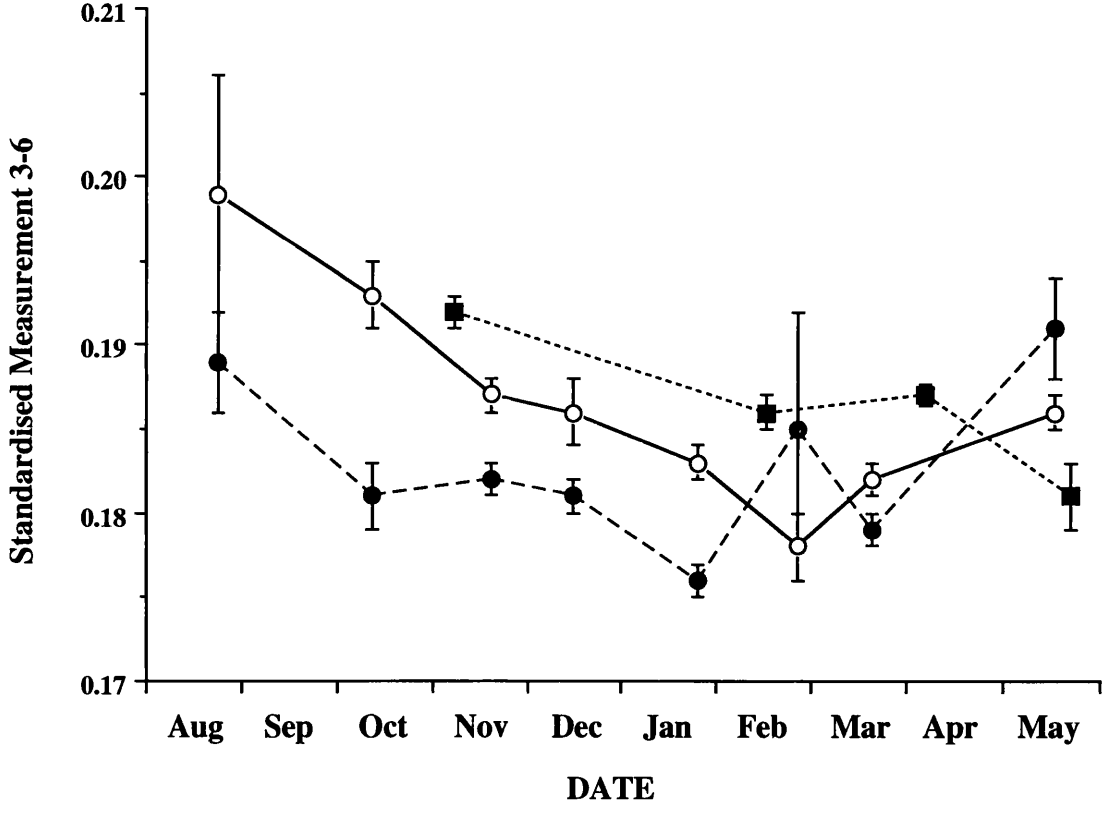
$1^{+}$LMG fish showed a similar pattern to $0^{+}$LMG fish in having longer heads and jaws than $0^{+}$UMG fish until smolting, although they also tended to have deeper heads than $0^{+}$UMG fish. At smolting these differences had disappeared suggesting that there is no difference in relative head size between $0^{+}$UMG and $1^{+}$LMG smolts.

**Trunk Measurements** (figs. 6.18 - 6.25)

*Anterior trunk measurements*

The relative ventral-dorsal anterior trunk diagonal (3-6, fig 6.18) tended to decrease in both $0^{+}$UMG and $0^{+}$LMG fish between summer and mid-winter ($H=26.9$, $P<0.0005$ and $H=23.5$, $P<0.005$ for UMG and LMG fish respectively). Over the whole period of sampling $0^{+}$UMG fish tended to have greater standardised measurements than $0^{+}$LMG fish (2-way ANOVA $F1,109=10.074$, $P=0.002$). However, when the period of smolting was analysed separately this difference was no longer evident.

Figure 6.18. Mean values for the standardised lengths of the ventral-dorsal anterior trunk diagonal (3-6) of $0^{+}$UMG (——○——), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


Standardised Measurement 3-6
0.21
0.20
0.19
0.18
0.17
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Unlike $0^+$UMG or $0^+$LMG fish which showed an increase through spring, $1^+$LMG fish tended to decrease from October to May (H=19.565, P>0.0005), but did not differ from $0^+$UMG fish (2-way ANOVA F1,67=7.507, P=0.008). Despite appearances from figure 6.18 $1^+$LMG fish also showed no difference from $0^+$LMG fish (2-way ANOVA $F_{1,66}$=2.176, P=0.145), although any effect might have been masked by the large amount of variation seen during February in $0^+$LMG fish. By May $1^+$LMG fish were not significantly different from $0^+$UMG or $0^+$LMG fish.

*Mid-trunk measurements*

The mid trunk standardised measurements (mid trunk depth 5-6, ventral mid trunk length 5-7, dorsal-ventral mid trunk diagonal 6-7, and ventral-dorsal mid trunk diagonal 5-8) showed generally similar variations in $0^+$UMG and $0^+$LMG fish throughout the sampling period. All tended to decrease from the level of the summer samples and then remained stable during the winter months (Kruskall-Wallis: H=37.4, P<0.000005; H=33.0, P<0.00005; H=22.8, P<0.002; H=32.0, P<0.00005; H=17.1, P<0.02*; H=19.5, P<0.007; H=25.9,P<0.001; and H=22.6, P<0.002 for standardised measurements for $0^+$UMG and $0^+$LMG fish of 5-6, 5-7 6-7, and 5-8 respectively). The exception was the dorsal-ventral mid trunk diagonal (6-7) in $0^+$UMG fish (*) which was above the significance level set at P<0.01. Over the whole year the standardised measurements of $0^+$UMG fish were larger than those of $0^+$LMG fish (2-way ANOVAs $F_{1,109}$=9.465,

$P<0.003$; $F_{1,109}=29.001$, $P<0.0001$; $F_{1,109}=10.881$, $P=0.0031$; $F_{1,109}=20.145$, $P<0.0001$ for measurements 5-6, 5-7, 6-7 and 5-8 respectively, figs 6.19 - 6.22). $0^+$UMG fish had relatively longer ventral mid trunk lengths and a longer dorsal-ventral mid trunk diagonal than $0^+$LMG fish in May ($H=16.5$, $P<0.0005$ and $H=13.6$, $P<0.005$ respectively).

In $1^+$LMG fish only mid trunk depth (5-6, fig 6.19) and the ventral-dorsal mid trunk diagonal (5-8, fig 6.22) showed significant decreases throughout the sampling months ($H=15.5$, $P<0.005$ and $H=14.8$, $P<0.005$ respectively), and only the dorsal-ventral mid trunk diagonal (6-7, fig 6.21) showed any difference in size over the sampling period, being significantly higher than LMG fish and lower than $0^+$UMG fish (2-way ANOVAs $F_{1,66}=12.270$, $P=0.0008$ and $F_{1,67}=13.460$, $P=0.0005$ respectively). However, at smolting the $1^+$LMG measurements showed no significant difference to either $0^+$UMG or $0^+$LMG fish.

The length of the dorsal fin attachment (6-8) could be considered as a length measurement of the mid trunk cell, but I consider it better to treat it separately. There was no real seasonal effect on the standardised lengths of dorsal fin attachment (6-8) in $0^+$UMG, $0^+$LMG or $1^+$LMG fish (fig. 6.23). However, over the whole sampling period the length of $0^+$UMG fish again tended to be greater than that of $0^+$LMG fish (2-way ANOVA $F_{1,109}=10.580$, $P=0.0015$) although by smolting the length of UMG fish had decreased and that of the $0^+$LMG

Figure 6.19. Mean values for the standardised mid trunk depth (5-6) of $0^{+}$UMG (—○—), $0^{+}$LMG (–●–) and $1^{+}$LMG (----■---) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

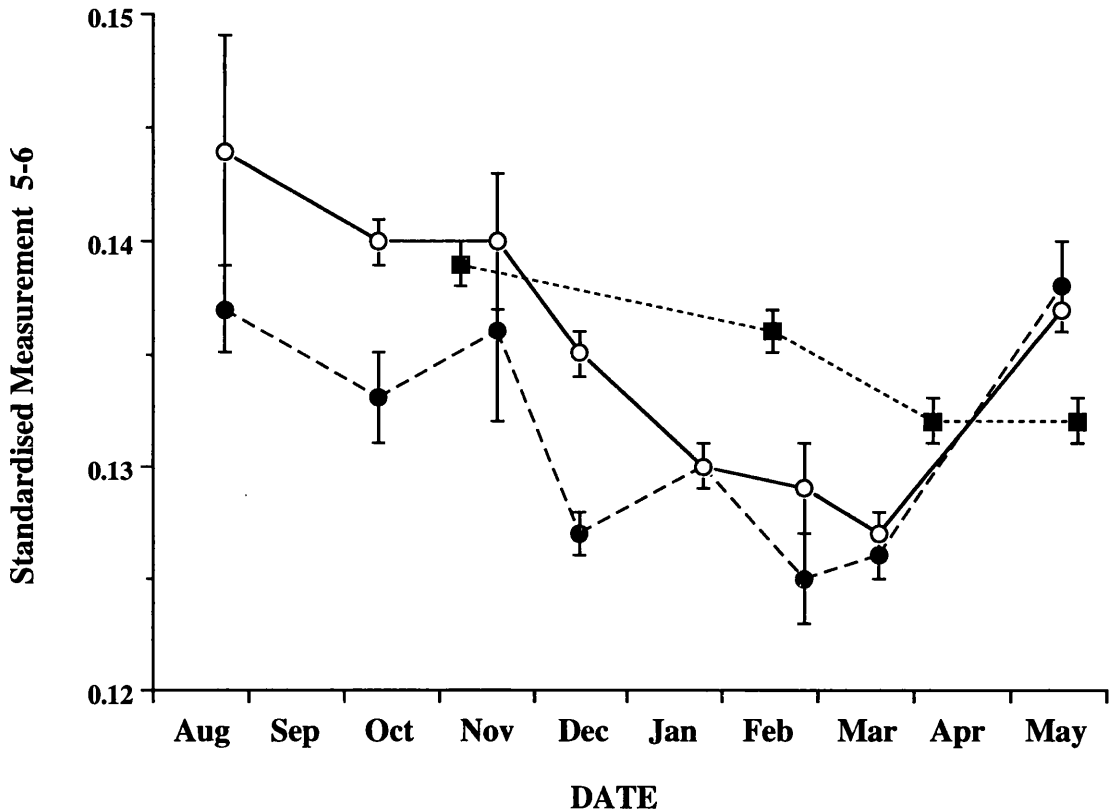

Standardised Measurement 5-6
0.15
0.14
0.13
0.12
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.20. Mean values for the standardised ventral mid trunk length (5-7) of $0^{+}$UMG (—○—), $0^{+}$LMG (— ● —) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

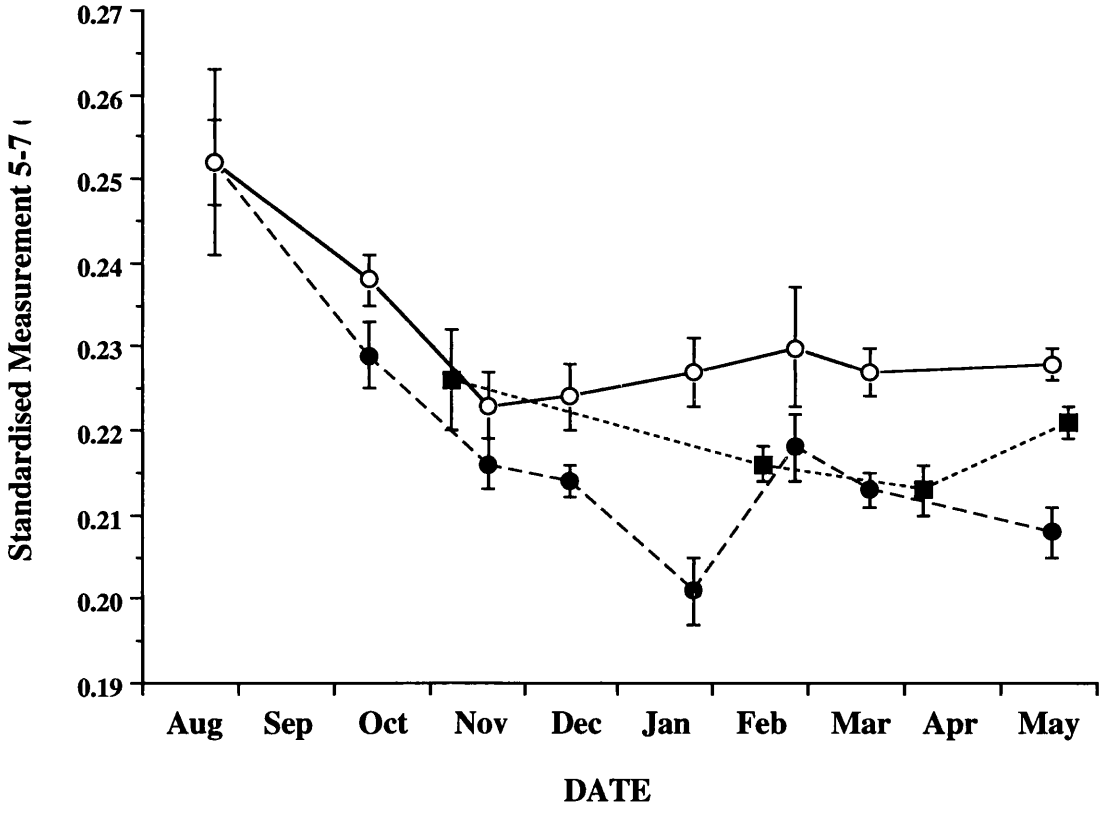

0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.20
0.19
Standardised Measurement 5-7
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.21. Mean values for the standardised lengths of the dorsal-ventral mid trunk diagonal (6-7) of $0^{+}$UMG (—○—), $0^{+}$LMG (- -●- -) and $1^{+}$LMG (·---■---·) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

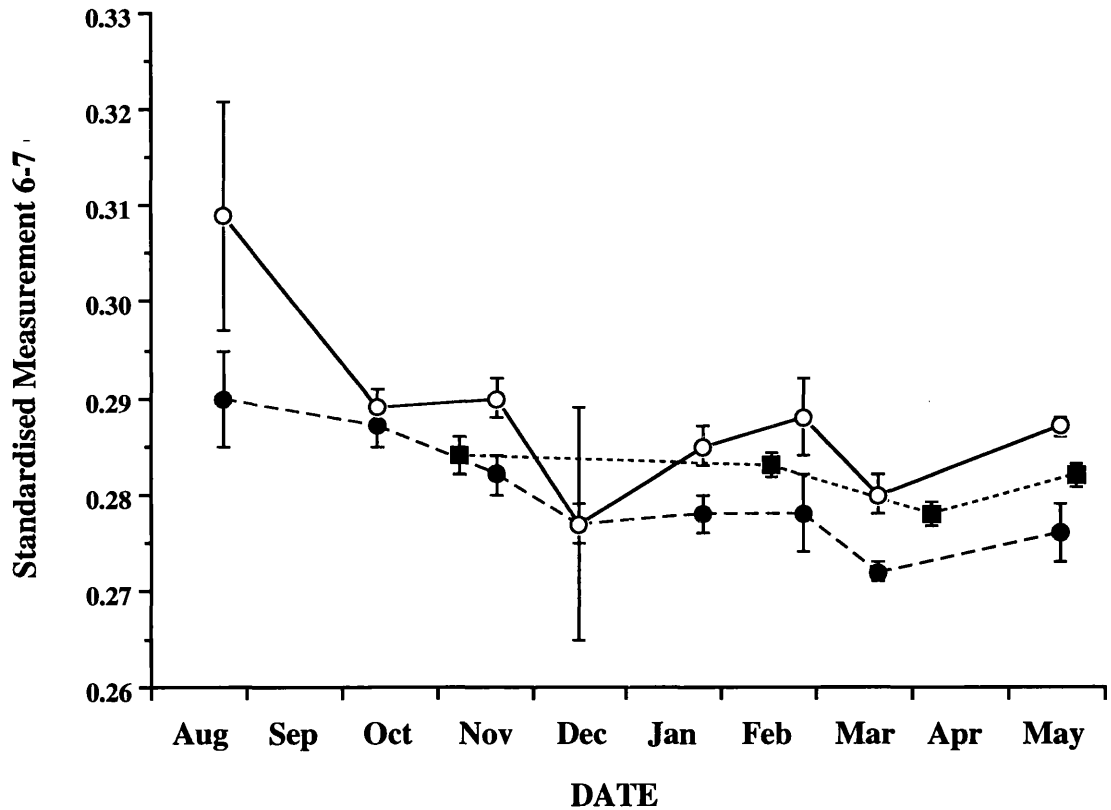

Standardised Measurement 6-7
0.33
0.32
0.31
0.30
0.29
0.28
0.27
0.26
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.22. Mean values for the standardised lengths of the ventral-dorsal mid trunk diagonal (5-8) of $0^{+}$UMG (——○——), $0^{+}$LMG (– –●– –) and $1^{+}$LMG (·--■--·) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

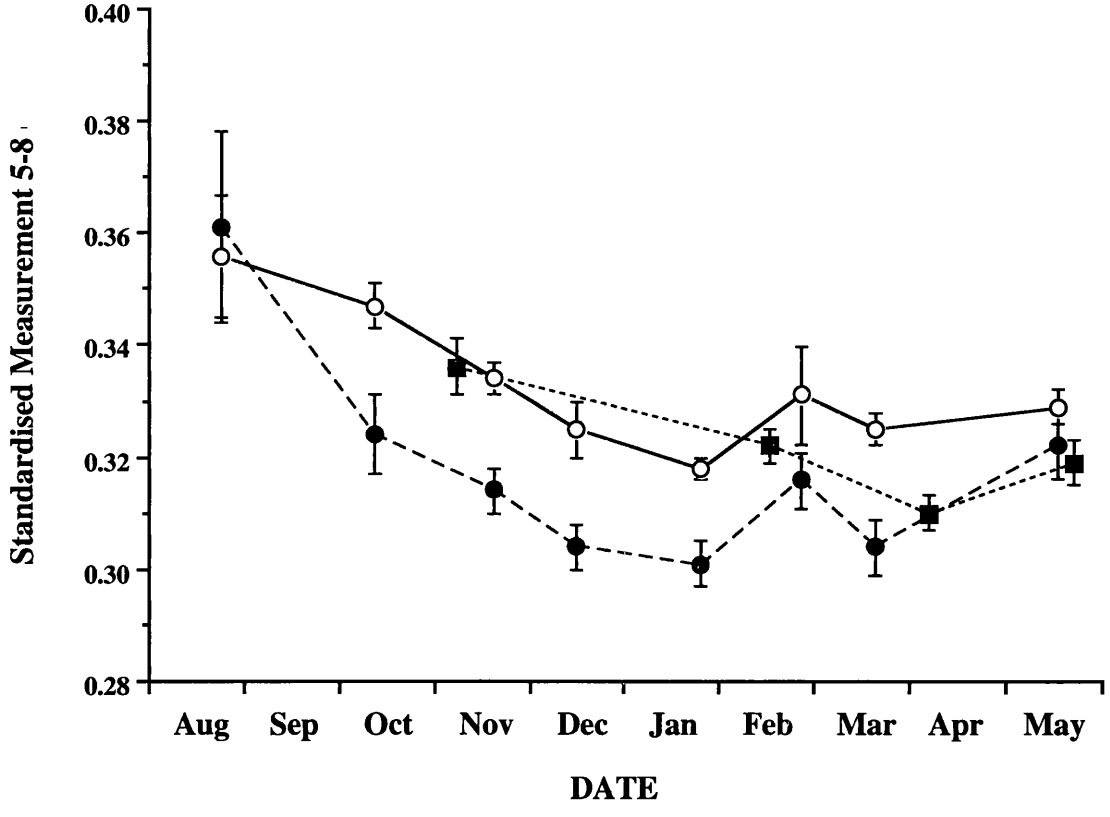
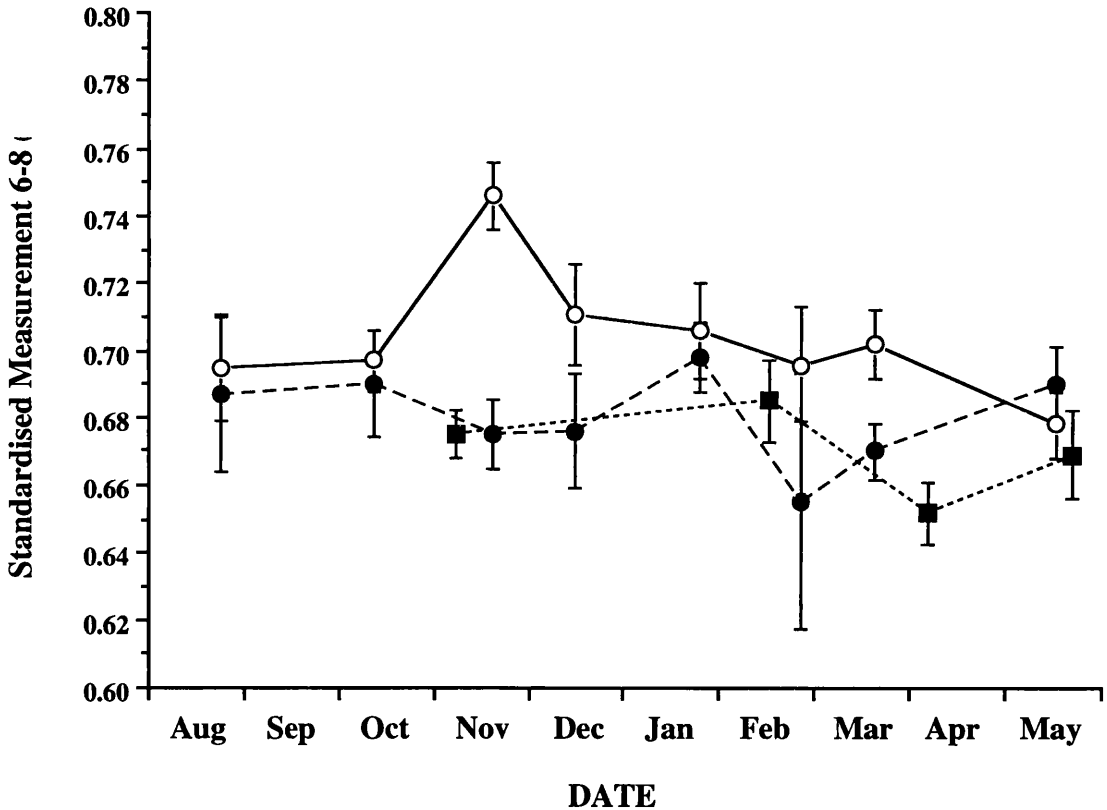

0.40
0.38
0.36
0.34
0.32
0.30
0.28
Standardised Measurement 5-8
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.23. Mean values for the standardised lengths of dorsal fin attachment (6-8) of $0^{+}$UMG (—○—), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (·--■--·) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


Standardised Measurement 6-8
0.80
0.78
0.76
0.74
0.72
0.70
0.68
0.66
0.64
0.62
0.60
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

fish had increased resulting in no significant difference between the modal groups at this time.

$1^+$LMG fish were similar to $0^+$LMG fish (2-way ANOVA $F_{1,66}=0.217$, $P=0.648$) and also had shorter dorsal fins than $0^+$UMG fish (2-way ANOVA $F_{1,67}=26.259$, $P<0.0001$), but again by smolting any differences had disappeared.

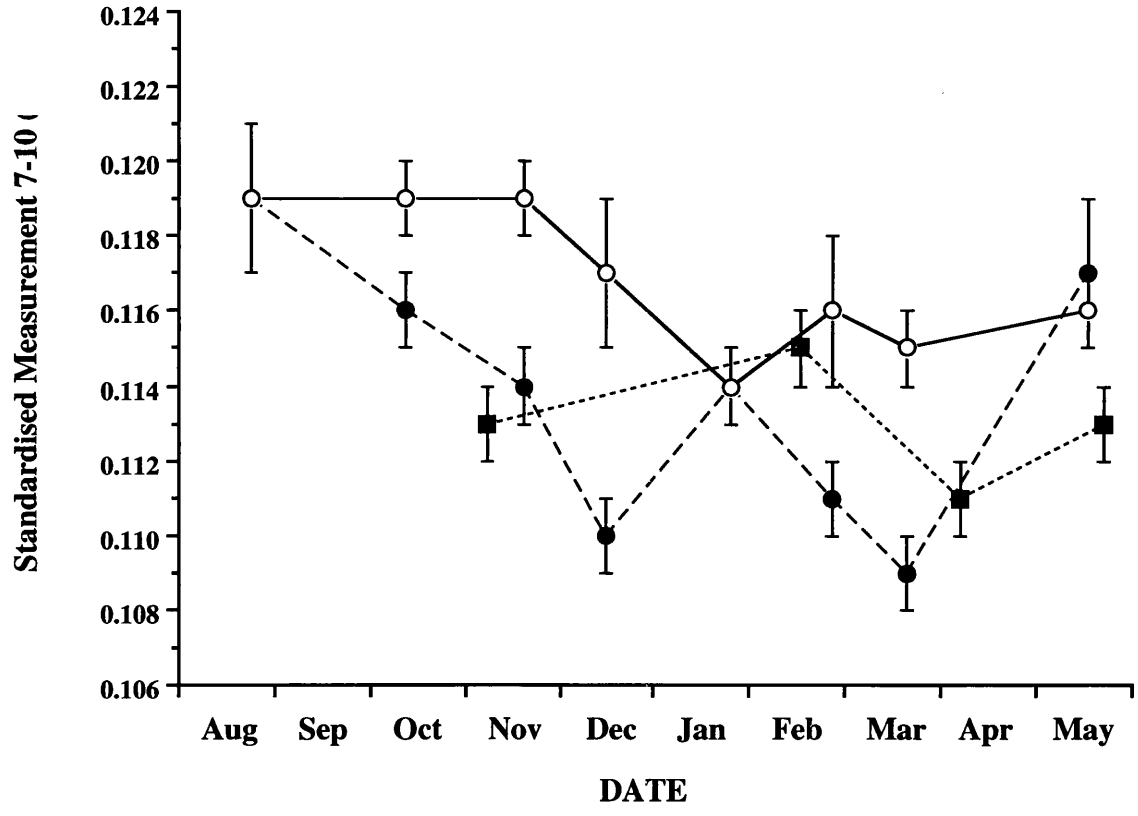
*Posterior trunk*

The ventral-dorsal posterior trunk diagonal (7-10, fig 6.24) did not differ over the sampling period in $0^+$UMG fish (Kruskal-Wallis $H=15.1$, $P=0.035$) despite a slight decline. However, in $0^+$LMG fish it did tend to drop between summer and winter before increasing again in the spring ($H=29.7$, $P<0.0005$). $0^+$UMG fish had significantly larger measurements than $0^+$LMG fish over most of the year (2-way ANOVA $F_{1,109}=22.038$, $P<0.0001$), exceptions being January and May (by which time the spring increase seen in $0^+$LMG fish had cancelled out any difference in standardised lengths).

$1^+$LMG fish did not differ significantly between August and May (Kruskall-Wallis $H=8.4$, $P=0.038$), although they were similar to LMG fish (2-way ANOVA $F_{1,66}=0.069$, $P=0.8$) and tended to have shorter posterior trunk diagonals than $0^+$UMG fish (2-way ANOVA $F_{1,67}=24.118$, $P<0.0001$) except during February. $1^+$LMG fish did not differ from $0^+$UMG or $0^+$LMG fish (Kruskall-Wallis $H=4.3$, $P=0.117$) during May.

Relative anal fin lengths (7-9, fig. 6.25) from both $0^+$UMG and $0^+$LMG fish increased from August to October,

Figure 6.24. Mean values for the standardised lengths of the ventral-dorsal posterior trunk diagonal (7-10) of $0^{+}$UMG (—○—), $0^{+}$LMG (– –●– –) and $1^{+}$LMG (- - ■ - -) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

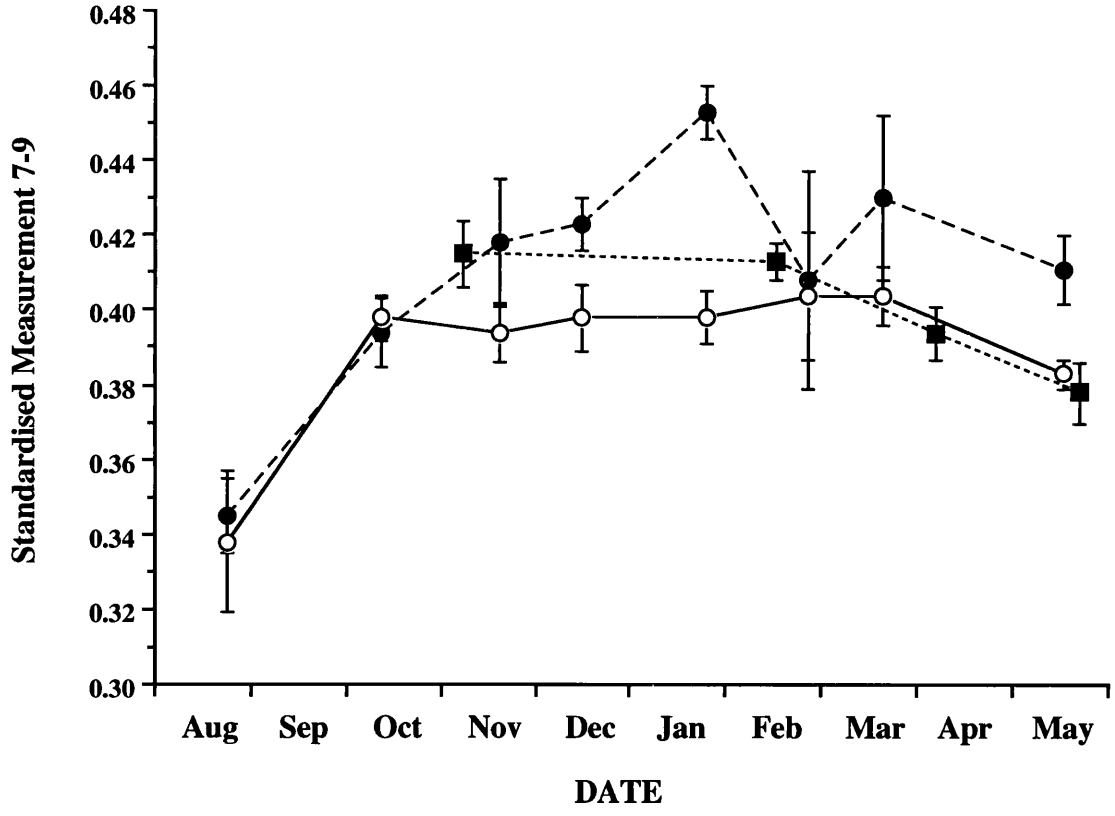

Standardised Measurement 7-10
0.124
0.122
0.120
0.118
0.116
0.114
0.112
0.110
0.108
0.106
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

but at this point those of $0^+$UMG fish levelled out and the overall effect of this was that there was no significant change over the sampling period. However, the anal fin lengths of the $0^+$LMG fish increased dramatically between August and January after which it remained relatively constant through to May. This resulted in $0^+$LMG fish having significantly longer anal fins than $0^+$UMG fish (2-way ANOVA $F_{1,109}=11.158$, $P=0.001$), but due to the slight decrease seen in $0^+$LMG anal fin lengths during spring, this was no longer apparent by May.

The anal fin lengths of $1^+$LMG fish remained constant throughout most of the sampling period before declining through the spring ($H=12.8$, $P<0.005$). This pattern was similar to that of $0^+$UMG fish (fig. 6.25) and there was no significant difference with either $0^+$UMG or $0^+$LMG fish (2-way ANOVA $F_{1,67}=0.376$, $P=0.548$ and $F_{1,66}=3.840$, $P=0.054$ respectively) and this continued throughout to the period of smolting.

The general pattern in the overall trunk area seemed to be that although the relative size of this area of the fish tends to decrease into the winter in all modal groups, $0^+$UMG fish have longer, deeper trunks than $0^+$LMG fish. This difference persists, albeit at a lesser level, into smolting. $1^+$LMG fish tend to remain intermediate between $0^+$UMG and $0^+$LMG fish throughout the sampling period and into smolting.

Figure 6.25. Mean values for the standardised lengths of anal fin attachment (7-9) of $0^{+}$UMG (—○—), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


Standardised Measurement 7-9
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

*Post anal region* (figs. 6.26 - 6.27)

Both $0^+$UMG and $0^+$LMG fish showed a dramatic decrease in ventral post anal length (9-11, fig 6.26) between August and October. In $0^+$UMG fish the length then remained stable for the rest of the year; the overall effect being that there was no difference during the sampling period (Kruskall-Wallis H=18.4, P=0.01).

The $0^+$LMG fish continued to decrease in ventral-post anal length until January when they increased to February and then decreased through spring. There was no significance difference between $0^+$UMG and $0^+$LMG fish overall (2-way ANOVA $F_{1,109}$=0.003, P=0.96) and this remained the case at smolting by $0^+$UMG fish.

$1^+$LMG fish varied little over the winter months, and were similar to $0^+$LMG fish (2-way ANOVA $F_{1,66}$=3.663, P=0.06) but had shorter ventral post anal lengths than $0^+$UMG fish (2-way ANOVA $F_{1,67}$=9.245, P=0.0034). However, their ventral post anal length increased significantly (H=13.1, P<0.005) between February and May resulting in there being no significant difference at smolting.

The ventral post anal length could be viewed simply as the antithesis of anal fin length or *vice versa*. However, anal fin length was greater in $0^+$LMG than $0^+$UMG fish, whereas there were no modal differences in post anal length implying that these two morphological measurements were unrelated.

Figure 6.26. Mean values for the standardised ventral post anal length (9-11) of $0^{+}$UMG (——○——), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

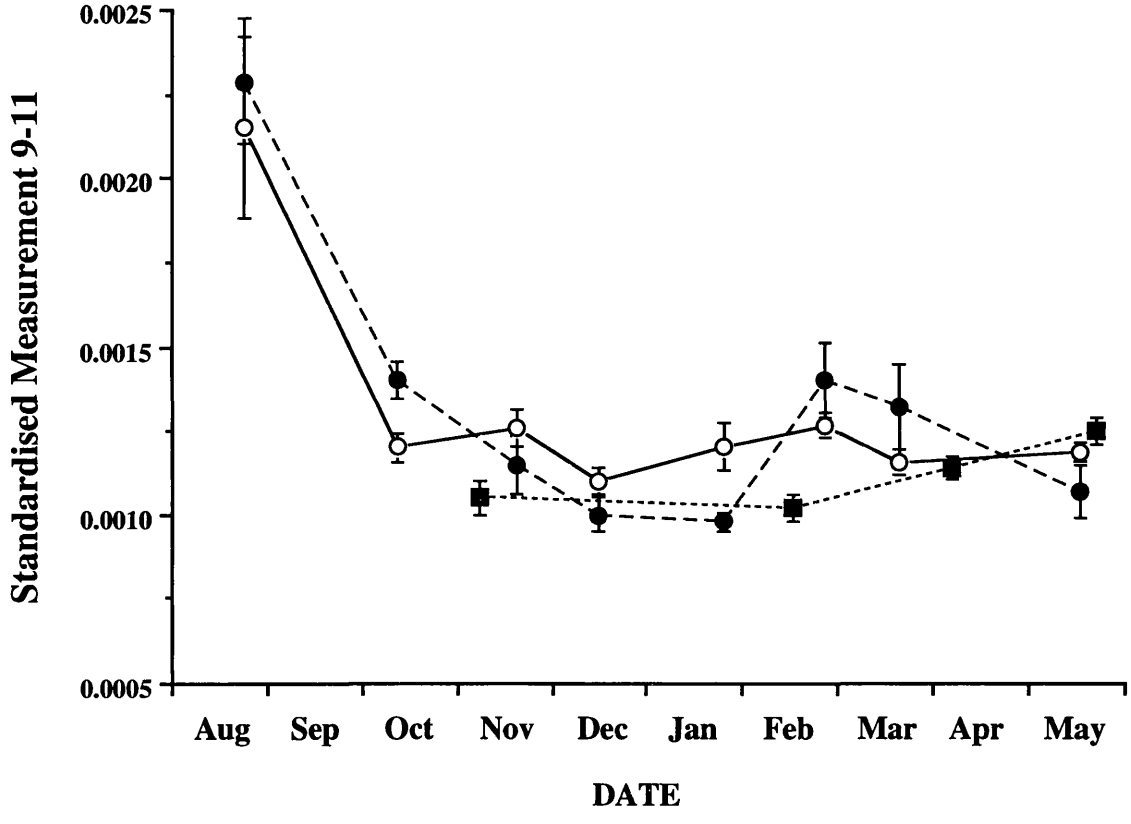
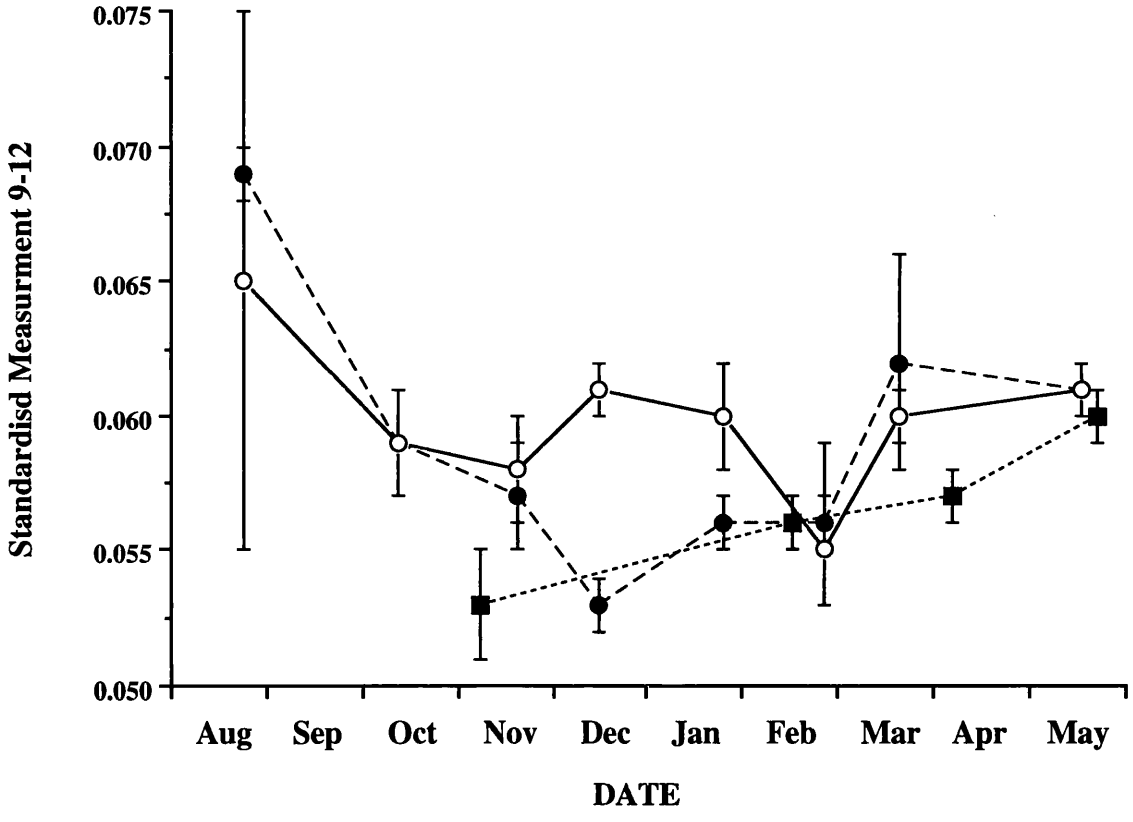

Standardised Measurement 9-11
0.0025
0.0020
0.0015
0.0010
0.0005
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.27. Mean values for the standardised lengths of the ventral-dorsal post anal diagonal (9-12) of $0^{+}$UMG (——○——), $0^{+}$LMG (— —●— —) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


Standardisd Measurment 9-12
0.075
0.070
0.065
0.060
0.055
0.050
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

*Caudal fin* (figs. 6.28 - 6.30)

Neither $0^+$UMG or $0^+$LMG fish showed any significant seasonal trend in their ventral-dorsal caudal fin diagonals (fig. 6.28). Through the year $0^+$UMG fish tended to have longer caudal fins than $0^+$LMG fish (2-way ANOVA $F_{1,109}=22.937$, $P<0.0001$), but due to the spring increase seen in $0^+$LMG fish, by May this had disappeared.

$1^+$LMG fish showed no seasonal variation in this tail length measurement (Kruskal-Wallis $H=4.3$, $P=0.23$) and were similar to $0^+$LMG fish (2-way ANOVA $F_{1,66}=3.614$, $P=0.06$) and also had shorter caudal fins than $0^+$UMG fish (2-way ANOVA $F_{1,67}=38.295$, $P<0.0001$). There may have been a significant difference at smolting between $1^+$LMG and both $0^+$UMG and $0^+$LMG fish, but this may be due to the greater amount of fin erosion seen in some $1^+$LMG fish.

The same general pattern is displayed by the other dorsal-ventral caudal fin diagonal (12-13, fig 6.29) throughout the sampling period in all modal groups. $0^+$UMG fish had significantly greater measurements than $0^+$LMG fish (2-way ANOVA $F_{1,109}=11.428$, $P=0.001$) and $1^+$LMG fish (2-way ANOVA $F_{1,67}=25.709$, $P<0.0001$), which were similar to $0^+$LMG fish (2-way ANOVA $F_{1,66}=6.381$, $P=0.014$). By May there was no difference between any of the fish.

$0^+$UMG and $0^+$LMG fish showed a remarkably similar pattern in caudal fin depth (13-14) characterised by a slight, and non-significant increase until January

Figure 6.28. Mean values for the standardised lengths of the ventral-dorsal caudal fin diagonal (11-14) of $0^{+}$UMG (—○—), $0^{+}$LMG (— ● —) and $1^{+}$LMG (--■--) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

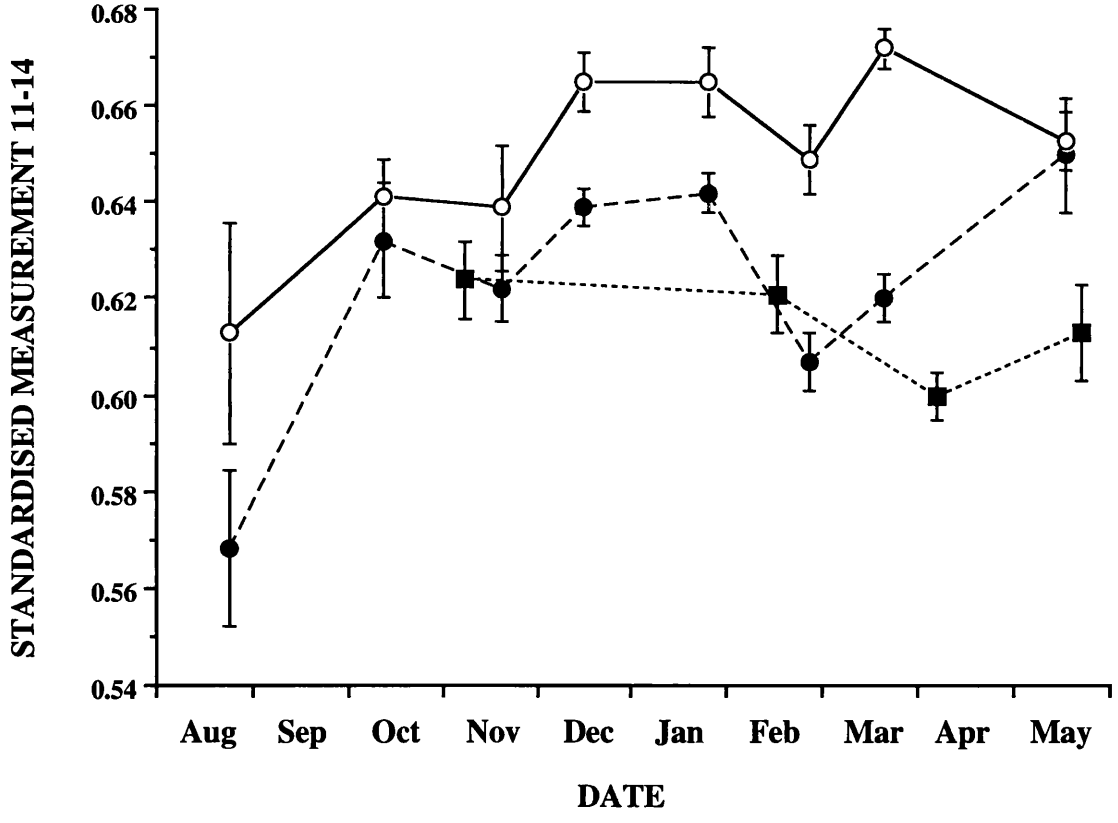

STANDARDISED MEASUREMENT 11-14
0.68
0.66
0.64
0.62
0.60
0.58
0.56
0.54
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.29. Mean values for the standardised lengths of the dorsal-ventral caudal fin diagonal (12-13) of $0^{+}$UMG (——○——), $0^{+}$LMG ( – –●– – ) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.

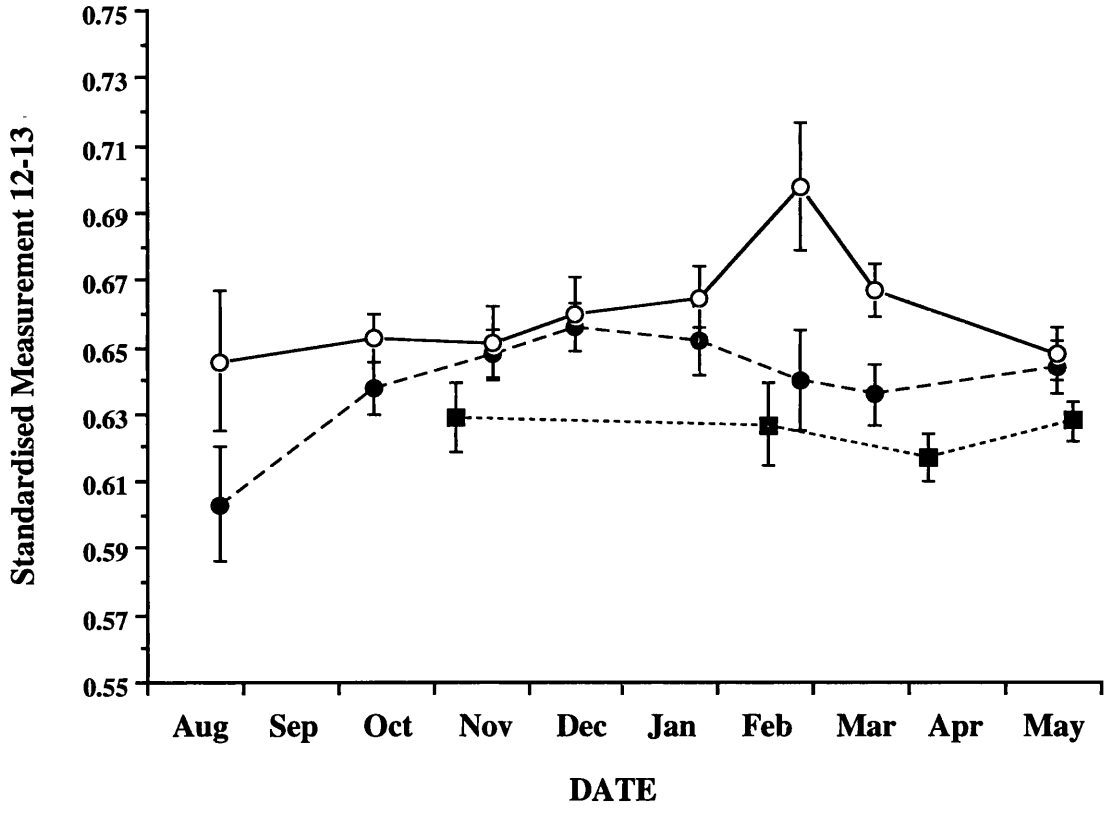
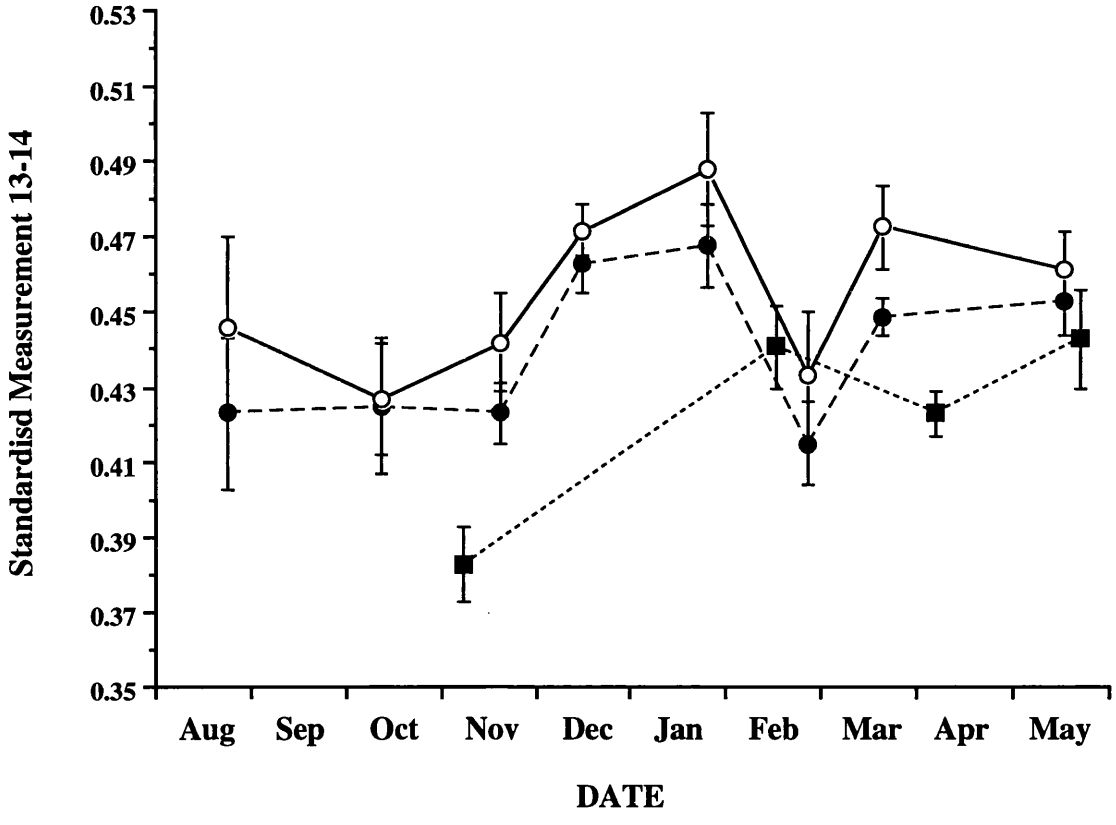

Standardised Measurement 12-13
0.75
0.73
0.71
0.69
0.67
0.65
0.63
0.61
0.59
0.57
0.55
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Figure 6.30. Mean values for the standardised caudal fin depths (13-14) of $0^{+}$UMG (—○—), $0^{+}$LMG (– –●– –) and $1^{+}$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


Standardisd Measurement 13-14
0.53
0.51
0.49
0.47
0.45
0.43
0.41
0.39
0.37
0.35
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

followed by a sudden drop to February and an increase through spring (fig. 6.30). There was no difference in tail depth between $0^+$UMG and $0^+$LMG fish (2-way ANOVA $F_{1,109}=4.829$, $P=0.03$) and this remained true during $0^+$UMG smolting.

$1^+$LMG fish showed an increase between November and February ($H=15.3$, $P<0.005$) then no change through to May. They had smaller caudal fin depths than $0^+$UMG fish (2-way ANOVA $F_{1,67}=18.408$, $P=0.0001$) but were no different from $0^+$LMG fish (2-way ANOVA $F_{1,66}=5.031$ $P=0.028$). However, their relative increase through spring resulted in no significant difference between $1^+$LMG, $0^+$UMG and $0^+$LMG tail depths at smolting (Kruskall-Wallis $H=2.5$, $P=0.28$).

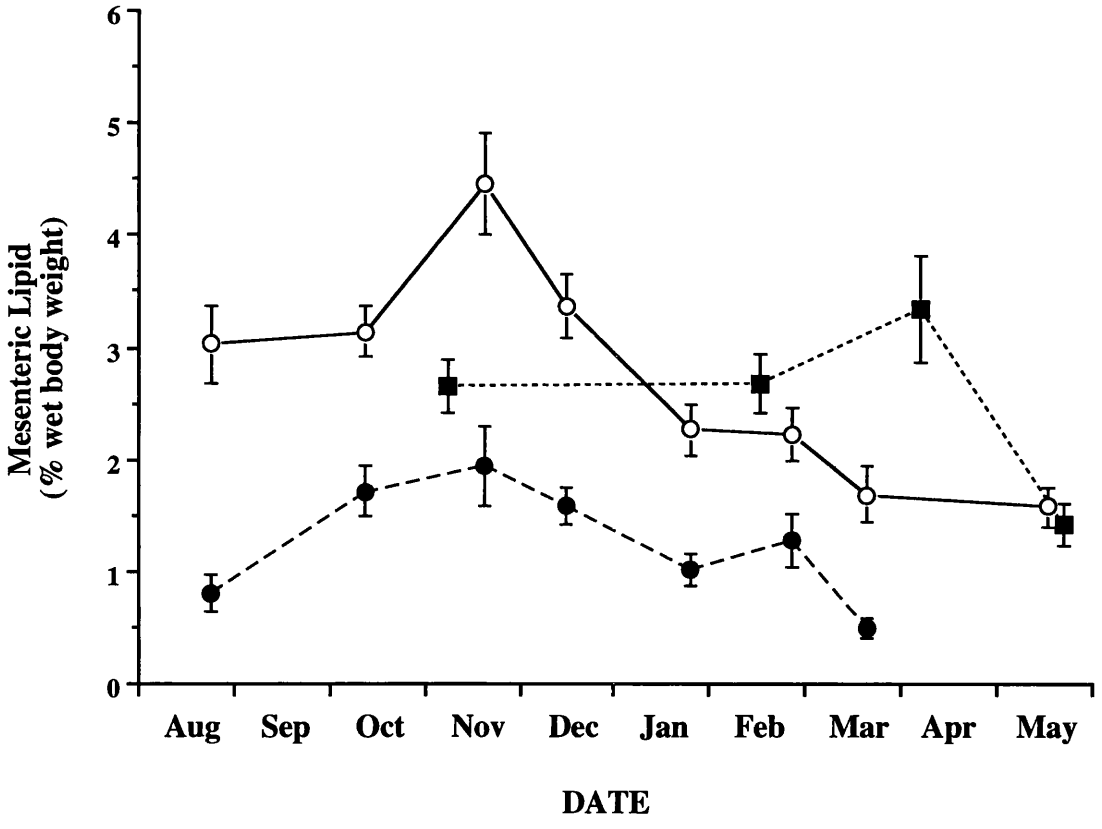
Overall $0^+$UMG fish had longer, but not deeper, caudal fins than $0^+$LMG fish until smolting, and $1^+$LMG caudal fin lengths seemed to show the same pattern as those of $0^+$LMG fish. It may be unwise, however, to put too much significance on these measurements from $1^+$LMG fish since after 2 years in freshwater tanks caudal fin erosion can be quite high. This would especially affect the caudal fin depth (13-14) and to a lesser extent the diagonals (11-14 and 12-13).

**Mesenteric Lipid Levels**

*Seasonal patterns*

The level of mesenteric lipid (expressed as a percentage of whole body wet weight) is shown for $0^+$UMG, $0^+$LMG and $1^+$LMG juvenile salmon in figure 6.31.

Figure 6.31. Mean values for mesenteric lipid (expressed as a percentage of wet body weight) for $0^+$UMG (—○—), $0^+$LMG (– –●– –) and $1^+$LMG (----■----) juvenile salmon over the sampling period from August 1990 to May 1991. Vertical bars represent one standard error.


Mesenteric Lipid
(% wet body weight)
6
5
4
3
2
1
0
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
DATE

Kruskal-Wallis analyses of variance showed that there was a significant effect of date on the mesenteric lipid level of all modal groups ($H=36.47$, $P<0.00001$; $H=25.77$, $P<0.0005$; and $H=8.89$, $P<0.05$ for $0^+$UMG, $0^+$LMG and $1^+$LMG fish respectively).

$0^+$UMG fish showed an increase in mesenteric lipid levels between October and November, followed by a decrease through to May. $0^+$LMG fish had a similar seasonal pattern but at a lower level (2-way ANOVAs: effect of modal group $F_{1,87}=161.244$, $P<0.0001$; interaction between modal group and time of year $F_{6,87}=1.530$, $P=0.18$). The peak in November was not as pronounced as that seen in $0^+$UMG fish, and again there was a general decrease throughout the rest of the year, except for the period between January and February, when there was no decrease in either $0^+$UMG or $0^+$LMG fish.

The levels of mesenteric lipid seen in $1^+$LMG fish were at the same general level as $0^+$UMG fish (2-way ANOVA: effect of mode $F_{1,50}=0.117$, $P=0.737$), but the pattern of seasonal change was different (2-way ANOVA: interaction between season and mode $F_{3,50}=10.703$, $P<0.0001$). There was no significant variation in $1^+$LMG mesenteric lipid levels throughout the sampling period (up to April), but between April and May the levels dropped. This resulted in $0^+$UMG and $1^+$LMG fish having similar relative levels of mesenteric lipid levels during the period of seaward migration (Mann-Whitney U-test, $Z=-0.642$, $P=0.52$).

*Effect of size*

When the level of mesenteric fat was regressed against forklength (mm) for each modal group and within each sampling period, there were no significant results, probably due to the small sample sizes (≤12).

There was no significant correlation of forklength with mesenteric lipid in $1^{+}$LMG fish ($r=0.075$, $P=0.68$).

*Prediction of mesenteric lipid levels*

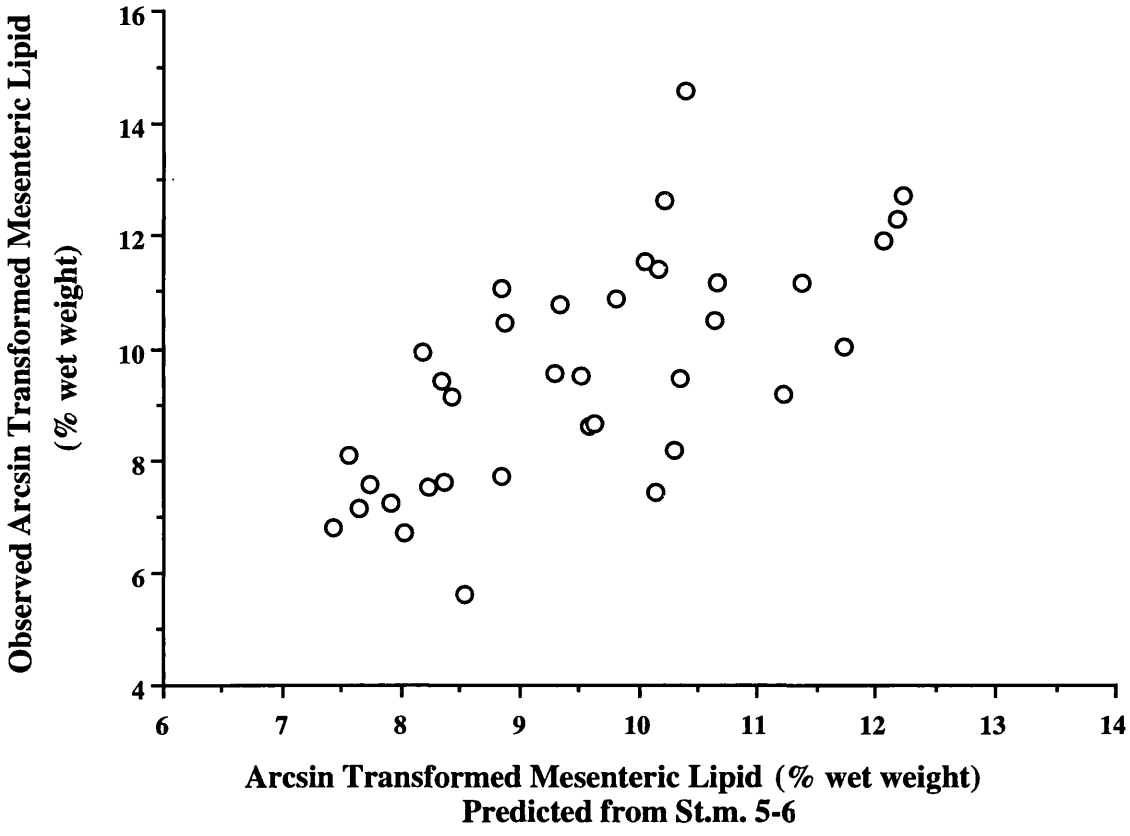
Stepwise multiple regressions were used to indicate which, if any, of the standardised measurements were correlated with the level of mesenteric lipid. Only measurements from the anterior and mid-trunks (opercular depth 3-4, ventral anterior trunk depth 3-5, ventral-dorsal anterior trunk diagonal 3-6, mid trunk depth 5-6, ventral mid trunk depth 5-7, ventral-dorsal mid trunk diagonal 5-8, dorsal-ventral mid trunk diagonal 6-7, length of dorsal fin attachment 6-8 and posterior trunk depth 7-8) were investigated since the mesentery lies within this area. Analyses were carried out on separate data sets for $0^{+}$UMG, $0^{+}$LMG and $1^{+}$LMG fish, and for all fish combined.

In $0^{+}$UMG fish only mid trunk depth 5-6 was a significant predictor of mesenteric lipid ($r^2=0.448$, $n=36$ $P<0.0001$). The final model is given in equation I and shown in figure 6.32.

$$\text{arcsin transformed lipid} = 260.00\ \text{mtd} - 25.26 \qquad \text{(I)}$$

where mtd = mid trunk depth (5-6)

Figure 6.32. The correlation between predicted and observed values of arcsin transformed lipid data based on standardised measurement 5-6 for $0^{+}$UMG juvenile salmon.


Observed Arcsin Transformed Mesenteric Lipid
(% wet weight)
16
14
12
10
8
6
4
6
7
8
9
10
11
12
13
14
Arcsin Transformed Mesenteric Lipid (% wet weight)
Predicted from St.m. 5-6

In $0^+$LMG fish only posterior trunk depth (7-8) was significantly correlated with mesenteric lipid ($r^2$=0.166, n=31 P<0.05), which although a measurement of trunk depth was from a different area of the body. The model for prediction of mesenteric lipid in $0^+$LMG fish is given in equation II and shown in figure 6.33.

arcsin transformed lipid = 203.62 ptd - 5.39 **(II)**

where ptd = posterior trunk depth (7-8)

Standardised trunk measurements of $1^+$ LMG fish showed no correlation with mesenteric lipid levels, which may have been due to the relatively small size range of this sample (90 - 133 mm FL).

When data from all fish were combined, 3 trunk measurements (combining ventral anterior trunk length 3-5, length of dorsal fin attachment 6-8 and mid trunk depth 5-6) were significantly correlated with arcsin transformed data on relative mesenteric lipid level (3-6, t=5.34, P<0.0001; 6-8, t=5.12, P<0.0001; 7-8, t=2.60, P=0.011). The final model given in equation III was significantly correlated with mesenteric lipid (fig. 6.34, $r^2$=0.42, n = 92 P<0.0001).

arcsin transformed lipid = 211.78 ptd
+ 179.27 atd + 27.37 dfa - 55.78 **(III)**

where ptd = posterior trunk depth (7-8), atd = ventral-dorsal anterior trunk diagonal (3-6) and dfa = length of dorsal fin attachment (6-8)

The correlation between condition factor ($W/L^3$) and mesenteric lipid was also tested, this time using simple linear regression. $0^+$UMG, $0^+$LMG and $1^+$LMG fish

Figure 6.33. The correlation between predicted and observed values of arcsin transformed lipid data based on standardised measurement 7-8 for $0^{+}$LMG juvenile salmon.

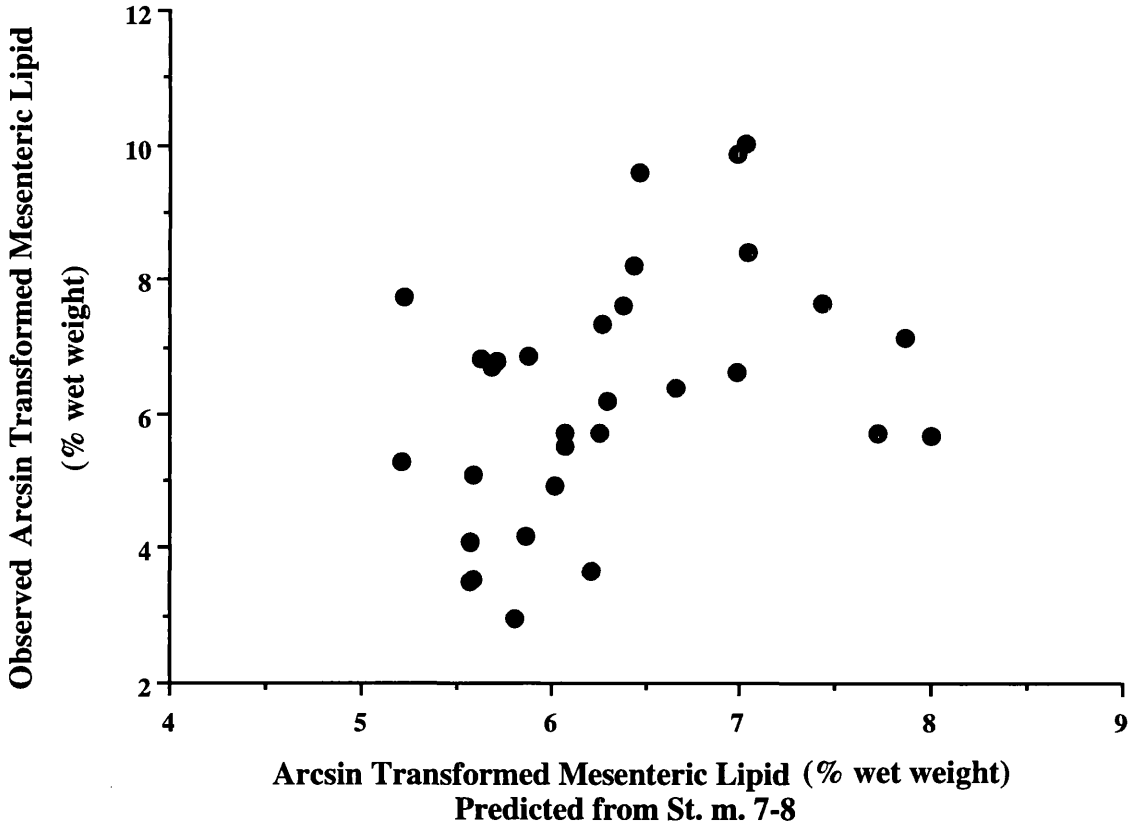
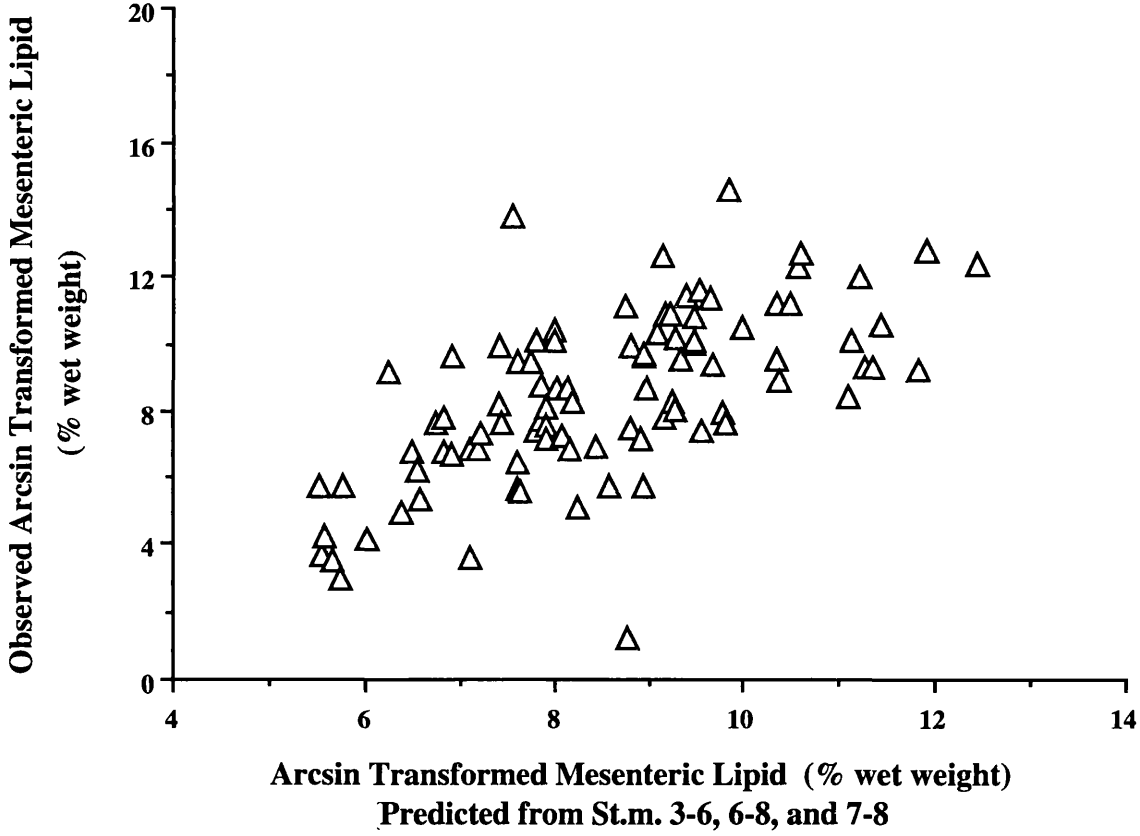

Observed Arcsin Transformed Mesenteric Lipid
(% wet weight)
12
10
8
6
4
2
4
5
6
7
8
9
Arcsin Transformed Mesenteric Lipid (% wet weight)
Predicted from St. m. 7-8

Figure 6.34. The correlation between predicted and observed values of arcsin transformed lipid data based on standardised measurements 3-6, 6-8, and 7-8 for all juvenile salmon.


Observed Arcsin Transformed Mesenteric Lipid
(% wet weight)
20
16
12
8
4
0
4
6
8
10
12
14
Arcsin Transformed Mesenteric Lipid (% wet weight)
Predicted from St.m. 3-6, 6-8, and 7-8

were each analysed, but data from all modal groups were not combined since condition factor differed significantly between modal groups.

Only $0^{+}$UMG fish showed a significant correlation between condition factor and mesenteric lipid ($r=0.627$, $P<0.0001$), resulting in the final model given in equation IV and figure 6.35.

arcsin transformed lipid = 2546.44 cf - 19.41 **(IV)**

where cf = condition factor

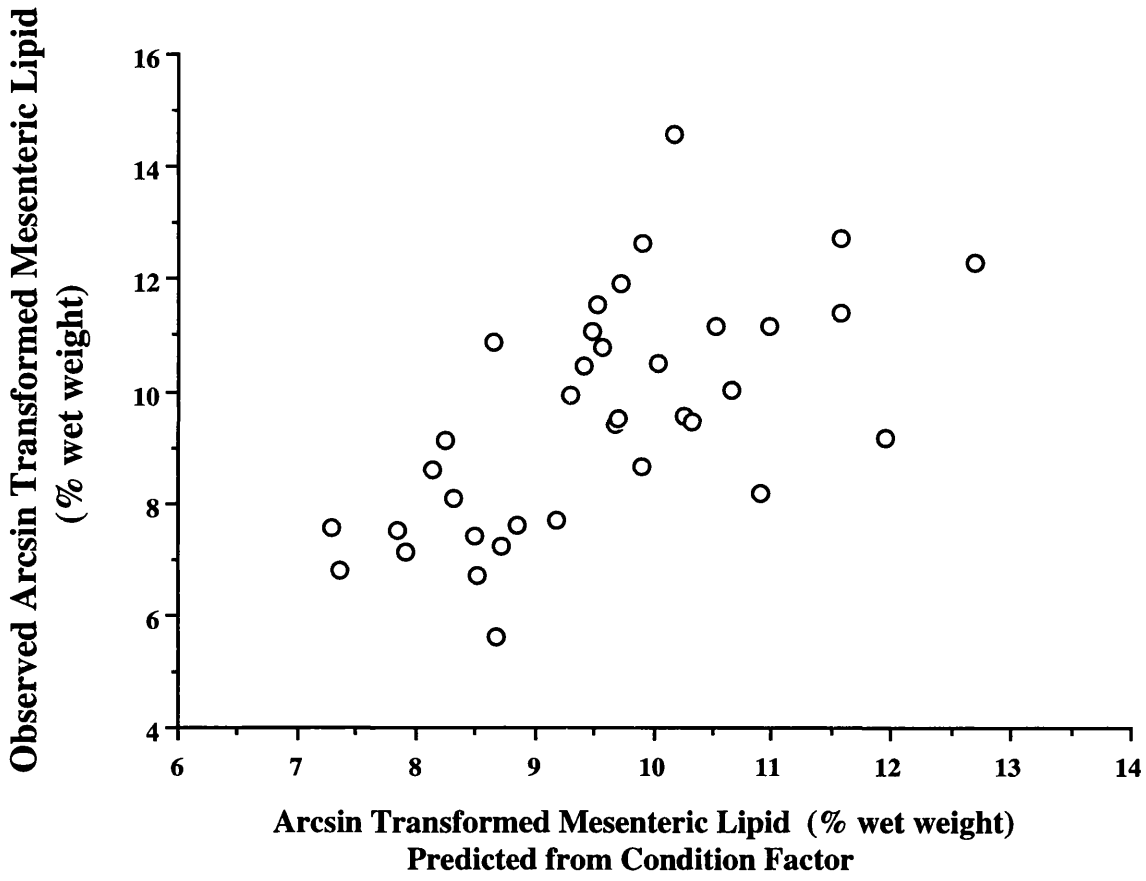
However, neither $0^{+}$LMG or $1^{+}$LMG fish showed a correlation of condition factor with mesenteric lipid ($r=0.321$, $P=0.08$ and $r=0.078$, $P=0.71$ respectively).

*Relationship between body morphology and mesenteric lipid level with CHV*

Both body shape and the level of mesenteric lipid were investigated as possible predictors of relative CHV (chapter 3) over the whole sampling period and for each modal group. In both cases in order to control for the effect of temperature, the relative CHV value for each fish was expressed as a percentage of the mean CHV in the respective month for each modal group. The results for $0^{+}$UMG and $1^{+}$LMG fish during May were excluded due to their relative CHV being lower at the time of smolting (chapter 3).

These CHV results corrected for temperature were then regressed against each standardised length and depth measurement from the trunk and post-anal regions of the fish (3-4, 3-5, 4-6, 5-6, 5-7, 6-8, 7-8, 7-9, 8-10, 9-

Figure 6.35. The correlation between predicted and observed values of arcsin transformed lipid data based on condition factors for $0^{+}$UMG juvenile salmon.


Observed Arcsin Transformed Mesenteric Lipid
(% wet weight)
16
14
12
10
8
6
4
6
7
8
9
10
11
12
13
14
Arcsin Transformed Mesenteric Lipid (% wet weight)
Predicted from Condition Factor

10, 9-11,10-12, and 11-12 fig. 6.1) for each modal group separately. Caudal fin measurements were not included, since this area is not greatly involved in holding station on the substrate. Due to the large number of linear regression analyses that were involved (39) the significance level was set at $P<0.01$.

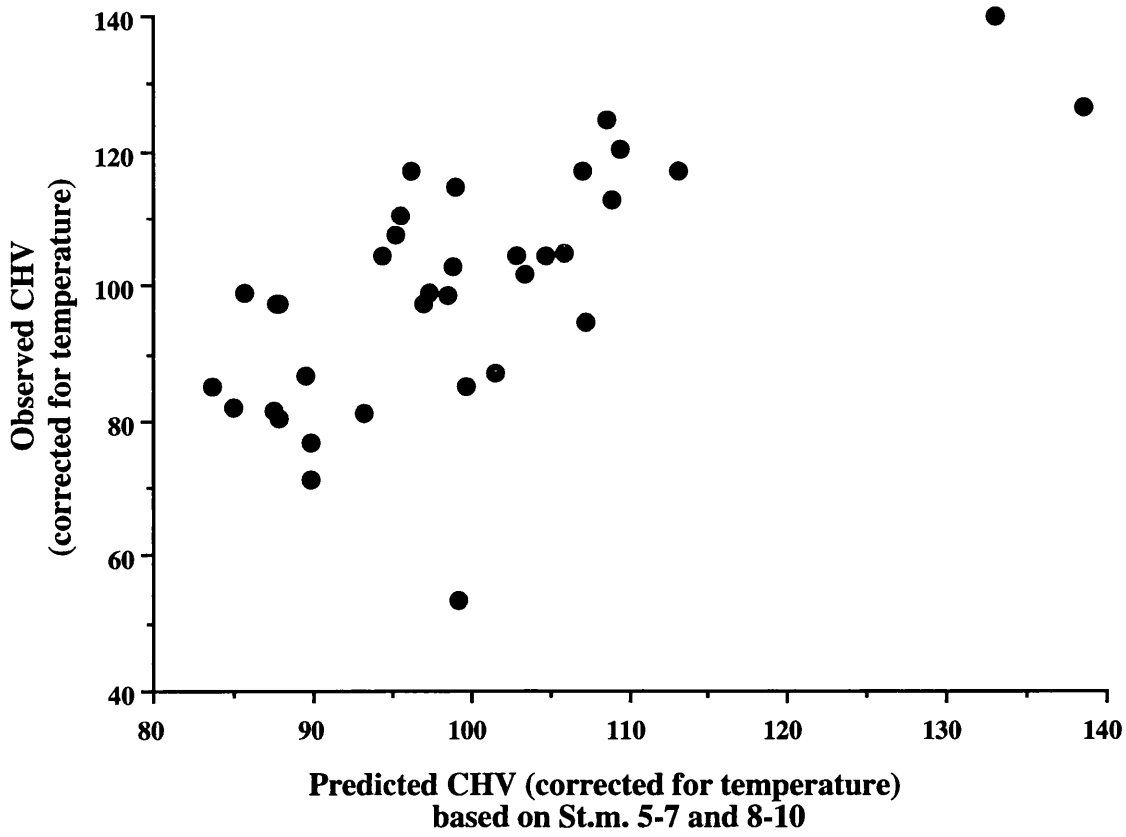
Only $0^{+}$LMG fish showed a significant correlation, the ventral mid-trunk length (5-7) being positively correlated with relative CHV ($r=0.493$, $P<0.005$) and the dorsal posterior trunk depth (8-10) being negatively correlated ($r=-0.543$, $P<0.001$). Stepwise multiple regression was used to determine a model for predicting of relative CHV (corrected for temperature) in $0^{+}$LMG fish using these two standardised measurements. The results are given in equation V and figure 6.36.

$$CHV(bl.s^{-1}) = 119.12 - 9126.99\ ptd + 262.36\ mtl \quad \textbf{(V)}$$

where ptd = posterior trunk depth (8-10), and mtl = mid trunk length (5-7).

To assess the relationship between mesenteric lipid (expressed as a percentage of whole wet body weight) and relative CHV (corrected for temperature), mesenteric lipid values were subjected to arcsin transformation prior to use in linear regression analyses. $0^{+}$UMG, $0^{+}$LMG and $1^{+}$LMG fish were all analysed separately and then in combination, but no significant correlations were found (correlation coefficients = 0.01, 0.05, -0.01 and -0.1 respectively).

Figure 6.36. The correlation between predicted and observed values of CHV based on standardised measurements 5-7 and 8-10 for $0^{+}$LMG juvenile salmon.


Observed CHV
(corrected for temperature)
140
120
100
80
60
40
80
90
100
110
120
130
140
Predicted CHV (corrected for temperature)
based on St.m. 5-7 and 8-10

## Discussion

*Principal components analysis*

The use of principal components analysis can be a very effective tool for discrimination between different stocks or groupings of fish by their overall relative body shape. However, due to the intrinsic complexity of each PC this procedure should not be used to describe definitive changes in any given area of the body. Winans (1984) and Winans and Nishioka (1987) respectively proposed that smolting in chinook and coho salmon was associated with differential growth of the caudal peduncle [i.e. post-anal area], indicated by differences in their PCII. However, in both these studies, PCII contained strong positive and negative loadings for measurements in all areas of the body, and thus changes in this PC cannot be unequivocally attributed to growth in any one part of the body. Therefore, in the present study, after an initial investigation of body shape in juvenile Atlantic salmon using PCA highlighted this problem, the individual measurements (fig. 6.1) were analysed separately in order to describe accurately changes in body shape.

*Changes in body shape of $0^+$ juvenile Atlantic salmon*

Previous studies on the morphology of juvenile salmonids have tended to focus on the period immediately prior to and during seaward migration, but in the present study the whole period of fresh water residence was covered. Distances between morphological

features (fig. 6.1) were standardised so that if an area of the fish grew at the same rate as the whole fish (ie. isometric growth) then there would have been no variation over the sampling period. Given this fact a surprising number of standardised measurements did vary over the sampling period, especially outwith the period prior to seaward migration. For juvenile salmon irrespective of modal group, the relative proportions of different body areas changed from their first summer onwards. Relative head size tended to remain the same, the trunk and post-anal regions made up relatively less of the total length, whereas the length of the caudal fin increased out of proportion to the rest of the fish.

Thorpe (1977) demonstrated in hatchery-reared juvenile Atlantic salmon different life-history strategies which were characterized by the length of fresh water residence. Although this is now a generally accepted phenomenon, and has been demonstrated in wild populations (Heggenes and Metcalfe 1991; Nicieza *et al.* 1991), the present study is the first detailed comparison of the actual body shape of fish from these two modal groups.

Figures 6.14 - 6.30 show that the fish sampled in August (when the bimodal length frequency distribution had only just become apparent) had standardised measurements that were similar for both modal groups. Although in some body measurements $0^{+}$LMG fish at this time were relatively shorter than $0^{+}$UMG fish, this was

never significant and so both modal groups in August can be regarded as having similar shaped bodies. However, after this summer sample a divergence in the shape of the two modal groups became apparent. Prior to smolting by the $0^+$UMG fish, differences in body shape between the $0^+$ modal groups could be characterized thus: $0^+$LMG fish had relatively larger jaws and longer, but not deeper, heads than $0^+$UMG fish; $0^+$UMG fish maintained longer, deeper trunk regions than $0^+$LMG fish; $0^+$UMG fish had longer sites of attachment of dorsal fins, but shorter sites of attachment of anal fins than $0^+$LMG fish; and fish from the $0^+$UMG had longer caudal fins than the $0^+$LMG fish. One curious pattern is the sudden, temporary change seen in the body proportions of the $0^+$LMG fish in February. Jaw size, head depth and head length (standardised measurement 2-4), and tail length decreased, whereas anterior trunk length and post-anal length both increased. It is not clear whether this is in any way adaptive or whether it is simply an artefact inherent in the sample taken at this time.

By the time of seaward migration in S1 smolts (previously $0^+$UMG), there were fewer differences between the standardised measures of the modal groups. $0^+$UMG fish had relatively deeper, but not longer heads and still maintained deeper mid trunks than $0^+$LMG fish, although the increase in relative growth rate in the latter group had cancelled out most of the previous differences. The length of the post-anal region was

similar in smolts and $0^{+}$LMG fish as it had been throughout the sampling period, but a spring increase in tail growth in $0^{+}$LMG fish cancelled out the previous difference in tail length between smolts and $0^{+}$LMG fish.

*Influences on body shape*

Romanov (1976; 1980) demonstrated that the shape and proportion of bones in the skulls of Pacific salmon varied throughout the development of the fish, from embryos to adults. Romanov (1984) also showed that differences in skull bones that related to feeding differed between hatchery and wild populations of juvenile masu salmon (*O. masou*). He suggested that the size of the mouth cavity was smaller in hatchery reared fish due to the relatively smaller size of food particles they consumed compared to wild fish. This supported the view of Aleev (1963) that it was primarily the size of food particles that determined the relative size of the mouth in fish. If this is true it reflects a very specific and sensitive growth response in relation to the environmental variable of diet composition, and could provide a possible reason for the divergence in jaw sizes seen between the modal groups within the hatchery-reared population in this study. Wankowski and Thorpe (1979) found that maximum growth of juvenile Atlantic salmon was achieved with food particles with a diameter of 0.02 to 0.026 of the forklength of the fish, and that this was true for fish

throughout their freshwater life (ie. of differing sizes).

Such a morphological response to diet occurs to an extreme degree in the population of Arctic charr (*Salvelinus alpinus*) in lake Thingvallavatn, Iceland. Genetically indistinguishable individuals have been shown to form different morphs dependant upon habitat utilization and prey type (Malmquist *et al.* 1992). Benthic morphs, which tended to prey on snails, had deeper bodies, larger pectoral fins and a mouth on the underside of the head. On the other hand limnetic morphs which concentrated on zooplankton when small and were piscivorous when larger, were more fusiform, had smaller pectoral fins, a terminally-situated mouth, a larger number of gill rakers, and may even have had eyes which were better adapted to the detection of moving prey (cited in Malmquist *et al.* 1992).

The growth of subordinate juvenile Atlantic salmon is suppressed by dominant individuals and more competitive fish are more likely to enter the UMG (Metcalfe 1989 and 1991; Metcalfe *et al.* 1989). Hence, the smaller relative jaw size of the dominant $0^{+}$UMG fish prior to smolting may be due to these fish feeding on the optimum prey size whereas, due to their relatively subordinate positions in the dominance hierarchy $0^{+}$LMG fish may not enjoy the luxury of selecting particles of an optimal size. In this situation it would make more sense for $0^{+}$LMG fish to have a large jaw so as to allow capture and consumption

of as large a range of prey sizes as possible. It would be energetically more profitable for LMG fish to concentrate on larger food particles to as great an extent as possible since, although rarer, these have a higher energy content and thus provide a better energetic return on the energy expended in capturing the food particle (Wankowski and Thorpe 1979). In addition, if LMG fish were to concentrate on capturing smaller food particles there would be the added danger that the relatively low amount of energy then available may be insufficient to offset the cost of capture. It must be remembered, however, that in absolute terms the jaw size of $0^{+}$UMG fish remained larger than that of $0^{+}$LMG fish throughout the sampling period. Alternatively <u>if</u> jaw size does change in response to diet this difference in relative jaw size could be due to the hatchery practice of matching pellet size to the <u>mean</u> size of the tank population. Thus $0^{+}$UMG fish would get smaller than optimum pellet size and $0^{+}$LMG fish would get larger than optimum pellet size. One quick method of testing the latter theory would be to compare the relative jaw size of $0^{+}$ salmon from a wild environment where the size and shape of prey items is much more variable.

Romanov (1984) also suggested that a reduction in part of the skull of hatchery fish demonstrated advanced development of the muscle associated with swimming ability. However, this supposition is questionable since it has been shown for a variety of salmonids that

wild fish can withstand higher water velocities than hatchery-reared fish (Bams 1967; Rimmer et al. 1985). Winz (1986) demonstrated a 1 bodylength per second difference in CHV in favour of wild strains of rainbow trout compared with domesticated strains and suggested that this could be due to a reduced selection for sustained swimming in hatchery environments.

Further evidence for very specific growth responses to food availability was provided by Currens *et al.* (1989). They showed that in chinook salmon and rainbow trout (*Oncorhynchus mykiss*) trunk depth (but not length) was most affected by food availability, whereas the post-anal region was relatively unaffected. (The size of the caudal fin region was not investigated in this study). These results are in agreement with the observations that the dimensions of body shape, especially body depth, indicate nutritional status (Theilacker 1978) and that the trunk region was the site of greatest lipid deposition and loss during periods of feeding and fasting respectively (Miglavs and Jobling 1989). Also lipid deposits in the viscera were the first to be mobilized at the onset of starvation (Jezierska et al. 1982), which could result in further changes in trunk depth in addition to those caused by a differing growth rate of this area. Although mesenteric fat is the most mobile of lipid depots, no effect of mesenteric lipid level was detected in the ability to hold station against a water current.

*Mesenteric lipid levels*

The seasonal pattern of mesenteric lipid storage from the present study (fig. 6.31) was in agreement with the afore mentioned studies in being closely associated with the differing feeding behaviours observed between modal groups. The higher feeding rate seen in $0^{+}$UMG fish (Higgins and Talbot 1985) resulted in the level of mesenteric lipid being significantly higher in these fish than in $0^{+}$LMG fish. The increased level of lipid reported in larger fish (Groves 1970; Jensen 1980) was also observed in $0^{+}$UMG fish compared to $0^{+}$LMG fish (fig. 6.31). Also the peak in mesenteric lipid seen in November in $0^{+}$UMG fish is closely associated with the sudden increase in feeding and growth rates seen in these fish during October (Kristinsson *et al.* 1985; Metcalfe *et al.* 1988). The relatively greater amount of mesenteric lipid seen in $0^{+}$UMG fish may, therefore, be partly responsible for the increased standardised trunk measurements of these fish (figs 6.14 - 6.30).

*Functional consequences of body shape*

Webb (1978) suggested that deeper, more robust bodies yield higher burst swimming velocities, and this has been demonstrated in coho salmon (Taylor and McPhail 1985), whereas more fusiform individuals have a greater sustained swimming performance. Obviously, in order to feed at a higher rate than LMG fish, the UMG fish would have to perform burst swimming more frequently to intercept a larger number of prey items, an activity to

which their deeper bodies would seem well suited. If the level of activity is correlated with morphology, the potential for natural selection to affect the body shape of UMG fish for the reasons outlined above is apparent. $0^+$LMG fish show a greatly reduced feeding rate over winter (Higgins and Talbot 1985) which results in little or no growth, and typifies the general cost-minimising strategy that these fish demonstrate. Indeed there is circumstantial evidence to suggest that $0^+$LMG fish would spend a much greater proportion of time at low temperatures sheltering in crevices in the substrate. Rimmer et al. (1983) showed that when the temperature in a Canadian stream dropped below 10°C, over 90% of $1^+$ and $2^+$ juvenile Atlantic salmon moved into shelters within the streambed. Since the mean age of Atlantic salmon smolts in this region is around $3^+$ (Metcalfe and Thorpe 1990), it is reasonable to assume that the fish that sheltered in the substrate were those that would remain in fresh water for a further year before migrating to the sea. Recent initial observations on $0^+$UMG and $0^+$LMG juvenile Atlantic salmon have confirmed that it is LMG fish that show a greater sheltering over winter at low temperatures (Huntingford and Thorpe pers. obs.). The greater amount of sheltering, lower metabolic rate (Higgins 1985), reduced feeding and general reduction in activity on the part of $0^+$LMG fish may lead to body morphology being less affected by the environment than in UMG fish during their first year.

However, Fraser (1994) has shown that relative burst swimming velocities were greater in $0^{+}$LMG Atlantic salmon than in $0^{+}$UMG fish at higher temperatures, but at low temperatures there was no difference. Hence, whether there is a functional consequence of the differing body shapes of the modal groups in juvenile Atlantic salmon remains unclear.

Juvenile Pacific salmon tend to oppose the water current by swimming actively (Hoar 1976; Taylor and Foote 1991), whereas juvenile Atlantic salmon tend to hold position on the substrate (Keenleyside 1962; Rimmer *et al.* 1985) by angling their pectoral fins to provide negative lift (Arnold and Webb 1991). This functional difference between the genera leads to an important difference in the size related ability to oppose water currents. In both anadromous and non-anadromous sockeye salmon (*O. nerka*) larger individuals had higher relative swimming velocities than smaller individuals (Brett and Glass 1973; Taylor and Foote 1991), whereas juvenile Atlantic salmon displayed no difference in relative CHV between the different sized modal groups (chapter 3). Taylor and Foote (1991) also showed that the anadromous forms of sockeye salmon had a superior sustained swimming ability compared to the non-anadromous forms, and suggested that this was due to the anadromous fish having shallower trunks and longer caudal (post-anal) regions than non-anadromous forms, although again tail size was not investigated.

A superior sustained swimming ability has also been shown for the more streamlined inland populations of juvenile coho salmon in comparison with the deeper bodied coastal populations, which in turn had greater burst swimming velocities. It was suggested that these differing morphologies and the associated different swimming characteristics were adaptations to the long migration distances encountered by the interior populations, and the possibly greater predation pressure experienced by the coastal populations (Taylor and McPhail 1985). Swain and Holtby (1989) also showed greater sustained swimming velocities of juvenile coho salmon in the fusiform, stream-dwelling type compared to the deep-bodied lacustrine forms. In view of these studies it was maybe not surprising that in the present study it was not the $0^{+}$UMG or $1^{+}$LMG fish but the $0^{+}$LMG fish that demonstrated a relationship between body shape and the ability to hold station on the substrate. Fish with relatively longer mid-trunk regions and shorter posterior trunk regions had higher CHV values, possibly since the mid-trunk region is in contact with the substrate to a greater degree than the posterior trunk region during station holding.

In Pacific salmon the posterior segments are involved in propulsive undulations during swimming (Taylor and McPhail 1985). Taylor and Foote (1991) suggested that the increased caudal length of anadromous sockeye salmon and sustained superior swimming performance relates to the long seaward migrations undertaken by

these species. This is also the view taken by Thorpe *et al.* (1981), who suggested that selection may have favoured more active migration in lacustrine species because of the lack of water movements as a strong directional cue in downstream migration from lakes. In contrast, figure 6.26 shows that there was no difference in the post-anal regions between $0^{+}$UMG and $0^{+}$LMG juvenile Atlantic salmon, reflecting the lack of reliance on actual swimming to oppose water current that is demonstrated by this species. What is of great interest in the present study is the lack of difference between modal groups in what has been assumed to be an important area in relation to the differing life history strategies. "Smolts are differentiated in the first place by considerably greater length of the wrist (a feature, as is well known, which has great importance for overcoming resistance to currents: a short thick wrist makes for naturally good swimming when living in a rapid current)" Nikolskii *et al.* (1947). Figure 6.26 also graphically illustrates that it is not the post-anal region that elongates when $0^{+}$UMG parr become smolts.

*Comparison of body shape with other salmonids*

Smolting salmonids, including Atlantic salmon, have been reported to become more streamlined as a result of a fall in the length to weight ratio, which is demonstrated as a decrease in the condition factor (Wankowski and Thorpe 1979). In some species of Pacific

salmon (Winans 1984; Winans and Nishioka 1987; Taylor and Foote 1991) and Arctic charr (Damsgard 1991) smolting has been associated with the elongation of the post-anal region, and this has also been reported for some populations of Atlantic salmon (Nikolskii et al. 1947; Evropeitseva 1957). However, the results from the present study indicated no such phenomenon in juvenile Atlantic salmon from the River Almond (Scotland). The condition factors of $0^{+}$UMG fish showed a general decline through to February and then remained unchanged through to May, whereas the mean condition factor of $0^{+}$LMG fish showed a drop over the winter months, before increasing through spring (fig. 6.5). This resulted in smolts and $0^{+}$LMG fish having similar condition factors by May, although this does not necessarily mean that these fish were by this time developing along similar lines; those of the $0^{+}$UMG fish had remained at a continuous level since February, whereas the condition factor of $0^{+}$LMG fish had continually increased over the same time period. However, figure 6.25 shows that there was no sudden increase in the post-anal lengths of the migrating fish in May, and so the only differences in smolting fish relative to the $0^{+}$UMG parr of previous months were an increased ventral-dorsal anterior trunk diagonal (3-6, fig. 6.18) and mid-trunk depth (5-6, fig. 6.19). Fessler and Wagner (1969) showed that the spring decrease in the relative caudal peduncle depth occurred both in migrant and non-migrant steelhead trout and was not temporally associated with smolting.

The cause of the dichotomy between the lack of change in post-anal lengths in smolts in this study and the increased post-anal lengths seen in smolting Atlantic salmon in the Pechora River, in the Urals of Russia (Nikolskii *et al.* 1947) is not known. It could be a genetic effect that is associated with the length of seaward migrations within each population, or further evidence of the developmental plasticity of juvenile Atlantic salmon observed in this and other studies. For example Hoar (1939) showed that length-weight relationships in juvenile Atlantic salmon varied considerably from place to place, year to year and also within a year.

From the data collected in the present study it appeared that $0^{+}$UMG parr over most of the sampling period were typified by longer and deeper anterior trunks (figs 6.18 and 6.19) in comparison with $0^{+}$LMG parr. However, increased growth in the anterior, mid-, and posterior trunk regions, and the tail regions in $0^{+}$LMG fish resulted in both migrating and non-migrating fish having generally similar shaped bodies in May, although smolts did retain slightly longer mid-trunks. The major morphological differences between modal groups were seen over autumn and winter, when the behavioural differences associated with the life-history strategies were most pronounced, rather than during the period of seaward migration of the upper modal group.

*Body shape and morphology of $1^+$ juvenile Atlantic salmon*

$1^+$LMG fish showed a seasonal pattern of changes in condition factor (fig. 6.5) that corresponded to the "classical" idea of how length-weight relationships vary in smolting salmonids. Over the winter months the mean condition factor of $1^+$LMG fish remained unchanged and was also significantly higher than the condition factors of either $0^+$UMG or $0^+$LMG fish. This indicated that $1^+$LMG fish were relatively heavier than $0^+$UMG fish (both destined to smolt in the following May).

It has been suggested that one of the causes of reported decreases in condition factor in smolting fish is the loss of lipids (Hoar 1939; Fessler and Wagner 1969; Woo *et al.* 1978). The results from the present study indicate that this is not the case when considering mesenteric lipid levels alone, since the increasing lipid levels in November and April (fig. 6.31) do not produce corresponding changes in condition factors (fig. 6.5) for $0^+$UMG and $1^+$LMG fish respectively. The seasonal levels in mesenteric lipid in $1^+$LMG fish were also different to those seen in either of the $0^+$ modal groups, and may reflect an age-dependant pattern of energy use and storage in juvenile salmon.

Okland *et al.* (1993) showed that slower-growing Atlantic salmon within a population (analogous to LMG fish) smolted as older and larger fish than the faster growing (UMG) fish, due to their spending a further

year feeding in fresh water. They suggested that the advantage of this slower growing strategy, which helped to balance the cost (in terms of lost opportunity for growth) of this extra period in fresh water, was that larger fish coped better with the osmotic stress and increased metabolic demands experienced during smolting. Feltham (1990) has also shown that, maybe surprisingly, the larger smolts migrating downstream were preyed on less by an avian predator than were the smaller smolts and parr.

The overall body shape of $1^{+}$LMG fish tended to show less seasonal variation than those of the $0^{+}$ fish, however, there were still intermodal size differences apparent.

Figures 6.14 - 6.17 showed that the standardised measurements of jaw size, head length and head depth of $1^{+}$LMG fish were similar to $0^{+}$LMG fish and greater than those of $0^{+}$UMG fish. The reason for this pattern is not clear, but it may again be related to the dominance-related feeding behaviour postulated above. Clearly behavioural tests should be undertaken to decide whether the subordinate role of $0^{+}$LMG fish is carried on into their second year of freshwater residence.

In terms of measurements from the trunk regions, there were few instances of $1^{+}$LMG fish differing from either of the other types of fish - even when there were significant differences between $0^{+}$UMG and $0^{+}$LMG fish. In general, $1^{+}$LMG fish could be viewed as intermediate in the shape of their anterior and mid-trunks. The

posterior trunks of $1^{+}$LMG fish were, however, shorter than those of $0^{+}$UMG fish and similar to $0^{+}$LMG fish. The same pattern was seen in the length of the site of attachment of the dorsal fin, with $0^{+}$ and $1^{+}$ LMG fish again having relatively shorter fins than $0^{+}$UMG fish. Again no obvious reason is apparent for this difference, although it may be worth noting that dorsal fins are used in territorial displays in White Cloud Mountain minnows, *Tanichthys albonubes* (Magurran and Bendelow 1990).

As has been previously stated, it is maybe unwise to infer too much from the pattern of caudal fin size seen in $1^{+}$LMG fish due to the amount of erosion that can be present after almost two years in a tank environment.

So it would appear that in many ways, even after a further year, LMG fish are still different from UMG fish, although these differences largely disappear by smolting.

*Significance of present results*

The data presented in this study indicate a difference in body shape of juvenile Atlantic salmon at smolting compared to the results of previous studies. The condition factors of $0^{+}$UMG and $1^{+}$LMG fish in this study showed a decline by May, and the levels over the whole sampling period (1.0 - 1.3) were in the same region as those previously reported for Atlantic salmon parr over the period of freshwater residence by Komourdijian *et al.* (1976) and Kristinsson et al. (1985), but larger

than the range of condition factors (0.8 - 1.1) reported for parr by Saunders and Henderson (1970) and Sosiak (1982). It is possible that the fish in the present study were actually undergoing a process of "desmolting" by the time of the May sample, or alternatively had been following the strategy which leads to sexual maturity whilst in fresh water. Although the latter possibility cannot be ruled out completely for $1^{+}$LMG fish, $0^{+}$ fish from the same population and sampled at the same time as the $0^{+}$UMG fish in this study showed no evidence of sexual maturity on dissection. The CHV results presented in chapter 3 also showed that both $0^{+}$UMG and $1^{+}$LMG fish displayed an identical and typically smolt-like reduction in their relative current holding ability during May compared to the $0^{+}$LMG fish. Therefore, although the levels of mean condition factor and mesenteric lipid in $0^{+}$UMG and $1^{+}$LMG fish did not appear "smolt-like" in comparison to other studies, the pattern of CHV and the lack of gonadal growth (that was apparent during the dissection of these fish to provide tissue samples for chapters 4 and 5) are typical of smolting fish.

Another possible explanation for the difference between this and other studies is that it reflects the plasticity of growth and development in this species in response to differing environmental conditions; this plasticity is not only evident in populations inhabiting different geographical areas, but also in

different individuals within the same population. Thus, for the Atlantic salmon stock used in this study, the major morphological difference between potentially migrating and non-migrating salmon was not seen at "smolting" but throughout the first year in fresh water. This may be functionally related to the relatively short river down which these fish must migrate, and to the different behaviours exhibited by juvenile salmon following different life history strategies.

## References

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# CHAPTER 7

# GENERAL DISCUSSION

## Summary

This thesis was designed to investigate various aspects of biochemistry or a physiological process that might be related both to the seasonal ecology of juvenile salmon as a whole and to the different life history strategies apparent within a single sibling population. Investigations were carried out into 3 main areas concerning the physiological ecology of juvenile salmon:

1. **The ability to hold station against a water current** - this is related to habitat utilization and provides a means for comparing the relative physiological performances of the modal groups.

2. **The biochemical adaptation to seasonally varying temperatures** - this was related to the genetic make-up of Atlantic salmon and allowed the demonstration of a system of temperature adaptation that has been described and/or suggested for other salmonids. It also focussed on two enzymes (LDH and G6PDH) which are each involved in metabolic pathways that can be directly related to ecological aspects of juvenile salmon behaviour.

3. **The development of body shape and the level of mesenteric lipid throughout the period of fresh water residence** - this allowed a comparison of how

the different developmental decisions affected the gross morphology of these fish, how differences in body shape actually related to the differing modal strategies, and a comparison of seasonal energy storage between modal groups.

All modal groups showed a seasonal temperature-related variation in the ability to hold station on the substrate against a water current. $0^{+}$UMG fish had consistently higher absolute holding velocities (until the period of smolting) than $0^{+}$LMG fish, with the resultant possibility of differential habitat utilization. There was no difference between the different sized modal groups in their relative ability to hold station between August and April, indicating that $0^{+}$LMG fish are as physiologically capable as UMG fish. In May, however, during the period when UMG fish would have been migrating downstream as smolts, LMG fish had significantly higher relative current holding velocities. This was due to the CHV of $0^{+}$LMG fish increasing with temperature over the spring months, whereas that of $0^{+}$UMG fish did not differ from the level recorded throughout winter. $1^{+}$LMG fish displayed the same pattern of relative CHV throughout their second year in fresh water and into the period of smolting as that seen in $0^{+}$UMG fish.

In terms of the efficiency of metabolic pathways, juvenile salmon produced enzymes (LDH and G6PDH) which showed seasonal changes in kinetic characteristics such

that the efficiency of these enzymes was greatest at the environmental temperature the time of sampling, indicating the production of different isozymes. There was no difference in the efficiency of LDH between modal groups, and both modal groups displayed an increased efficiency at lower environmental temperatures. Only during winter was there a difference in G6PDH between modal groups, with UMG fish having an apparently less efficient enzyme than LMG fish. This could possibly have been adaptive in relation to the differing ecologies of these fish.

The morphology of juvenile salmon indicated a large variation in body shape both within and between modal groups over the sampling period (August - May). The major difference between modal groups seemed to occur over the winter period when UMG fish tended to have longer, deeper trunks than the LMG fish. Another interesting aspect of body morphology was that throughout the sampling period LMG fish had relatively larger jaws than their UMG sibs.

**Behavioural consequences of life-history strategies**

The phenomenon of different life history strategies which results in different ages at smolting in juvenile Atlantic salmon has been known for some time (Thorpe 1977; Bailey *et al.* 1980), but it is only more recently that this has become a generally accepted fact. However, there is still debate as to the length of the critical period (the smolting window) during which the

salmon make the physiological "decision" as to which growth strategy they will follow during their next year in fresh water. Villareal (1983), Metcalfe *et al.* (1986) and Thorpe *et al.* (1989) have reported that for salmon in Scotland the divergence of strategies into modal groups occurs shortly after midsummer and become fixed soon after. However, rather than basing the future year's growth strategy on a physiological decision made over a short period during summer and dependant upon the then current status of energy provisions (Thorpe 1986), it would seem to make sense for juvenile salmon to be able to react to changes in environmental conditions. Thus LMG fish confronted with exceptionally mild conditions (such as high winter temperatures) and/or increased food availability (either through increased amounts of food or an increased opportunity to feed) might be expected to take advantage of these improved conditions to maintain growth over the winter and migrate to sea as smolts in the following spring: in effect changing to the fast growth strategy typified by UMG fish. The problem with this theory is that pre-LMG fish actually begin to show a reduction in appetite (Metcalfe *et al.* 1986, 1988) prior to the onset of the harsher winter conditions. Also once the developmental strategy has become fixed LMG fish will maintain a low level of appetite even when presented with food in abundance (Metcalfe *et al.* 1986), and so any improvement in environmental conditions would have to act as a "trigger" on the

neuroendocrine system to allow LMG fish to increase their growth rate.

The opposite situation is maybe more likely to result in modal cross-over. The energy stores of immature brown trout are most severely depleted by late winter (Cunjak 1988). Thus if UMG fish are faced with a very harsh and prolonged winter it could be argued that it would make sense for those with lower levels of energy reserves not to continue with this strategy since their reserves may become severely depleted, or to undertake migration since the process of smolting is considered energetically expensive.

A further option for UMG fish faced with harsh conditions might be to migrate downstream during May, but then spend time feeding in the estuary and increasing energy reserves prior to a decision on whether to complete the smolting process either during the autumn or following spring (Cunjak *et al.* 1990).

Under hatchery conditions the differing behaviour and ecology demonstrated by the modal groups tend to act against modal cross-over. The UMG fish are more dominant, compete for food more effectively and are thus able to maintain growth throughout the year. In contrast the more subordinate LMG fish, do not compete as effectively for food and, as has been described above, show a reduced food intake due to a reduction in appetite and also a reduced metabolic rate over autumn and winter. Their refusal of food (Metcalfe and Thorpe 1992) is indicative of the cost-minimising strategy

that these fish exhibit, and would not be sufficient for an increase in growth rate to the point where these fish would in effect switch modal groups.

Stewart *et al.* (1990) have shown that increased daylengths over autumn and winter caused an increase in the proportion of fish entering the upper modal group due to the increased photoperiod stimulating the appetite of fish in the middle size class. Whitesel (1993) reared LMG fish (separately from UMG fish) at temperatures of 10°C and then segregated these fish in February into two groups around the mean forklength. The result was that the larger fish continued to grow and, after being introduced into a stream in April, migrated to sea in May as S1 smolts.

These studies demonstrated that the smolting window is open to environmental manipulation, and although photoperiod is highly unlikely to alter in the wild, it is not inconceivable that high winter temperatures may occur, and indeed the recruitment of LMG fish into the UMG has been shown to occur at water temperatures of 10°C (Kristinsson *et al.* 1985). In conjunction with high water temperatures, a reduction in competition for food may also arise either from the smaller LMG fish emigrating to feed in different areas of the stream, or from an increased food supply or opportunity to feed, thus partially negating the effect of dominance hierarchies on food intake.

The higher densities and more stable environmental conditions found in hatcheries may cause life history

strategies to be more pronounced and less flexible, and thus the factors causing the adoption of a specific life history strategy would constantly be reinforced. In contrast, the more diverse environmental and social conditions possible in a wild environment may allow a greater flexibility of life history strategy on the part of each individual juvenile Atlantic salmon - a suggestion shared by Nicieza *et al.* (1991).

**Physiological considerations**

The results of the investigations on enzyme catalysis (chapters 4 and 5) indicate that there was virtually no difference in the efficiency of LDH or G6PDH between the modal groups throughout the first year of fresh water residence. The only difference was seen in G6PDH in winter when UMG fish had higher Km values than LMG fish. This enzyme is part of the pentose phosphate pathway which is involved in lipid biosynthesis. Since UMG fish show a higher temperature-specific food intake with consequent higher growth rates and higher relative levels of body lipid, the higher winter $K_m$ values may simply be an adaptation to the higher substrate levels. Hence the greater temperature-related reduction in metabolic rate, gut evacuation rate and lower conversion efficiency displayed by LMG salmon in comparison with UMG fish (Higgins 1985) appears to be as a result of the adoption of this cost-minimising strategy rather than part of the cause.

**Adoption of life-history strategies**

If held under good growth conditions (elevated water temperatures, good food supply, low levels of competition), it is possible for the vast majority of a sibling population either to become mature when still in fresh water or to become S1 smolts and migrate to sea after 1 year in fresh water (Thorpe 1989). Similarly, if pre-LMG fish are segregated before the developmental pathway becomes fixed and then reared separately from the UMG fish, a proportion of these previously LMG fish will smolt in their first spring (Whitesel 1993). This indicates that all juvenile salmon have the potential to maintain appetite and, therefore, growth and smolt after 1 year, since their development is determined largely by environmental conditions (reviewed in Thorpe *et al.* 1992). LMG fish contain genes that code for enzymes that are as efficient as those in UMG fish, and LMG fish do not "switch off" because they are intrinsically physiologically less capable than their more dominant siblings. Further evidence for this point of view comes from two unrelated studies on muscle growth (Higgins 1985) and critical holding velocity (chapter 3).

Initial muscle growth in unimodal juvenile Atlantic salmon was by an increase in fibre size (hypertrophy), whereas after the developmental split has occurred growth of UMG fish was by an increase in fibre numbers (hyperplasia) with mean fibre size remaining relatively constant (Higgins 1985). Over the same period LMG fish

only showed relatively slight increases in fibre numbers and little change in fibre size, which was consistant with their cost-minimising strategy of little or no growth over their first autumn and winter. However, $1^{+}$LMG fish (i.e. in their second and final year prior to smolting) demonstrated the same trend of hyperplasia throughout the year as that seen in $0^{+}$UMG fish.

Similarly figures 3.2b and 3.3b show that between August and April there was no difference in the relative ability to hold station against a water current in $0^{+}$UMG, $0^{+}$LMG and $1^{+}$LMG fish despite the large size differences between these groups. The similarity between $0^{+}$UMG and $1^{+}$LMG fish is particularly striking in May when both show a decline in CHV concurrent with the period of seaward migration relative to the LMG fish.

However, despite the fact that all juvenile salmon are potentially capable of sustaining growth at low temperatures, this does not mean that metabolic rate does not have an effect on the adoption of developmental pathway. Ferguson *et al.* (1987) have shown that rainbow trout, *O. mykiss*, that possess the mutant allele Pgm1-t(b) in their liver display a vastly increased activity of the enzyme phosphoglucomutase (PGM: EC. 5.4.2.5) and display earlier hatching and emergence from the redd, as well as being larger and more aggressive than their siblings that do not possess this allele. The earlier hatching and emergence is due

to the increased glycolytic flux in the livers of embryos containing Pgm1-t(b), which results in increased developmental rates of these fish (Allendorf *et al.* 1983). It is this faster development in embryos that may ultimately lead to dominance hierarchies among parr. Earlier emerging fry would have a greater chance of obtaining and then defending a feeding territory and thus would be dominant in relation to the later emerging fry, since resident animals have been shown to display greater aggression than intruding animals when defending their territories (Huntingford and Turner 1987). The aspect of natural selection acting against runaway earlier emergence of fry is the greater threat of predation on these more obvious individuals (Ferguson *et al.* 1987) compared to the dilution effect obtained from mass synchronized hatchings (Brånnas 1988).

Such an effect, although maybe more pronounced by the effect of a mutant allele, may also be possible simply via natural variation in metabolic rates. Recently juvenile Atlantic salmon with higher inherent metabolic rates have been shown to be more dominant with respect to those with lower metabolic rates regardless of hatching date (Metcalfe *et al.* in press) suggesting that higher metabolic rates may make fry intrinsically better "competitors". However, these experiments were conducted in paired trials, so when hatching in a redd, those fish with higher developmental rates would also tend to emerge earlier. Thus the phenomenon of prior

ownership of territories described above would serve to enhance the aggression of these fish and, therefore, their higher status within the dominance hierarchy (Metcalfe and Thorpe 1992b).

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