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EARLY THERMAL EXPERIENCE HAS DIFFERENT EFFECTS ON GROWTH AND MUSCLE FIBRE RECRUITMENT IN SPRING- AND AUTUMN-RUNNING ATLANTIC SALMON POPULATIONS

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Summary

The consequence of early thermal experience for subsequent growth patterns was investigated in Atlantic salmon (*Salmo salar* L.). Spring- and autumn-running salmon were caught in upland (Baddoch) and lowland (Sheeoch) tributaries of the River Dee, Aberdeenshire, Scotland, respectively, on the final stages of their spawning migrations. The eggs were incubated at the simulated natural temperature regime of each stream, which was on average 2.8 °C lower for the Baddoch. The offspring, representing 11 families per population, were transferred at first feeding to constant environmental conditions (12–14 °C; 16 h:8 h light:dark photoperiod) and reared in replicate tanks. Salmon of both populations were longer and heavier at 6 and 12 weeks in fish initially reared under the cooler Baddoch regime. Length frequency distributions became bimodal after 18 weeks, and only the upper growth mode was studied. Modelling of length distributions at 40 weeks revealed significantly different patterns of muscle growth according to initial temperature regime, but only for the Sheeoch salmon. In fish of Sheeoch origin, significantly more white muscle fibres were recruited per mm^2 increase in myotomal cross-sectional area at Sheeoch than at Baddoch temperatures (P<0.01). After 40 weeks, the density of white fibres was 10.4 % higher in fish initially reared at the Sheeoch (533±6 mm^{-2}) than at the Baddoch (483±5 mm^{-2}) thermal regimes (means ± S.E.M., 16 fish per group; P<0.001). Muscle satellite cells were identified using an antibody to *c*-met. At 24 weeks, the density of muscle satellite cells was 29 % higher in Sheeoch salmon reared to first feeding at the temperature of their natal stream than at cooler Baddoch temperatures (P<0.01). In contrast, the number and size distributions of white muscle fibres in the myotomes of Baddoch salmon were independent of early thermal experience.

Key words: skeletal muscle, growth, muscle fibre recruitment, muscle satellite cell, myogenic regulatory factor, temperature, fish population, Atlantic salmon, *Salmo salar*.

Introduction

Atlantic salmon (*Salmo salar* L.) usually migrate back to their natal rivers to spawn (Stabell, 1984). Within large river systems, there is often a patchy distribution of sites suitable for spawning and as habitat for fry. Tagging and radio-tracking studies indicate that the fidelity of the homing migration extends to particular tributaries within the river system (Saunders, 1967; Youngson et al., 1994). Within the Aberdeenshire Dee, spring-running fish tend to spawn in upland tributaries of the catchment, whereas fish that enter the river later in the year spawn in lowland areas (Youngson et al., 1994). The reproductive isolation of populations allows for genetic divergence and might favour the evolution of traits that maximize fitness under different selection regimes (Verspoor, 1997; Nielsen, 1998). The existence of multiple populations of salmon within river catchments is strongly supported by studies of variation in allozymes, mitochondrial DNA and DNA microsatellites (see Verspoor, 1997). Evidence for the local adaptation of populations both between and within rivers is more controversial and based on inference. For example, heritable differences in body morphology have been correlated with tributary flow characteristics, with fish originating from faster-flowing streams having more streamlined bodies and larger fins than fish from slower-flowing streams (Riddell and Leggett, 1981; Riddell et al., 1981). Heritable differences in egg mortality at low pH have also been found between populations of salmon from the Kyles of Sutherland river system in north-east Scotland that were correlated with differences in water chemistry within the catchment (Donaghy and Verspoor, 1997).

Temperature also varies in a systematic fashion within the catchment of the major rivers in north-east Scotland because of differences in altitude. For example, within the

Aberdeenshire Dee, headwater streams are significantly cooler than streams lower down in the catchment except during mid-summer (Shackley and Donaghy, 1992). Webb and McLay (1996) studied spawning and fry hatch times in relation to temperature at five sites over 120 km of the River Dee ranging from 400 m altitude in a headwater tributary to 5–10 m above sea-level near the river mouth. They found that spawning occurred progressively later at sites farther down the river system, with no overlap in the spawning period between the most distant sites. Differences in spawning date to a large extent compensated for the colder water temperature in the upland streams (Webb and McLay, 1996). It was suggested that these differences between populations were of adaptive significance for maximizing growth and reflect strong selective pressure on fry emergence to coincide with favourable conditions of temperature, water flow and food availability.

Muscle composition in salmon fry has also been shown to vary with egg incubation temperature (Stickland et al., 1988; Johnston and McLay, 1997). Eggs reared at low ambient temperatures produce fry with a higher number of white muscle fibres than eggs reared in heated water (Stickland et al., 1988; Usher et al., 1994; Johnston and McLay, 1997). In the accompanying paper (Johnston et al., 2000b), the response of muscle cellularity to temperature in alevins was found to vary with source population. Eggs from salmon spawning in a highland (Baddoch) and lowland (Sheeoch) tributary of the River Dee were incubated at the temperature of each stream, which was on average 2.8 °C cooler for the Baddoch. At first feeding, the cross-sectional area of white muscle at the adipose fin was relatively independent of the thermal regime in Sheeoch salmon, but was 30.5 % greater at Baddoch than at Sheeoch temperatures in fish of Baddoch origin (Johnston et al., 2000b). Both the mean diameter and the number of white muscle fibres per trunk cross-sectional area were significantly greater at the cooler regime in Baddoch fish. In contrast, the density of muscle satellite cells was independent of egg incubation temperature in the Baddoch salmon, but significantly greater at Sheeoch than at Baddoch temperatures in fish of Sheeoch origin (Johnston et al., 2000b). Thus, the formation of embryonic muscle fibres and satellite cells showed different responses to temperature in alevins, which varied according to source population.

Muscle fibre recruitment continues throughout freshwater life in salmon (Higgins and Thorpe, 1990; Johnston et al., 1999) and involves the proliferation of the muscle satellite cells, a population of undifferentiated myoblasts formed in the late embryo (Koumans et al., 1991; Johnston et al., 1995; Stoiber and Sänger, 1996). The division products of the satellite cells provide nuclei both for myotube formation and for fibre hypertrophy. Triploid Atlantic salmon have fewer satellite cells than their diploid counterparts and also recruit fewer muscle fibres per mm^2 increase in muscle cross-sectional area (Johnston et al., 1999). In Atlantic herring (*Clupea harengus* L.), the rearing temperature to first feeding has been shown to influence both the density of presumptive muscle satellite cells (Johnston, 1993) and subsequent patterns of muscle fibre recruitment (Johnston et al., 1998).

The aim of the present study was to investigate the consequences of egg incubation temperature for muscle growth patterns in salmon of Baddoch and Sheeoch origin. In particular, we wished to test the hypothesis that variations in muscle satellite cell density in alevins would be reflected in altered patterns of muscle fibre recruitment during freshwater growth. Fish incubated at the simulated natural temperature regimes of the Baddoch and Sheeoch until first feeding were therefore transferred to constant environmental conditions to reveal any intrinsic differences in growth potential.

Materials and methods

Fish

The Baddoch Burn, an upper-catchment spawning tributary of the River Dee in Aberdeenshire, Scotland, is equipped with a permanent fish trap. In autumn 1996, 11 female two-sea-winter salmon and 11 males, a mixture of one-sea-winter and two-sea-winter fish, were trapped on the final stages of their spawning migration. The Sheeoch Burn is a lower-catchment spawning tributary. Broodstock, a mixture of one- and two-sea-winter fish (11 males and 11 females) were caught between 2 and 20 November by electro-fishing and netting a 750 m stretch of the stream. Fish were held in a temporary enclosure in the stream until ready to strip. Eggs from each female were fertilised with milt from a single male to produce 11 families from each source population. The eggs from each pairing were then split into two groups and reared at the Scottish Office's Almondbank Hatchery under the simulated temperature regime of either the Baddoch or the Sheeoch tributary, as described in H. A. McLay, I. A. Johnston, J. H. Webb and D. Robins (in preparation) and in the accompanying paper (Johnston et al., 2000b). The temperature regimes used were derived from temperatures recorded in the Baddoch and Sheeoch between October 1992 and June 1996. The data from each tributary were smoothed and used as a temperature time series for the incubation up to the point that fish were ready to feed.

At first feeding, families were combined according to population incubation temperature group (four groups), and two batches of 775 fry from each group were transferred to replicate 1 m diameter circular tanks (eight tanks) within a light-proof enclosure. The water supply to each tank, flowing at 2 l min^{-1}, was maintained at between 12 and 14 °C. The photoperiod regime was 16 h:18 h light:dark. Fish were fed to excess (5 % body mass day^{-1}) on a pelleted salmon diet. Automatic feeders delivered food during the light period. Growth was monitored at intervals up to 40 weeks post first feeding. First measurements were taken 6 weeks post first feeding. Fifty fish were netted at random from each tank, lightly anaesthetized in MS222, and fork length and body mass were recorded. Fish were then allowed to recover and returned to the tank. The mean fork length was calculated for fish in each tank, and eight fish of average length were selected from each. These fish were transferred alive to the Gatty Marine Laboratory, St Andrews, Scotland, and sampled immediately

for studies of muscle structure. This procedure was repeated 12 and 18 weeks post first feeding except that the mean body length and mass of fish in each tank were determined by weighing 100 individuals. At 20 weeks after transfer, the numbers of fish in each tank were reduced to 250 randomly selected individuals. Further measurements and samples were taken at 24, 32 and 40 weeks post first feeding. Beyond 18 weeks, the length frequency distribution of fish in each tank became bimodal, fish in the upper growth mode progressively developing the characteristics of smolts (Hoar, 1976). On these sampling occasions, length frequency distributions were constructed on the basis of measurements of 100 randomly selected fish from each tank, and the mean fork lengths of the upper and lower growth modes were calculated. Eight fish per tank, corresponding to the average fork length of the upper growth mode, were selectively sampled for analysis of muscle structure.

Analyses of muscle structure

Fish were killed by an overdose of anaesthetic (MS222) and by pithing the central nervous system. A 3 mm thick transverse section of the trunk was cut just anterior to the adipose fin. The section was divided into a series of labelled blocks that were frozen on cork strips in 2-methylbutane cooled to near its freezing point in liquid nitrogen (−159 °C). The blocks were stored in a liquid nitrogen refrigerator until they could be processed. Transverse 7 μm frozen sections were cut on a cryostat, air-dried and either stained with Scarba Red or haematoxylin/eosin or processed for immunohistochemistry. The outlines of white muscle fibres and the total cross-sectional area of white muscle tissue were digitized using an image-analysis system, and the equivalent muscle fibre diameters were calculated (Kontron, Switzerland).

Immunohistochemistry

The following anti-rabbit polyclonal IgG primary antibodies were used: *m*-met, MyoD, myf-5, myogenin and myf-6 (all supplied by Santa Cruz Biotechnology, Inc). Primary antibodies were diluted 1:20 (v/v) for *m*-met and 1:100 (v/v) for myogenic regulatory factors (MRFs) prior to use in a solution containing 1 % (v/v) Triton X-100 and 1 % (m/v) bovine serum albumin (BSA) (Sigma Chemicals, Poole, UK) in phosphate-buffered saline (PBS).

Sections were fixed for 10 min in 4 % (m/v) paraformaldehyde in PBS and processed for immunohistochemistry as described previously (Johnston et al., 1999). Dual labelling was carried out with two different chromagens with *m*-met as the first primary antibody and mixed MRFs as the second primary antibody (Johnston et al., 2000b). Duplicate sections were counterstained in Mayer's haematoxylin to visualise total myonuclei. Counts of the numbers of cells with cytoplasm stained red for *m*-met and the number of nuclei stained blue/black for MRFs were made from a minimum of six fields of 50 muscle fibres per fish and related to the cross-sectional area of the muscle. Nuclear counts were corrected for section thickness and the mean diameter of the nuclei determined from electron micrographs (Abercrombie, 1946).

Statistical analyses

The effects of incubation temperature regime, source population and tank replicate on the length and mass of fish measured at 6 and 12 weeks post first feeding were examined using residual maximum likelihood (see Robinson, 1987) using the statistical package Genstat 5. Temperature regime and source population were treated as fixed effects and tank replicates as random effects. Beyond 18 weeks, data were non-normally distributed; by 40 weeks, the distributions of both length and mass were bimodal.

To model the length distributions at 40 weeks, we fitted a double normal distribution to the fish lengths from each tank. The double normal distribution is a mixture of two normal distributions, with five parameters: the mean and variance of each normal distribution, and a split parameter that measures the probability that an observation comes from one or other of the distributions. Fitting a double normal distribution to each tank can be thought of as fitting a full model in the sense that it encompasses all possible differences between temperature, source and tank. However, the full model has 40 parameters (five for each tank), which is too many to be biologically useful. To simplify the model, we first examined evidence of any replicate effect by constraining the parameters from each pair of replicates to be equal and comparing the likelihood of the full model with that of the simplified model. We then examined the effect of temperature and stock by constraining further sets of parameters to be equal and again comparing models using likelihood ratio tests. A similar approach was used to model the log(mass) distribution at 40 weeks.

The data on length and mass of fish sampled for muscle structure and muscle cellularity variables were analysed using analysis of covariance (ANCOVA) with population source and thermal regime as fixed effects and growth period, fork length or muscle cross-sectional area as a covariate, followed by Tukey's *post-hoc* tests. The densities of immunopositive cells were compared using a Student's *t*-test or a Mann–Whitney rank sum test in cases where the data failed tests for normality and equal variance.

Non-parametric statistical techniques were used to fit smoothed probability density functions to the measured fibre diameters using the kernel approach. The statistical methods used are described by Silverman (1986) and Bowman and Azzalini (1997), and their application to the study of muscle fibre size distribution by Johnston et al. (1999). The average smooth probability density functions were estimated using the diameters pooled over each group and the mean optimal smoothing parameter (Bowman and Azzalini, 1997). Values for the smoothing parameter were 0.17 for the Baddoch and 0.18 for the Sheeoch population. To restrict diameters to positive values, density functions were estimated for the natural logarithm of diameter and then transformed back to the original scale. A two-stage sampling procedure was used to account for the hierarchical nature of the data. First, an equal

number *n* of fish was selected at random from the two populations at each sample period and then 500 muscle fibres were selected at random per fish. The kernel estimator was applied to the fibre diameters pooled over the group. To obtain consistency when comparing tail percentiles, the maximal diameter within groups was fixed at 110 % of the maximum diameter sampled. The fifth, tenth, fiftieth, ninety-fifth and ninety-ninth percentiles of muscle fibre diameter were calculated from the distributions. A rank sum two-sample non-parametric test was used to test the hypothesis that the median value of the specified percentile was equivalent between temperature groups.

Results

Growth post first feeding

The mean length and mass of fish measured 6 and 12 weeks post first feeding are summarised in Table 1. Analysis revealed highly significant temperature effects for both length (Wald statistic 178.2, d.f.=1; $P<0.001$) and mass (Wald statistic 193.6, d.f.=1; $P<0.001$) at 6 weeks but no significant differences between populations or the size of fish in replicate tanks. At 12 weeks post first feeding, temperature effects on length (Wald statistic 79.4, d.f.=1; $P<0.001$) and mass (Wald statistic 100.3, d.f.=1; $P<0.001$) were also highly significant, fish previously reared under the cooler Baddoch regime being larger, and there was also evidence of significant population×temperature interactions for both length (Wald statistic 41.16, d.f.=1; $P<0.001$) and mass (Wald statistic 44.54), reflecting different effects in the population/temperature groups (Table 1).

The mass and length frequency distributions of fish in the different population/temperature groups at 40 weeks post first feeding are illustrated in Figs 1 and 2 respectively. Comparison of models fitted to combinations of length distributions revealed no significant differences between tank replicates but

Table 1. *Body length and mass in Baddoch and Sheeoch salmon 6 and 12 weeks post first feeding*

	Sheeoch population		Baddoch population	
	Sheeoch regime	Baddoch regime	Sheeoch regime	Baddoch regime
Length (mm)				
6 weeks	39.6±3.57	44.1±3.2	39.8±2.5	43.1±2.40
12 weeks	58.3±6.1	59.3±6.4	55.2±3.6	61.2±5.4
Mass (g)				
6 weeks	0.59±0.16	0.86±0.21	0.58±0.11	0.77±0.14
12 weeks	2.18±0.75	2.35±0.82	1.74±0.36	2.53±0.75

Eggs were incubated under the stimulated natural temperature regimes of the Baddoch and Sheeoch streams until hatch (for details, see Johnston et al., 2000b), and the fish were transferred to constant conditions at first feeding.

Values are means ± S.D. A total of 100 and 200 fish were measured in each population/temperature group at 6 weeks and 12 weeks, respectively.

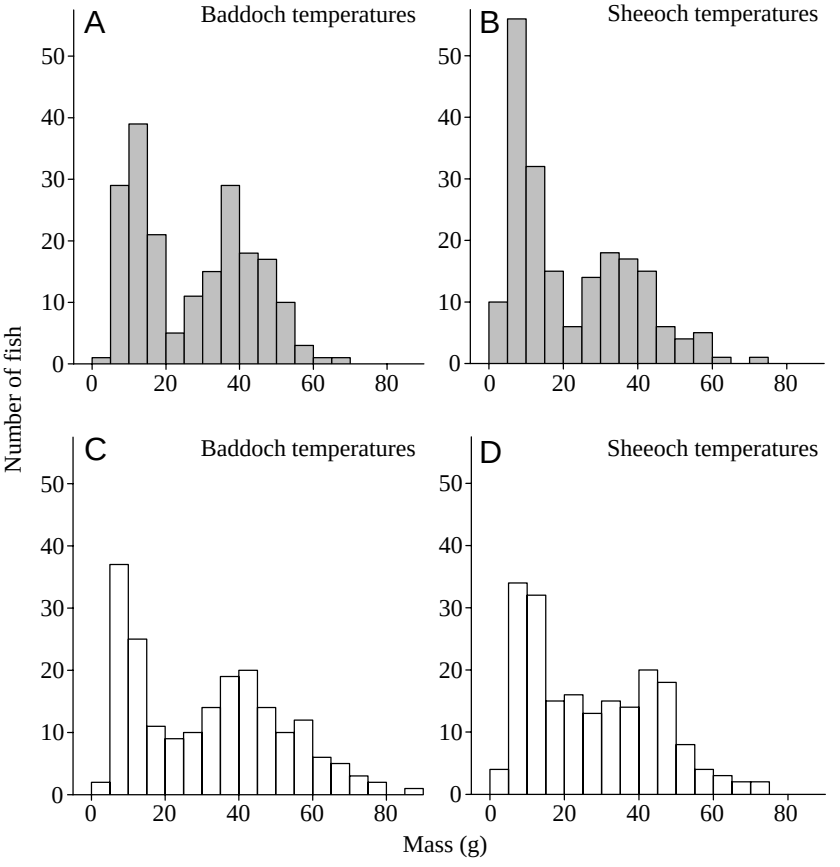

Fig. 1. Histograms showing the body mass of Atlantic salmon (*Salmo salar* L.) at 40 weeks after transfer to constant environmental conditions (see text for details). (A,B) The progeny of fish from the Sheeoch source population reared at the simulated natural temperature regime of the Baddoch (A) and Sheeoch (B) tributaries until first feeding. (C,D) The progeny of fish from the Baddoch source population reared at the simulated natural temperature regime of the Baddoch (C) and Sheeoch (D) tributaries until first feeding.

indicated that the populations previously reared under different incubation temperature regimes differed in their patterns of growth. Comparisons of simplified models fitted to length distributions among population/temperature groups indicated significant differences in distributions between Sheeoch fish reared under the Sheeoch regime and Sheeoch fish reared under the Baddoch regime (likelihood ratio statistic, LrStat, 25.7, d.f.=5; $P<0.001$), Baddoch and Sheeoch fish reared under the Baddoch regime (LrStat 29.4, d.f.=5; $P<0.001$) and Baddoch and Sheeoch fish reared under the Sheeoch regime (LrStat 22.03, d.f.=5; $P<0.01$). There was also some evidence for an effect of incubation temperature on the length distribution of Baddoch fish (LrStat 25.7, d.f.=5; $P=0.021$) but, when the *P*-value was adjusted (multiplied by 4) to account for multiple comparisons, this was not significant. Comparisons of models fitted to log(mass) data produced similar results: there were differences in log(mass) distribution between Sheeoch fish incubated under the Sheeoch regime compared with Sheeoch fish incubated under the Baddoch regime (LrStat 23.26, d.f.=5; $P<0.001$), Baddoch and Sheeoch fish incubated under the Baddoch regime (LrStat 23.39, d.f.=5; $P<0.001$) and Baddoch and Sheeoch fish incubated under the Sheeoch regime (LrStat 19.36, d.f.=5; $P<0.01$). The adjusted probability for comparison between the log(mass) distributions for Baddoch fish reared under the Baddoch regime and Baddoch fish reared under the Sheeoch regime compared with the full model ($P>0.05$) suggested that incubation temperature did not significantly affect mass of Baddoch fish at 40 weeks.

In an attempt to determine where temperature related differences lay, we examined the parameters fitted to the distributions of length and mass in the different population temperature groups (Table 2). Means estimated for both distributions (upper and lower modes) tended to be higher in fish previously reared under the cooler Baddoch regime, and mean length and mass were generally greater in Baddoch than in Sheeoch fish. The split parameter, which estimates the probability of an observation falling within the distribution with the lower mean (the lower mode), suggested that proportionally more Baddoch fish developed as smolts.

Muscle structure

The fish selected for analysis of muscle structure up to 18 weeks post first feeding were of the same mean fork length as the fish in the tanks from which they were drawn. There were no systematic differences between replicates, and data were combined into four population/temperature groups: fish of Sheeoch and Baddoch origin that had been reared at either the Sheeoch or Baddoch thermal regime until first feeding. After 18 weeks, the length frequency distribution of fish became bimodal, and only fish from the upper growth mode were sampled.

Although there were some small differences in body size

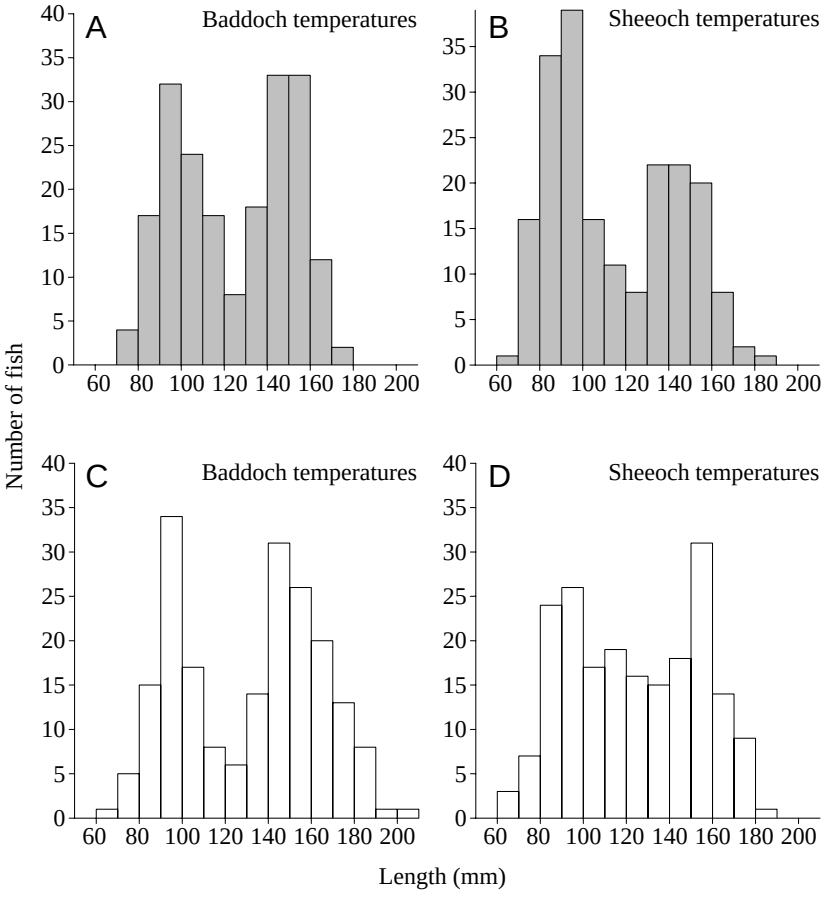

Fig. 2. Histograms showing the body length of Atlantic salmon (*Salmo salar* L.) at 40 weeks after transfer to constant environmental conditions (see text for details). (A,B) The progeny of fish from the Sheeoch source population reared at the simulated natural temperature regime of the Baddoch (A) and Sheeoch (B) tributaries until first feeding. (C,D) The progeny of fish from the Baddoch source population reared at the simulated natural temperature regime of the Baddoch (C) and Sheeoch (D) tributaries until first feeding.

Table 2. *Estimates of parameters of distributions fitted to length and mass in the offspring of Atlantic salmon (*Salmo salar *L.) from the Sheeoch and Baddoch populations 40 weeks post first feeding*

	Sheeoch population		Baddoch population	
	Sheeoch regime	Baddoch regime	Sheeoch regime	Baddoch regime
Length distributing parameters				
Mean upper mode (mm)	143.9±1.0	148.2±1.3	150.3±1.4	152.9±1.8
Variance upper mode	14.1±1.5	10.8±1.0	14.9±2.2	17.2±1.5
Mean lower mode (mm)	90.7±1.5	98.6±1.5	98.3±2.6	94.5±1.8
Variance lower mode	10.9±0.9	12.0±1.1	14.1±1.9	10.6±1.2
Split parameter: probability of an observation lying in the lower mode	0.56±0.042	0.48±0.043	0.52±0.068	0.38±0.041
Mass distribution parameters				
Mean upper mode (g)	34.5±1.7	38.8±1.3	36.0±1.6	42.3±1.6
Variance upper mode	12.4±1.2	10.4±1.0	14.4±1.0	15.4±1.2
Mean lower mode (g)	9.1±0.4	11.5±0.5	9.8±0.6	9.8±0.5
Variance lower mode	3.2±0.4	3.7±4.0	3.4±0.5	3.1±0.4
Split parameter: probability of an observation lying in the lower mode	0.49±0.046	0.45±0.042	0.33±0.045	0.33±0.039

Eggs were incubated under the stimulated natural temperature regimes of the Baddoch and Sheeoch streams until hatch (for details, see Johnston et al., 2000b), and the fish were transferred to constant conditions at first feeding. Distributions were fitted to measurements made on 200 fish from each population/temperature group.

Values are estimates of parameters ± S.E.M.

between groups, there was no significant difference in the total cross-sectional area of white muscle at the level of the adipose fin either between populations or with initial temperature regime (ANCOVA with growth period post-transfer as a covariate). However, muscle growth patterns were found to differ both with population origin and with early thermal experience for fish grown under identical conditions post first feeding. The relationship between the number of muscle fibres and the cross-sectional area of white muscle is shown in Fig. 3A,B. An ANCOVA with the number of white muscle fibres per myotome as dependent variable and muscle cross-sectional area as covariate revealed significant differences between populations ($F_{1,215}$=12.6; P<0.001) and early thermal experience ($F_{1,215}$=4.96; P=0.05) and a significant two-way population×temperature interaction ($F_{1,215}$=16.76; P<0.001). Tukey's *post-hoc* tests revealed that the differences between

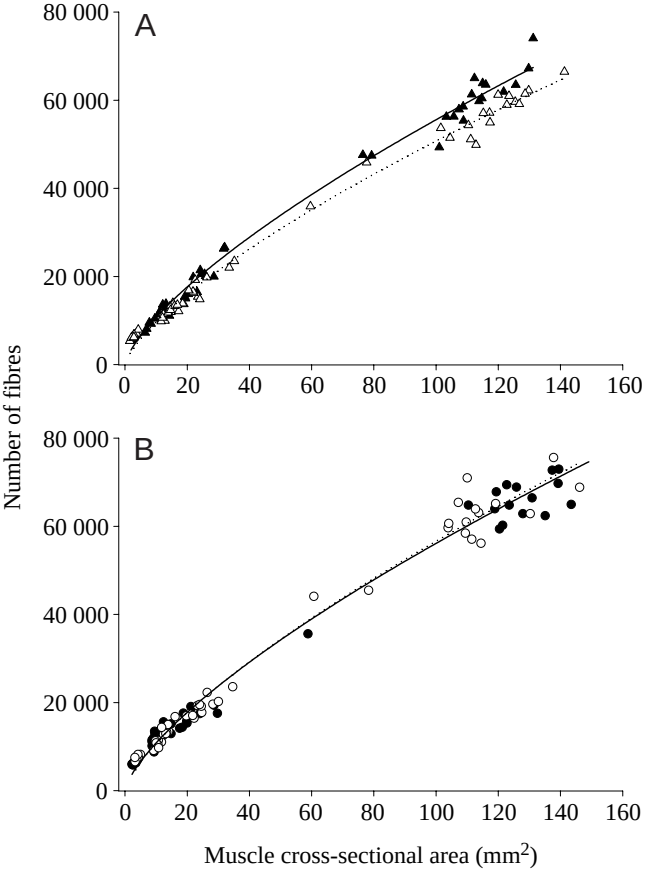

Fig. 3. The relationship between the number of white muscle fibres and the cross-sectional area of white muscle at the level of the adipose fin in the progeny of Atlantic salmon (*Salmo salar* L.) originating from (A) the Sheeoch (triangles) and (B) the Baddoch (circles) tributaries of the River Dee system, Aberdeenshire, Scotland, and grown at constant temperature and photoperiod. Prior to first feeding, the fish had been incubated under the simulated natural temperature regimes of the Baddoch (open symbols; broken line) and Sheeoch (filled symbols; solid line) tributaries (see text for details). Each point represents an individual fish. The data was fitted to a power equation of the form $n=aA^b$, where n is fibre number and A is muscle cross-sectional area. The constants for the different groups were as follows: Sheeoch fish at Sheeoch temperatures, a=2084±138, b=0.71±0.015, adjusted r^2=0.99, N=53, P<0.001; Sheeoch fish at Baddoch temperatures, a=1856±116, b=0.72±0.014, adjusted r^2=0.99, N=53, P<0.001; Baddoch fish at Baddoch temperatures, a=2053±175, b=0.72±0.019, adjusted r^2=0.99, N=51, P<0.001; Baddoch fish at Sheeoch temperatures, a=2096±202, b=0.71±0.020, adjusted r^2=0.99, N=54, P<0.001 (means ± S.E.M.).

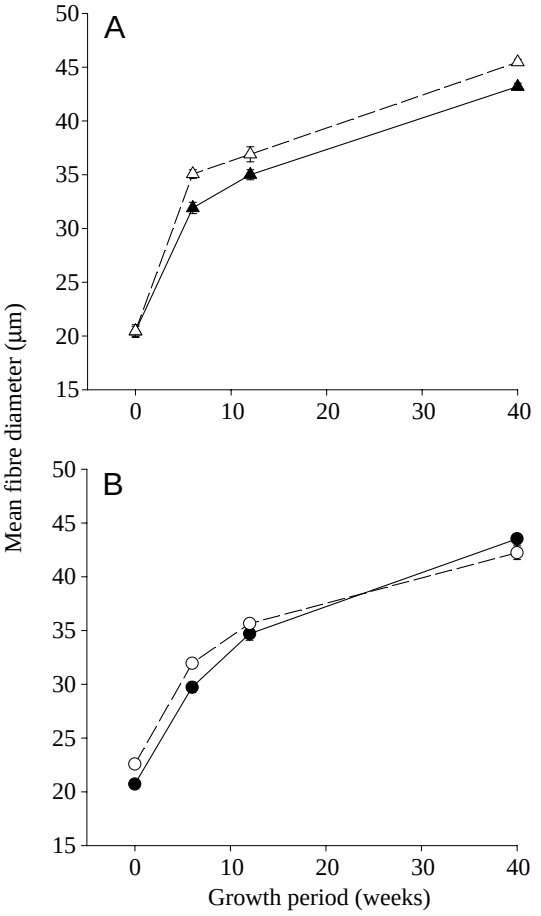

Fig. 4. The relationship between the mean diameter of white muscle fibres and growth period under constant environmental conditions for the progeny of Atlantic salmon (*Salmo salar* L.) originating from (A) the Sheeoch (triangles) and (B) the Baddoch (circles) tributaries of the River Dee system, Aberdeenshire, Scotland. Prior to first feeding, the fish had been incubated under the simulated natural temperature regimes of the Baddoch (open symbols; broken line) and Sheeoch (filled symbols; solid line) tributaries (see text for details). The values represent means ± S.E.M. for 16 fish per group, except for the first sample, which contained 10 fish per group.

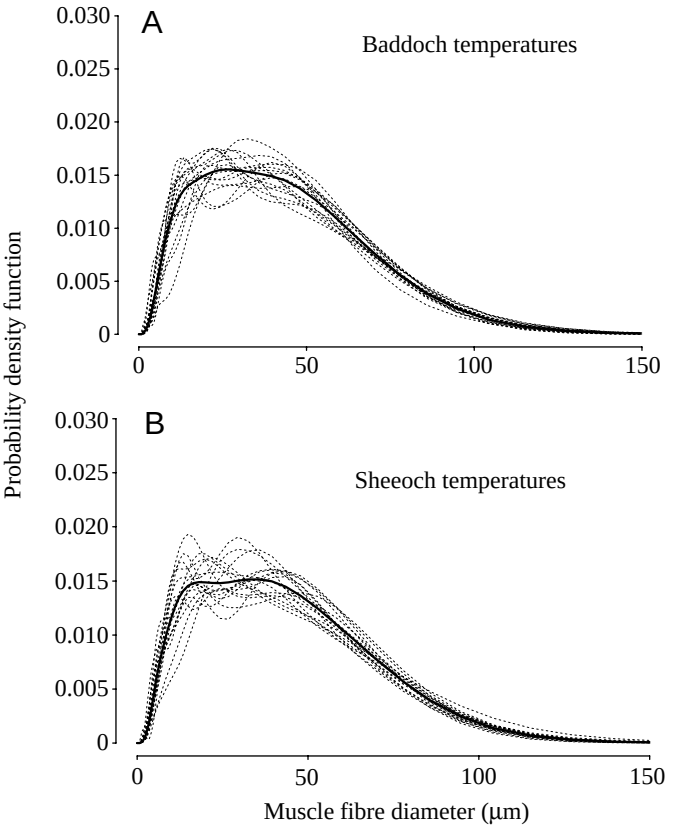

Fig. 5. The smooth probability density function of white muscle fibre diameter in the progeny of Atlantic salmon (*Salmo salar* L.) originating from the lower catchment of the River Dee (Sheeoch tributary). The fish were reared at the simulated natural temperature regime of (A) the Baddoch and (B) the Sheeoch tributaries until first feeding and then grown for 40 weeks at 12–14 °C. The dotted lines represent the smooth distributions of probability for individual fish, and the solid line is the mean for each group.

thermal regimes were highly significant for Sheeoch (P<0.0001) but not for Baddoch fish. Thus, an effect of early thermal regime on the subsequent pattern of muscle fibre recruitment was observed for the Scheeoch but not the Baddoch populations (Fig. 3A,B). For the offspring of the Sheeoch population, the number of fibres for a given cross-sectional area of white muscle was higher for fish initially reared at the Sheeoch than at the Baddoch temperature regime (Fig. 3A). In Sheeoch fish sampled at 40 weeks, the fibre density was 10.4 % higher in fish reared under the Sheeoch (533±6 m^{-2}) than the Baddoch (483±5 m^{-2}) temperature regime (means ± S.E.M., 16 fish per group) (t-test; P<0.001).

An ANCOVA with the mean diameter of white muscle fibres as the dependent variable and growth period as covariate revealed significant main effects of early thermal experience ($F_{1,215}$=7.08: P<0.01), but not of population origin. *Post-hoc* tests showed that the temperature effect was apparent only in the Sheeoch population (P<0.01) (Fig. 4A,B). The mean white fibre diameter was significantly higher in Sheeoch fish reared under the cooler Baddoch than the warmer Sheeoch temperature regime (Fig. 4A).

Smooth probability densities of muscle fibre diameter at 40 weeks following transfer are shown for individual fish (dotted lines) and for each group (solid lines) from the Sheeoch (Fig. 5) and Baddoch (Fig. 6) populations. For most individuals, the distributions of fibre diameter were unimodal with a broad peak between 10 and 40 μm diameter (Figs 5, 6). The largest, and presumably oldest, cohort of muscle fibres was around 120 μm in diameter (Table 3). The fifth, tenth, ninety-fifth and ninety-ninth percentiles of muscle fibre diameter were significantly greater for Sheeoch fish reared under the cooler Baddoch regime than under their warmer native thermal regime (Table 3). In contrast, the fifth to the ninety-ninth percentiles of muscle fibre diameter were independent of initial rearing temperature in the Baddoch population (Table 3).

The density of total muscle nuclei decreased with increasing fish length for both populations (Fig. 7). An ANCOVA showed

Table 3. *Comparison of the percentiles for the mean probability density functions of muscle fibre diameter in Sheeoch and Baddoch populations of Atlantic salmon (*Salmo salmo *L.)*

	Muscle fibre diameter (μm)			
	Sheeoch population		Baddoch population	
Percentile	Sheeoch regime	Baddoch regime	Sheeoch regime	Baddoch regime
5	10.5±0.3	12.2±0.3***	10.1±0.3	10.4±0.4
10	14.5±0.4	16.2±0.4**	14.0±0.4	14.3±0.4
50	40.2±0.5	42.4±0.4**	40.7±0.6	40.6±0.5
95	91.7±0.7	95.7±0.6***	92.3±1.0	92.6±0.9
99	115.8±1.3	123.2±1.3***	116.4±1.8	117.2±1.4

Eggs were incubated under the simulated natural temperature regimes of the Sheeoch and Baddoch streams until hatch, and the fish were transferred to constant environmental conditions at first feeding.

Asterisks mark statistically significant differences between thermal regimes within a population: $^{**}P<0.01$; $^{***}P<0.001$.

Values are means ± S.E.M. of the percentile values of the probability density estimate of muscle fibre diameter from 16 fish per population per temperature group.

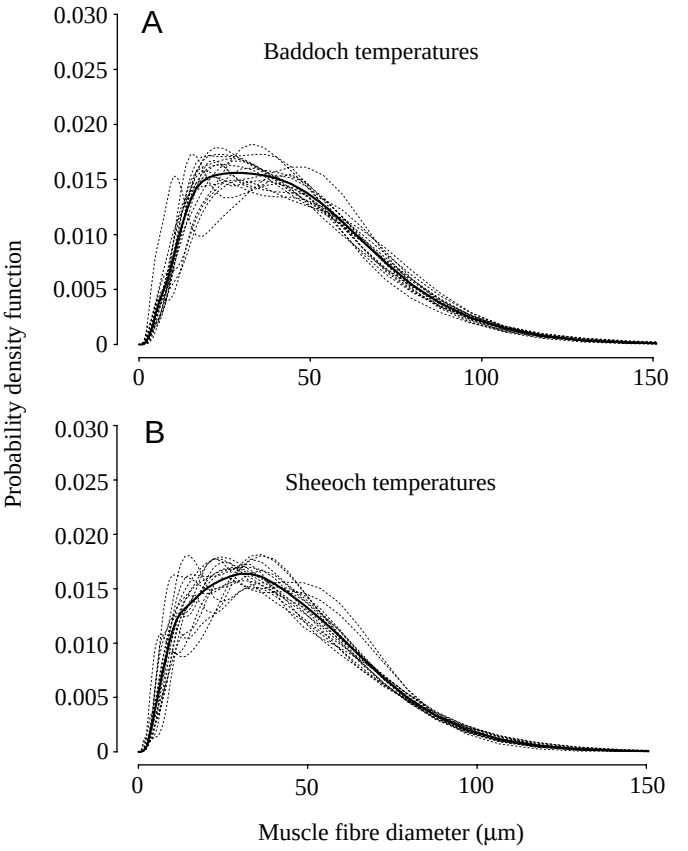

Fig. 6. The smooth probability density function of white muscle fibre diameter in the progeny of Atlantic salmon (*Salmo salar* L.) originating from the upper catchment of the River Dee (Baddoch tributary). The fish were reared at the simulated natural temperature regime of (A) the Baddoch and (B) the Sheeoch tributaries until first feeding and then grown for 40 weeks at 12–14 °C. The dotted lines represent the smooth distributions of probability for individual fish, and the solid line is the mean for each group.

significant main effects of thermal regime ($F_{1,219}$=10.08; P<0.001), but not population. *Post-hoc* tests revealed that the temperature effect resulted from differences within the Sheeoch (P<0.05) but not the Baddoch populations. In the population of Sheeoch origin at the 24 week sample, the density of mononuclear cells immunopositive for *c*-met was 29 % higher for fish reared until first feeding at the Sheeoch (10 985±523 mm^{-3}) than at the Baddoch (8516±425 mm^{-3}) thermal regime (means ± S.E.M., six fish per group) (*t*-test; P<0.01) (Fig. 8). The density of *c*-met-positive cells also staining for one or more myogenic regulatory factor (MyoD, myf-5, myogenin or myf-6) showed a similar trend but was not significantly different between temperature groups (Fig. 8).

Discussion

The sites at which Atlantic salmon spawn in large river systems such as the Aberdeenshire Dee vary with respect to environmental factors such as temperature regime and/or water chemistry (Shackley and Donaghy, 1992; Webb and McLay, 1996). For example, eggs laid in the Baddoch, a high-altitude tributary, experience lower temperatures throughout the period of embryonic development than eggs spawned and incubated in the Sheeoch (Webb and McLay, 1996; H. A. McLay, I. A. Johnston, J. H. Webb and D. Robins, in preparation). In the present study, the temperature regime experienced prior to first feeding by the offspring of Sheeoch salmon was found to influence the pattern of muscle growth from the fry to the smolt stage of development (Figs 3A, 4A). For Sheeoch salmon reared under their native temperature regime, the contribution of fibre recruitment was greater, and the contribution of fibre hypertrophy to muscle growth was smaller, than in fish initially reared at cooler Baddoch temperatures (Figs 3A, 4A). However, the muscle growth pattern to the smolt stage was similar in the offspring of spring-running fish from the Baddoch burn irrespective of egg incubation temperature (Figs 3B, 4B).

Length and mass measurements made on all the fish sampled in each tank provided evidence of a persistent effect of early thermal experience on body size and patterns of growth up to 40 weeks post first feeding. Fish of both Baddoch and Sheeoch origin reared under the cooler Baddoch regime were both longer and heavier at 6 and 12 weeks post first feeding. Incubation temperature also affected patterns of growth at 40 weeks, but evidence of significant temperature effects was only found in Sheeoch fish. However, what is not clear is the extent to which these differences reflect the small differences in size at first feeding (H. A. McLay, I. A. Johnston, J. H. Webb and D. Robins, in preparation) or differences in rates or patterns of growth in the intervening period. Fowler (1972) found that differences in the size of fish, arising from differences in egg

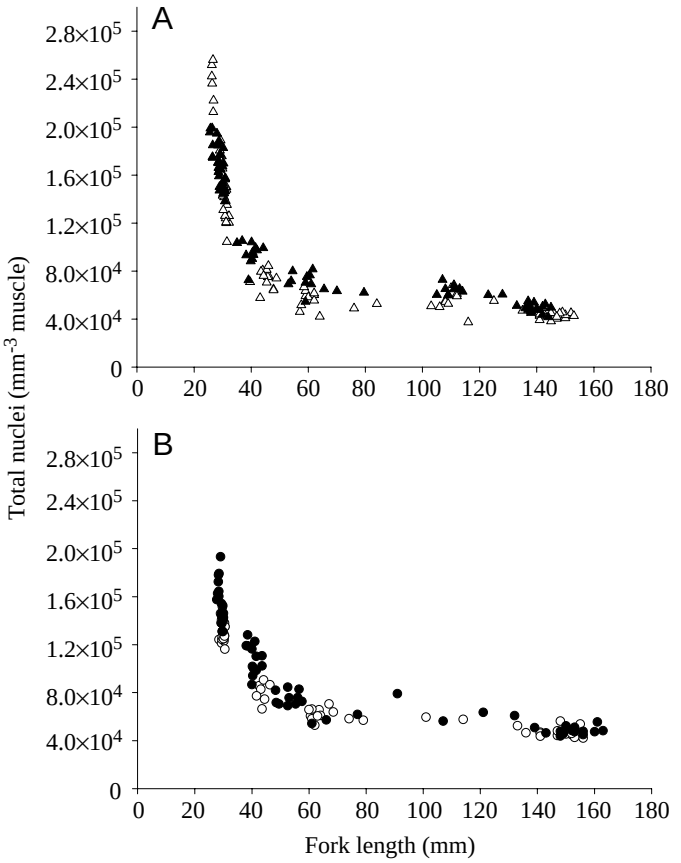

Fig. 7. The relationship between the total density of muscle nuclei and fork length for the progeny of Atlantic salmon (*Salmo salar* L.) originating from (A) the Sheeoch (triangles) and (B) the Baddoch (circles) tributaries of the River Dee system, Aberdeenshire, Scotland, grown under constant environmental conditions. Prior to first feeding, the fish had been incubated under the simulated natural temperature regimes of the Baddoch (open symbols) and Sheeoch (filled symbols) tributaries. Each point represents an individual fish.

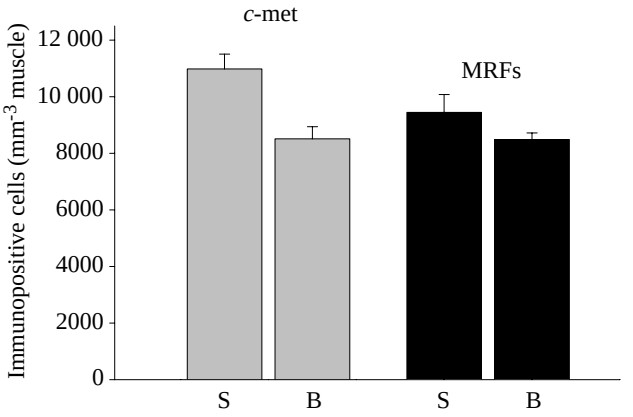

Fig. 8. The density of mononuclear cells in Atlantic salmon (*Salmo salar* L.) of Sheeoch origin stained with antibodies against *c*-met, a marker of muscle satellite cells, and mixed myogenic regulatory factors (MRFs). Fish were incubated at either the simulated Sheeoch (S) or Baddoch (B) regimes prior to first feeding and then transferred to constant environmental conditions for 24 weeks, when they were sampled. The values represent means + S.E.M. for six fish per temperature group.

size, may persist for as long as 12 weeks after first feeding, whereas Hayes and Armstrong (1942) found that differences disappeared over a 35-day period.

H. A. McLay, I. A. Johnston, J. H. Webb and D. Robins (in preparation) found that Baddoch fish reared under the Baddoch regime were larger at first feeding than Sheeoch fish reared under the Baddoch regime. This size difference may have allowed proportionally more Baddoch fish to reach some critical length threshold and develop as smolts (Metcalfe et al., 1988; Kristinsson et al., 1995). Skilbrei (1991) concluded that the decision to smolt the following spring was strongly dependent on length at the time of the winter light stimulus. In the present study, fish reared under constant conditions that favoured rapid growth smolted in the absence of any change in day length, indicating that size thresholds, or possibly related social status, may be more important in determining whether fish in culture adapt a fast developmental strategy.

The egg incubation temperature affected the cellularity of embryonic muscle in both populations (Johnston et al., 2000b). At hatching, the cross-sectional area of trunk muscle occupied by white muscle fibres was found to be similar at the different thermal regimes in Sheeoch salmon, but 18.9% greater in Baddoch salmon reared at Baddoch than at Sheeoch temperatures (Johnston et al., 2000b). These differences in Baddoch salmon were the result of the higher number of embryonic muscle fibres of larger mean diameter in fish reared under the cooler regime (Johnston et al., 2000b). Thus, the incubation temperature regimes had different effects on muscle growth in the embryo and yolk-sac stages than during the fry and parr stages, and these responses varied between different components of the Dee stock. Other aspects of the thermal phenotype have also been observed to vary between the populations, including development period and fry emergence times (Donaghy and Verspoor, 1997; Webb and McLay, 1996; H. A. McLay, I. A. Johnston, J. H. Webb and D. Robins, in preparation). Variation in the expression of *MEP-2**, a gene that codes for malic enzyme, has also been related to temperature, with significant variation in allele and genotype frequencies among populations both within and between river systems (Verspoor and Jordan, 1989).

Post-embryonic muscle growth in teleost fish involves undifferentiated mononuclear cells called satellite cells (Koumans et al., 1991; Johnston et al., 1995; Rowlerson et al., 1995). Satellite cells are thought to represent a stem cell population and their division products committed to terminal differentiation (Cornelison and Wold, 1997; Johnston et al., 1999). The division products of the satellite cells are able to proliferate for a limited period before leaving the cell cycle (Schultz, 1996). In fish, these cells are either absorbed into growing muscle fibres or they fuse together on the surface of an existing fibre to form a new myotube (Johnston et al., 1995, 1998). Tissue culture studies indicate that there may be different populations of satellite cells (Koumans et al., 1993), although whether this is related to the different fate of nuclei

during growth is unknown. All the cells committed to differentiation express one or more members of the MyoD family of muscle transcription factors (MyoD, myogenin, myf-5, myf-6) (Cornelison and Wold, 1997; Johnston et al., 1999, 2000b; Delalande and Rescan, 1999). In the rainbow trout (*Oncorhynchus mykiss*), mononuclear cells were first observed after the formation of the embryonic muscle fibres, but significantly before hatching (Stoiber and Sänger, 1996). It has been suggested, on the basis of ultrastructural observations, that they divide in, and originate from, the adjacent mesenchymal lining and enter the muscle *via* the myosepta (Stoiber and Sänger, 1996). In Atlantic salmon, dual immunolabelling of muscle sections with *c*-met and proliferating cell nuclear antigen, a marker of cell division, indicates that approximately 80 % of the mononuclear cells correspond to satellite cells at first feeding (Johnston et al., 2000b). Of these, only 15–20 % correspond to the muscle stem cells since the remainder expressed one or more of the myogenic regulatory factors, indicating their commitment to differentiation (Johnston et al., 2000b).

The density of mononuclear cells staining for the *c*-met tyrosine kinase receptor, a molecular marker of satellite cells (Cornelison and Wold, 1997; Johnston et al., 1999) was approximately 20 % higher at first feeding in Sheeoch fish reared under the warmer than the cooler temperature regime (Johnston et al., 2000b). These differences in the density of *c*-met-immunoreactive cells between temperature groups were still apparent 24 weeks after transfer to constant temperature and photoperiod (Fig. 8). In contrast, the density of cells expressing *c*-met and myogenic regulatory factors was similar between temperature groups for the Baddoch population at first feeding (Johnston et al., 2000b). Thus, there was an association between the density of satellite cells and the relative importance of fibre recruitment to growth. A similar association was noted in Atlantic salmon following ploidy manipulation. The density of satellite cells was approximately 24 % lower in triploid than in diploid individuals, and triploids recruited one-third fewer fibres for each mm^2 increase in white muscle cross-sectional area (Johnston et al., 1999).

There was also a difference in fibre hypertrophy in Sheeoch fish incubated at the two temperature regimes, with cooler egg incubation temperatures associated with larger fibre diameters following transfer to constant temperature (Fig. 4A; Table 3). In mammals, exercise- and stretch-induced fibre hypertrophy involves muscle insulin growth factor-1 and the calcineurin signalling pathway (Abbott et al., 1998). Activation of the calcineurin pathway results in a rise in intracellular [Ca^{2+}] and the nuclear translocation of the transcription factors NF-ATc1 (nuclear factor of activated T cells) and GATA-2 to the nucleus, leading to the transcription of genes necessary for fibre hypertrophy (Musarò et al., 1999; Semsarian et al., 1999). The role of the calcineurin signalling pathway in the regulation of fibre size during the normal growth of fish has yet to be established. It is known that as fibre diameter increases more nuclei are absorbed to maintain the nuclear-to-cytoplasmic volume ratio within set limits (Koumans et al., 1991). However, there was no correlation between the density of satellite cells committed to differentiation (MRF-positive cells) and mean fibre diameter in Sheeoch fish, and it is possible that the mean ratio of nuclear to cytoplasmic volume differed between temperature groups.

The present study was designed to investigate longer-term effects of early thermal experience on muscle growth patterns; however, it provides few insights into their ecological significance or any consequences for life history decisions. The density of mononuclear cells decreases as the fish increases in length as a result of an increase in the number and diameter of the fibres; however, it is generally thought that the number of muscle stem cells remains approximately constant (Koumans and Akster, 1995; Koumans et al., 1991, 1994). It is likely, therefore, that the effects of egg incubation temperature on muscle fibre recruitment will persist throughout life. In Atlantic salmon, the number of muscle fibres continues to increase in sea water, reaching approximately 180 000 in seawater-adapted smolts, 650 000 in one-sea-winter fish and 1.2 million in two-sea-winter fish (Johnston, 1999; Johnston et al., 1999, 2000a).

Inter-specific studies have shown a positive correlation between muscle fibre recruitment and growth rate and ultimate body size (Weatherley et al., 1988). For example, the dwarf lacustrine form of the southern smelt (*Retropina retropina* Richardson) stops recruiting new white muscle fibres at a significantly shorter fork length than the larger riverine form (Meyer-Rochow and Ingram, 1993). Similarly, female Argentine hake (*Merluccius hubbsi*) reach a larger body size than males, and this is associated with a greater relative contribution of fibre recruitment to muscle growth (Calvo, 1989). The maximum diameter for white muscle fibres in Atlantic salmon is approximately 240 μm, perhaps reflecting some constraint on the diffusion of metabolites (Johnston et al., 1999). Small-diameter fibres can be expected to grow more quickly than large-diameter fibres because of their higher surface-to-volume ratio and greater capacity to assimilate nutrients (Weatherley et al., 1988). More importantly, a high fibre number could potentially overcome the constraint on body size associated with the maximum diameter of the fibres. Although age and size at sexual maturity undoubtedly have a large genetic component, they may well be influenced by thermal experience in the early life stages.

The results of the present study indicate that the temperature prior to hatching can influence the number of satellite cells and, hence, the relative importance of fibre recruitment and hypertrophy to muscle growth at subsequent stages of the life cycle. Similar findings have been reported for spring-spawning populations of Atlantic herring (Johnston, 1993; Johnston et al., 1998). The white muscle of larvae hatching from herring eggs incubated at 8 °C had significantly more mononuclear cells than that of larvae hatching at 5 °C (Johnston, 1993). In a subsequent experiment, herring were reared at either 5 °C or 8 °C until first feeding and then transferred to ambient conditions. Fish initially reared at 8 °C had a significantly higher cross-sectional area of white muscle after 80 days than

fish reared at 5 °C, in this case because of an increase in both fibre number and diameter. Different stocks of Atlantic herring spawn in almost every month of the year in the North Sea and Baltic at temperatures ranging from 2 to 16 °C. Although there is little genetic structuring of the stocks, they show significant differences in size at sexual maturity and in the number of white muscle fibres per trunk cross-sectional area (Greer-Walker et al., 1972). Phenotypic effects of development temperature on muscle satellite cells provide a possible mechanism for generating the observed differences in fibre number between stocks, although genetic differences are probably also important. Development temperature has been shown to produce other lasting effects on phenotype in fish, including meristic characters (Tåning, 1952) and sex (Lagormarisino and Conover, 1993; Strüssmann et al., 1996). Egg temperature has also been shown to be linearly and negatively correlated with growth rate in both male and female snapping turtles (*Chelydra serpentina*) (O'Steen, 1998). There is growing evidence that the temperature regime during early development is important for determining subsequent growth performance and temperature preference in a range of ectotherms.

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