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The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L.)

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Summary. From fertilisation to hatching one group of salmon embryos was reared at ambient temperatures (fluctuating around 1.6° C) and another at 10° C. At Gorodilov stages 28, 30 and 33 transverse sections of whole embryos were obtained for light and electron microscopy. Total cross-sectional areas, fibre numbers, fibre diameters and myofibrillar areas of the white muscle of m. lateralis were measured. At hatching (stage 33, which occurred much earlier at the higher temperature), the higher temperature embryos had significantly larger ($P<0.01$) but fewer ($P<0.05$) muscle fibres. These larger fibres contained significantly more myofibrillar material ($P<0.05$) than the smaller fibres of the lower temperature embryos. Lesser differences were found at pre-hatching stages. Higher temperatures caused myofibre hypertrophy to increase at a greater rate than hyperplasia. Hence, the cellularity of the tissue produced under the different temperature regimes was quite different.

Key words: Salmon – Myogenesis – Temperature

Introduction

It has long been known (Krogh 1914) that the rate of embryonic development is influenced by temperature in a variety of animals, and in the salmon this was demonstrated by Hayes et al. (1953). However, less is known about the effects of temperature on cellular aspects of developing tissues. Embryonic stages of myogenesis in fish are associated with myofibre hyperplasia as well as hypertrophy. The present study investigated the effects of temperature on these two features of embryonic muscle development in Atlantic salmon (*Salmo salar* L.).

Materials and methods

Eggs were stripped from a 4-year-old female salmon on 28 November 1985, and fertilised with sperm from a 4-year-old male. They were transferred to perspex hatching trays in mesh baskets, which were kept in hatching troughs through which water flowed at 1.5–2.0 l/min, at the DAFS Smolt Rearing Station, Almondbank, Perthshire. Half the eggs were incubated at 10° C, maintained constant by means of a proportional electronic thermostat. The other half were kept at ambient temperature which fluctuated around a daily median of 1.6° C (S.E. 0.2) for the study period (127 days from fertilisation).

From the onset of somitogenesis, samples of 10 eggs from each temperature regime were sent by train to London at defined intervals, wrapped in cotton wool soaked in river water, inside a jar cooled with ice. All eggs arrived alive.

Development stages were defined according to Gorodilov's (1983) system. Stages 28, 30 and 33 were used, corresponding to ages 83, 103, and 127, and 34, 40 and 54 days after fertilisation for the ambient and 10° C embryos respectively. For both regimes, stage 33 were newly hatched.

The embryos were removed from their shells, decapitated and the first 15-somite region placed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate (at pH 7.3) for 2–3 h. Then they were post-fixed in 1% osmium tetroxide, dehydrated in acetones and embedded in araldite. Semi-thin (0.5–1 μm) transverse sections were cut and stained with methylene blue. These complete body-sections, taken between the 10th and 15th somites, were used for the light microscopic analyses. Ultrathin sections were obtained from stage 33 for electron microscopy.

A total of 18 embryos (3 at each stage, under each regime) were analysed. The whole transverse sectional area of muscle (m. lateralis) on one side was measured using a VIDS II image analyser (Analytical Measuring Systems Ltd). The number of fibres in this area was counted from photomicrographs (Fig. 1). Superficial red muscle was excluded from all analyses. Cell size analysis was made from a region dorsal to the lateral line, excluding the most superficial white fibres (Fig. 1). As it was impossible to identify fibre boundaries in the light microscope sections, mean myofibrillar areas (readily seen within individual fibres) were measured in up to 100 fibres in each region. To assess variation in these areas across the muscle blocks, myofibrillar areas were measured across a transect for all the stage 33 fish (Fig. 1).

Electron micrographs (from the square sample area, Fig. 1) of 10 fibres were analysed by the VIDS system for all stage 33 fish, to assess the relationship between muscle fibre area and myofibrillar area for each temperature regime. The significances of differences in various parameters were determined by Student's *t*-test between temperature regimes.

Results

The salmon embryos developed much faster at the higher temperature (Fig. 2).

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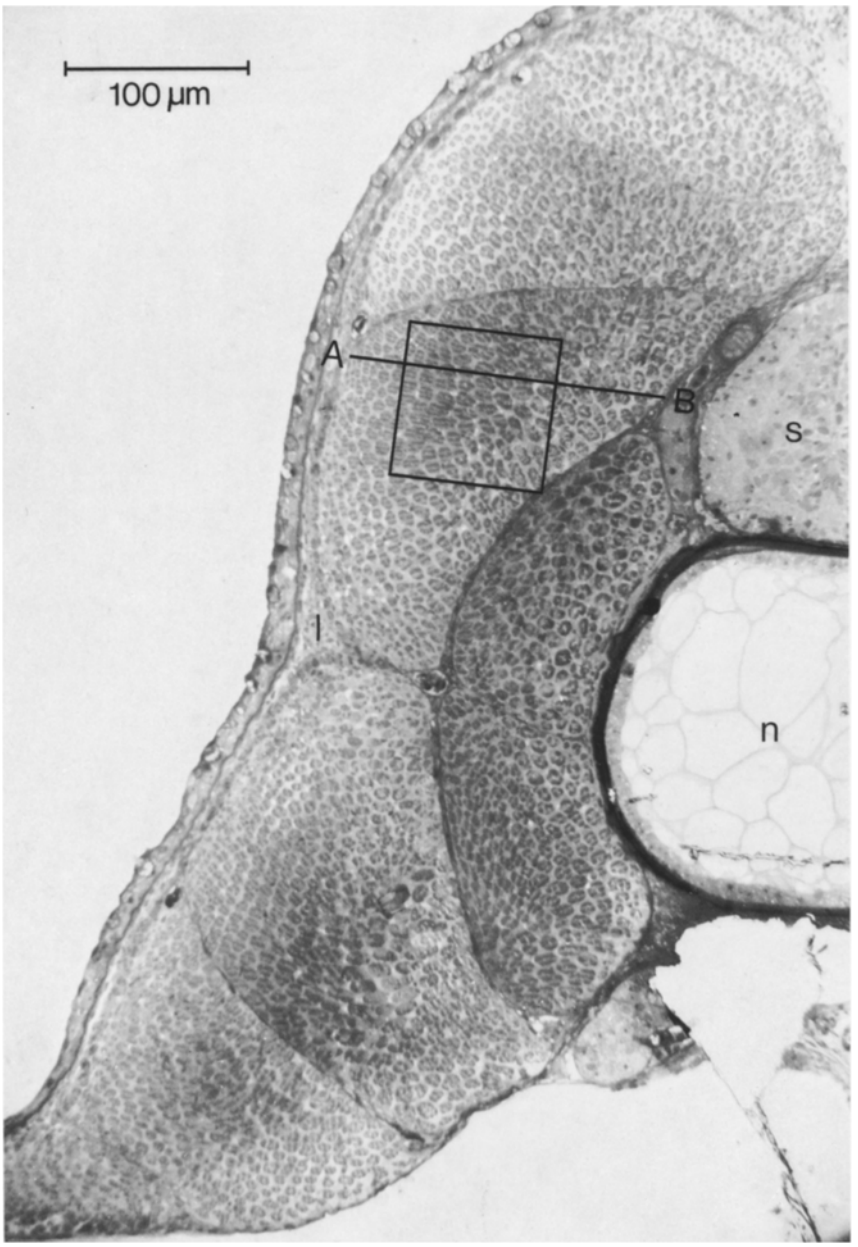


Fig. 1. A complete transverse section of one side of a newly hatched fish reared at ambient temperature. The line *AB* indicates the position of a typical transect along which all myofibrillar areas were measured. The box indicates the region where measurements were made to estimate mean muscle fibre cross-sectional areas and myofibrillar areas. *l*, lateral line; *s*, spinal cord; *n*, notochord

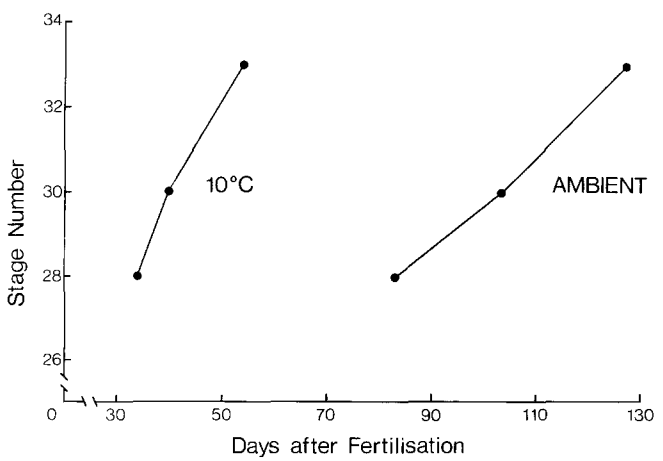


Fig. 2. Shows the relationship between stage number and days after fertilisation for each temperature regime

The total muscle cross-sectional area (of one side) of the ambient embryos was slightly but not significantly larger than the 10° C embryos at all stages (Fig. 3). Also, there was no significant difference between the lengths of ten ambient and ten 10° C embryos at stage 33. At stages 28 and 30, there were no significant differences between treatments in the total number of muscle fibres in the m. lateralis of one side, but these increased at a much greater rate with respect to stage number in the ambient embryos (Fig. 4) so that at hatching the 10° C embryos contained about 30% fewer fibres. Mean myofibrillar areas were significantly larger in the 10° C embryos at stages 28 and 33 (80% and 50% larger respectively) (Fig. 5).

Myofibrillar areas from a transect (Fig. 1) of representative ambient and 10° C embryos at stage 33 gradually increased in size from the lateral towards the middle region of the muscle (Fig. 6). The size of these areas then levelled off before falling rapidly at the most medial border. The

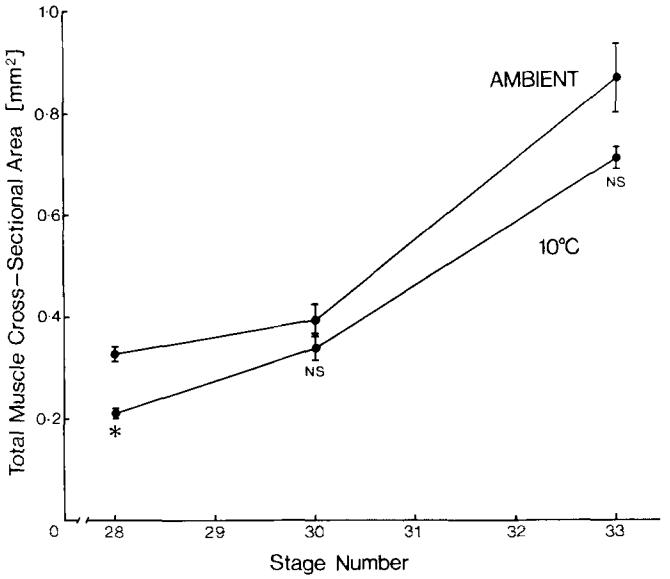


Fig. 3. Shows the relationship between total muscle cross-sectional area (for m. lateralis on one side) and stage number for each temperature regime. Significance of differences at each stage are indicated: *NS*, Non-significant; *, $P<0.02$

larger size and fewer number of fibres was also evident in the 10° C embryos.

Electron micrograph measurements on the stage 33 embryos showed that myofibrils occupied significantly more ($P<0.05$) of the fibre areas in the 10° C embryos (64.8% ± 3.9) than in the ambient ones (59.3% ± 1.5) (Fig. 7).

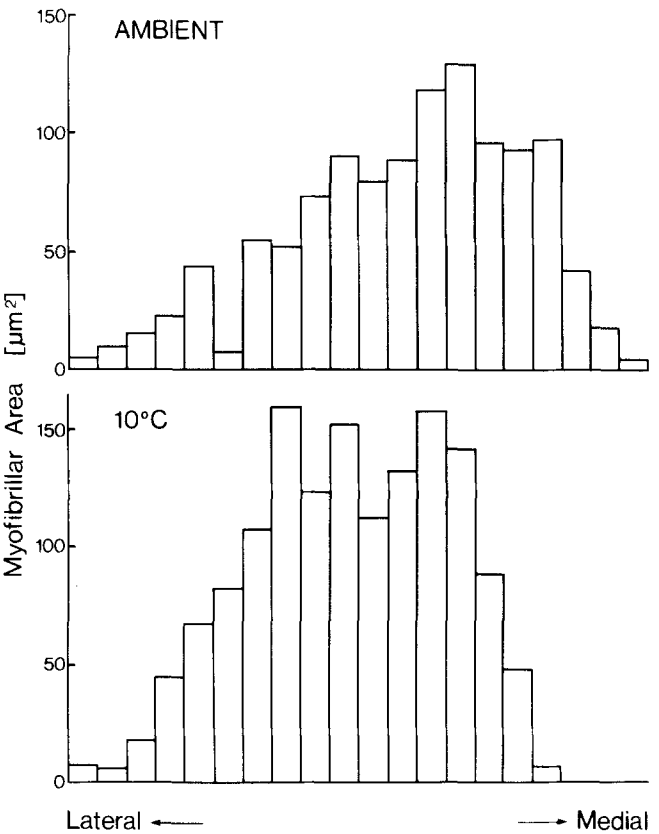
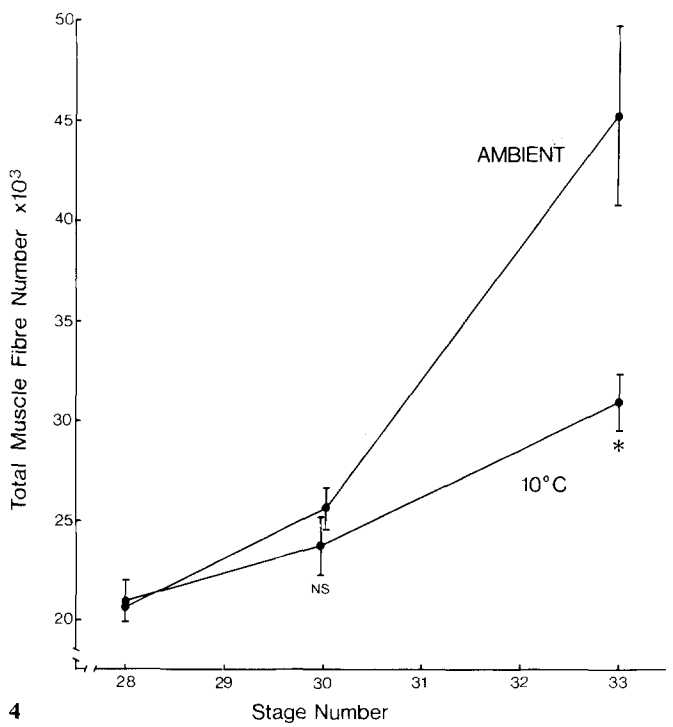


Fig. 6. Shows the change in myofibrillar area across m. lateralis (of one side) from the most lateral to most medial aspect



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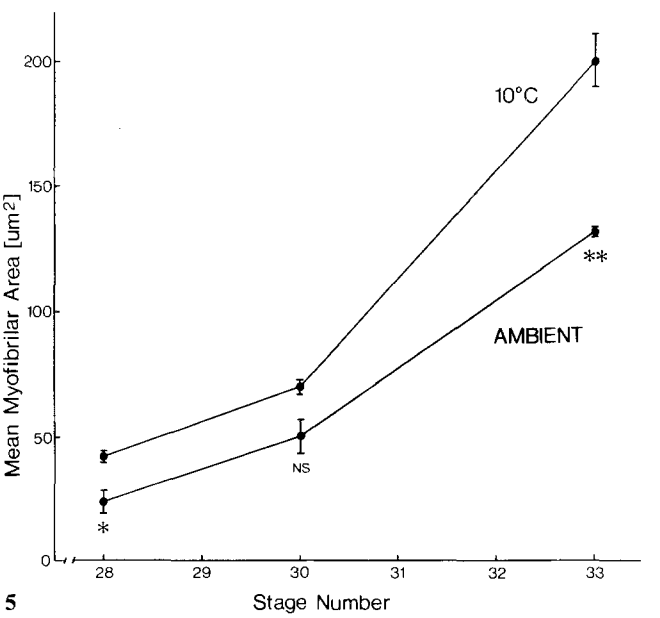

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Fig. 4. Shows the relationship between total muscle fibre number (in m. lateralis of one side) and stage number for each temperature regime. Significance of differences at each stage are indicated: *NS*, Non-significant; *, $P<0.05$

Fig. 5. Shows the relationship between mean myofibrillar area and stage number for each temperature regime. Significance of differences at each stage are indicated: *NS*, Non-significant; *, $P<0.05$; **, $P<0.01$

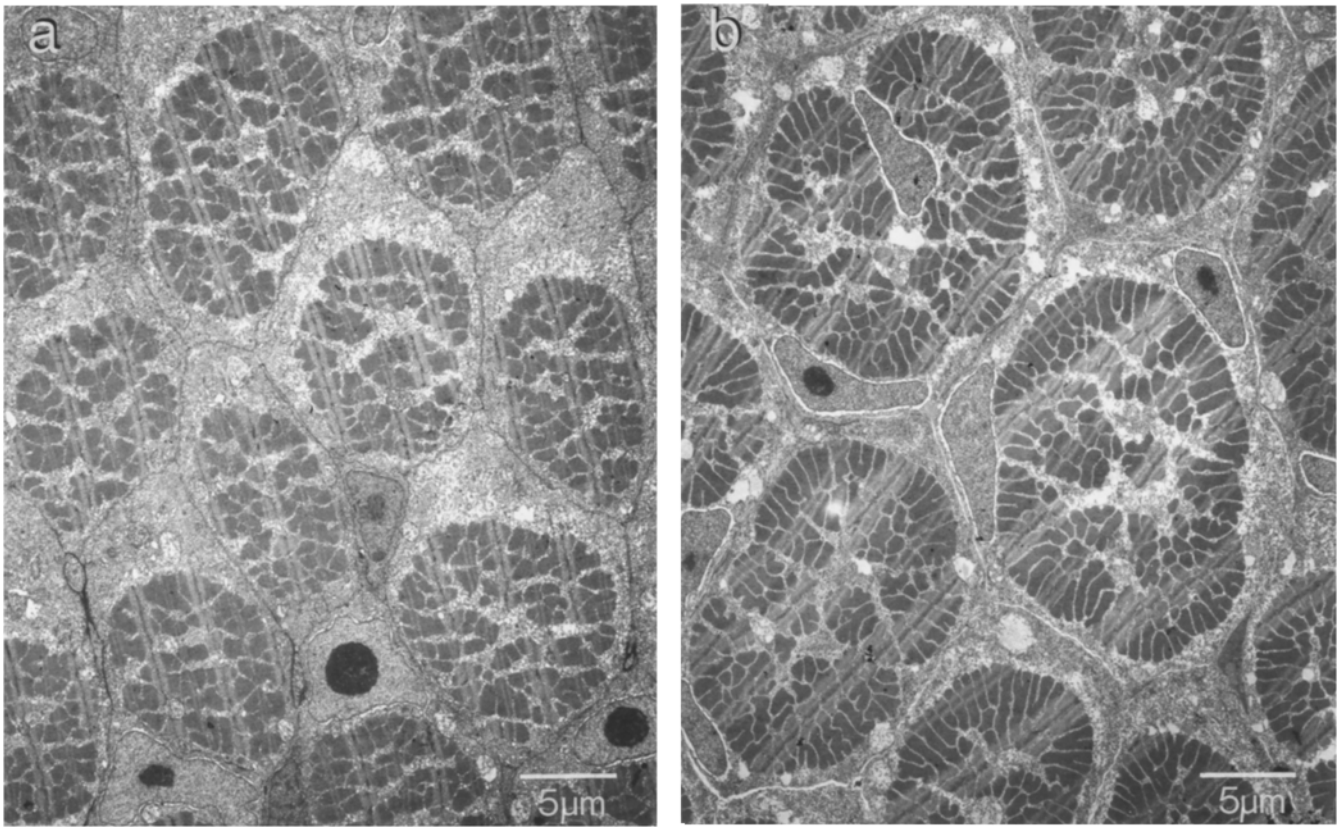

Fig. 7. Electron micrographs of muscle taken from newly hatched fish reared at (**a**) ambient temperature and (**b**) 10° C

Discussion

Higher rearing temperatures increase the developmental rate of fish embryos and advance the time of hatching (Hayes et al. 1953; Kinne and Kinne 1962; Balon 1980), and the present study confirms this. It is also clear that the developmental rate of all the muscle parameters measured has been increased by the higher rearing temperature. However, to assess differential effects of temperature on muscle parameter development the embryos have to be compared at the same developmental stage. Hayes et al. (1953) suggested that the order of appearance of anatomical features in salmon morphogenesis might be altered by temperature, but Gorodilov (1983) showed a constancy in this sequence independent of temperature between 0.5 and 11° C. We therefore used developmental stages as defined by Gorodilov as the instants for quantitative comparison of myogenesis under the contrasted temperature regimes.

Both fibre hyperplasia and hypertrophy occurred during salmon myogenesis in this study. Hyperplasia was evidenced by an increase with age in the total number of myofibres seen in the complete body sections. Hypertrophy was seen as an increase in both fibre cross-sectional area and in myofibrillar content. Both fibre hyperplasia and hypertrophy continue in post-hatch salmonid muscle growth (Weatherley et al. 1979, 1980; Stickland 1983; Villarreal 1983; Higgins 1985), whereas in mammals hyperplasia ceases near the time of birth (Stickland 1981; Wigmore and Stickland 1983). If it is accepted that the newest fibres are the smallest, then the transect histograms (Fig. 6) indicate that new fibres form at the lateral borders of the myotome. Waterman (1969) found a similar situation in *Brachydanio rerio*. There also appear to be zones of proliferation dorsally and ventrally in the salmon myotome (Fig. 1). In post-hatch salmon this lateral proliferation zone is not evident, and very small fibres are found throughout the myotome (Brooks, personal communication).

The most interesting result is the finding that temperature had a greater effect on the rate of embryonic fibre hypertrophy (as measured by myofibrillar area increase) than on hyperplasia. It would seem that temperature had a differential effect on cell division and protein synthesis. By the time of hatching the higher temperature embryos had fewer but 40% larger muscle fibres than the ambient embryos. Also, as their myofibrillar areas were 50% greater, the 10° C embryos had relatively more myofibrillar material in their fibres at hatching ($P<0.01$). This may be due to increased temperatures causing increased embryonic movements (Fluchter and Rosenthal 1965), in turn stimulating myofibril production. However, this contrasts with the general finding that fibre hyperplasia is greater among faster than among slower growing fishes (Weatherley and Gill 1987), and requires further study.

In summary, higher incubation temperature produced muscles at hatching with fewer but larger fibres, with relatively higher myofibrillar content. So although higher temperatures increased the development rate, not all mechanisms of myogenesis were increased to the same extent. Hence the cellularity of the tissue produced differed under different temperature regimes.

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