

Muscle growth in the Antarctic teleost, *Notothenia neglecta* (Nybelin)

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Abstract: A histochemical and electron microscopy study was carried out on muscle growth in demersal stages of the Antarctic teleost *Notothenia neglecta* Nybelin. The total number of myotomal muscle fibres was similar in fish ranging in body mass from 11.9g to 889g. Post-anal myotomes contained around 17,000 slow muscle fibres and 70,000 fast muscle fibres. Myosatellite cells were extremely rare. The diameter of fast fibres varied from <10µm to 130µm in the smallest, and from >40µm to 450µm in the largest fish studied. Slow muscle fibre diameters in the largest fish ranged from >30µm to 260µm. Even the largest diameter slow fibres contained significant numbers of mitochondria, which suggests that the diffusion of oxygen does not limit metabolism. The results confirm that muscle fibre hyperplasia ceases prior to the demersal stages of the life history, and that subsequent muscle growth is entirely via the hypertrophy of existing fibres. Comparative studies suggest that this may be one of the factors contributing to the relatively slow rate of somatic growth in this species.

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Introduction

Longevity and slow rates of annual growth are characteristic of many Antarctic fish (Freytag 1980, North & White 1987, Radtke *et al.* 1989). Although feeding occurs throughout the year in many species (Daniels 1982), there is evidence that growth is highly seasonal. For example, maximum growth rates of the post-yolk sac larvae of several nototheniids were 2–4 times faster in summer than winter (North 1990). It seems likely that slow winter growth is related to the reduced availability and quality of prey organisms (Foxton 1956, Clarke 1988) and to the lower light intensities and shorter day-lengths which decrease the feeding opportunities of larvae (Kellermann 1986). Interestingly, the maximum summer growth rates of larval Nototheniids from South Georgia at <4°C are within the range of temperate species at >14°C (North 1990). Larvae and early juveniles have appreciably faster growth rates than latter stages (Burchett 1983, Radtke *et al.* 1989). This also suggests that factors other than low temperature might limit maximum growth rates in Antarctic fish.

There is evidence that the ability of species for rapid somatic growth is closely linked to the dynamics of muscle increase (Weatherley *et al.* 1988). Muscle growth occurs through a combination of the recruitment of new fibres (hyperplasia) and the hypertrophy of existing fibres (Willemse & Van Den Berg 1978, Weatherley *et al.* 1979). Weatherley *et al.* (1988) studied 10 species of freshwater fish and found that both growth rate and ultimate size were positively

correlated with the length at which the recruitment of new fibres ceased. In general, muscle fibre number stabilized at around 44% of the ultimate body length, and thereafter further growth was by the hypertrophy of existing fibres (Weatherley & Gill 1984, Weatherley *et al.* 1988). Support for this hypothesis comes from a study of muscle growth in the Argentine hake, *Merluccius hubbsi*, which shows significant differences in maximum size between the sexes (Calvo 1989). Fast muscle fibres, <10µm diameter, were absent from the myotomes of 52–53 cm males whereas females, which attain a larger ultimate mass, retained fibres in this size class until 60–63 cm (Calvo 1989).

There are several reports that the myotomes of Antarctic fish contain a high proportion of very large diameter muscle fibres (Smialowska & Kilarski 1981, Dunn *et al.* 1989). This suggests that muscle hyperplasia may cease relatively early during development in Antarctic species, contributing to the slow growth of late juvenile and adult stages. The present study investigates this possibility for demersal stages of *Notothenia neglecta* from the South Orkney Islands.

Materials and methods

Fish

Specimens of the Antarctic fish, *Notothenia neglecta* (Nybelin) were caught by trammel net from the inshore waters around Signy Island, South Orkney Islands. Fish were subsequently transported to the UK and maintained for several months in

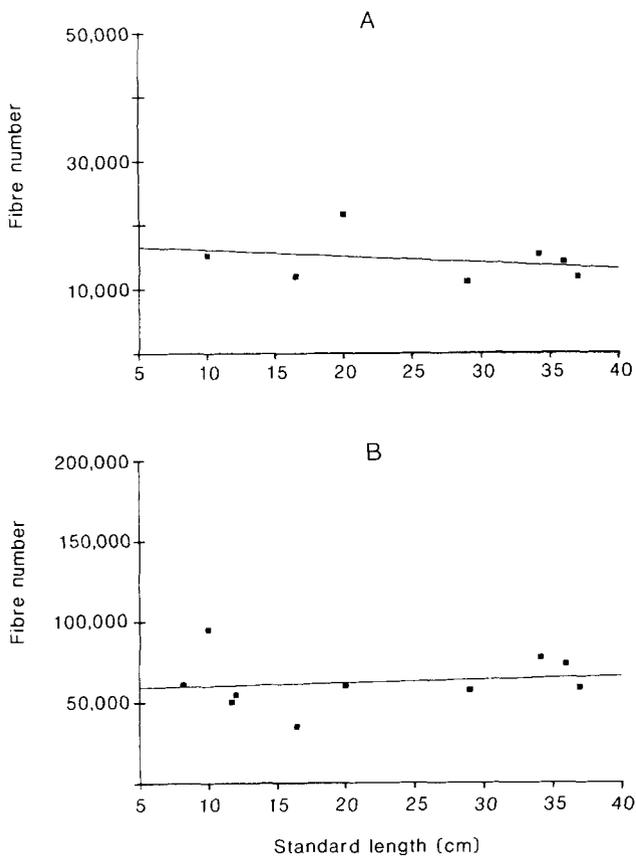


Fig. 1. Graph of myotomal fibre number versus standard length for *N. neglecta* of varying sizes. A. slow muscle fibres, regression coefficient $r = 0.14$; B. fast muscle fibres, $r = 0.28$.

tanks of filtered, re-circulated seawater at $0.5\text{--}1.0^\circ\text{C}$ (12h light:12h dark). They were fed to satiation several times a week on a mixed diet of squid, krill and fish. Fish were killed by stunning and decapitation and their body mass and standard length (SL) recorded.

Determination of muscle fibre number and diameter

Two 0.5 cm sections were cut through the trunk with a sharp knife immediately posterior to the urogenital opening. The first section was used to determine the cross-sectional area of red (slow) and white (fast) muscle (Johnston & Camm 1987), and the other was used for histochemical studies. Typically 2–3 blocks were sufficient to sample the entire slow muscle strip in the region of the lateral line nerve. The much larger mass of fast muscle fibres required 5–12 blocks from representative areas. Tissue samples were orientated transversely on cryostat chucks and frozen in semi-solid isopentane (2-methylbutane) cooled to near its freezing point in liquid nitrogen. Frozen sections of $10\ \mu\text{m}$ were cut, air-dried and stained for glycogen by the periodic acid-Schiff method. Slow muscle fibres were identified by their intense staining reaction for glycogen. The cross-sectional

area (CSA) and mean diameter from ten diameters on each muscle fibre were determined using a microscope drawing arm and digital planimeter interfaced to a Hewlett-Packard 86B microcomputer. In small fish (CSA c $120\text{--}200\ \text{mm}^2$) muscle blocks from the whole of the CSA were taken, in medium sized fish (CSA $200\text{--}450\ \text{mm}^2$) blocks from one half of the area were taken, and in the larger fish (CSA $>450\ \text{mm}^2$) random blocks from one sixth of the area were taken. Measurements from each section were made using a randomly placed 8 cm square grid, and at least two grids per section were sampled (Egginton, 1988). Whole fibres falling inside the grid, and those overlapping the left hand and lower sides were counted. Those overlapping the upper and right hand sides were omitted (Egginton, 1988).

Electron microscopy

Small bundles of fast and slow fibres were fixed for 12h at their resting length *in situ* in 2% glutaraldehyde, 1 mM CaCl_2 , 0.15M cacodylate buffer pH 7.4 (at 4°C). Samples were subsequently post-fixed in 1% osmium tetroxide, washed in distilled water, dehydrated through a series of alcohols, and cleared in 1,2-epoxypropane prior to embedding in Araldite CY212 resin (EMscope, Trent, England). Ultrathin ($60\ \text{nm}$) sections were mounted on 150 mesh pyroxyline coated copper grids, stained with uranyl acetate and Reynolds lead citrate and viewed with a Phillips 301 Transmission Microscope at 60 kV.

Statistical analyses

Regression analysis was carried out on the data for total fibre number according to Sokal and Rohlf (1969).

Results

A total of 11 *Notothenia neglecta* were studied ranging from the earliest demersal stages around 11.9g body mass (10 cm SL) to sexually mature specimens 889g body mass (37 cm SL). Males and females were used in our analysis, since it was not possible to sex the immature specimens. The number of fast and slow muscle fibres present in post-anal myotomes was similar in all the fish studied (Fig. 1).

Fast muscle fibres in the smallest size class, $0\text{--}10\ \mu\text{m}$, were only present in fish $<20\ \text{g}$ body mass (Fig. 2). Between the smallest and largest body mass there was a modal progression of fibre diameter from $42\ \mu\text{m}$ to $152\ \mu\text{m}$ in fast muscle (Fig. 2) and from $19\ \mu\text{m}$ to $121\ \mu\text{m}$ in slow muscle (Fig. 3). In the largest specimens sampled, there were no fast fibres $<40\ \mu\text{m}$ diameter (Fig. 2A), and no slow fibres $<20\ \mu\text{m}$ diameter (Fig. 3A). The maximum diameter of fast muscle fibres ranged from $130\ \mu\text{m}$ in the 11.9g fish to $>450\ \mu\text{m}$ in the 754g fish (Fig. 2). In slow muscle the maximum fibre diameter ranged from $50\ \mu\text{m}$ to $>250\ \mu\text{m}$ between the smallest and largest fish studied (Fig. 3).

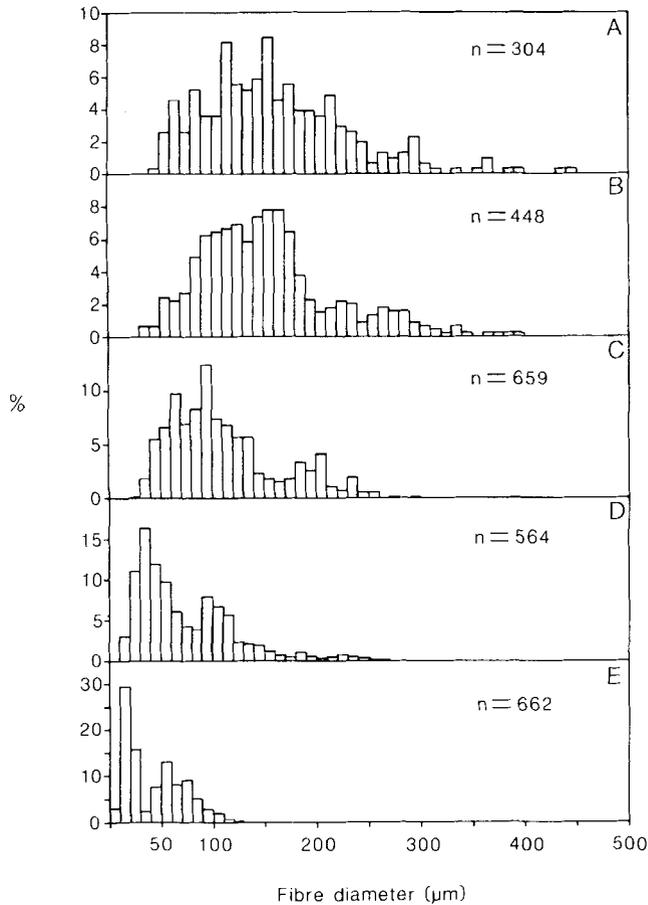


Fig. 2. Frequency distributions of the diameters of myotomal fast muscle fibres in *N. neglecta*.

- A. body mass = 754.8g, Standard Length (SL) = 34.0cm, mean diameter = 142.2 μ m, Standard Deviation (SD) = 71.9, skewness = 0.99, kurtosis = 4.46.
 B. body mass = 435.7g, SL = 29.0cm, mean dia.= 152.7 μ m, SD = 65.9, skewness = 0.9, kurtosis = 3.9.
 C. body mass = 77 1g, SL = 16.5cm, mean dia.= 112.7 μ m, SD = 0.85, skewness = 0.85, kurtosis = 3.00
 D. body mass = 27.3g, SL = 11.7cm, mean dia.= 61.5 μ m, SD = 39.9, skewness = 0.98, kurtosis = 2.7
 E. body mass = 11.9g, SL = 8.2cm, mean dia.= 42 μ m, SD = 27.49, skewness = 0.58, kurtosis = 2.23.

Electron microscopy

Myosatellite cells were extremely rare in both fast and slow muscles. Although sections from >40 blocks from 11 fish were examined very few myosatellite cells were observed. Both small, <40 μ m, and large, >100 μ m, diameter slow muscle fibres contained numerous mitochondria (Fig. 4), and mitochondria were present in the central core of even the largest diameter slow fibres.

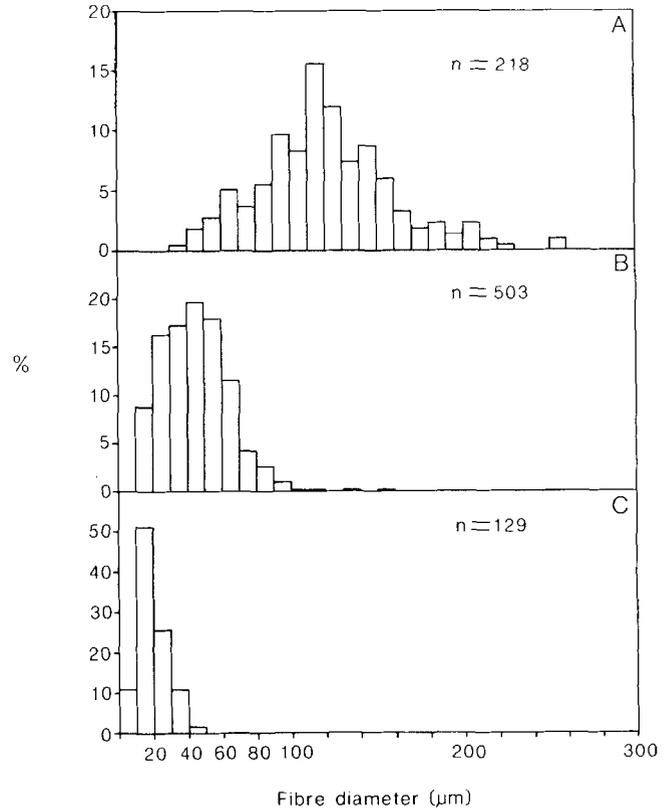


Fig. 3. Frequency distributions of the diameters of myotomal slow muscle fibres in *N. neglecta*.

- A. body mass = 789.9g, SL = 34.2cm, mean dia.= 121.4 μ m, SD = 39.27, skewness = 0.52, kurtosis = 3.6.
 B. body mass = 77 1g, SL = 16.5cm, mean dia.= 49.4 μ m, SD = 21.63, skewness = 0.61, kurtosis = 4.2.
 C. body mass = 20 1g, SL = 10.0cm, mean dia.= 19.4 μ m, SD = 8.6, skewness = 0.9, kurtosis = 3 15.

Discussion

The pelagic blue-phase fingerling stage of *Notothenia neglecta* migrates inshore at around 15 months and undergoes a transition to a demersal juvenile (Everson, 1968). This secondary metamorphosis involves characteristic changes in body morphology (Norman, 1938), and a reduction in the relative proportion of slow muscle fibres in the myotomes (Johnston & Camm, 1987). The main finding of the present study is that muscle growth is entirely via the hypertrophy of existing fibres, even in the earliest demersal stages. Weatherley *et al* (1988) found that, for temperate fish, the recruitment of new muscle fibres ceased at around 44% of the ultimate length. Since *N. neglecta* reach at least 55cm SL and 1.8 kg body mass at Signy Island (Fitch 1986), and the number of fibres does not increase after the fish enter the demersal phase (Fig. 1), it is clear that muscle fibre recruitment ceases at a much earlier stage during development than in any of the temperate species previously studied (Davison 1983,

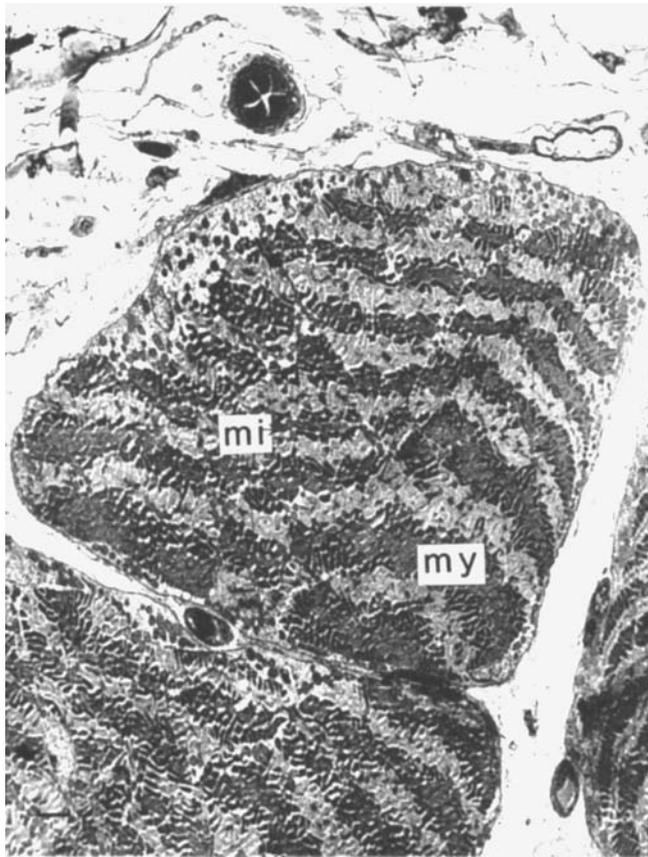


Fig. 4. Electron micrograph of a slow muscle fibre from *Notothenia neglecta* in the mid-line region. Mitochondria are present at the centre of the fibre. Fibre diameter = 200 μm . Body mass = 789.9g, SL = 34.2cm. mi = mitochondria, my = myofibrils. Bar = 10 μm .

Weatherley *et al.* 1988, Calvo 1989). The origin of new muscle fibres has been variously ascribed to the division of myosatellite cells (Nag & Nurshall 1972), or the budding of existing fibres (Willemse & Lieuwma-Noordanus 1984). Myosatellite cells were extremely rare in juvenile *N. neglecta*, consistent with the absence of any muscle cell division. The rate of growth is greater for small than large diameter fibres (Weatherley *et al.* 1988). Thus, the relatively high proportions of large diameter muscle fibres in juvenile *N. neglecta* may significantly contribute to the observed slow rates of annual somatic growth.

Weatherley *et al.* (1988) suggested that there is a maximum diameter for muscle fibres, beyond which nutrient assimilation and metabolite removal become limiting. Fast twitch fibres in *N. neglecta* largely depend on the rapid hydrolysis of phosphocreatine to supply the high rates of energy required for contraction (Dunn & Johnston 1986). Fast fibres contained a distinct layer of sub-sarcolemmal mitochondria, which may have a role in the restoration of phosphocreatine stores by aerobic metabolism (Johnston & Camm 1987). The

maximum diameter of fast muscle fibres in 70cm muskellunge (*Esox masguinongy*), a fast growing temperate fish, is about 120 μm (Weatherley & Gill 1985), while fast fibres in *Lepomis gibbosus* can reach 210 μm in diameter (Weatherley *et al.* 1988). Fast muscle fibres in the size class 400–500 μm are relatively common in mature specimens of most Antarctic fish species studied (this study, Smialowski & Kilarski 1981, Dunn *et al.* 1989). This probably represents the upper size limit for anaerobic muscle fibre types in vertebrates. Although fibres of 1–2mm diameter are found in some invertebrate muscles, such as the barnacle *Balanus nubilus* (Ashley & Ridgway 1970), these fibres are invaginated with deep clefts and channels to facilitate diffusion (Hoyle & Smyth 1963). Slow muscle fibres operate at slower frequencies and have a substantial lower maximum mechanical power output than fast fibres (Altringham & Johnston 1990). The energy supply for contraction in slow fibres is largely supplied by aerobic metabolism (Bone 1978, Johnston 1981). Typically, slow muscle fibres in relatively active temperate fish species have diameters in the range 20–60 μm (Johnston 1989). Dunn *et al.* (1989) reported slow muscle fibres >120 μm diameter in *Pseudochaenichthys georgianus*, and *Psilodraco breviceps*, and >220 μm in *Pagothenia hansonii*, and *Champsocephalus gunnari*. Archer & Johnston (1990) found that the density of mitochondria adjacent to capillaries is not significantly different from that in the central core of slow fibres in *Chaenocephalus aceratus*, and mitochondria are also present in the central core of slow fibres >150 μm diameter in *N. neglecta* (Fig. 4), which suggests that the diffusion of oxygen is not limiting. The pelagic fingerling stages of *Notothenia neglecta* utilise sub-carangiform locomotion for sustained activity and have a well developed layer of slow myotomal muscle containing a relatively high volume density of mitochondria (0.37: Johnston & Camm 1987). The transition to the demersal life-style is associated with the adoption of a drag-based labriform mode of locomotion for low speed swimming. The demersal stages only contain a small strip of slow myotomal muscle with a reduced mitochondrial content (0.13: Johnston & Camm 1987), and the trunk appears to function largely as a rudder in order to facilitate directional changes (Archer & Johnston 1989). This relatively low intensity exercise together with cold temperatures perhaps explains why slow myotomal fibres can reach such large diameters in these fish.

There is now substantial evidence that physiological processes involving very high ATP flux are limited by low temperature eg. burst swimming activity (Johnston 1989). The situation for processes involving much lower rates of ATP utilisation such as growth is less clear. Whilst low temperature undoubtedly imposes a general constraint to growth in Antarctic fish, it seems likely that other factors such as seasonal variations in food availability (Clarke 1988) and the reliance on fibre hypertrophy for muscle growth also limit maximum rates. If all notothenioids are derived from the same ancestral benthic stock (DeWitt

1971), the limits to growth by hypertrophy alone may have been set before the radiation of the Notothenioidei. Slow growth may therefore be a consequence of phylogeny, in addition to the problems imposed by cold temperatures.

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