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Freshwater environment affects growth rate and muscle fibre recruitment in seawater stages of Atlantic salmon (*Salmo salar* L.)

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Summary

The influence of freshwater environment on muscle growth in seawater was investigated in an inbred population of farmed Atlantic salmon (*Salmo salar* L.). The offspring from a minimum of 64 families per group were incubated at either ambient temperature (ambient treatment) or in heated water (heated treatment). Growth was investigated using a mixed-effect statistical model with repeated measures, which included terms for treatment effect and random fish effects for individual growth rate (α) and the instantaneous growth rate per unit change in temperature (γ). Prior to seawater transfer, fish were heavier in the heated (61.6±1.0 g; *N*=298) than in the ambient (34.1±0.4 g; *N*=206) treatments, reflecting their greater growth opportunity: 4872 degree-days and 4281 degree-days, respectively. However, the subsequent growth rate of the heated group was lower, such that treatments had a similar body mass (3.7–3.9 kg) after approximately 450 days in seawater. The total cross-sectional area of fast muscle and the number (*FN*) and size distribution of the fibres was determined in a subset of the fish. We tested the hypothesis that freshwater temperature regime affected the rate of recruitment and hypertrophy of muscle fibres. There were differences in *FN* between treatments and a significant age×treatment interaction but no significant cage effect (ANOVA). Cessation of fibre recruitment was identified by the absence of fibres of <10 μm diameter. The maximum fibre number was 22.4% more in the ambient ($9.3\times10^5\pm2.0\times10^4$ than in the heated ($7.6\times10^5\pm1.5\times10^4$) treatments (*N*=44 and 40 fish, respectively; *P*<0.001). For fish that had completed fibre recruitment, there was a significant correlation between *FN* and individual growth rate, explaining 35% of the total variation. The density of myogenic progenitor cells was quantified using an antibody to c-met and was approximately 2-fold higher in the ambient than in the heated group, equivalent to 2–3% of the total muscle nuclei. The number of myonuclei in isolated fibre segments showed a linear relationship with fibre diameter. On average, there were 20.6% more myonuclei in 200-μm-diameter fibres isolated from the ambient (3734 myonuclei cm^{-1}) than from the heated (3097 myonuclei cm^{-1}) treatments. The maximum fibre diameter was greater in heated than in ambient groups, whereas the age×treatment interaction was not significantly different (ANCOVA). There were also no consistent differences in the rate of hypertrophy of muscle fibres between treatments. It was concluded that freshwater temperature regime affected fibre number and the nuclear content of fast muscle in seawater but not the rate of fibre hypertrophy. The mechanisms and life history consequences of developmental plasticity in fibre number are discussed.

Key words: muscle growth, myogenic cells, muscle fibre recruitment, temperature, growth, developmental plasticity, fish, *Salmo salar*.

Introduction

Many species of teleost fish exhibit indeterminate growth (Weatherley and Gill, 1987). Muscle fibres reach a maximum diameter, which is dictated by metabolic demands and diffusional constraints related to nutrient assimilation and/or metabolite removal. An important consequence of indeterminate growth is that the number of fibres needs to increase throughout much of the life cycle as the muscle mass increases, involving a prolonged period of postembryonic

hyperplasia (Greer-Walker, 1970; Stickland, 1983). Species that reach a large ultimate body size require more muscle fibres than do small species, and therefore hyperplasia continues for longer in the life cycle (Weatherley et al., 1988). Variations in the duration of muscle fibre recruitment also underlie sexual dimorphism in body size. For example, in female Argentine hake (*Merluccius hubbsi*), fibre recruitment ceased at 55–60 cm total length (TL) compared with 45–52 cm TL in males, reflecting their greater maximum size (Calvo, 1989). Postembryonic muscle growth in fish involves a highly proliferative population of myogenic progenitor cells that have many of the characteristics of the satellite cells found beneath the basal lamina of muscle fibres in mammals (Koumans and Akster, 1995; Fauconneau and Paboeuf, 2001). However, some myogenic cells are located outside the basal lamina in the larval and juvenile stages of teleosts (Veggetti et al., 1990; Johnston, 1993). The pool of proliferating myoblasts donates a large number of nuclei to muscle fibres as they expand in diameter (hypertrophic growth; Koumans et al., 1991).

Two main processes of fibre recruitment have been identified in teleost myotomal muscle. In the late embryo, larval and early juvenile stages, muscle fibres are added from discrete germinal zones (Veggetti et al., 1990; Rowlerson et al., 1995; Johnston et al., 1998; Barresi et al., 2001) in a process that has been termed 'stratified hyperplasia' (Rowlerson and Veggetti, 2001). The anatomical location of these germinal zones varies among muscle fibre types and species (Rowlerson et al., 1995; Johnston et al., 1998). In most fish, the final and most important mechanism of muscle expansion is 'mosaic hyperplasia' (Rowlerson and Veggetti, 2001). Mosaic hyperplasia involves the widespread activation of myogenic precursors scattered throughout the myotome (Koumans and Akster, 1995; Johnston et al., 1999; Johnston, 2001). Proliferating myoblasts withdraw from the cell cycle and fuse to form myotubes on the surface of existing muscle fibres (Veggetti et al., 1990; Johnston et al., 1998, 2000a). In the fast muscle, immature muscle fibres have a higher content of glycogen and aerobic enzymes than do mature ones, resulting in a characteristic mosaic pattern of fibre diameter and histochemical staining characteristics (Johnston et al., 1975; Matsuoka and Iwai, 1984; Rowlerson et al., 1995). Cells with the ultrastructural characteristics of undifferentiated myoblasts are first observed after the completion of segmentation and the formation of the embryonic muscle (Atlantic herring *Clupea harengus*, Vieira and Johnston, 1992; rainbow trout *Oncorhynchus mykiss* and pearl fish *Rutilus frisii meidingeri*, Stoiber and Sänger, 1996).

The number and diameter of muscle fibres present in the myotomes from hatching to first feeding has been shown to vary with egg incubation temperature in such diverse species as Atlantic salmon (*Salmo salar*; Stickland et al., 1988; Johnston and McLay, 1997), whitefish (*Coregonus lavaretus*; Hanel et al., 1996), rainbow trout (Matschak et al., 1998), Atlantic herring (Vieira and Johnston, 1992), plaice (*Pleuronectes platessa*; Brooks and Johnston, 1993), turbot (*Scophthalmus maximus*; Gibson and Johnston, 1995) and Atlantic cod (*Gadus morhua*; Galloway et al., 1998). The muscle fibres present in these early stages of ontogeny reflect the processes of embryonic myogenesis (Devoto et al., 1996; Johnston, 1993; Veggetti et al., 1990) and fibre recruitment by stratified hyperplasia (Johnston et al., 1998; Barresi et al., 2001). The number of myogenic precursors identified by ultrastructural criteria was found to be significantly higher in 1-day-old Atlantic herring larvae reared at 8°C than at 5°C (Johnston, 1993). Furthermore, after 80 days at ambient temperature, there were more fast muscle fibres in herring larvae hatching from eggs incubated at 8°C than at 5°C, consistent with an effect of development temperature on mosaic hyperplasia (Johnston et al., 1998, 2001). The number of fast and slow muscle fibres has also been reported to vary with development temperature throughout the larval stages of sea bass *Dicentrarchus labrax* (Ayala et al., 2001) and in the freshwater parr stages of Atlantic salmon (Johnston et al., 2000b). For salmon, the treatment group with the highest fibre number also had the highest density of cells expressing c-met (Johnston et al., 2000a,b), a molecular marker of myogenic precursor cells (Cornelison and Wold, 1997). There is therefore evidence that development temperature has persistent effects on all three phases of muscle growth.

The recruitment and hypertrophy of fast muscle fibres was shown to vary with growth rate in rainbow trout fed different ration levels (Rasmussen and Ostenfeld, 2000). Valente et al. (1999) found evidence for a faster rate of fibre recruitment in a fast-growing strain of rainbow trout compared with a slow-growing strain, although there was a similar number of muscle fibres at any given body length. Numerous structural and regulatory genetic loci are likely to influence growth potential in teleosts (Mommsen and Moon, 2001). Inter-specific comparisons indicate that a high growth rate and a large body size are related to the capacity to recruit new muscle fibres (Weatherley et al., 1988).

Salmo salar L. is a migratory species with a remarkably plastic and complex life history (Stabell, 1984). Adult stages spawn in freshwater rivers, where the parr remain for 1–5 years before smolting and migrating to the sea. The salmon then spend 1–4 years on oceanic feeding grounds in the Arctic before sexually maturing and migrating back to their natal stream to spawn. In the present study, we have exploited the availability of an inbred population of farmed salmon to test two hypotheses concerning postembryonic growth. Our first hypothesis was that relatively small differences in freshwater temperature regime have persistent effects on the recruitment and hypertrophy of fast muscle fibres during growth in seawater. The second hypothesis was that in fish fed to appetite there would be a correlation between the final number of muscles recruited and individual growth rate. In order to test these hypotheses it was necessary to develop a statistical model that accounted for the effects of scale and temperature on growth rate.

Materials and methods

Fish

Atlantic salmon (*Salmo salar* L.) were from the genetic improvement programme operated by Marine Harvest (Scotland) Ltd. The hen salmon used were originally derived from several batches of eggs stripped from wild-caught fish from the River Shin, Scotland in the late 1960s and early 1970s. The founder population had been inbred for 9–10 generations. The male fish used were derived from a single batch of eggs from a late maturing strain of Norwegian salmon and had been subject to a selective inbreeding programme for seven generations (since 1973). In order to randomise genetic effects between treatments, 3-sea winter males were crossed with 2-sea winter females and a large number of families were generated for the populations studied. 45 hens and 8 cock fish were stripped to produce 360 families for the 'heated' group, and 32 hens and 2 cocks were stripped to produce 64 families for the 'ambient' group. The eggs were fertilised on 21 November and 30 November 1998 for the heated and ambient groups, respectively. Following water hardening, the fertilised eggs were placed in standard concrete hatchery trays at a flow rate of 10 l min^{-1}. The water supply to the heated group was maintained at approximately 8.3°C, which was 1–3°C above that of the ambient group during the period from fertilisation to pigmentation of the eyes. The heated and ambient groups hatched 55 days (447 degree-days) and 59 days (457 degree-days) after fertilisation, respectively. The temperature history of the two treatment groups throughout the entire period in freshwater is shown in Fig. 1. The heated group were maintained at warmer temperatures than the ambient group, except for a period in the summer. The cumulative degree-days in freshwater were 4872 for the heated group compared with 4281 for the ambient group.

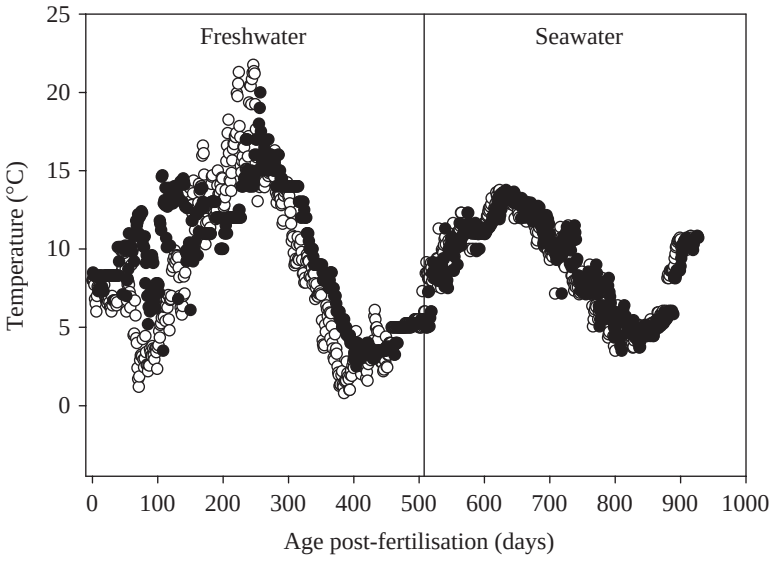

Fig. 1. The temperature regime experienced by the ambient (open circles) and heated (filled circles) treatment groups of Atlantic salmon.

Table 1. *Numbers of fish by treatment and seawater cage that were repeatedly weighed to assess growth performance*

Cage	Ambient	Heated	Total
1	120	155	275
2	86	143	229
Total	206	298	504

Fish were maintained according to standard hatchery practice and fed a commercial diet (BioMar Ltd, Grangemouth, Scotland, UK) in duplicate 2 m and then 4 m circular tanks. The fish were branded when they reached a mass of 10 g and individually PIT tagged (passive integrated transponder tagged; Fish Eagle Co., Gloucester, UK) prior to seawater transfer to identify groups. Smolts were stocked into two steel construction sea cages (5 m×5 m×5 m) with a 12 mm mesh cube net at Loch Eil, Scotland, UK on 17/18 April 2000. Each cage was stocked with 600 smolts, representing a random selection of the available fish from the heated and ambient treatments, which were age 506 days and 515 days post-fertilisation, respectively. Fish were fed to appetite using an automated system (AKVAsmart, UK Ltd, Glasgow, Scotland, UK) with a standard commercial ration of the Ecolife® series (BioMar Ltd) manufactured in five pellet sizes (3 mm, 6.5 mm, 9 mm and 12 mm) as the fish increased in size throughout the experiment. The sea cages were stocked with Goldsinney wrasse (*Ctenolabrus rupestris*; 1 per 50 salmon) to control sea lice infestations. Additional treatments with Excis [1% cypermethrin (m/v); Novartis Animal Health, Litlington, UK] for 1 h were performed on three occasions. In all treatments, the net pens were raised to a depth of 1 m and enclosed in a tarpaulin with oxygen provided to ensure that a minimum level of 7 p.p.m. was maintained.

Sampling strategy

A total of 206 fish from the ambient treatment and 298 fish from the heated treatment were weighed 11 times in seawater to investigate growth performance (Table 1). In addition, a set of fish was randomly sampled for studies of muscle structure on 9 June 1999 (freshwater stage) and 12 July, 3 August and 1 November 2000 and 10 January, 7 March and 4 June 2001 (seawater stages). A non-random selection of the largest fish in each cage was also made on 4 June and 22 August 2001. Fish were identified by brand and cross-referenced against PIT-tag number, and their mass and fork length were recorded.

Analysis of muscle growth

The fish were lightly anaesthetized in benzocane (20 mg l^{-1}) and killed with a blow to the head. Muscle blocks were prepared immediately. A 0.7 cm thick steak was prepared at the level of the first dorsal fin ray using a sharp knife. The entire steak was sampled for fry. For the seawater stages, the trunk

cross-section was traced onto acetate sheets in triplicate using a fine pen, identifying slow and fast myotomal muscle, the fin muscles and non-muscle components. The fast myotomal muscle component of the steak from one side of the body was divided into a series of evenly spaced blocks ranging from three per individual in the smallest fish to 12 per individual in the largest fish. Blocks were mounted on cork sheets and frozen in 2-methyl butane cooled to near its freezing point (–159°C) in liquid nitrogen. The blocks were wrapped in tin foil and stored in a liquid nitrogen refrigerator until they could be processed. The blocks were equilibrated to –20°C, and 7 μm transverse frozen sections were cut, mounted on poly-L-lysine-coated slides, air dried and either stained with Mayer's haematoxylin or used for immunohistochemistry. Randomly selected fields containing 100–300 muscle fibres per block were digitised using an image analysis system (SigmaScan software; SPSS Inc., Chicago, IL, USA), and the mean fibre diameter was calculated. A minimum of 800 and an average of 1000 muscle fibres were measured per fish, and the fibre number was estimated from the total cross-sectional area as described previously (Johnston et al., 1999). The maximum fibre diameter in each fish was determined from the mean of the 10 largest measured diameters in the sampled fibres.

Immunocytochemistry

Myogenic cells were identified by their expression of c-met (Johnston et al., 1999), and their location was determined using an antibody to laminin (Koumans et al., 1991), a major component of the basal lamina of muscle fibres. 18-μm-thick frozen sections were fixed in acetone for 10 min and then air dried for 10 min. Non-specific binding sites were blocked in a solution containing 20% (v/v) normal goat serum, 1.5% (m/v) bovine serum albumin (BSA) and 1% (v/v) Triton X-100 in phosphate-buffered saline (PBS) (all from Sigma Chemicals, Poole, UK). Anti-rabbit polyclonal immunoglobulin G (IgG) antibodies to laminin and m-met were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Both antibodies were shown to cross-react with the salmon muscle proteins. The antibodies were diluted 1:100 (v/v) (laminin) or 1:20 (m-met) in 1% Triton X-100, 1.5% (m/v) BSA, 10% (v/v) normal goat serum in PBS. The sections were rinsed in PBS for 5 min and incubated in the primary antibody overnight at 4°C. The sections were washed in PBS and incubated in a 1:800 (v/v) dilution of the secondary antibody (biotin-conjugated anti-rabbit IgG; Sigma). The sections were rinsed in PBS and were incubated for 1 h in the dark in a 1:100 (v/v) dilution of extravidin–Cy3 conjugate (Sigma). The sections were equilibrated in 2×SSC (300 mmol l^{-1} NaCl, 30 mmol l^{-1} sodium citrate, pH 7.0 at 20°C) for 5 min, counterstained in Sytox green® (Molecular Probes Inc., Leiden, The Netherlands) and then mounted in a fluorescent medium (DAKO Corp., Carpinteria, CA, USA). The sections were viewed with a laser confocal microscope (BioRad Radiance 2000). The density of myonuclei (stained green with Sytox green) and c-met^{+ve} cells (stained yellow) were quantified in sequential scanning mode in five or six fields of 0.37 mm^2 tissue section per fish using LaserPix v.4.0 software (BioRad, Hemel Hempstead, UK). Nuclear counts were corrected for section thickness and the mean diameter of nuclei (Abercrombie, 1946) previously determined from electron micrographs (Johnston et al., 2000a).

Nuclear content of isolated muscle fibres

Small bundles of fast muscle fibres were isolated from the dorsal myotome just behind the region sampled for histology. Fibre bundles were pinned at their resting length on strips of Sylgard (RS Ltd, Corby, UK) and fixed for 6–10 h in 4% (m/v) paraformaldehyde in PBS. Single muscle fibres freed from connective tissue were isolated in PBS using a binocular microscope fitted with dark-field illumination. Fibres were suspended in 1% (m/v) saponin in PBS for 3 h, washed three times in PBS and treated with 2 μg ml^{-1} units RNase (Sigma). Following further washes in PBS, the nuclei were stained with 30 μmol l^{-1} Sytox green in PBS for 5 min in the dark. Fibres were mounted on glass slides using fluorescent mounting medium (DAKO) and viewed with a laser confocal microscope (BioRad Radiance 2000). The density of fluorescent myonuclei was quantified in fibre segments 0.3–0.6 mm long using a z-series of 1 μm optical thick sections and LaserPix v.4.0 software.

The growth model

The specific growth rate (GR_t) for a period of time t is defined as:

$$GR_t = \frac{\log\left(\frac{M_t}{M_0}\right)}{t} \times 100 , \quad (1)$$

where M_0 is the initial body mass and M_t is the mass after t days.

Mooij et al. (1994) modelled the expected instantaneous growth rate of a fish ($d\hat{M}_t/dt$) as a temperature-dependent power function of M_t as follows:

$$\frac{d\hat{M}_t}{dt} = [\alpha + \gamma(T - T_{avg})]M_t^{\beta} , \quad (2)$$

where the parameters are listed in Table 2. For constant temperatures, Elliott (1975) gave the following mass–time function (equation 3) as a solution to equation 2:

$$\hat{M}_t = \{M_0^{1-\beta} + t(1-\beta)[\alpha + \gamma(T - T_{avg})]\}^{\frac{1}{1-\beta}} , \quad (3)$$

where the expected mass after t days, $\hat{M}_t$, can be calculated given M_0 and the other parameters. After a further t' days, the expected mass will be given by:

$$\hat{M}_{t+t'} = \{\hat{M}_t^{1-\beta} + t'(1-\beta)[\alpha + \gamma(T - T_{avg})]\}^{\frac{1}{1-\beta}} . \quad (4)$$

Mooij et al. (1994) assumed that β had the value 0.6 and estimated the parameters α and γ by minimising the sum of the squared differences between the log-transformed observed and predicted masses as shown in equation 5, where N is the

Table 2. *List of variables and parameters of the equations used in the statistical model of growth*

Observed variables	
t	Time (days)
M_0	Initial mass (g)
M_t	Observed mass after t days (g)
T	Mean environmental temperature for t days (°C)
T_{avg}	Long-term mean environmental temperature (°C)
Parameter estimated *a priori*	
β	Power of the growth-rate differential equation (assumed to be 0.6)
Parameters to be estimated	
α	Instantaneous growth rate of a fish weighing 1 g at T=T_{avg}
γ	Change in instantaneous growth rate per unit change in temperature

number of fish and k is the number of observations for each fish:

$$\sum_N \sum_k [\log(M_t) - \log(\hat{M}_t)]^2 . \quad (5)$$

Rearranging equation 4 and using observed, rather than predicted, masses gives:

$$\frac{M_{t+t'}{}^{1-\beta} - M_t{}^{1-\beta}}{1-\beta} = \alpha t' + \gamma t'(T - T_{avg}) . \quad (6)$$

Thus, the change in mass from time t to t' is a function of time t', temperature and the growth rates α and γ.

A standard multiple regression equation has the form:

$$y = a + bx_1 + cx_2 + \varepsilon , \quad (7)$$

where y is the response term, x_1 and x_2 are the explanatory variables, a is the intercept, b and c are the regression coefficients and ε is the error, or residual, term.

Letting y=($M_{t+t'}{}^{1-\beta}$–$M_t{}^{1-\beta}$)/(1–β), x_1=t' and x_2=t'(T–T_{avg}) allows equation 6 to be specified as shown below in model 1:

$$y = \alpha x_1 + \gamma x_2 + \varepsilon . \quad \text{(Model 1)}$$

Here, we assume that the intercept term is zero so that when time equals zero, the change in mass will also equal zero. The regression coefficients of model 1 are the growth rates α and γ (defined in Table 2), and these can be estimated using multiple linear regression. The units of α and γ are $g^{1-\beta}$ day^{-1} and $g^{1-\beta}$ day^{-1} $deg.^{-1}$, respectively. Models were fitted using computer programmes written in R, an open source dialect of S-plus.

Model 1 estimates a mean value of the growth rates α and γ, but a treatment effect can be included as shown in model 2:

$$y = \alpha_i x_1 + \gamma_i x_2 + \varepsilon , \quad \text{(Model 2)}$$

where i=1,...,n and n is the number of treatments. The change in mass is now a function of time, temperature, growth rates and treatment. Analysis of variance (ANOVA) was used to determine whether including a treatment effect significantly improved the fit of the model, thus indicating whether there was a statistical difference in the growth rate between the treatments. Similarly, the effects of the duplicate sea cages can be assessed. In model 3, sea cage was included so that the growth rates α and γ were now functions of both treatment i and cage k:

$$y = \alpha_{ik} x_1 + \gamma_{ik} x_2 + \varepsilon . \quad \text{(Model 3)}$$

The parameter of interest is the growth rate α and this will vary from fish to fish. Thus, for an individual fish j, it can be thought of as being composed of a component due to treatment i, α_i from model 2 (or α_{ik} from model 3), plus a random component, A_j. Model 4 incorporates a random effect for fish:

$$y = (\alpha_i + A_j)x_1 + \gamma_i x_2 + \varepsilon . \quad \text{(Model 4)}$$

The random component A_j can then be thought of as a random variable with a distribution that is assumed to be Normal with a mean of zero and a variance σ^2_A [$A_j \sim N(0,\sigma^2_A)$]. Essentially, the term α_i is the mean growth rate for treatment i, and A_j represents the difference between the growth rate of the jth fish and the mean growth rate for a fish. Thus, the change in mass is expressed as a function of time, temperature, growth rates, treatment and fish. Model 4 also takes into account the repeated nature of the observations; the data consist of several measurements on the same fish and so each growth period for a particular fish cannot strictly be treated as an independent piece of information. Including fish as a random component in this way takes the repeated measurement aspect into account. Since model 4 includes both random and fixed effects it represents a mixed-effects model.

Model 4 included a random fish effect for α but we would also expect that γ, the change in instantaneous growth rate per unit change in temperature, varies randomly for each fish. This is included in model 5:

$$y = (\alpha_i + A_j)x_1 + (\gamma_i + G_j)x_2 + \varepsilon , \quad \text{(Model 5)}$$

where G_j is the random component and is distributed as $N(0,\sigma^2_G)$.

Statistical analyses

The effect of treatment group on fibre number (*FN*) and the maximum fibre diameter in relation to age post-fertilisation was investigated with a General Linear Model ANOVA with a normal error structure using sequential sums of squares (Minitab™ statistical software; Minitab Inc., State College, USA). The model fitted had treatment, age and an age–treatment interaction term as fixed factors. Cage was nested in treatment, with cage as a random effect and age post-fertilisation as a covariate. The *FN* data were square-root transformed to normalise the residuals. The effect of treatment and total muscle cross-sectional area (TCA) on *FN* was analysed using a similar model with a TCA^3–treatment interaction term, and with TCA as a covariate with cage as a random factor nested in treatment. The data on the density of nuclei and c-met^{+ve} cells were analysed using a one-way analysis of covariance (ANCOVA). *Post-hoc* testing was by Fisher's least-significant difference test. Plots of residuals *versus* fitted values, normal probability *versus* residuals and histograms of the residuals were examined for each of the data sets.

Nonparametric statistical techniques were used to fit smoothed probability density functions to the measured diameters using a kernel function as described in Bowman and Azzalini (1997). The application of these methods to the analysis of muscle fibre diameters has been described in detail previously (Johnston et al., 1999). Values for the smoothing parameter *h* (Bowman and Azzalini, 1997) were in the range of 0.13 to 0.20 with no systematic differences between groups. Bootstrap techniques were used to distinguish underlying structure in the distributions from random variation (Bowman and Azzalini, 1997; Davison and Hinkley, 1997; Johnston et al., 1999). The Kolmogorov–Smirnov two-sample test statistic was used to test the null hypothesis that the probability density functions of treatment groups were equal over all diameters. A Wilcoxen nonparametric test was used to test the hypothesis that the median value of the 50th percentile was equivalent between groups.

Results

Effect of freshwater treatment on seawater growth

The seawater growth of the treatment groups is illustrated in Fig. 2. Prior to seawater transfer, fish were heavier in the heated (61.6±1.0 g; *N*=298) than in the ambient (34.1±0.4 g; *N*=206) treatments (mean ± S.E.M.), but their subsequent growth rate was lower. As a result, by the final sample in July 2001, the masses of the treatments were similar; 3718±75 g for the ambient group (*N*=176) and 3879±60 g (*N*=267) for the heated group. A multiple linear regression approach was used to model growth rate, where *y*, the response variable, and x_1 and x_2, the explanatory variables, were defined as shown below in equation 8:

$$y = \frac{M_{t+t'}^{1-\beta} - M_t^{1-\beta}}{1-\beta}, \quad x_1 = t', \quad \text{and} \quad x_2 = t'(T - T_{avg}). \tag{8}$$

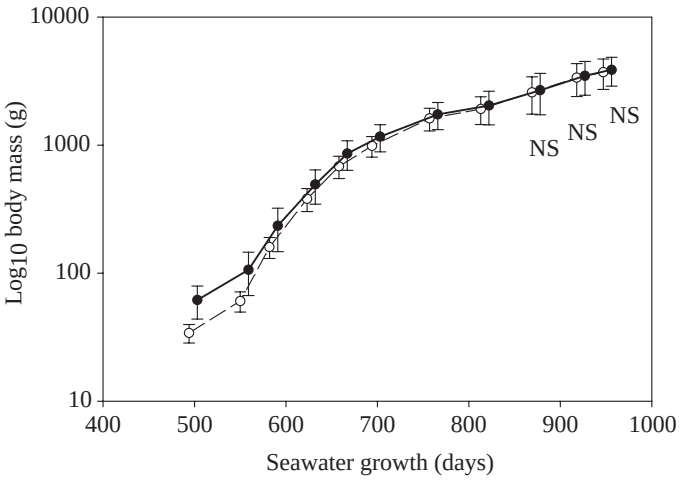

Fig. 2. The growth performance of fish from the ambient (open circles; *N*=206) and heated (filled circles; *N*=298) treatment groups of Atlantic salmon. The values represent means ± S.D. NS, no significant difference between treatments.

The analysis of variance for models 1 and 2 is shown in Table 3. Model 1 estimated a mean value of the growth rates of all fish, whereas model 2 estimated different values of the growth rate and the temperature sensitivity of growth rate for each treatment (Table 4). An *F* test indicated a significant treatment effect at the $P<0.05$ level. The results for model 3 indicate that there was a significant cage effect (Table 3) but, in practice, the effect of including cage was not large (Table 4); thus, we chose to ignore cage effect when fitting model 4 and model 5. The values of the growth rate α in Table 4 show that the regression coefficient for cage 1 of the heated group was similar to cage 2 of the ambient group. Model 4 included a random fish effect for growth rate α, and model 5 included a random fish effect for both α and γ. A log-likelihood ratio test

Table 3. *Analysis of variance table for models 1, 2 and 3*

Model	Source of variation	d.f.	SS	MSS	*F* ratio
Model 1	Regression	2	161 050	80 525	16 296.2*
	Residual	4671	23 081	5	
	Total	4673	184 131		
Model 2	Regression	4	161 107	40 277	8055.4*
	Residual	4669	23 024	5	
	Total	4673	184 131		
Model 2–model 1	Due to treatment	2	57	28.5	5.8*
	Residual	4669	23 024	5	
Model 3	Regression	8	161 537	20 192	4168.9*
	Residual	4665	22 594	5	
	Total	4673	184 131		
Model 3–model 2	Due to cage	4	430	107.5	22.2*
	Residual	4665	22 594	5	

d.f., degrees of freedom; SS, sums of squares; MSS, mean SS. The asterisks indicate that the regression is significant at the $P=0.05$ significance level.

Table 4. *Estimates of the regression coefficients (α and γ) for models 2, 3 and 5*

Model	Treatment	Cage	$\hat{\alpha}$	$\hat{\sigma}_A$	$\hat{\gamma}$	$\hat{\sigma}_G$
Model 1			0.1207		0.01470	
Model 2	Ambient		0.1232		0.01448	
	Heated		0.1189		0.01487	
Model 3	Ambient	1	0.1244		0.01221	
		2	0.1214		0.01758	
	Heated	1	0.1203		0.01300	
		2	0.1174		0.01689	
Model 5	Ambient		0.1231	0.0082	0.01454	0.0048
	Heated		0.1188		0.01495	

The parameters $\hat{\sigma}_A$ and $\hat{\sigma}_G$ are the standard deviations of random components A_j and G_j, respectively.

Table 5. *Log-likelihood ratio test of model 4 versus model 5*

Model	d.f.	Log-likelihood	*L* Ratio
Model 4	6	–10 379.2	
Model 5	8	–10 305.8	146.8*

d.f., degrees of freedom. The asterisks indicate that the regression is significant at the P=0.05 significance level.

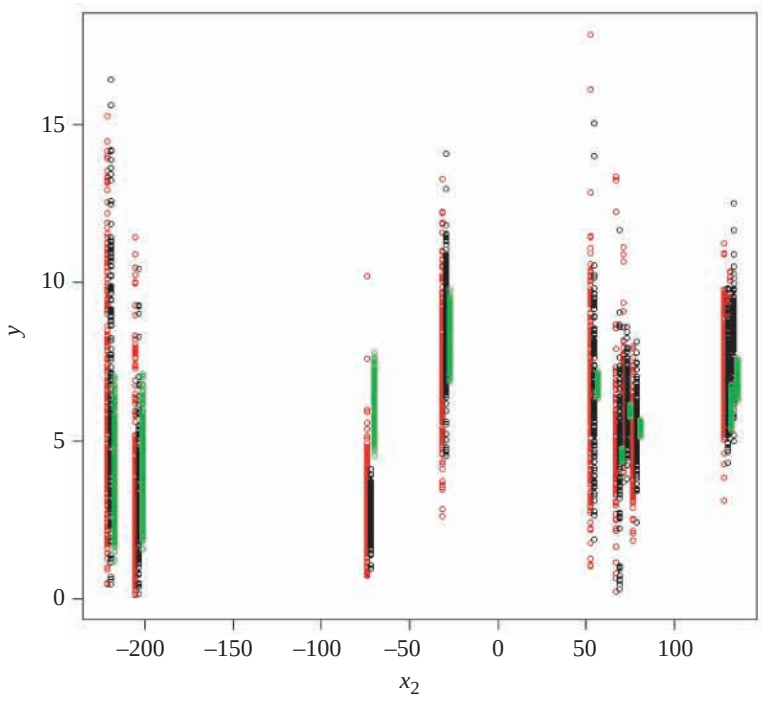

Fig. 3. Fit of the growth model to the observed data of body mass in seawater. In the figure, y is plotted against x_2, where $y=(M_{t+t'}^{1-\beta}-M_t^{1-\beta})/(1-\beta)$ and $x_2=t'(T-T_{avg})$. The variables are defined in Table 2. Black dots represent observed values for the ambient group, red dots represent observed values for the heated group, and the fitted values of y from model 5 are shown in green. The values have been offset slightly along the x-axis so that they can be seen more clearly.

was used to test whether the more-specific model 5 was required over model 4 (Table 5). Model 5 was preferred over model 4, and the estimates of the regression coefficients for model 5 are shown in Table 4. The fitted values of y for model 5 plotted against x_2 are displayed in Fig. 3. The green dots indicate the range in the fitted values because of including a small random value for each fish. The fit of the model to the observations was generally good but less so for the first seawater sample (Figs 3, 4). There was a significant difference between the treatment groups at weighings 1–8 (335 days), but there was no difference at weighings 9–11. Both instantaneous growth rate (α) and the change in instantaneous growth rate per unit change in temperature (γ) were significantly higher for the ambient than the heated treatment groups (Table 4).

Muscle fibre recruitment

The relationship between fibre number (*FN*) and age post-fertilisation for a random subset of fish is shown in Fig. 5A. The final random sample was taken on 4 June 2001; 904 days and 913 days post-fertilisation for the ambient and heated groups, respectively. Muscle fibre recruitment was investigated using ANCOVA (Table 6; model A). The analysis revealed a highly significant difference in *FN* between treatment groups (P=0.038, N=129) but no significant difference between cages. The age×treatment interaction term was also significant, consistent with a significant effect of treatment on the rate of muscle fibre recruitment (Table 6; model A). The relationship between *FN* and the total cross-sectional area (TCA) of fast muscle for the random and non-random (enclosed in box) samples is shown in Fig. 5B. An ANOVA with *FN* as dependent variable, and treatment, TCA and TCA^3×treatment as fixed effects is also shown in Table 6 (model B). The treatment and interaction terms were both significant. A non-random sample of the largest fish in each cage was also taken in June and on 22 August 2001. Smooth distributions were fitted to the measurements of fibre diameter in each sample; an example is shown in Fig. 6. The end of the fibre recruitment phase of growth was identified by the absence of immature fibres (<10 μm diameter). The maximum number of fast fibres (FN_{max}) was calculated by pooling all the fish that did not contain fibres of <10 μm diameter. The maximum fibre number was 22.4% greater in the ambient ($9.3\times10^5\pm2.0\times10^4$) than in the heated ($7.6\times10^5\pm1.5\times10^4$) treatments ($N$=44 and 40 fish, respectively; $F_{1,82}$=45.83, P<0.001; one-way ANOVA). There was a significant correlation between FN_{max} and the individual growth rate calculated from model 4 (June 2001 sample; r^2=0.35, $F_{1,56}$=29.8, P<0.001; Fig. 7).

Muscle fibre hypertrophy

The first step in the analysis was to investigate the smooth distributions of fibre diameter. Bootstrap sampling was used to generate a variability band

corresponding to the combined treatments, and this was compared with the mean probability densities in the heated and ambient groups. The data for the first and final seawater samples are illustrated in Fig. 8. In the first seawater sample (July 2000), the maximum diameter was approximately 165 μm for the heated group and 145 μm for the ambient group (Fig. 8A). The right-hand tail of the distribution, representing the oldest cohorts of fibres recruited in freshwater, occurred at higher diameters in the heated group than in the ambient group, reflecting the higher mean body mass of the heated group (225 g) compared with the ambient group (147 g). The maximum density of fibres was at 25 μm, and this large peak probably represents fibres recruited following seawater transfer. The peak density in the ambient treatment was displaced slightly to the left compared with the heated group (Fig. 8A). Nonparametric Kolmogorov–Smirnov tests revealed significant differences in the fibre size distributions between treatments in each of the seawater samples (P<0.05). In the final sample, illustrated in Fig. 8B, the mean body mass of the heated group (4449 g) was not significantly different from that of the ambient group (4414 g). The right-hand tail of the distribution was at higher fibre diameters in the heated group

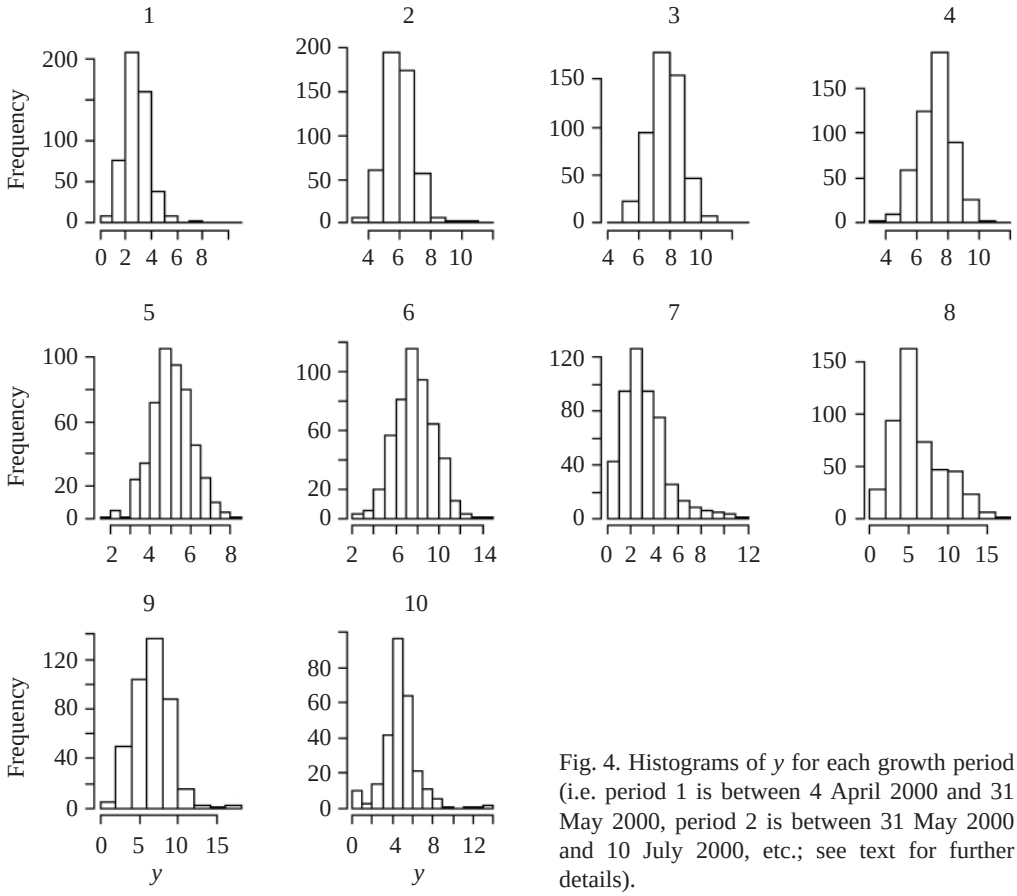

Fig. 4. Histograms of y for each growth period (i.e. period 1 is between 4 April 2000 and 31 May 2000, period 2 is between 31 May 2000 and 10 July 2000, etc.; see text for further details).

Table 6. *Analysis of variance with the number of fast muscle fibres (FN) as a dependent variable using the method of sequential sums of squares for tests*

Source	d.f.	Seq. SS	Seq. MS	F	P
Model A					
Treatment	1	8.936×10^4	8.936×10^4	25.93	0.038
Age	1	6.333×10^6	6.333×10^6	4539.0	0.0005
Treatment–age	1	3.420×10^4	3.420×10^4	8.31	0.005
Cage (treatment)	2	7100	3550	0.84	0.425
Error	124	5.10×10^5	4115		
Total	129	6.974×10^6			
Model B					
Treatment	1	2.574×10^{11}	2.574×10^{11}	67.67	0.016
TCA	1	6.601×10^{12}	6.601×10^{12}	1998.6	0.0005
TCA^3×treatment	1	9.888×10^{10}	9.888×10^{10}	29.94	0.0005
Cage (treatment)	1	7.591×10^9	3.796×10^9	1.15	0.32
Error	120	3.963×10^{11}			
Total	129	9.086×10^{12}			

d.f., degrees of freedom; seq. SS, sequential sums of squares; seq. MS, sequential mean squares; F, variance ratio; P, probability. √FN was fitted as the dependent variable to normalise the residuals.

than in the ambient group (Fig. 8B). The peak density of fibres occurred at approximately 125 μm in the ambient group and 130 μm in the heated group. The mean probability densities on the left-hand side of the distribution were very different for heated and ambient groups. The ambient groups had a higher density of fibres in the range of 50–90 μm (Fig. 8B), reflecting the greater intensity of fibre recruitment during seawater growth (Fig. 5A,B). To investigate the differences in fibre size further, the 50th percentile (median) fibre diameter (D_{50}) and the maximum diameter (D_{max}) were calculated. Across the entire experiment, median fibre diameter was higher for the heated group compared with the ambient group (Wilcoxen test; $P<0.05$). The relationship between D_{max} and age post-fertilisation for the randomly sampled fish is illustrated in Fig. 9A. Fast muscle fibres approached their maximum diameter of approximately 200 μm at around 800 days post-fertilisation. An ANCOVA with D_{max} as the dependent variable showed a significant effect of treatment but no significant treatment × age interaction (Table 7). D_{max} was higher in heated than in ambient treatments with respect to body mass (Fig. 9B). There was no consistent difference in the mean rate of increase in D_{max} in seawater between ambient (0.27 ± 0.06 μm day^{-1}) and heated (0.22 ± 0.06 μm day^{-1}) treatments. We also used the mean values of fibre number per group to estimate the number of fibres recruited between successive sample points. The 800–1000 fibres measured at each sample were ranked by diameter and then the estimated proportion of fibres recruited since the last sample was subtracted and the mean diameter of the remaining fibres were calculated. The estimated rate of fibre hypertrophy of the fibres present at seawater transfer also showed no consistent differences between treatment groups (Fig. 10).

Nuclear content of isolated muscle fibres

An isolated single fast-muscle fibre stained for total myonuclei is shown in Fig. 11A. Connective tissue was carefully removed from the fibres prior to counting the nuclei. There was a linear relationship

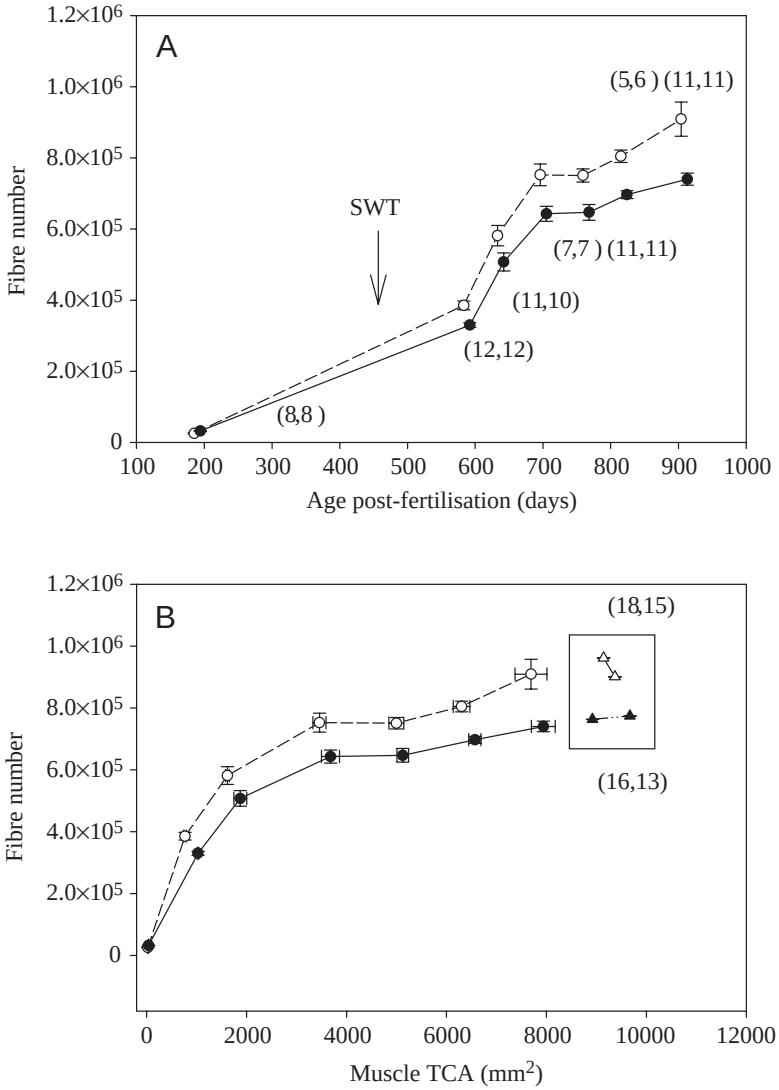

Fig. 5. The number of fast muscle fibres per trunk cross-section (*FN*) for the ambient (open circles) and heated (filled circles) treatment groups of Atlantic salmon. (A). The relationship between *FN* and age post-fertilisation for the randomly sampled fish. The numbers in brackets represent the number of fish sampled for the ambient and heated groups, respectively. The arrow shows the age at which smolts were transferred to seawater cages (SWT). (B) The relationship between *FN* and the total cross-sectional area (TCA) of fast muscle at the level of the first dorsal fin ray. The values represent means ± S.E.M. Fish were selected at random, except for the last two samples (box), which represents a selection of the largest individuals available in each cage. The numbers in brackets represent the number of fish sampled for the ambient and heated groups, respectively.

Table 7. *Analysis of covariance of the maximum diameter of fast muscle fibres in Atlantic salmon using the method of sequential sums of squares for tests*

Source	d.f.	Seq. SS	Seq. MS	*F*	*P*
Treatment	1	5116	5116	120.53	0.008
Age	1	2.289×10^5	2.289×10^6	1161.3	0.0005
Treatment × age	1	14	14	0.07	0.790
Cage (treatment)	2	85	42	0.21	0.807
Error	134	26417	197		
Total	139	244987			

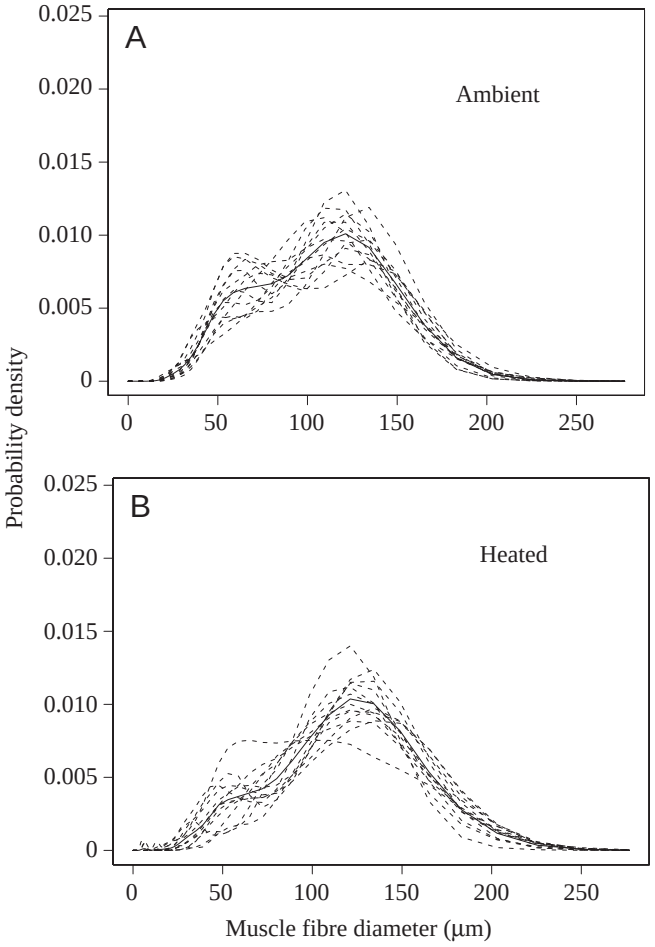

Fig. 6. The distribution of muscle fibre diameter in the fast myotomal muscle of Atlantic salmon: (A) the final sample of the ambient treatment group sampled 988 days post-fertilisation; (B) the final sample of the heated treatment group sampled 997 days post-fertilisation. Smooth distributions were fitted to 800 measurements of fibre diameter per fish using a nonparametric kernel function. The broken lines represent the probability density for individual fish, and the solid line represents the mean probability density function for each group.

Table 8. *Density of nuclei and cells immunopositive for c-met in the fast myotomal muscle of seawater stages of Atlantic salmon reared under 'ambient' and 'heated' regimes in freshwater*

	Freshwater temperature regime	
Parameter	Ambient	Heated
Sample July 2000		
Number of fish	7	8
Fork length (cm)	23.7±0.4	27.8±0.5
Body mass (g)	141.7±7.0	229.4±12.5
Nuclei per mm^3 muscle	66,920±2070	44,830±1870
c-met^{+ve} cells per mm^3 muscle	1819±110	930±60
MC/N (%)	2.70	2.07
Sample March 2001		
Number of fish	7	7
Fork length (cm)	56.2±0.4	58.9±0.7
Body mass (g)	2247±48	2414±58
Nuclei per mm^3 muscle	37,830±1940	27,700±1840
c-met^{+ve} cells per mm^3 muscle	1214±70	670±114
MC/N (%)	3.21	2.42

Values represent means ± S.E.M.
N, nuclei per mm^3 muscle; MC, c-met^{+ve} cells per mm^3 muscle.

between the number of nuclei in a 1 cm segment of fibre and fibre diameter (Fig. 11B). A General Linear Model ANOVA with fibre diameter as covariate and nuclear content as dependent variable revealed a significant difference between treatment groups ($F_{1,154}$=37.48, P<0.001). The regression equations were as follows: nuclei = 314 + 17.1 fibre diameter (ambient group; r^2=89.3; ANOVA; $F_{1,75}$=626.0, P<0.001) and nuclei = 237 + 14.3 fibre diameter (heated group; r^2=83.6; ANOVA; $F_{1,78}$=398.9, P<0.001). For fast-muscle fibres of 200 μm diameter, there were 20.6% more nuclei in the ambient treatment (3734 myonuclei cm^{-1}) than in the heated (3097 myonuclei cm^{-1}) treatment.

Myogenic progenitor cells

In some cases, myogenic cells were surrounded by the basal lamina of muscle fibres (yellow arrow in Fig. 12A) whilst in others cases they were not (yellow arrow in Fig. 12B). The density of myogenic cells and nuclei was determined for the July 2000 and March 2001 samples (Table 8). ANOVA with treatment group and body mass as fixed factors and a treatment–body mass interaction term with body mass as covariate revealed significant differences between

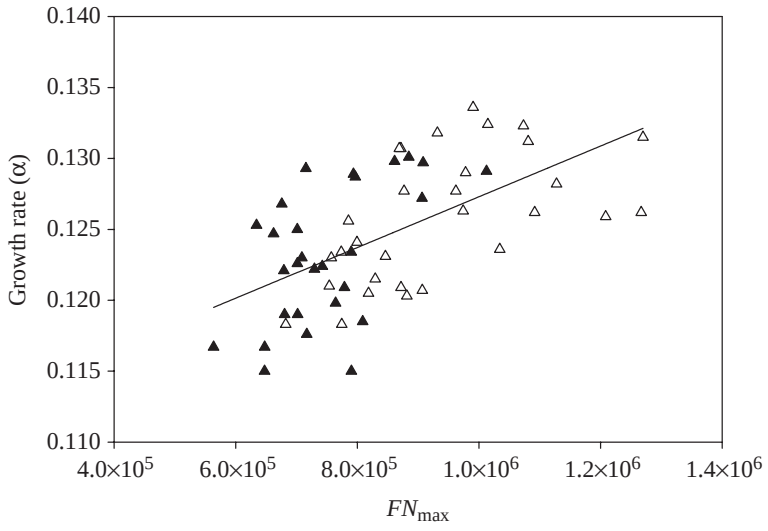

Fig. 7. The relationship between fibre number (FN_{max}) and individual growth rate (α) in fish that had completed fibre recruitment in the June 2001 samples. Filled and open symbols represent the heated and ambient treatments, respectively. A first-order linear regression was fitted to the data (r^2=0.35; α=0.109+1.79×10^{-8} (FN_{max})).

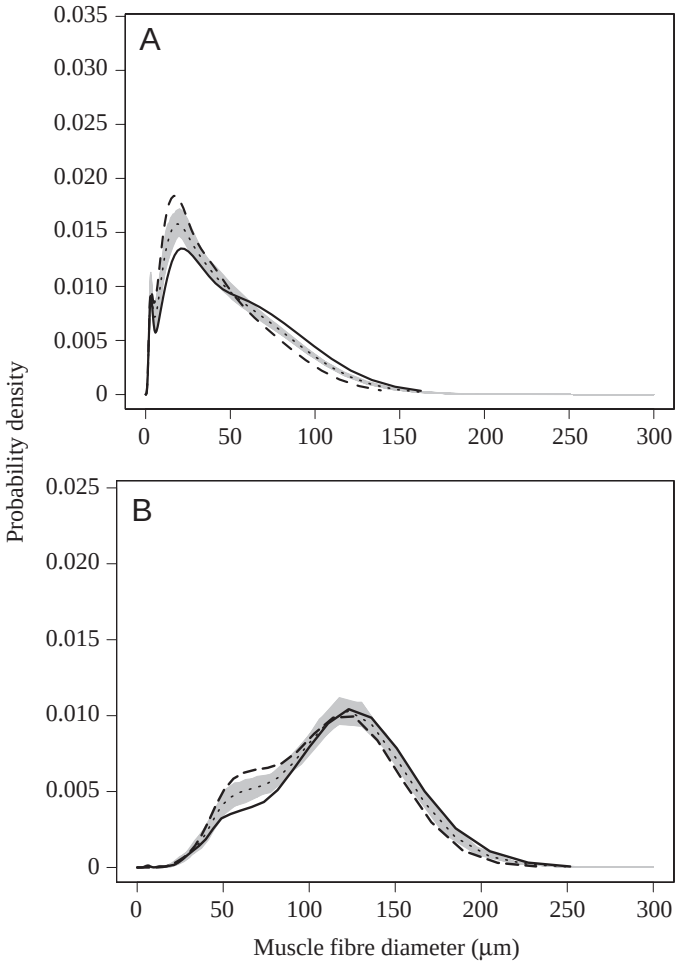

Fig. 8. The mean probability density of muscle fibre diameter for heated (solid line) and ambient (dashed line) treatment groups for the first (A) and last (B) seawater samples taken in July 2000 and August 2001, respectively. The shaded polygon represents 100 bootstrap estimates of the combined populations of ambient and heated fish, and the dotted line represents the mean probability density function of the pooled groups. Regions where the mean probability density function fell outside the shaded polygon provided graphical evidence for a difference between the populations.

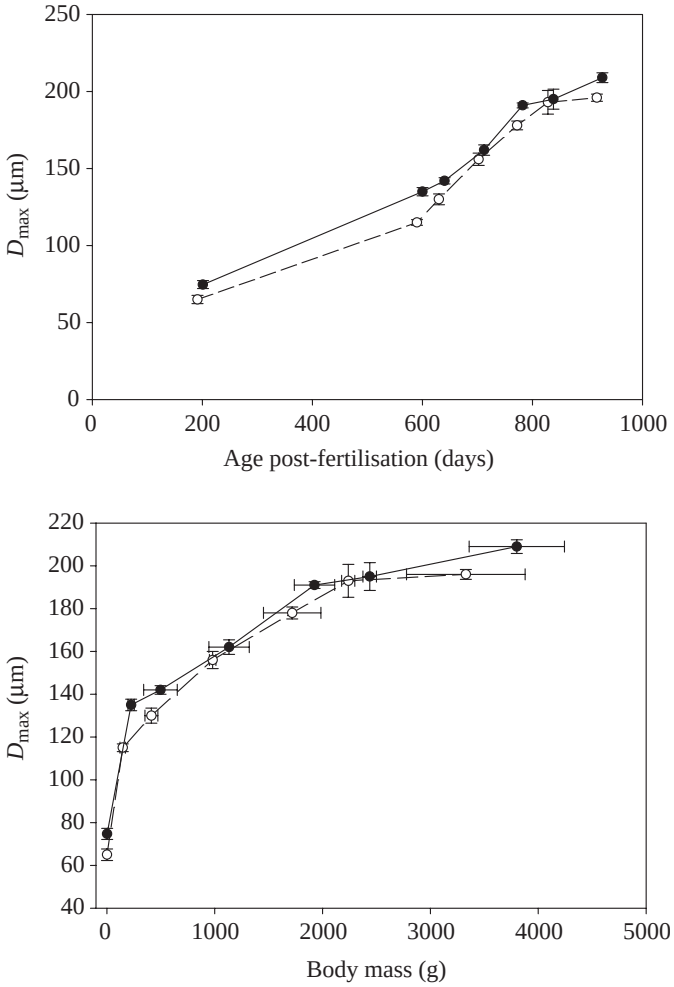

Fig. 9. The relationship between maximum fibre diameter (D_{max}) and (A) age post-fertilisation and (B) body mass for the heated (filled circles) and ambient (open circles) treatment groups of Atlantic salmon. Values represent means ± S.E.M. The number of fish sampled is as in Fig. 5A.

treatment groups with respect to total nuclear density and the density of c-met^{+ve} cells (Table 8). Differences in nuclear density per mm^2 muscle cross-sectional area reflected differences in fibre size distribution (Fig. 8) and differences in the nuclear content of individual muscle fibres (Fig. 11B). The density of c-met^{+ve} cells was almost 2-fold higher in the ambient than in the heated groups, representing 2–3% of the total muscle nuclei (Table 8).

Discussion

For fish fed to appetite, the two most important parameters governing growth rate are temperature and body size (Jobling, 1983). Growth usually shows a distinct optimum related to the life history stage and thermal tolerance of the species (Weatherley and Gill, 1987). For example, in Atlantic salmon, the growth rate of juveniles increased between 6°C and 15.9°C and then declined as temperature increased to 22.5°C (Elliott and Hurley, 1997). The specific growth rate of the fish decreases with increasing body size (Jobling, 1983). Several growth models have been developed that provide an estimate of instantaneous growth rate independent of the effects of temperature and scale (Ricker, 1979; Elliott and Hurley, 1997; Mooij et al., 1994; Mooij and van Ness, 1998). The multiple regression model described in the present study incorporated effects for treatment and cage, and random factors for individual growth rate (α) and individual variation in the temperature sensitivity of growth (γ). The models provided a good fit to the observed data on fish masses except for the first period following seawater transfer (Figs 3, 4). It is possible that the stress associated with the implantation of PIT tags and seawater transfer depressed growth performance over this first period.

Early thermal experience has been shown to alter the

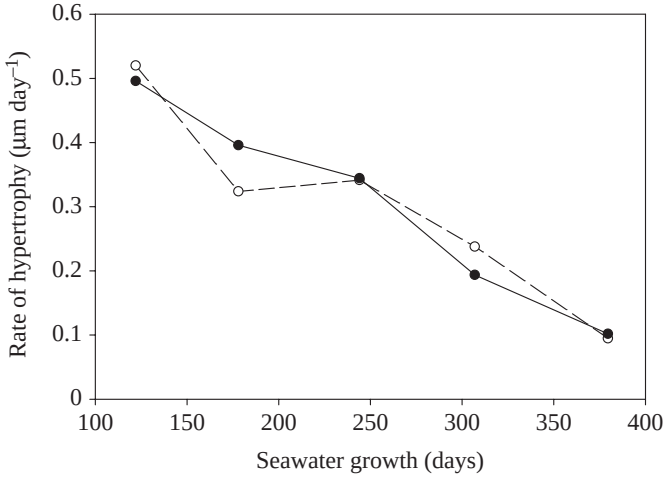

Fig. 10. The rate of muscle fibre hypertrophy of fast muscle fibres between successive sample points plotted against seawater growth in Atlantic salmon from the heated (filled circles) and ambient (open circles) treatment groups. The rate of hypertrophy has been plotted at the midpoint of the time period over which it was calculated. Values represent the mean of the difference between the observed fibre diameter and the mean value of the fibre diameter in the preceding sample.

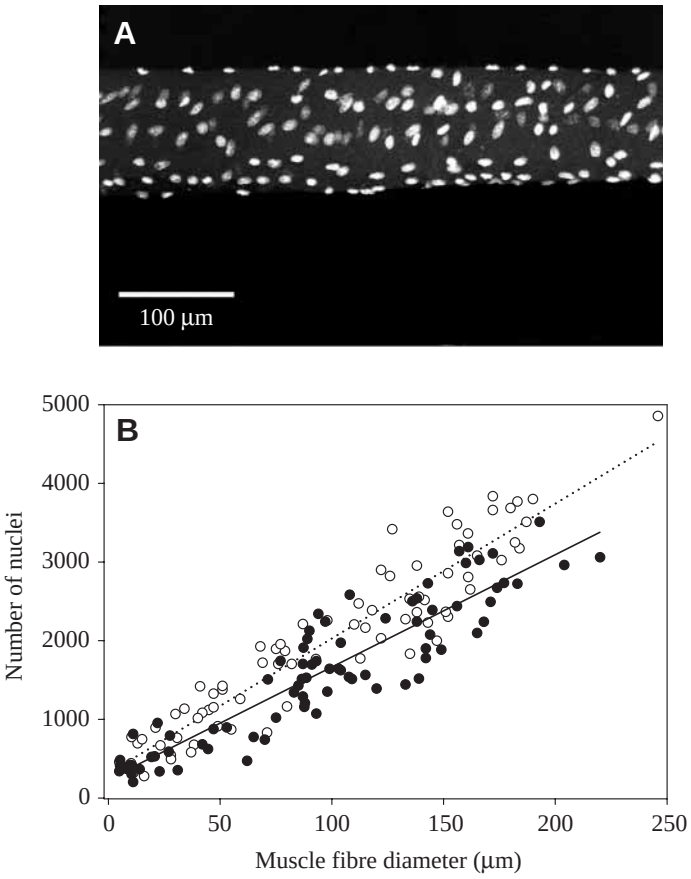

Fig. 11. The nuclear content of isolated muscle fibres. (A) A fast muscle fibre stained with the fluorescent DNA stain Sytox green. The confocal image represents a projection of 1 μm sections through the fibre. (B) The number of myonuclei in single muscle fibres of 1 cm length in relation to muscle fibre diameter. Open circles represent the ambient group and filled circles represent the heated group. The lines represent linear regressions (see text for details).

number, size distribution and nuclear content of muscle fibres in juvenile Atlantic salmon (Stickland et al., 1988; Nathanailides et al., 1995; Johnston and McLay, 1997; Johnston et al., 2000a,b). It has been suggested that such effects are, in part, related to associated changes in oxygen tension due to the large diameter of salmonid eggs (Matschak et al., 1998). A difference in mean egg incubation temperature of 2.8°C was sufficient to produce changes in muscle cellularity throughout the freshwater parr stages (Johnston et al., 2000a,b). In the present study, we tested the hypothesis that freshwater temperature regime would continue to have effects on the recruitment and hypertrophy of muscle fibres once the fish smolted and began a phase of rapid growth in seawater. Salmon were maintained either in heated water or at ambient temperature until smoltification. The cumulative degree-days in freshwater were 13.8% higher in the heated groups than in the ambient groups, providing a significantly greater growth opportunity. Shortly before seawater transfer, the mean body mass of the heated groups was, on average, 44.6% more than that of the ambient groups. Following seawater transfer, the treatment groups were fed to appetite and reared together in the same cages, providing an equal growth opportunity.

We tested the hypothesis that freshwater treatment would alter the rate of muscle fibre recruitment and hypertrophy in seawater. In the first seawater sample, muscle TCA, D_{50} and D_{max} were all significantly greater in the heated than in the ambient groups, reflecting the larger body mass of the heated fish. However, the number of muscle fibres per trunk cross-section was 14.5% higher in the ambient ($3.9\times10^5\pm1.1\times10^4$) than in the heated groups ($3.3\times10^5\pm6\times10^3$) ($P<0.05$; Fig. 5A). An ANOVA revealed significant effects of freshwater environment on the number of fast muscle fibres throughout the period of seawater growth (Table 6). The end of the recruitment phase of growth was identified by the absence of fibres less than 10 μm in diameter. The final fibre number (FN_{max}) was 22.4% greater in fish reared in ambient compared with heated water temperatures. These changes in FN_{max} represent a freshwater treatment effect on mosaic hyperplasia, since this is the only mechanism of fibre expansion still active in seawater stages.

Significant differences in the distribution of muscle fibre diameter were observed between treatments (Fig. 8); however, the reasons for this are complex. The median and mean fibre diameter were higher in the heated than in the ambient group throughout the experiment. The median and mean fibre diameters are a reflection of two opposing processes, namely fibre recruitment and hypertrophy, which tend to decrease and increase fibre diameter, respectively. It is not possible to measure the rate of fibre hypertrophy in an individual fish. However, there were several lines of evidence to suggest that

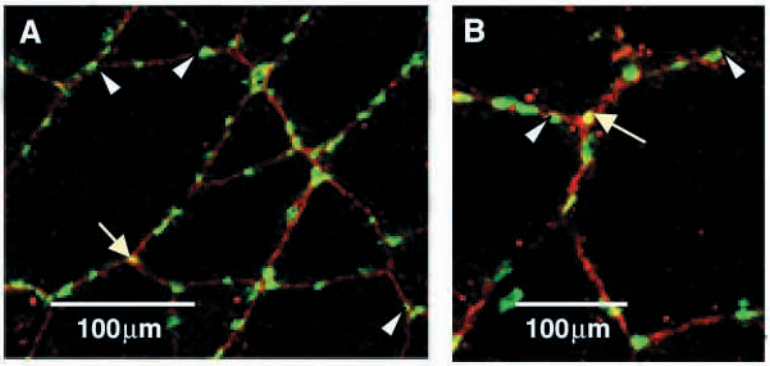

Fig. 12. (A,B) Immunohistochemistry showing sections of salmon fast myotomal muscle double-stained with primary antibodies to laminin and c-met using Cy-3 as the secondary antibody and Sytox green as a nuclear counterstain. Nuclei are stained green (white arrowheads), and c-met^{+ve} cells (yellow arrows) and the basal lamina are stained red.

the rate of fibre hypertrophy in seawater was not affected by freshwater treatment. Firstly, the average increase in D_{max} between samples showed no consistent trend between treatments. Secondly the age–treatment interaction term for D_{max} was not significantly different (Table 7). Thus, our first hypothesis concerning the effect of freshwater environment on seawater growth was accepted for fibre recruitment but rejected for the rate of hypertrophic growth.

FN_{max} is an important anatomical trait because it can potentially influence both growth rate and maximum body size. The rate of hypertrophy of individual muscle fibres decreases with age as their maximum diameter is approached (Weatherley and Gill, 1987). We found a positive correlation between FN_{max} and individual growth rate (α), explaining around 35% of the total variation (Fig. 7). Similar relationships between growth rate and fibre number within species have been reported previously for mammals (reviewed in Rehfeldt et al., 1999). In the present study, the higher fibre number in ambient compared with heated groups can explain at least some of the difference in growth rate between treatments, although other causes, including developmental effects on the endocrine system regulating growth, cannot be excluded.

The mononuclear cells that express the cell surface receptor c-met are thought to represent a mixture of muscle stem cells and their progeny at various points along the pathway to terminal differentiation (Hawke and Garry, 2001; Zammit and Beauchamp, 2001). In fish muscle, the majority of c-met^{+ve} cells also express members of the MyoD gene family, which suggests most are already committed to terminal differentiation (Johnston et al., 2000a; Brodeur et al., 2002). The progeny of the stem cell population either fuse to form myotubes or are absorbed into existing fibres as they expand in diameter. A significant fraction of the myogenic cells will also be involved in nuclear turnover (Schmalbruch and Lewis, 2000). It is not known at what stage the fate of the cells participating in myotube formation and hypertrophic growth is determined. In the present study, the number of nuclei in isolated fibre segments was found to be 20.6% higher in the ambient than in the heated treatments, indicating that the freshwater temperature regime affected the total production of muscle nuclei. The number of mononuclear c-met^{+ve} cells representing myogenic precursors was 2-fold higher in the ambient than in the heated treatments, equivalent to 2–3% of the muscle nuclei (Table 8). The proportion of c-met cells to total nuclei was within the range previously reported for myosatellite cells identified by electron microscopy in adult common carp (*Cyprinus carpio*; Koumans et al., 1994).

A simple explanation for our results is that the treatment groups differed in the number of muscle stem cells, resulting in proportional increases in differentiating myogenic nuclei. However, it is also possible that freshwater treatment affected some aspect of the signalling pathways that regulate the proliferation and/or differentiation of myogenic cells. The number of times the myogenic cells divide prior to terminal differentiation is known to be influenced by a complex network of hormones, growth factors and transcription factors, most of which have been poorly characterised in fish (Johnston et al., 2002). Growth hormone is a powerful stimulator of insulin-like growth factor-1 (IGF-1) genes, which have multiple actions on myogenic cells (Florini et al., 1991; Mommsen and Moon, 2001). Myostatin (Mstn), a member of the TGF-β (transforming growth factor β) superfamily, is a potent negative regulator of muscle growth (McPherron et al., 1997) and is highly conserved across the vertebrates (Rodgers and Weber, 2001). Mice carrying a targeted disruption of the Mstn-encoding gene show a 2-fold increase in muscle mass arising from a combination of increased muscle fibre hyperplasia and hypertrophy. Mstn-deficient mice also show a suppression of body fat (McPherron and Lee, 2002), which was not observed in the present study (I. A. Johnston, unpublished results). The lack of an effect of treatment on fibre hypertrophy also argues against a role for myostatin and IGF-1 genes (McPherron et al., 1997; Barton-Davis et al., 1999).

Previous studies have shown that variations in embryonic temperature regime alone are sufficient to produce changes in muscle cellularity in fish. There is evidence that the sensitivity of fibre number to egg incubation temperature varies between different spawning populations of Atlantic salmon (Johnston et al., 2000b). Johnston et al. (2000a) collected eggs from Atlantic salmon spawning in lowland and upland tributaries of the River Dee, Scotland, UK. The embryos were incubated at the simulated temperature regimes of each tributary, which was, on average, 2.8°C cooler for the upland than the lowland stream. For the lowland fish, *FN* was approximately 10% higher when eggs were incubated at the temperature of their natal stream, whereas in the upland fish *FN* was similar at both thermal regimes (Johnston et al., 2000b). In the present study, eggs from an inbred population of farmed salmon were incubated at somewhat higher and more constant temperatures. The choice of constant or fluctuating temperature regime has been shown to influence the number of muscle fibres in larval

pearl fish (Stoiber et al., 2002). Without information on the shape of the reaction norm relating fibre number to development temperature, it is difficult to interpret differences in the direction of responses among studies. For example, a bell-shaped reaction norm could produce either an increase, a decrease or no change in fibre number depending on where on the temperature range the eggs were incubated.

It has been estimated that approximately one-third of the variation in fibre number in limb muscles of the pig is phenotypic and not related to genetic origin (Rehfeldt et al., 1999). Several studies have shown that in mammals poor maternal nutrition causes a low birth mass and a reduction of the number of secondary myotubes, resulting in a permanent reduction in the number of muscle fibres (Wilson et al., 1988; Dwyer et al., 1995). Developmental plasticity of muscle growth has been reported previously in birds prior to the establishment of effective thermoregulation. In this case, mild heat exposure in young broiler chickens (*Gallus domesticus*) resulted in a transient growth halt followed by immediate compensatory growth (Yahav and Hurwitz, 1996). Such thermal conditioning was associated with an immediate increase in circulating IGF-1 concentration followed by satellite cell proliferation (Halevy et al., 2001).

The temperature prior to hatching is critical for determining the number of myogenic cells (Johnston et al., 2000a) and fibre number post-hatch in Atlantic salmon (Stickland et al., 1988; Johnston and McLay, 1997; Johnston et al., 2000b). Adverse conditions during early development have a negative impact on subsequent growth in a wide range of species (Lindström, 1999). The present study found that FN_{max} was reduced by 18.3% for a relatively modest rise in temperature during freshwater development. The heated treatment could not be considered stressful since growth was increased relative to the ambient group. Such phenotypic variation in FN_{max} in fish for ecologically relevant temperature changes has the potential to influence a range of life history characteristics, including growth rate and ultimate body size. However, an effect of FN_{max} on ultimate size would require an independence of fibre number and size. Studies in mammals suggest that this may not be the case because in animals that have stopped growing, fibre number and mean diameter are inversely correlated (Rehfeldt et al., 1999). Developmental plasticity in growth characteristics may be relatively commonplace in ectotherms. For example, egg incubation temperature was shown to influence subsequent growth rate and body size at defined developmental stages in lizards (Braña and Ji, 2000) and turtles (O'Steen, 1998; Rhen and Lang, 1999), although in these studies the effects on muscle fibre number were not investigated.

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