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THE MAGELLAN-ANTARCTIC CONNECTION: LINKS AND FRONTIERS AT HIGH SOUTHERN LATITUDES.
W.E. ARNTZ, G.A. LOVRICH and S. THATJE (eds.)

Muscle growth in Antarctic and Subantarctic notothenioid fishes*

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SUMMARY: The suborder Notothenioidei comprises 122 species divided into 8 families, with members of 6 of the families living outside Antarctic waters. The Antarctic species underwent an extensive radiation from a small demersal ancestor to occupy different ecological niches and levels in the water column. The axial muscle of Antarctic and some Subantarctic notothenioids is unusual in containing very large diameter muscle fibres and a low muscle fibre number. Maximum fibre diameters are greater than 500 μm in many species. There is no indication of systematic differences in fibre number, fibre type composition, ATPase activity, time of cessation of fibre recruitment (hyperplasia) and swimming performance between Antarctic and Subantarctic species. Instead, fibre number is significantly decreased in species belonging to the most derived families relative to the more basal families (a trend that also correlates with an increase in the diameter of the fibres). The length of the cell cycle of the muscle fibres shows cold compensation in the Antarctic species *H. antarcticus* relative to the closely related Subantarctic one (*H. bispinis*). Feeding after a starvation period results in a strong stimulation of the proliferation of muscle fiber progenitors in *H. bispinis*. Similar studies have not yet been performed on any Antarctic species.

Keywords: Antarctic notothenioids, Subantarctic notothenioids, muscle development, muscle growth, temperature, hypertrophy, hyperplasia.

RESUMEN: CRECIMIENTO MUSCULAR EN NOTOTÉNIDOS ANTÁRTICOS Y SUBANTÁRTICOS. – El suborden Notothenioidei comprende 122 especies divididas en 8 familias, incluyendo miembros de 6 de las familias que viven en aguas subantárticas. Las especies antárticas sufrieron una impresionante radiación a partir de un ancestro pequeño y demersal que les permitió ocupar diferentes nichos ecológicos y niveles en la columna de agua. El número total de fibras de la musculatura axial de nototénidos antárticos y algunos subantárticos es escaso y las fibras presentan un tamaño inusualmente grande, alcanzando diámetros máximos mayores a 500 μm en muchas de las especies estudiadas. No existen diferencias sistemáticas entre la musculatura axial de nototénidos antárticos y subantárticos en el número de fibras musculares, los tipos de fibras musculares, la actividad ATPasa, el momento de cese de incorporación de fibras musculares (hiperplasia), ni en la capacidad (performance) natatoria a diferentes temperaturas. De hecho, el número de fibras musculares es significativamente menor en las especies pertenecientes a las familias más derivadas en comparación con especies de las familias más basales (una tendencia que también se correlaciona con un incremento en el diámetro de las fibras). La duración del ciclo celular muestra una compensación al frío en la especie antártica *H. antarcticus* en comparación con la especie subantártica más relacionada (*H. bispinis*). La alimentación luego de un período de ayuno produce una fuerte estimulación en la proliferación de progenitores de las fibras musculares en *H. bispinis*. No se han realizado aún estudios similares en especies antárticas.

Palabras clave: nototénidos antárticos, nototénidos subantárticos, desarrollo muscular, crecimiento muscular, temperatura, hipertrofia, hiperplasia.

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THE SOUTHERN OCEAN

Antarctica is certainly the most isolated and extreme continent in terms of the environmental conditions to which terrestrial and marine organisms are exposed. The Antarctic continent is surrounded by the Southern Ocean, one of the deepest and coldest seas in the world. The Southern Ocean is fairly stable in terms of water temperature, with the continental shelf waters typically at or below the freezing point of seawater (Eastman, 1993) and with warming episodes during summer time (Hunt *et al.*, 2003). Up to half of the Southern Ocean is covered with pack ice during the maximum coverage period in the region south of the Antarctic Polar Front (APF). The isolation of Antarctica is a very interesting and long process that took place over more than a hundred million years and culminated after the establishment of the APF, initiating a long period of climatic cooling that persists up to the present (Eastman, 1993). The understanding of the geological evolution of the Antarctic continent is very important in order to understand the natural history of the extant fauna.

By the end of the Cretaceous (75-65 Ma) the Antarctic continent was still connected to Australia and South-America. Geological and geophysical evidence indicates that the link to Australia, the South Tasman Rise, may have been submerged by as early as 64 Ma and fully separated from Antarctica by deep water around 50 Ma. The separation from South America, the opening of the Drake Passage, occurred later, but the exact time of occurrence is still uncertain. Seafloor spreading produced magnetic anomalies in the Drake Passage region by at least 28 Ma. Moreover, palaeoceanographic evidence suggests the existence of a significant marine opening, probably shallow waters, as early as 36 Ma (reviewed in Crame, 1999).

Changes in the palaeotemperature of the globe have been inferred from the ratio of the oxygen isotopes in the calcium carbonate of microfossil foraminiferal shells (Clarke and Johnston, 1996, Zachos *et al.*, 2001) (Fig. 1). Major cooling of marine temperatures occurred after the Eocene Climatic Optimum, with much of the change occurring over the early-middle (50 to 48 Ma) and late Eocene (40 to 36 Ma), the early Oligocene (35 to 34 Ma), and the middle-late Miocene (14 to 10 Ma).

The southernmost part of South America is the closest landmass to Antarctica. Under present-day conditions the interchange of fauna between them is restricted by deep water, but that was not the case for a long period of time prior to the establishment of the APF.

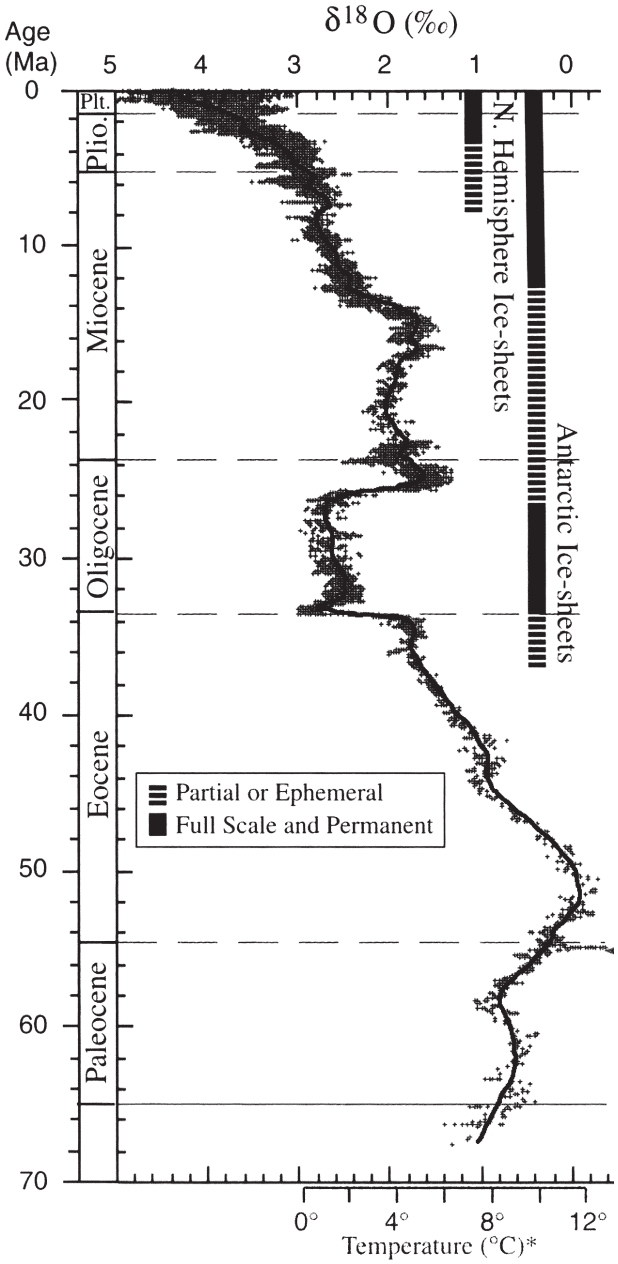

FIG. 1. – Schematic representation of the variation of the deep-sea water temperatures inferred from palaeoclimate data. Modified from Zachos *et al.* (2001).

THE SUBORDER NOTOTHENIOIDEI

The highly endemic adaptive radiation of the suborder Notothenioidei (Perciformes) is the best example of extensive radiation in a marine fish group (Eastman and Eakin, 2000) and can be con-

sidered as a species flock similar to the cottoid species flock in Lake Baikal (Eastman and McCune, 2000). The Notothenioidei dominate the fish fauna of the continental shelf of the Southern Ocean. The suborder comprises 8 families, 43 genera and 122 species, with 26 species belonging to 6 of the families living outside Antarctic waters (Eastman and Eakin, 2000).

The ancestral form of the notothenioids is generally considered to have been a small temperate bottom-living species without a swimbladder. Despite the lack of swimbladder they have undergone an extensive ecological diversification, currently occupying a variety of niches in the water column. Moreover, they show a large diversification in terms of body size, body colour, buoyancy, etc. (Eastman, 1993). The key physiological feature that allowed the Notothenioidei to diversify and become dominant in the fish fauna of the Southern Ocean was almost certainly the development of antifreeze glycoproteins (AFGPs) (Cheng and DeVries, 1991; Eastman, 1993). There are many other important features that are associated unequivocally with the AFGPs, or more widely with living at very low water temperatures: higher blood osmolarity, aglomerular kidneys, low haematocrit counts, etc. The key ecological feature that permitted the diversification of the suborder was probably the weak competition Notothenioids experienced due to the extinction events related to the cooling conditions and the isolation of Antarctica (Eastman, 1993).

Phylogeny of notothenioids

Morphological and molecular-based phylogenies have been constructed in order to understand the radiation that the suborder accomplished. Morphological based phylogenies have been built since the eighties (Eakin, 1981; Balushkin, 1990, 1992, 2000; Eakin *et al.*, 2001), including cladistic analysis (Andersen, 1984; Iwami, 1985). In the absence of a unique osteological character, the suborder was diagnosed by a combination of morphological characters. It was not possible to identify a sister group, with blennioids and zoarcioids as likely candidates. Therefore, characters have often been polarised relative to the Bovichtidae (functional outgroup).

The molecular approach only started about a decade ago, making use of DNA sequence data from mitochondrial and nuclear genes. It mostly supported the analysis based on morphological data. Nevertheless, it contradicts the morphological studies in some instances, for example proposing that Bovichtidae (*Bovichtus* and *Cottoperca*) is the basal family of the suborder (Lecointre *et al.*, 1997) instead of the family Pseudaphritidae (Balushkin, 2000). However, the more basal families (Bovichtidae, Pseudaphritidae and Eleginopsidae) are well established at the base of the tree and except for a single species of bovichtid are represented exclusively by non-Antarctic species (Fig. 2).

Divergence times have been calculated by different authors and methods, and at present there is a

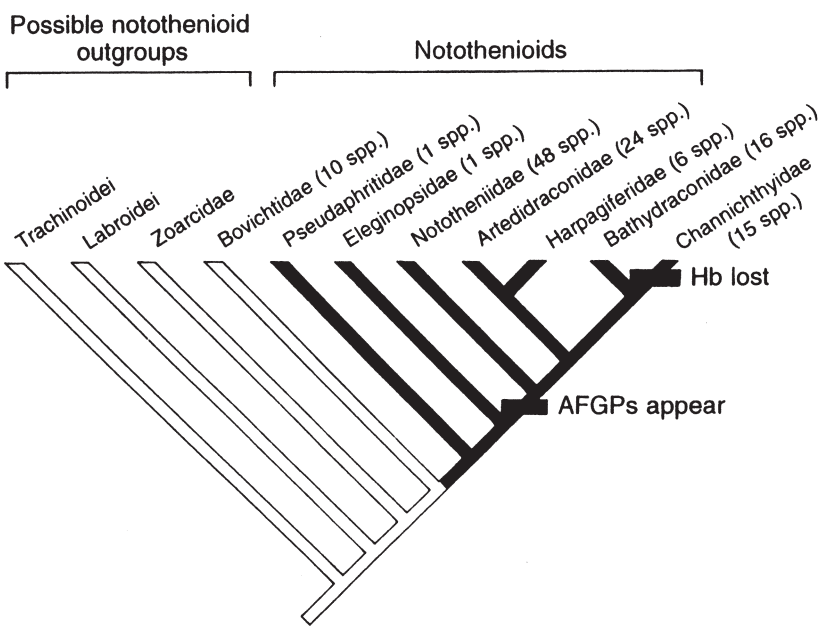

FIG. 2. – Phylogenetic tree of the 8 families of the suborder Notothenioidei showing the appearance of antifreeze glycoproteins (AFGPs) and the loss of haemoglobin (Hb). From Eastman and Clarke (1998).

lack of consensus about the time of occurrence of some important events. For example, the radiation of the AFGP-bearing Antarctic notothenioids took place only about 7-15 Ma (Bargelloni *et al*., 1994) or 24 Ma ago (Near, 2004) depending on the calibration of the molecular clock. The appearance of the AFGPs was mapped in the tree of the suborder and evidence was found that they evolved only once in the group, after the divergence of the Eleginopsidae (Eastman and Clarke, 1998) that occurred 27 Ma (Bargelloni *et al*., 1994) or 40 Ma ago (Near, 2004) (Fig. 2). Independently of the divergence times, the number of species in the families derived after the appearance of AFGPs is much higher than that in the basal families (109 versus 12, calculated from Fig. 2), stressing the fact that the acquisition of AFGP was the main characteristic that allowed diversification.

MUSCLE DEVELOPMENT AND GROWTH IN FISH

Four main phases of muscle growth have been described in teleosts, one corresponding to embryonic growth and the other three to post-embryonic growth (reviewed in Rowlerson and Veggetti, 2001). Postembryonic growth can be divided, according to the predominant process that is taking place at a given time of muscle growth, as stratified hyperplasia, mosaic hyperplasia and hypertrophy. Not all phases are present in all fish species, with some species lacking mosaic and/or stratified hyperplasia. The final size of a given species is strongly regulated by the duration of the hyperplastic phases, since that is the period of time when the final number of muscle fibers is established. The final number of fibres restrains the final size of the species because the maximum size of a given fibre has physiological constraints, probably due to limitations in diffusion rates. Only one teleost group, the notothenioids, have shown evidence of an increase in the maximum size of the muscle fibres, probably representing a relaxation of diffusional constraints in a very cold and stable environment.

Embryonic growth

In vertebrates, including fish, the skeletal muscles of the trunk and limb are derived from the somites. The somites are formed from the paraxial mesoderm in a rostral to caudal sequence and their number varies greatly in teleost fish, from 26 in the platy fish to more than 200 in some eels (Richardson *et al*., 1998). The basic helix-loop-helix family of transcription factors (bHLH), *MyoD*, *Myf5*, *myogenin* and *MRF4* play a major regulatory role in myogenesis (Megeney and Rudnicki, 1995; Ordahl and Williams, 1998). *MyoD* and *Myf5* are somehow redundant but necessary for myogenic determination, and the loss of both in double mutant mouse results in myoblasts failing to form skeletal muscle (Rudnicki *et al*., 1993). Myogenin and MRF4 act afterwards in myogenic differentiation (Tajbakhsh and Buckingham, 2000).

The patterning of the embryonic muscle is regulated by interplay of midline signals, including at least three different families of proteins: Hedgehog (Hh), bone morphogenic protein (BMP) and Wnt. These signals play an important role in the regulatory network that controls *Myf5* and *MyoD* activation in muscle progenitors (Pownall *et al*., 2002). Devoto *et al*. (1996) demonstrated that sonic hedgehog induces slow muscle fate in zebrafish. More recently, it has been shown that sonic hedgehog behaves as a morphogen, inducing four different cell types in a concentration-dependent manner. The induction is not restricted to the slow muscle lineage, with a subset of fast muscle fibres also depending on sonic hedgehog expression (Wolff *et al*., 2003).

The patterning of the paraxial mesoderm has been extensively studied in zebrafish. Two populations of muscle precursors are present in the embryo: the adaxial and the lateral presomitic cells. The adaxial cells are medial cuboidal cells adjacent to the notochord that express *MyoD* and slow myosin isoforms early in the embryo development, prior to their morphological change and migration to the surface of the myotome to form the monolayer of slow muscle fibres (Devoto *et al*., 1996). The migration of the adaxial cells to form the monolayer of slow muscle fibres has also been described for other teleost such as pearlfish (*Rutilus frisii meidingeri*) (Stoiber *et al*., 1998), rainbow trout (*Oncorhynchus mykiss*) (Rescan *et al*., 2001), Atlantic herring (*Clupea harengus*) (Temple *et al*., 2001), and sea bream (*Sparus aurata*) (Tan and Du, 2002). A subset of the adaxial cells, the muscle pioneers, expresses *Engrailed* proteins and remains attached to the notochord, extending from the notochord to the surface of the somite. The lateral presomitic cells are smaller, have an irregular shape and are separated from the notochord by the adaxial cells. They only express *MyoD* after somite forma-

tion and will form in the embryo the bulk of fast muscle fibres (Devoto *et al*., 1996).

Post-embryonic growth

Two processes occur during postembryonic growth: the addition of new fibres (hyperplasia) and the growth in size of the existing ones (hypertrophy). The proliferation of a population of myogenic progenitors is the source of nuclei for fibre recruitment and hypertrophy (Koumans and Akster, 1995). These progenitors correspond to the cells that had first been identified as "satellite cells" in frog by Mauro (1961) and afterwards in avian and mammalian muscle (reviewed in Bischoff, 1994). Muscle is a postmitotic tissue and all growth that occurs after the embryo formation, as well as regeneration after injury episodes, is due to the activation of the satellite cell population. The progeny of these cells either fuse to each other forming a new muscle fibre (hyperplasia) or fuse to the surface of existing fibres to increase their size (hypertrophy). Several quiescent and satellite cell markers, including *MNF, c-met, Pax-7, M-cadherin* for the former and desmin, *MyoD and Myf5* for the latter, have been identified in recent years (reviewed in Hawke and Garry, 2001). Hyperplasia is without doubt the most important process for determination of the final size of a given species. New fibres can be added in a specific area (stratified hyperplasia) or all over the myotome (mosaic hyperplasia). The process of stratified hyperplasia presupposes the existence of germinal zones that have been described in the myotome of several marine teleost larvae, including anchovy (*Engraulis mordax*) (O'Connel, 1981); Atlantic herring (*Clupea harengus*) (Johnston, 1993); cod (*Gadus morhua*) (Galloway *et al*., 1999a); halibut (*Hyppoglossus hyppoglossus*) (Galloway *et al*., 1999b); plaice (*Pleuronectres platessa*) (Brooks and Johnston, 1993); sea bass (*Dicentrarchus labrax*) (Veggetti *et al*., 1990); sea bream (*Sparus aurata*) (Rowlerson *et al*., 1995); turbot (*Scophthalmus maximus*) (Gibson and Johnston, 1995); and zebrafish (*Danio rerio*) (Waterman, 1969). There are two main models proposed for stratified growth: A) In zebrafish new slow and fast muscle fibres are first added at the end of the segmentation period from growth zones near the dorsal and ventral extremes of the myotome and close to the horizontal septum. This mode of muscle growth has been proven to continue into larval life, by traditional fibre size measurement, BruU labeling and *in situ* hybridisation (Barresi *et al*., 2001; Fernández, unpublished results, Fig. 3). This mechanism of growth is the one described for most teleost fishes. B) Rowlerson and Vegetti (2001) proposed another model based especially on sea bream and sea bass (Sparidae). This model postulates the existence of a proliferation zone underneath the monolayer of slow muscle

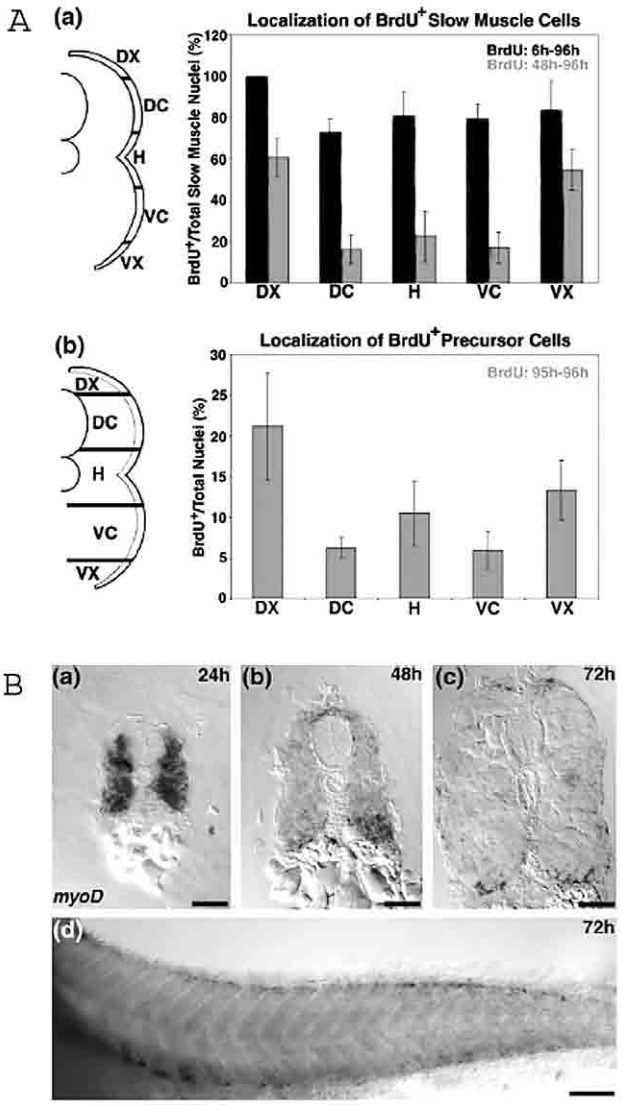

FIG. 3. – A) Dividing cells in the dorsal and ventral extremes of the myotome give rise to slow-muscle fibres after 48 h in zebrafish. BrdU positive nuclei were quantified in the slow muscle fibre layer (a) and the whole myotome (b) of 96 h larvae. (a) 6 to 96 h BrdU incubation showed an even distribution of BrdU-positive nuclei throughout the slow-muscle monolayer (dark bars). However, 48h to 96 h BrdU incubation showed a higher percentage of BrdU nuclei at the dorsal and ventral extremes of the monolayer (light bars). (b) The percentage of BrdU nuclei was higher at the dorsal and ventral extremes of the myotome in larvae incubated in BrdU for one hour (95 to 96 h). B) (a,b,c) Dorsal and ventral growth zones after 48 h in zebrafish. Transverse sections of embryos probed for *MyoD* expression at 24, 48, and 72 h of development show that while *MyoD* is expressed in the whole myotome at 24 h, it is restricted to the dorsal and ventral extreme of the myotome at 48 and 72 h. (d) Lateral view of whole-mount showing *MyoD* expression in every somite of a 72 h embryo. Modified from Barresi *et al*. (2001).

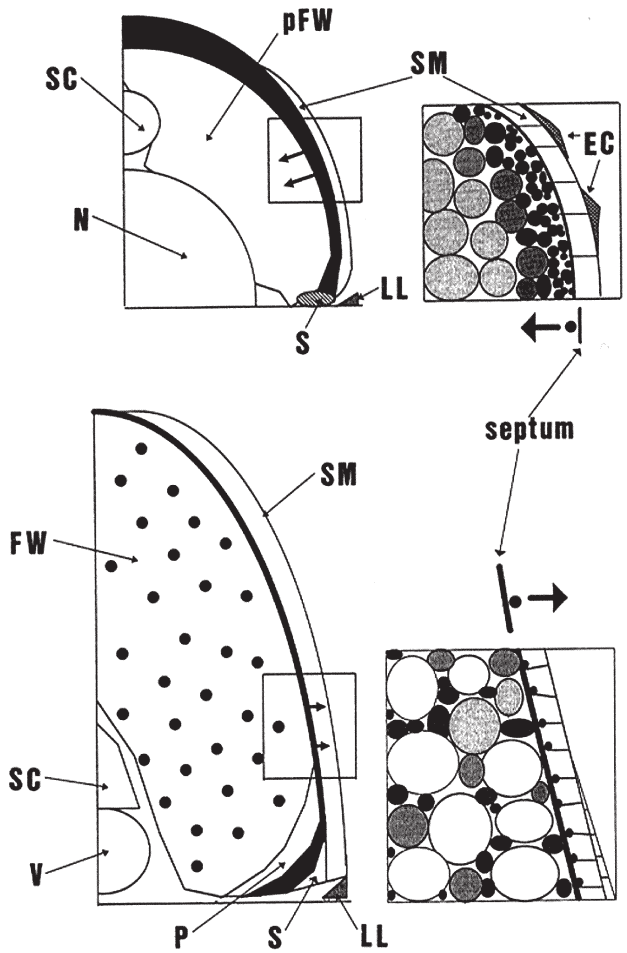

FIG. 4. – Schematic representation of post-embryonic growth in an epaxial quadrant of lateral muscle. Above: in the mosaic hyperplastic phase new fast muscle fibres are generated throughout the myotome generating the characteristic mosaic pattern of fibre diameters in cross-sections. The previous proliferation zone persists for a while but giving rise now to slow muscle fibres and probably to intermediate fibres (also called pink fibres) in the region close to the lateral line. SC= spinal cord, N= notochord, FW= fast muscle layer, pFW= presumptive FW muscle layer, P= pink fibres, V= vertebral column. Below: Stratified hyperplasia occurs in a restricted region of the myotome indicated in black, producing mainly new fibres that contribute to the fast muscle population (see arrows). At the surface of the monolayer of slow muscle fibres (SM), external cells (EC) may be present and may contribute to an additional layer of slow muscle fibres. Phenotypically slow muscle fibres (S) start to appear close to the lateral line (LL). From Rowlerson and Veggetti (2001).

fibres that gives rise to the new fast fibres. At the same time, the new slow fibres could be generated from external cells, appearing mainly close to the horizontal septum (Fig. 4). Other authors have also described external cells but considered that they were not myogenic due to their transient appearance (Johnston, 1993) or to their ultrastructure (Stoiber and Sanger, 1996); their exact role is still uncertain.

The last phase of post-embryonic growth is the one due exclusively to hypertrophy, probably in the same way that muscle growth occurs in mammals after birth. Muscle fibres increase in diameter due to the absorption of additional nuclei, maintaining the nuclei:cytoplasmic ratio between certain limits (Koumans *et al*., 1993). As stated above, it is likely that a pluripotent stem cell population gives rise to the precursor myogenic cells involved in hyperplasia, hypertrophy and muscle repair, though the embryological origin of these populations remains to be determined.

NOTOTHENIOIDEI MUSCLE

Muscle development in notothenioids

Little is known about muscle development of notothenioid fishes. Embryological development has not been studied yet, while larvae have shown the usual one or two fibre thick superficial layer of slow muscle fibres and a bulk of fast muscle fibres (Dunn *et al*., 1989, Calvo *et al*, 1995). Muscle development in notothenioids using molecular tools has only started to be studied recently. Partial sequences of M*yoD* were cloned in some species of Antarctic and Subantarctic notothenioids (Fernández *et al*., unpublished data, GenBank accession nos. AF 396675-80; Johnston *et al*., 2002) and they showed a very high similarity to the zebrafish sequence (Fig. 5).

Fibre type distribution in the myotome and pectoral fin muscle

At least four different fibre types have been characterised in the axial muscles of Subantarctic notothenioids by means of histochemical techniques for myosin ATPase, succinic dehydrogenase (SDHase), glycogen and lipid: slow, tonic, intermediate and fast (Fernández *et al*., 2000). Slow and fast muscle fibres show respectively high and low activities of SDHase, reflecting their different mitochondrial contents. The myosin ATPase of slow and fast muscle fibres shows a different susceptibility to inactivation by low (pH 4.3) or high pH (10.4). Preincubation at different pHs prior to staining for myosin ATPase can be used to characterise different fibre types when performed at room temperature in Subantarctic notothenioids (Fernández *et al*., 2000), but fibres are not well differentiated at 4-6ºC (Fernández *et al*., unpublished data). Similar results were previously reported for Antarctic notothenioids (Davison and MacDonald, 1985; Harrison *et*

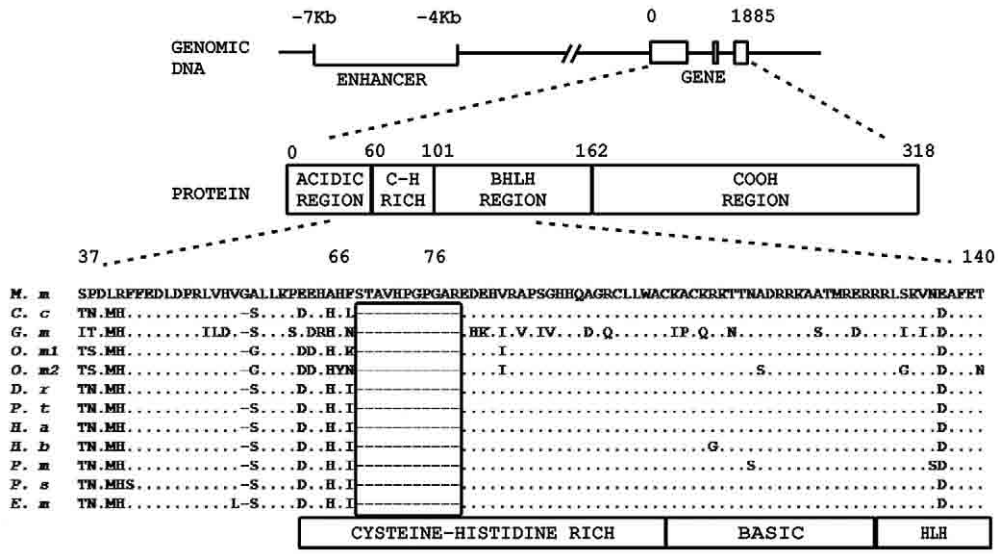

FIG. 5. – Schematic representation of mouse MyoD genomic DNA and protein structure aligned with ten teleost fish species. All fish proteins show an eleven amino acid deletion in the cysteine-histidine (C-H) rich region. *M.m, Mus musculus; C.c, Cyprinus carpio; G.m, Gadus morhua; O.m1, Om2, Oncorhynchus mykiss; D.r Danio rerio; P.t, Patagonotothen tessellata; H.a, Harpagifer antarcticus; H.b, Harpagifer bispinis; P.m, Paranotothenia magellanica; P.s, Patagonotothen sima; E.m, Eleginops maclovinus*. From Johnston *et al*. (2002).

al., 1987; Dunn *et al*., 1989). The order of inactivation of the different fibre types with alkaline preincubation in notothenioids was fast > intermediate > slow (Fig. 6), different to the order found for temperate species which is slow > fast > intermediate (Johnston *et al*., 1974). Therefore, in general the pH-sensitivity of the ATPase activity of fast muscle fibres in Antarctic and Subantarctic notothenioids was similar to that for slow muscle fibres in temperate or tropical species. However, Johnston (1987) measured the shortening speeds of live fibre bundles and demonstrated that the SDH^{+ve} and SDH^{-ve} muscle fibres in the notothenioid *Chaenocephalus aceratus* corresponded to slow and fast twitch muscle fibres respectively, as in other teleosts. The ATPase results could indicate the existence of different myosin isoforms in notothenioids compared to other teleosts. Interestingly, comparing the sequence and the structure of ATPase sites in myosins, Gauvry *et al*. (2000) found that there was a high similarity between the fast myosin of tropical species and the slow myosin of Antarctic species.

The different fibre types are relatively segregated in the myotome of notothenioids, with a superficial layer of tonic, slow and intermediate fibres surrounding a core of fast fibres. The same kind of segregation has been described for many other teleost species (Johnston *et al*., 1974; Smialowska and Kilarsky, 1981) and is strongly related to the needs for swimming. The different fibre types are specialised for working at different swimming speeds (Bone *et al*., 1978; Johnston and Altringham, 1991).

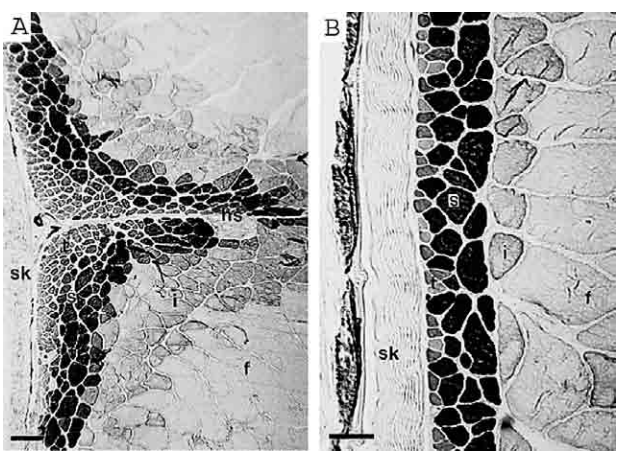

FIG. 6. – Myosin ATPase staining of myotomal muscles in the Subantarctic notothenioid *Patagonotothen tessellata* (13 cm) following pre-incubation at pH 10·6 for 30 s. Slow fibres (s) stain darkly, presumptive tonic fibres (t) and intermediate (i) have a residual activity and fast fibres (f) are completely inactivated. (A) Section at the level of the main horizontal septum; (B) section of the superficial muscle fibres. hs, major horizontal septum; sk, skin. Scale bars: (A) 100 μm, (B) 50 μm. From Fernández *et al*. (2000).

Electromyographic studies on common carp (*Cyprinus carpio*) established that the fibres are sequentially recruited, first slow, then intermediate and finally fast with increasing swimming speed (Johnston *et al*., 1977). Temperature affects fibre recruitment for swimming; for example, at lower temperatures the carp starts recruiting the fast fibres at lower swimming speeds (Rome *et al*., 1984). Maximum length specific velocity and acceleration as well as inertial power output during fast-starts varied significantly with temperature in the Subantarctic notothenioid *E. maclovinus* acclimated and tested at

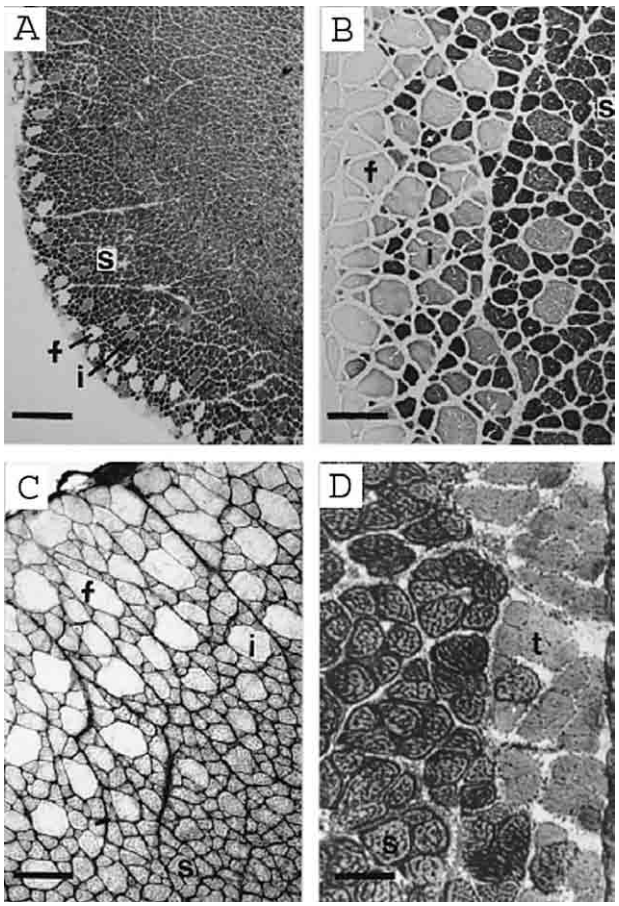

FIG. 7. – Histochemistry of pectoral fin adductor muscle in (A,B) *Eleginops maclovinus* (10 cm *L*T) and (C,D) *Patagonotothen tessellata* (14 cm *L*T). (A) Section stained for myosin ATPase following 30 s pre-incubation at pH 10·4. (B) Section stained for myosin ATPase following 90 s pre-incubation at pH 10·4. (C) Section stained for glycogen. (D) Section stained for succinic dehydrogenase. f, Fast muscle fibres; i, intermediate muscle fibres; s, slow muscle fibres; t, presumptive tonic muscle fibres. Scale bars: (A) 250 μm, (B) 100 μm, (C) 100 μm, (D) 50 μm. From Fernández *et al*. (2000).

2 to 10°C, being maximum at 8°C (Fernández *et al*., 2002). Burst swimming velocity also varied significantly with temperature for the Antarctic species *Trematomus bernachii* and *T. centronotus* tested between -1°C and 10°C, being maximum at 6°C (Wilson *et al*., 2001). The analysis of burst swimming after a 4-week acclimation period at -1°C and 4°C showed no significant difference for *Pagothenia borchgrevinki* (Wilson *et al*., 2001).

The same four fibre types have been described for the pectoral fin muscles (Fernández *et al*., 2000). The fibre type distribution in the *abductor profundis* muscle of all the species described was similar, comprising four different zones. The tonic fibres were found close to the pectoral girdle bones, followed by a core of slow muscle fibres, a zone of slow muscle fibres intermingled with fast ones, and finally a zone of fast fibres occupying the surface of the muscle (Fig. 7). Even though the distribution of the fibre types was conserved in all the species, there was a consistent variation in the proportion of the different zones from the proximal to the distal ends of the muscle (Fernández *et al*., 2000).

Special characteristics of notothenioid muscle

The axial muscle of the notothenioids is unusual in containing very large diameter muscle fibres in comparison to other teleosts (Smialowska and Kilarsky, 1981; Dunn *et al*., 1989; Battram and Johnston, 1991; Fernández *et al*., 2000; Johnston *et al*., 2003) and low muscle fibre numbers (Battram and Johnston, 1991). The maximum fibre diameters increase linearly with standard length (SL), reaching 500 μm in many of the species studied (Johnston *et al*., 2003) (Fig. 8). With regard to the fibre number, for example, *E. maclovinus,* a notothenioid with an unusually large number of fibres, has only 164,000 fibres as against 1,200,000 fibres in an Atlantic salmon (*Salmo salar*) of a similar size (Johnston *et al*., 2003). Phylogenetic Independent Contrast Analysis showed that fibre number differs significantly between species that belong to the most basal and the most derived families, suggesting a decreasing trend in fibre numbers during the evolution of the suborder (Fig. 9). Moreover, the decrease in the

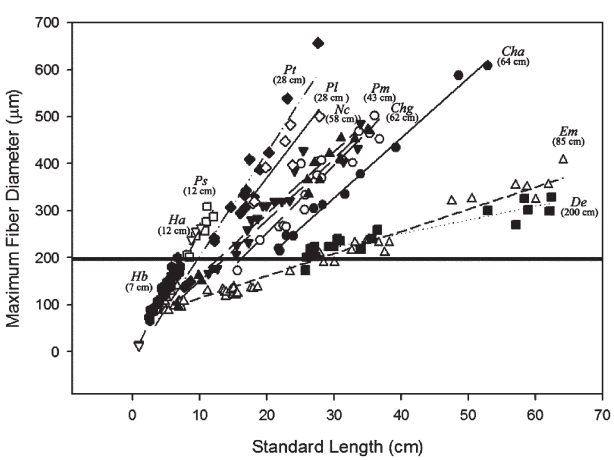

FIG. 8. – Relationship between the estimated maximum fast muscle fibre diameter and standard length in 11 species of notothenioid fishes from the Antarctic Ocean and Patagonian shelf. The slope of the regression line for each species correlates inversely to the final length of the species (in brackets close to the name of each species). *Notothenia coriiceps, Nc*; *Dissostichus eleginoides, De*; *Eleginops maclovinus, Em; Patagonotothen tessellata, Pt; P. longipes* sp., *Pl*; *P.sima, Ps; Chaenocephalus aceratus, Cha; Champsocephalus gunnari, Chg*; *Paranotothenia magellanica, Pm; Harpagifer antarcticus, Ha* and *Harpagifer bispinis, Hb*. The line drawn at 200 μm represents the common maximum fibre diameter for temperate and tropical teleosts. Modified from Johnston *et al*. (2003).

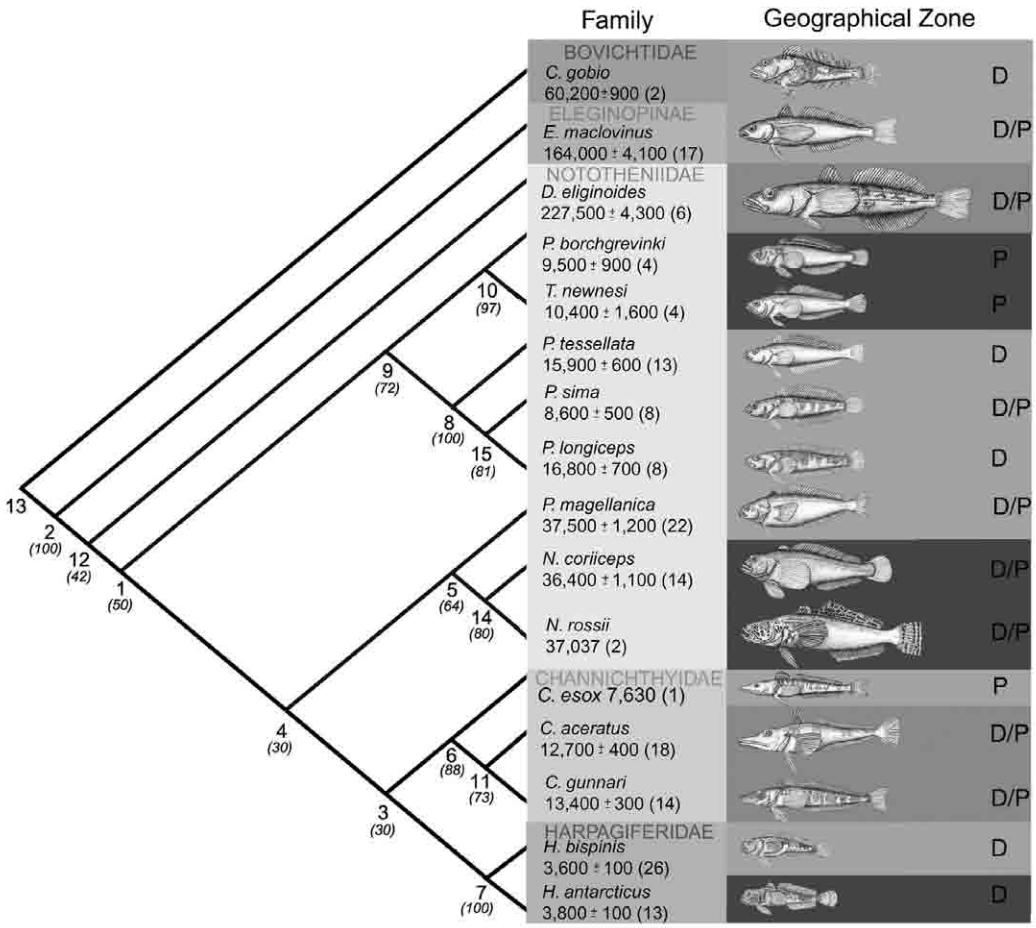

FIG. 9. – Maximum likelihood phylogenetic tree estimated from 12S mitochondrial rRNA sequences and the trait values for the final number of fast muscle fibres (FN_{max}) for the notothenioid fishes studied using Phylip. Values are means ± S.E.M. (number of individuals). The bootstrap support values obtained from the Phylip analysis are shown italicised in parentheses by the nodes. The fish are not drawn to scale. The locomotory habit of each species is also shown: D, demersal; D/P, demerso-pelagic and P, pelagic. The shading on the right-hand side shows the geographical zones of capture for each species: Beagle Channel (light grey), Shag Rocks, South Georgia (grey) and Antarctic Peninsula (dark grey). The shading on the left-hand side indicates the current taxonomic families, some of which are not monophyletic. Modified from Johnston *et al.* (2003).

number of fibres correlates with an increase in the diameter of the fibres (Johnston *et al.*, 2003). On the other hand, there is no evident relationship between the geographical zone (Antarctic and Subantarctic) and the maximum fibre diameter of the species. Therefore, a major conclusion from this work is that the special traits (low fibre number and giant fibre size) of the muscle of notothenioids have an important phylogenetical component, apart from the well-established relationship between low temperature and large fibre diameter.

Since muscle growth in teleosts involves the recruitment of new fibres (hyperplasia) and the increase in size of existing ones (hypertrophy), and the two processes are regulated through the activity of myosatellite cells, these two traits in the evolution of the suborder could be regulated through a single mechanism. The main phases in post-embryonic muscle growth are stratified and mosaic hyperplasia. The latter is absent in all the species of the more derived families that have already been studied (Harpagiferidae and Channichthyidae), giving a clue about how muscle fibre number has been adjusted during the evolution of this suborder (Johnston *et al.*, 2003).

The main process involved in muscle growth of notothenioids, hypertrophy, has been studied in adult *Harpagifer antarcticus* (Antarctic) and *Harpagifer bispinis* (Subantarctic) acclimated to summer and winter temperatures (Brodeur *et al.*, 2003a; Brodeur *et al.*, 2003b). These species are very good models for studying hypertrophy since hyperplasia no longer exists in adult fishes. Cell cycle times were estimated for *H. bispinis* at 10ºC (81.3 h) and 5ºC (150 h) and *H. antarcticus* at 0ºC (111 h). The longer duration of the cell cycle at 5ºC in *H. bispinis* than at 0 ºC in *H. antarcticus* indicates the existence of a cold compensation in the cell

cycle time of the Antarctic species, allowing them a substantial reduction in the cell cycle progression rate at low temperatures. It would be interesting to investigate whether this is a common feature for all Antarctic species.

Brodeur *et al*., (2003b) found evidence of a direct stimulation of myogenic cell proliferation by feeding at two different temperatures (about a two-fold increase in the c-met positive cells) in *H. bispinis*. The number of myogenic cells generated in response to feeding did not appear to be directly related to temperature. The main difference between the responses to feeding of fish acclimatised to simulated winter and summer conditions resided in the expression of myogenin, which was much less pronounced in summer. Interestingly, the delay between the ingestion of the meal and the activation of the myogenic progenitors (c-met positive cells) in *H. bispinis* was shorter than the cell cycle duration estimated for both summer and winter temperatures (150 and 81 h respectively). This result could indicate either that the cell cycle progression rate is accelerated by feeding or that a proportion of the activated cells were stopped at a checkpoint in the cell cycle and could therefore divide faster after activation since they had already progressed through part of the cell cycle (Walworth, 2000). The latter is in agreement with previous results on *Notothenia coriiceps* suggesting that myogenic cells activated by feeding were cells stopped at G1/S checkpoint of the cell cycle (Brodeur *et al*., 2002).

Eurythermal fish respond to cold-acclimation with almost a two-fold increase in the abundance of muscle mitochondria. Diverse adaptive explanations have been proposed to explain this fact, including the hypothesis that increases in mitochondrial volume density partially compensate for the reduced catalytic capacity at low temperatures (Johnston, 1982; Egginton and Sidell, 1989) or otherwise compensate the reduced diffusion coefficients of cytosolic metabolites (Tyler and Sidell, 1984; Sidell and Hazel, 1987). Antarctic and Subantarctic notothenioids, living regularly at very low temperature, also have abundant mitochondria in the slow muscle fibres (Johnston, 1987; Londraville and Sidell, 1990). For example, reported mitochondrial volume density values of slow muscle fibres were 0.56 for *Pleuragramma antarcticum* and 0.51 for *Champsocephalus esox,* amongst the highest recorded for vertebrates (Johnston *et al*., 1988; Johnston *et al*., 1998). Nevertheless, there seems to be variability in the mitochondrial volume density values due to species habits (Johnston *et al*., 1998). The same adaptive explanations proposed for cold-acclimation may apply for fishes living at low temperature like notothenioids, with increased volume and surface density of mitochondrial clusters as the main mechanism for enhancing the aerobic capacity of muscle in cold-water species (Johnston *et al*., 1998).

CONCLUSIONS

The comparison of the muscles of Antarctic and Subantarctic notothenioids have shown no differences in fibre type composition, ATPase activity, cessation of fibre recruitment (hyperplasia) and swimming performance at different temperatures. The length of the cell cycle of the muscle fibres shows cold compensation in the Antarctic species *H. antarcticus* relative to the closely related Subantarctic species (*H. bispinis*). Feeding after a starvation period resulted in an increased proliferation of muscle fibre progenitors in *H. bispinis*, but no such study has been performed yet in any Antarctic species.

The finding that the diversification of notothenioids was associated with a size-specific reduction in fibre numbers with a subsequent increase in fibre diameter gives us a great opportunity to study the variation of basic mechanisms of muscle growth in relation to temperature in an evolutionary context. Multiple scenarios are open to consideration. Is the stem cell population that gives rise to myogenic progenitors reduced at lower temperatures? Is the proliferation capacity of this population reduced? Is the interplay between midline signalling pathways affected by developmental temperature, setting in this form the special characteristics of the muscle of notothenioids?

The new field of Developmental Ecology has been growing intensively in the last twenty years. Notothenioids offer a unique opportunity to study the mechanisms of muscle development and growth in a particularly interesting group of diverse fishes exposed to extreme environmental conditions for at least the last 25 Ma.

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REFERENCES

Andersen, N. – 1984. Genera and subfamilies of the family Nototheniidae (Pisces, Perciformes) from the Antarctic and Sub-Antarctic. *Steenstrupia,* 10(1): 1-34.

Balushkin, A. – 1990. Review of blue notothenias of the genus *Paranotothenia* Balushkin (Nototheniidae) with description of a new species. *J. Ichthyol.,* 30(6): 132-147.

Balushkin, A. – 1992. Classification, phylogenetics and origins of the families of the suborder Notothenioidei (Perciformes). *J. Ichthyol.,* 32: 90-110.

Balushkin, A. – 2000. Morphology, classification, and evolution of notothenioid fishes of the Southern Ocean (Notothenioidei, Perciformes). *J. Ichthyol.,* 40(1): 74-109.

Bargelloni, L., P.A. Ritchie, T. Patarnello, B. Battaglia, D.M. Lambert and A. Meyer. – 1994. Molecular evolution at subzero temperatures: mitochondrial and nuclear phylogenies of fishes from Antarctica (suborder Notothenioidei), and the evolution of antifreeze glycopeptides. *Mol. Biol. Evol.*, 11(6): 854-863.

Barresi, M.J., J.A. D'Angelo, L.P. Hernandez and S.H. Devoto. – 2001. Distinct mechanisms regulate slow-muscle development. *Curr. Biol.,* 11(18): 1432-1438.

Battram, J.C. and I.A. Johnston. – 1991. Muscle growth in the Antarctic teleost, *Nothothenia neglecta* (Nybelin). *Antarct. Sci.,* 3(1): 29-33.

Bischoff, R. – 1994. The satellite cell and muscle regeneration. In: A.G. Engel and C. Franszini-Armstrong (eds.), *Myogenesis*, 2: 97-118. McGraw-Hill, New York.

Bone, Q., J. Kiceniuk and D.R. Jones. – 1978. On the role of the different fibre types in fish myotomes at intermediate swimming speeds. *Fishery Bull. Fish Wildl. Serv. U.S.*, 691-699.

Brodeur, J.C, L. Pèck and I.A. Johnston. – 2002. Feeding increases MyoD and PCNA expression in myogenic progenitor cells of *Notothenia coriiceps*. *J. Fish Biol.,* 60: 1475-1485.

Brodeur, J.C., J. Calvo, A. Clarke and I.A. Johnston. – 2003a. Myogenic cell cycle duration in *Harpagifer* species with sub-Antarctic and Antarctic distributions: evidence for cold compensation. *J. Exp. Biol.,* 206: 1011-1016.

Brodeur, J.C., J. Calvo and I.A. Johnston. – 2003b. Proliferation of myogenic progenitor cells following feeding in the sub-Antarctic notothenioid fish *Harpagifer bispinis*. *J. Exp. Biol.,* 206(1): 163-169.

Brooks, S. and I.A. Johnston. – 1993. Influence of development and rearing temperature on the distribution, ultrastructure and myosin sub-unit composition of myotomal muscle-fibre types in the plaice *Pleuronectes platessa*. *Mar. Biol.,* 117: 501-513.

Calvo, J., D.A. Fernández and I.A. Johnston. – 1995. Diferenciación de fibras musculares por inhibición de ATPasa en larvas de *Patagonotothen tessellata* (Notothenoideii). *VI Latin American Congress on Marine Sciences (COLACMAR)*. Mar del Plata, Argentina.

Cheng, C.C. and A.L. DeVries. – 1991. The role of antifreeze glycopeptides and peptides in the freezing avoidance of cold-water fish. In: G. Di Prisco (ed.), *Life under extreme conditions*, pp. 1-14. Springer-Verlag, Berlin-Heidelberg.

Clarke, A. and I.A. Johnston. – 1996. Evolution and adaptive radiation of Antarctic fishes. *TREE,* 11(5): 212-217.

Crame, J. – 1999. An evolutionary perspective on marine faunal connections between southernmost South America and Antarctica. *Sci. Mar.,* 63(Suppl. 1): 1-14.

Davison, W. and J.A. MacDonald. – 1985. A histochemical study of the swimming musculature of Antarctic fish. *N. Z. J. Zool.,* 12: 473-483.

Devoto, S.H., E. Melancon, J.S. Eisen and M. Westerfield. – 1996. Identification of separate slow and fast muscle precursor cells in vivo, prior to somite formation. *Development,* 122 (11): 3371-3380.

Dunn, J.F., S.D. Archer and I.A. Johnston. – 1989. Muscle fibre types and metabolism in post-larval and adult stages of notothenoid fish. *Polar Biol.,* 9: 213-223.

Eakin, R.R. – 1981. Osteology and relationships of the fishes of the Antarctic family Harpagiferidae (Pisces, Notothenioidei) from the Ross Sea. *Ant. Res. Ser.,* 31(5):155-159.

Eakin, R.R., J.T. Eastman and C.D. Jones. – 2001. Mental barbel variation in *Pogonophryne scotti* Regan (Pisces : Perciformes : Artedidraconidae). *Antarct. Sci.,* 13(4): 363-370.

Eastman, J.T. – 1993. *Antarctic fish biology: evolution in a unique environment*. Academic Press, San Diego.

Egginton, S. and B.D. Sidell. – 1989. Thermal acclimation induces adaptive changes in sub cellular structure of fish skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* 256: 1-9.

Eastman, J.T. and A. Clarke. – 1998. A comparison of adaptive radiations of Antarctic fish with those of nonAntarctic fish. In: G. Di Prisco, E. Pisano and A. Clarke (eds.), *Fishes of Antarctica: a biological overview*, pp. 3-26. Springer-Verlag, Berlin-Heidelberg.

Eastman, J.T. and R.R. Eakin. – 2000. An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments on Antarctic species. *Arch. Fish. Mar. Res.,* 48(1): 11-20.

Eastman, J.T. and A.R. McCune. – 2000. Fishes on the Antarctic continental shelf: evolution of a marine species flock? *J. Fish Biol. A,* 57: 84–102.

Fernández, D.A., J. Calvo, C.E. Franklin and I.A. Johnston. – 2000. Muscle fibre types and size distribution in sub-Antarctic notothenioid fishes. *J. Fish Biol.,* 56(6): 1295-1311.

Fernández, D.A., J. Calvo, J.M. Wakeling, F.A. Vanella and I.A. Johnston. – 2002. Escape performance in the sub-Antarctic notothenioid fish *Eleginops maclovinus*. *Polar Biol.*, 25, 914-920.

Galloway, T.F., E. Kjorsvik and H. Kryvi. – 1999a. Muscle growth and development in Atlantic cod larvae (*Gadus morhua* L.), related to different somatic growth rates. *J. Exp. Biol.,* 202(15): 2111-2120.

Galloway, T.F., E. Kjorsvik and H. Kryvi. – 1999b. Muscle growth in yolk-sac larvae of the Atlantic halibut as influenced by temperature in the egg and yolk-sac stage. *J. Fish Biol.,* 55(1): 26-43.

Gauvry, L., S. Ennion, C. Ettelaie and G. Goldspink. – 2000. Characterization of red and white muscle myosin heavy chain gene coding sequences from Antarctic and tropical fish. *Comp. Biochem. Physiol. B.,* 127(4): 575-588.

Gibson, S. and I.A. Johnston. – 1995. Temperature and development in larvae of the turbot *Scophthalmus maximus*. *Mar. Biol.,* 124: 17-25.

Harrison, P., C.J.M. Nicol and I.A. Johnston. – 1987. Gross morphology, fibre composition and mechanical properties of pectoral fin muscles in the Antartic teleost *Notothenia neglecta* Nybelin. *Proc. V Congr. Europ. Ichthyol., Stockholm*: 459-465.

Hawke, T.J. and D.J. Garry. – 2001. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.,* 91(2): 534-551.

Hunt, B.M., K. Hoefling and C. Cheng. – 2003. Annual warming episodes in seawater temperatures in McMurdo Sound in relationship to endogenous ice in notothenioid fish. *Ant. Sci.,* 15(3): 333–338.

Iwami, T. – 1985. Osteology and relationships of the family Channichthyidae. *Mem. Natl. Inst. Polar Res., Tokyo, Ser.,* 36: 1-69.

Johnston, I.A. – 1982. Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. *Cell Tissue Res.,* 222(2): 325-337.

Johnston, I.A. – 1987. Respiratory characteristics of muscle fibres in a fish (*Chaenocephalus aceratus*) that lacks haem pigments. *J. Exp. Biol.,* 133: 415-428.

Johnston, I.A. – 1993. Temperature influences muscle differentiation and the relative timing of organogenesis in herring (*Clupea harengus*) larvae. *Mar. Biol.,* 116: 363-379.

Johnston, I.A. and J. Altringham. – 1991. Movement in water: constraints and adaptations. In: P. Hochachka and T. Mommsen (eds.). *Biochemistry and molecular biology of fishes*, 1, 249-268. Elsevier, Amsterdam.

Johnston, I.A., S. Patterson, W. Ward and G. Goldspink. – 1974. The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. *Can. J. Zool.,* 52: 871-877.

Johnston, I.A., W. Davison and G. Goldspink. – 1977. Energy-metabolism of carp swimming muscles. *J. Comp. Physiol.,* 114(2): 203-216.

Johnston, I.A., J.P. Camm and M. White. – 1988. Specializations of swimming muscles in the pelagic Antarctic fish *Pleuragrama antarcticum*. *Mar. Biol.,* 100: 3-12.
Johnston, I.A., J. Calvo, H. Guderley, D. Fernández and L. Palmer. – 1998. Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *J. Exp. Biol.,* 201: 1-12.
Johnston, I.A., T.E. Hall and D.A. Fernández. – 2002. Genes regulating the growth of myotomal muscle in teleost fish. In: N. Shimizu, T. Aoki, I. Hirono and F. Takashima (eds.). *Aquatic genomics - steps towards a great future*, pp. 153-166. Springer-Verlag, Heidelberg.
Johnston, I.A., D.A. Fernández, J. Calvo, V.L. Vieira, A.W. North, M. Abercromby and T. Garland Jr. – 2003. Reduction in muscle fibre number during the adaptive radiation of notothenioid fishes: a phylogenetic perspective. *J. Exp. Biol.,* 206 (15): 2595-2609.
Koumans, J. and H. Akster. – 1995. Myogenic cells in development and growth of fish. *Comp. Biochem. Physiol. A, Mol. Integr. Physiol.,* 110: 3-20.
Koumans, J.T.M., G. Booms and J. Osse. – 1993. Growth of carp (*Cyprinus carpio*) white axial muscle; hyperplasia and hypertrophy in relation to the nucleus/sarcoplasm ratio and the occurrence of different subclasses of myogenic cells. *J. Fish Biol.,* 43: 69-80.
Lecointre, G., C. Bonillo, C. Ozouf-Costaz and J.C. Hureau. – 1997. Molecular evidence for the origins of Antarctic fishes: paraphyly of the Bovichthydae and no indication for the monophyly of the Notothenioidei (Teleostei). *Polar Biol.,* 18: 193-208.
Londraville, R.L. and B.D. Sidell. – 1990. Ultrastructure of aerobic muscle in Antarctic fishes may contribute to maintenance of diffusive fluxes. *J. Exp. Biol.,* 150: 205-220.
Mauro, A. – 1961. Satellite cells of skeletal muscle fibers. *J. Biophys. Biochem. Cytol.,* 9: 493-495.
Megeney, L.A. and M.A. Rudnicki. – 1995. Determination versus differentiation and the MyoD family of transcription factors. *Biochem. Cell Biol.,* 73(9-10): 723-732.
Near, T. J. – 2004. Estimating divergence times of notothenioid fishes using a fossil-calibrated molecular clock. *Antarct. Sci.,* 16(1): 37-44.
O'Connel, C.P. – 1981. Development of organ systems in the northern anchovy, *Engraulis mordax*, and other teleosts. *Am. Zool.,* 21: 429-446.
Ordahl, C.P. and B.A. Williams. – 1998. Knowing chops from chuck: roasting myoD redundancy. *Bioessays,* 20(5): 357-562.
Pownall, M.E., M.K. Gustafsson and C.P. Emerson Jr. – 2002. Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. *Annu. Rev. Cell Dev. Bi.,* 18: 747-783.
Rescan, P.Y., B. Collet, C. Ralliere, C. Cauty, J.M. Delalande, G. Goldspink and B. Fauconneau. – 2001. Red and white muscle development in the trout (*Oncorhynchus mykiss*) as shown by in situ hybridisation of fast and slow myosin heavy chain transcripts. *J. Exp. Biol.,* 204(12): 2097-2101.
Richardson, M.K., S.P. Allen, G.M. Wright, A. Raynaud and J. Hanken. – 1998. Somite number and vertebrate evolution. *Development,* 125(2): 151-60.
Rome, L.C., P.T. Loughna and G. Goldspink. – 1984. Muscle fibre recruitment as a function of swimming speed and muscle temperature in carp. *Am. J. Physiol.,* 247: 272-279.
Rowlerson, A., F. Mascarello, G. Radaelli and A. Veggetti. – 1995. Differentiation and growth of muscle in the fish *Sparus aurata* (L): II. Hyperplastic and hypertrophic growth of lateral muscle from hatching to adult. *J. Muscle Res. Cell M.,* 16(3): 223-236.
Rowlerson, A. and A. Veggetti. – 2001. Cellular mechanisms of post-embryonic growth in aquaculture especies. In: I.A. Johnston (ed.). *Muscle development and growth*, 18, pp. 103-128. Academic Press, San Diego.
Rudnicki, M.A., P.N. Schnegelsberg, R.H. Stead, T. Braun, H.H. Arnold and R. Jaenisch. – 1993. MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell,* 75(7): 1351-1359.
Sidell, B.D. and J.R. Hazel. – 1987. Temperature affects the diffusion of small molecules through cytosol of fish muscle. *J. Exp. Biol.,* 129: 191-203.
Smialowska, E. and W. Kilarsky. – 1981. Histological analysis of fibres in myotomes of Antarctic fish (Admiralty Bay, King George Islands, South Shetland Islands) I. Comparative analysis of muscle fibre sizes. *Pol. Polar Res.,* 2 (1-2): 109-129.
Stoiber, W., J.R. Haslett, A. Goldschmid and A.M. Sanger. – 1998. Patterns of superficial fibre formation in the European pearlfish (*Rutilus frisii meidingeri*) provide a general template for slow muscle development in teleost fish. *Anat. Embryol. (Berl),* 197(6): 485-496.
Stoiber, W. and A.M. Sanger. – 1996. An electron microscopic investigation into the possible source of new muscle fibres in teleost fish. *Anat Embryol (Berl),* 194(6): 569-579.
Tajbakhsh, S. and M. Buckingham. – 2000. The birth of muscle progenitor cells in the mouse: spatiotemporal considerations. *Curr. Top. Dev. Biol.,* 48: 225-268.
Tan, X. and J. Du. – 2002. Differential expression of two MyoD genes in fast and slow muscles of gilthead seabream (*Sparus aurata*). *Dev. Genes Evol.,* 212(5): 207-217.
Temple, G.K., N.J. Cole and I.A. Johnston. – 2001. Embryonic temperature and the relative timing of muscle-specific genes during development in herring (*Clupea harengus* L.). *J. Exp. Biol.,* 204(21): 3629-3637.
Tyler, S. and B.D. Sidell. – 1984. Changes in mitochondrial distribution and diffusion distances in muscle of goldfish upon acclimation to warm and cold temperature. *J. Exp. Zool.,* 232: 1-9.
Veggetti, A., F. Mascarello, P. Scapolo and A. Rowlerson. – 1990. Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax* (L.). *Anat. Embryol.,* 182: 1-10.
Walworth, N.C. – 2000. Cell-cycle checkpoint kinases: checking in on the cell cycle. *Curr. Opin. Cell Biol.,* 12(6): 697-704.
Waterman, R. – 1969. Development of the lateral musculature in the teleost *Brachydanio rerio*: a fine structural study. *Am. J. Anat.,* 125: 457-494.
Wilson, R., C. Franklin, W. Davison and P. Kraft. – 2001. Stenotherms at sub-zero temperature: thermal dependence of swimming performance in Antarctic fish. *J. Comp. Physiol., B*, 171: 263-269.
Wolff, C., S. Roy and P.W. Ingham. – 2003. Multiple muscle cell identities induced by distinct levels and timing of hedgehog activity in the zebrafish embryo. *Curr. Biol.,* 13(14): 1169-1181.
Zachos, J., M. Pagani, L. Sloan, E. Thomas and K. Billups. – 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science,* 292: 686-693.