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Review

Environment and plasticity of myogenesis in teleost fish

Ian A. Johnston

Gatty Marine Laboratory, School of Biology, University of St Andrews, St Andrews, Fife, KY16 8LB, Scotland, UK

e-mail: iaj@st-andrews.ac.uk

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Summary

Embryonic development in teleosts is profoundly affected by environmental conditions, particularly temperature and dissolved oxygen concentrations. The environment determines the rate of myogenesis, the composition of sub-cellular organelles, patterns of gene expression, and the number and size distribution of muscle fibres. During the embryonic and larval stages, muscle plasticity to the environment is usually irreversible due to the rapid pace of ontogenetic change. In the early life stages, muscle can affect locomotory performance and behaviour, with potential consequences for larval survival. Postembryonic growth involves myogenic progenitor cells (MPCs) that originate in the embryo. The embryonic temperature regime can have long-term consequences for the growth of skeletal muscle in some species, including the duration and intensity of myotube formation in adult stages. In juvenile and adult fish, abiotic (temperature, day-length, water flow characteristics, hypoxia) and biotic factors (food availability, parasitic infection) have complex effects on the signalling pathways regulating the proliferation and differentiation of MPCs, protein synthesis and degradation, and patterns of gene expression. The phenotypic responses observed to the environment frequently vary during ontogeny and are integrated with endogenous physiological rhythms, particularly sexual maturation. Studies with model teleosts provide opportunities for investigating the underlying genetic mechanisms of muscle plasticity that can subsequently be applied to non-model species of more ecological or commercial interest.

Key words: temperature, oxygen, myotomal muscle, environmental genomics, phenotypic plasticity, ectotherm, developmental plasticity, skeletal muscle.

Introduction

Teleost fish are found in fresh, brackish and marine habits that often show complex temporal-spatial variations in temperature, salinity, oxygen content, pH, light availability and water flow. Individually and in combination, environmental inputs affect all physiological systems including the skeletal musculature. Environment can have direct effects on the skeletal muscle, as with temperature and flow regime, or act *via* other physiological systems, as is the case with a variation in the chemical composition of the external medium (Fig. 1). Thus the gills detect changes in the chemical composition of the water (oxygen, pH, salinity, etc.) whereas altered day-length and/or light intensity are sensed *via* the retina and pineal gland (Fig. 1). The brain integrates all environmental inputs and modifies the outputs of the nervous, endocrine and cardiovascular systems in ways that alter phenotype and muscle function (Fig. 1). Resulting changes in behaviour may lead the fish to seek a new environment and/or over time result in muscle plasticity. Phenotypic plasticity can be defined as the ability of an organism to respond to an environmental input with a change of form, state, movement or rate of activity (West-Eberhard, 2003). The range of phenotypes that are expressed in response to environmental variation can be conveniently considered in terms of a reaction norm (Schlichting and Pigliucci, 1998). Muscle plasticity often involves structural changes in cellular organelles (Johnston and Maitland, 1980; Tyler and Sidell, 1984; Penney and Goldspink, 1980) or supporting structures such as capillaries (Johnston, 1982; Egginton and Sidell, 1989), and typically requires several weeks for a new steady state to be reached.

Abiotic factors often change in a coordinated and relatively predictable fashion with season, are integrated with endogenous physiological rhythms, and can be modified by other ecological factors, particularly food availability (Fig. 1). In wild fish, parasite infections of the skeletal muscle are common and can result in damage to the muscle and immune responses (Canning and Curry, 2005), both of which may modify phenotypic responses to environmental change and associated outputs (locomotion, metabolism and growth) (Fig. 1). Muscle plasticity and its consequences vary with the directional signal of environmental change. For example, in the threespine stickleback (*Gasterosteus aculeatus*) the effect of

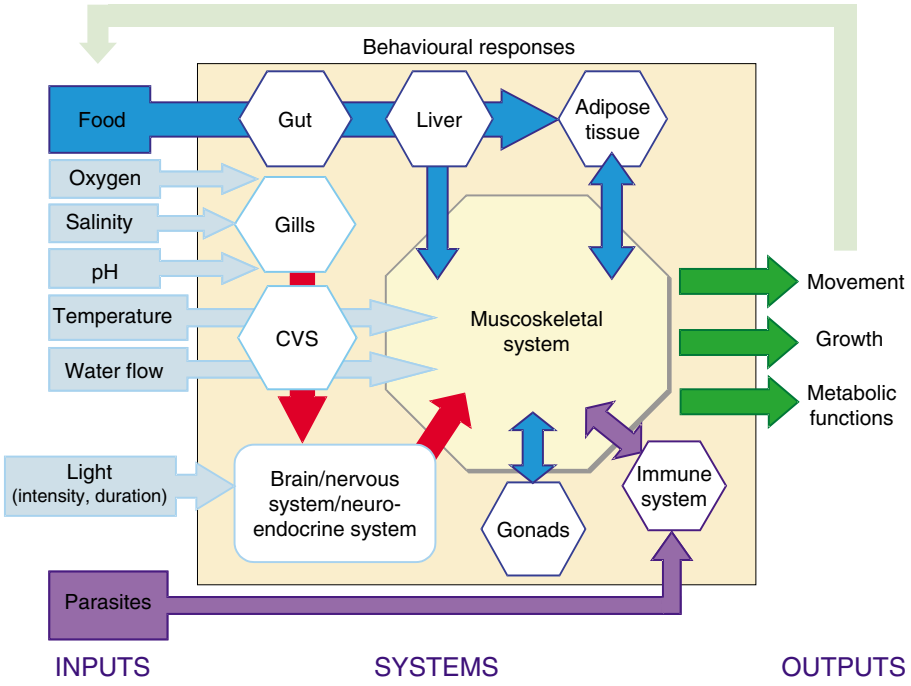

Fig. 1. The environmental inputs and physiological systems that affect the functional outputs of skeletal muscle in teleost fish. Altered environmental conditions can result in a behavioural response, i.e. movement to seek a new environment, or lead over time to muscle plasticity, which tunes the functional output of the muscle to the prevailing conditions.

temperature acclimation on fast-start performance was shown to differ between the spring and the autumn when the day length was increasing and decreasing, respectively (Guderley et al., 2001).

Two broad categories of muscle plasticity can be distinguished on the basis of the reversibility of the response (Fig. 2). During the embryonic and larval stages, muscle plasticity to the environment is usually irreversible due to the rapid pace of ontogenetic change. Following the establishment of the adult body plan, seasonal acclimatization to environmental change produces completely reversible changes in muscle phenotype. An exception is probably myogenesis because this continues well into adult life. Acclimatization responses require a stable environmental cue, may develop during ontogeny (Cole and Johnston, 2001), and are more pronounced in species from environments with highly seasonal climatic inputs (Johnston and Temple, 2002).

Studies on the escape swimming performance and predation behaviour of the short-horn sculpin (*Myoxocephalus scorpius* L.) from St Andrews Bay, Scotland illustrate how muscle plasticity and locomotory performance can vary during ontogeny as thermal niche changes and the fish sexually mature. Juvenile stages live in the sub-tidal and near-shore environments where temperature varies considerably on a daily basis. Temperature acclimation of juveniles reveals little or no plasticity of swimming behaviour or muscle contractile properties (Temple and Johnston, 1998). Muscle plasticity is acquired during ontogeny as adult stages migrate offshore to deeper water where the temperature is more stable over short time periods, but is markedly different between summer (~15°C) and winter (~5°C) (Johnston et al., 1995; Temple and Johnston, 1998). Maximum swimming performance at 15°C was improved under summer conditions whereas performance at 5°C was relatively little affected by acclimation. Tested at the maximum summer temperature of 20°C, length-specific speed and acceleration were 110% higher and 55% higher, respectively, in 15°C- than 5°C-acclimated individuals (Temple and Johnston, 1998), reflecting an increase in the maximum shortening speed and power output of fast muscle fibres (Johnston et al., 1995).

Following sexual maturation, seasonal acclimatization responses are closely coupled to reproductive cycles. In common with other species from the northern hemisphere, short-horn sculpin spawn in the early spring, but build up their gonads during the winter. In part, the materials for gonad development are obtained by mobilising energy reserves from the liver, adipose tissue and myotomal muscle, producing phenotypic changes that parallel starvation (Love, 1980). The eggs comprised 39% of body mass prior to spawning, producing increased drag forces on the body and stretching of fast muscle fibres in abdominal myotomes (James and Johnston, 1988). Compared to fish outside the spawning season the fast muscle fibres of gravid individuals had a lower resting membrane potential (Altringham and Johnston, 1988) and a faster maximum contraction speed, but a lower power output, as determined from the force–velocity relationship (James and Johnston, 1998). These physiological changes plus the added mass of eggs contributed to a decreased escape performance in gravid fish (James and Johnston, 1998).

The molecular mechanisms of plasticity of contractile properties with temperature acclimation have been extensively studied in the cyprinid species, common carp (*Cyprinus carpio*) and goldfish (*Carassius carassius*). These species show a classical trade-off in swimming performance between seasonal high and low temperatures with acclimatization (Fry and Hart, 1948; Johnson and Bennett, 1995), involving changes in myofibrillar ATPase activity (Johnston et al., 1975a; Johnson and Bennett, 1995), muscle shorting speed (Johnston et al., 1985), twitch duration (Fleming et al., 1990) and power output (Wakeling et al., 2000). Microarray studies indicate hundreds of protein targets (Gracey et al., 2004), including the class II myosins (Imai et al., 1997). A complex pattern of myosin heavy chain expression has been found in fast muscle, with various embryonic, slow- and fast-type

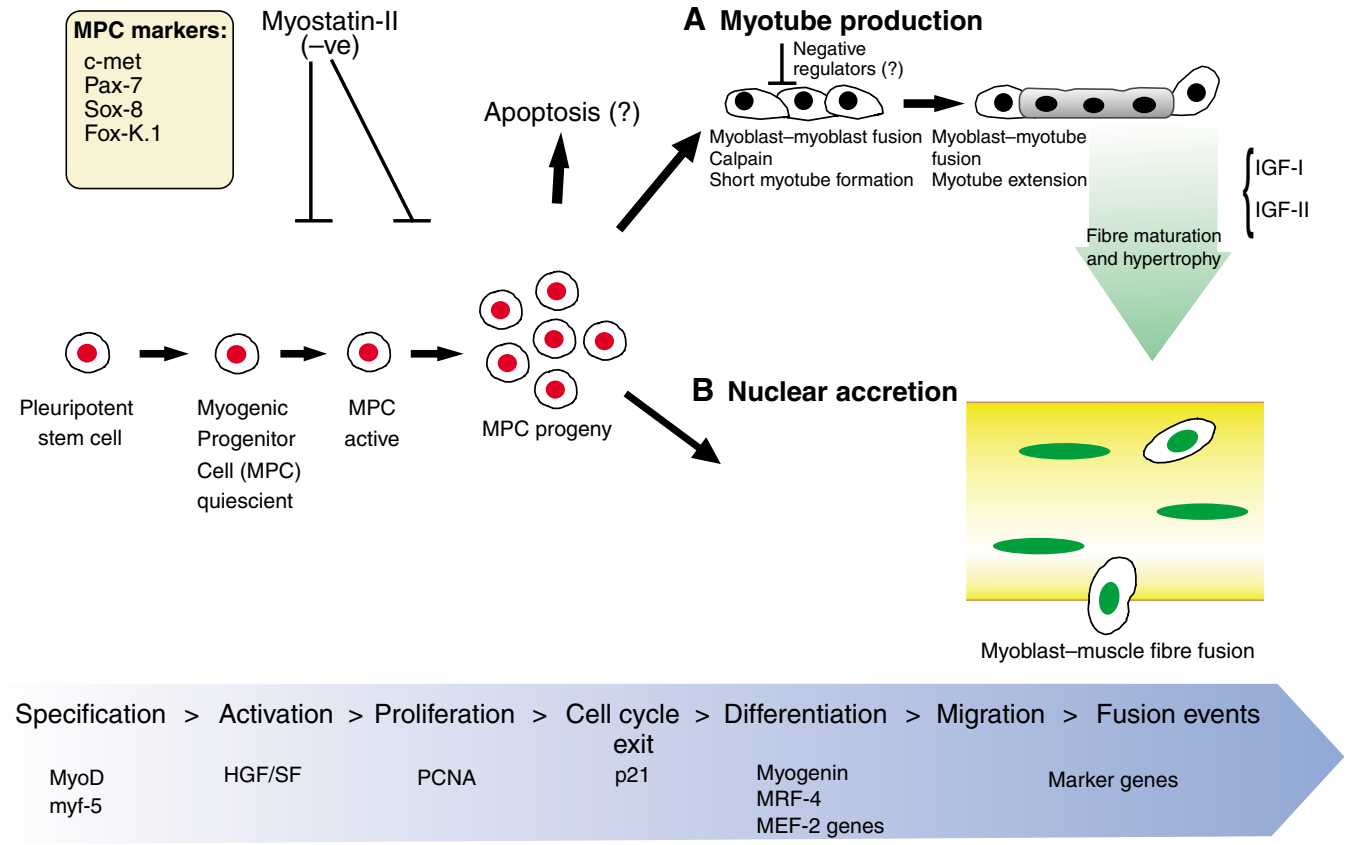

Fig. 2. A model describing the main events of myogenesis in teleost skeletal muscle. In this scheme pleuripotent stems cells become myoblasts, which are committed to a myogenic fate to form the Myogenic Progenitor Cell (MPC) population, involving the expression of the myogenic regulatory factors (MRFs) myoD (myoblast determination factor; there are at least two paralogues in teleosts) and myf-5. Following activation by Hepatocyte Growth Factor/Scatter Factor (HGF/SF) the MPCs are thought to undergo an asymmetric division to regenerate the MPC and provide a daughter cell committed to terminal differentiation. MPC markers (boxed) include c-met (the receptor for HGF/SF), paired-box protein 7 (Pax-7), and the transcription factors, sox-8 and Fox-K1. The MPC progeny undergo a proliferation phase [when proliferating cell nuclear antigen (PCNA), a DNA polymerase δ associated peptide, is upregulated] controlled by positive and negative signalling pathways. Myostatin-II is an important negative regulator of muscle growth and may also negatively regulate the activation of MPCs (cf. satellite cells in mammals) (McCroskery et al., 2003). Following cell cycle exit (and upregulation of p21), the MPC progeny initiate the differentiation programme involving the expression of the MRFs, myogenin and MRF-4 and MEF-2 gene family members. The MPC progeny can migrate through the muscle and have several fates. Until around 44% of the ultimate fish length, myoblasts in fast muscle can fuse to form short myotubes in a myoblast–myoblast fusion event, which probably involves calpain. Short myotubes can be extended by the fusion of additional myoblasts in a myoblast–myotube fusion event. Once formed myotubes initiate the programme of myofibrillargenesis and mature into muscle fibres. The regulation of fibre mass is thought to be controlled by signalling pathways involving insulin-like growth factor I (IGF-I) and IGF-II. At all stages of growth the MPC progeny can fuse with muscle fibres (myoblast–muscle fibre fusion) in the process of nuclear accretion. As muscle fibres increase in diameter and length additional nuclei are required to maintain the myonuclear domain (the volume of cytoplasm controlled by each nuclei) within certain limits.

isoforms being expressed at some acclimation temperatures, but not others (Nihei et al., 2006).

There are many barriers to gaining a deep understanding of the mechanisms of muscle plasticity induced by environmental change. These undoubtedly include the complexity of environmental interactions observed in nature, which are often difficult to replicate in an experimental setting. Indeed, the overwhelming majority of published studies have involved either seasonal acclimatization in wild fish (with only a few of the relevant abiotic or ecological factors recorded) or laboratory experiments, in which a single environmental variable has been manipulated with others held constant or unrecognised. The complexity of genotype–environmental interactions and the poorly characterised genetic background of most study organisms represent another set of challenges. Reproductive isolation of populations along a latitudinal gradient of distribution or between lakes that have become separated over time often leads to genetic differentiation and local adaptation between populations (Schluter, 1996; Quinn et al., 2000; Penn et al., 2002). Population level differentiation at many genetic loci and the complexity of environment–genotype interactions can lead to a poor appreciation, definition and/or control of critical variables affecting muscle plasticity.

There have been recent reviews on temperature acclimation responses and locomotory behaviour (Johnston and Temple, 2002) and there is a large literature on the effects of hypoxia on muscle metabolism in fish (e.g. Van den Thillart, 1982; Gracey et al., 2001). The focus of this short review will therefore be on the plasticity of myogenesis to environmental change. There is an extensive literature on the regulation of myogenesis in mammals, which might be expected to be a good starting point for unravelling the corresponding mechanisms in teleosts, particularly given the conservative features of vertebrate genomes. However, there are several reasons for exercising caution in extrapolating between vertebrate models. Firstly, myogenesis has some unique features in teleosts compared to amniotes: these include the earlier stage at which the fate of muscle cells is specified, the presence of adaxial cells, and the production of myotubes throughout much of ontogeny (Currie and Ingham, 2001; Rowlerson and Veggetti, 2001). Fish also have less control over their internal environment than amniotes and in nature are subject to marked seasonal fluctuations in food supply with conditions for growth varying throughout the year. These factors are likely to have profound consequences for the genetic mechanisms regulating myogenesis.

Common mechanisms of vertebrate myogenesis?

The fundamental events in myogenesis that are common to all vertebrates are the specification of stem cells to a myogenic lineage (myoblasts), proliferation, cell cycle exit, differentiation, migration and fusion (Fig. 2). The transcription factors (myogenic regulatory factors, MRFs) responsible for committing mesodermal cells to the muscle lineage (myoD and myf-5) and those involved in initiating and maintaining the muscle differentiation programme (myogenin, MRF4, MEF2 gene family members) are highly conserved in teleosts and amniotes (for a review, see Rescan, 2001). In mammals, the activities of MRFs are controlled by both negative (Id proteins, LIM proteins, Twist, I-mf proteins) and positive (CBP/p300, MEF2 proteins) regulatory factors (for reviews, see Buckingham, 2001; Brand-Saberi, 2005).

Myoblast to myoblast fusion creates short myotubes that can be extended by the absorption of additional myoblasts [the mammal literature has recently been reviewed (Horsley and Pavlath, 2004)] (Fig. 2). Muscle fibres also absorb myoblasts (myoblast-muscle fibre fusion) as they increase in diameter and length during growth (Fig. 2). A large number of factors that potentially regulate myoblast fusion have been identified in mammals, including caveolin-3, IL-4, PGF2α, calpain, calpastatin, NFATC2, and the transmembrane semaphorin Sema4c (Horsley and Pavlath, 2004; Ko et al., 2005). The binding of prostaglandin $F_{2\alpha}$ to its receptor produces an increase in intracellular calcium concentration, which activates the transcription factor NFATC2 required for nuclear accretion (Horsley and Pavlath, 2003). The factors regulating myoblast fusion and nuclear accretion in teleost fish have not yet been investigated, although orthologues of the key regulatory molecules found in mammals can be identified in fish genomes.

In mammals, insulin-like growth factor-1 (IGF-1) and the autocrine actions of IGF-II have major roles in regulating muscle mass and fibre size (Barton-Davies et al., 1999). IGF-I activates the Akt-mTor signalling pathway, which has a central role in regulating protein synthesis and degradation in muscle (Stitt et al., 2004; Ohanna et al., 2005). The autocrine IGF-II transcription required for skeletal myocyte differentiation is regulated by mTOR and the availability of amino acids (Erby et al., 2003). Thus the mTOR–IGF axis provides a molecular link between nutritional levels and protein synthesis leading to muscle fibre hypertrophy. Growth in fish is also primarily controlled by the availability of growth hormone, IGF-I and IGF-II and their respective receptors, and IGF-binding proteins (Moriyama et al., 2000; Castillo et al., 2002; Castillo et al., 2004; Peterson et al., 2004). In mammals, *IGF-II* mRNA decreased during postnatal development (Daughaday and Rotwein, 1989). In contrast, *IGF-II* mRNA was reported at higher levels than *IGF-1* mRNA in several tissues of adult fish, including muscle and liver (Gabillard et al., 2003; Peterson et al., 2004).

Myostatin-II (MSTN-II) is a negative regulator of muscle mass in prenatal mammals and belongs to the transforming growth factor beta (TGF-β) superfamily of signalling molecules. Myostatin-null mice and cattle with genetic mutations in *MSTN* genes show dramatic increases in skeletal muscle mass with increased fibre hyperplasia and hypertrophy (McPherron et al., 1997). It has been reported that myostatin inhibits myoblast proliferation by upregulating $p21^{Waf1,Cip1}$ and downregulating cyclin-E-Cdk2 activity (Thomas et al., 2000) and activates myoblast differentiation by downregulating the expression of MyoD, Myf5 and Pax-3 (Yang et al., 2005). Transgenic zebrafish that overexpressed MST-II showed a small but significant increase in fibre number relative to the wild type, but no change in fibre size, consistent with some role for myostatin as an inhibitor of embryonic hyperplasia (Xu et al., 2003). The role of MSTN in postembryonic growth is less clear. Administration of exogenous myostatin to adult mice induces muscle and fat loss analogous to human cachexia syndromes (Zimmers et al., 2002). The rainbow trout genome contains two paralogues of myostatin, TMyostatin 1 and TMyostatin 2 (Rescan et al., 2001). TMST 1 is ubiquitously expressed in all tissues whereas TMST 2 is specific to the brain and skeletal muscle, where it starts to be expressed in the free-swimming stages. The muscle wasting that accompanies sexual maturation produced no change in *TMST-1* mRNA transcript levels but was associated with a significant decease in *TMST 2* expression in both slow and fast muscle (Rescan et al., 2001).

Myotomal muscle in teleost fish

Fish swim using a combination of paired and unpaired fins and rhythmic contractions of the segmentally arranged

myotomes. Embryonic myotomes have a simple block shape in two-dimensional projections, which transforms to a V-shape in a rostral to caudal progression. In zebrafish, the adult myotome shape is not attained until 6 weeks after hatching (Raamsdonk et al., 1974). In adult stages, the myotomes have a W-shape in two dimensions, but consist of a series of overlapping cones in three dimensions (Van Leeuwen, 1999). In larval common carp, longitudinal reconstructions revealed that six myotomes were sectioned at different levels when preparing a transverse section through the trunk (Alami-Durante et al., 2000). Different muscle fibre types are organised into discrete layers within the myotome, greatly facilitating molecular and physiological studies and their interpretation. There is a superficial layer of aerobic slow twitch muscle that powers sustained activity (Johnston et al., 1977; Rome et al., 1984). Fast muscle fibres, which comprise the bulk of the myotome, develop 3–5 times higher power outputs than the superficial slow muscle fibres at higher tail-beat frequencies (Altringham and Johnston, 1990), reflecting their role in providing the power required for escape responses and predation behaviour. Intermediate muscle fibre types, where present, have relatively high aerobic and glycolytic capacities and intermediate contractile properties and recruitment patterns to slow and fast muscle fibres (Johnston et al., 1977; Mascerello et al., 1995). The shape, muscle mass and structural components of myotomes and their dynamic interactions vary along the length of the trunk, reflecting their particular roles in generating swimming movements (Davies et al., 1995; Thys et al., 1998; Coughlin et al., 2005).

Phases of myogenesis

The main phases of myogenesis in teleosts and their relative importance to muscle fibre production are illustrated in Fig. 3.

Embryonic myogenesis

The embryonic phase of myogenesis has been studied in some detail in the zebrafish *Danio rerio*. Cells first become committed to a myogenic fate at the end of gastrulation when specific cells express myoD (Weinberg et al., 1996). Somites condense from the paraxial mesoderm and segment in a rostral to caudal progression. Cell labelling and fate studies have shown the paraxial mesoderm originates from a specific region of the embryo identifiable just prior to gastrulation (Kimmel et al., 1990). MyoD expression starts in two triangular fields on either side of the forming axial mid-line (Weinberg et al., 1996). A 4×5 array of relatively large myoD expressing cuboidal cells can be identified on either side of the notochord prior to segmentation (Kimmel et al., 1995; Weinberg et al., 1996; Devoto et al., 1996). Adaxial cells are surrounded by more numerous and irregularly shaped cells of the presomitic mesoderm. A sub-set of these adaxial cells, the muscle pioneers, elongate to span the somite width, differentiate and develop striations (Felsenfeld et al., 1991). Myotubes derived from adaxial cells migrate through the myotome from their position at the notochord to form a superficial layer of slow muscle fibres (Devoto et al., 1996). Glycoproteins from the hedgehog family that are secreted from the notochord are thought to be responsible for slow muscle fibre specification (Devoto et al., 1996; Blagden et al., 1997). Sonic hedgehog sense mRNA injected into eggs results in the activation of myoD expression throughout the presomitic paraxial mesoderm and its subsequent differentiation into slow muscle fibres (Barresi et al., 2000). Mutations in the *u-boot* (*ubo*) gene cause the adaxial cells to abort their developmental programme, fail to migrate or express the slow fibre marker Prox 1 and adopt the fast muscle phenotype. The blocking of hedgehog signalling has been shown to disrupt the elongation of fast muscle fibres (Henry and Amacher, 2004). It has been suggested the medial to lateral migration of slow muscle fibres provides a morphogenetic signal that patterns fast muscle fibre elongation (Henry and Amacher, 2004). In rainbow trout, muscle-specific genes were shown to be expressed at approximately the 25-somite stage in the medial part of the somite where the slow muscle precursors are initially located, proceeding down the trunk in a rostral to caudal wave (Chauvigné et al., 2005). At the 30-somite stage the adaxial cells started to express the slow myosin light chain and the Lim protein SLIM1/FHL1. The adaxial cells migrated through the somite to a superficial position as described in the zebrafish. Double *in situ* hybridisations revealed a common myogenic programme during the early stages of myogenesis with co-expression of slow and fast isoforms throughout the myotome. Later during embryogenesis the inappropriate isoforms for particular fibre types were downregulated and other genes such as parvalbumins started to be expressed (Chauvigné et al., 2005).

Stratified hyperplasia

The number of embryonic slow muscle fibres in the initial wave of myogenesis is not sufficient to account for all those present at hatching (Barresi et al., 2001). Additional slow muscle myotubes are formed in the late embryo in discrete germinal zones at the lateral margins of the myotome (Barresi et al., 2001); a process termed stratified hyperplasia (Rowlerson and Veggetti, 2001). Normal striated hyperplasia of slow muscle is observed in the zebrafish slow-muscle-omitted gene mutant (smu–/–), which is deficient in Shh signalling. Thus stratified hyperplasia of slow muscle fibres in late embryonic stages is not dependent on the scaffold of adaxial cell-derived slow fibres formed earlier in development. Stratified hyperplasia is the main mechanism responsible for the increase in the number of slow muscle fibres after hatching (Veggetti et al., 1990; Rowlerson et al., 1995; Barresi et al., 2001). The formation of slow muscle myotubes from germinal zones apparently continues throughout life. For example, in the Arctic charr (*Salvelinus alpinus*) slow fibre number was found to increase with body length, scaling to body mass$^{0.45}$ (Johnston et al., 2004).

Labelling experiments with the thymidine analogue 5′-2-bromo-deoxyuridine (BrdU) have also identified germinal zones of myotube formation in the fast muscle that become

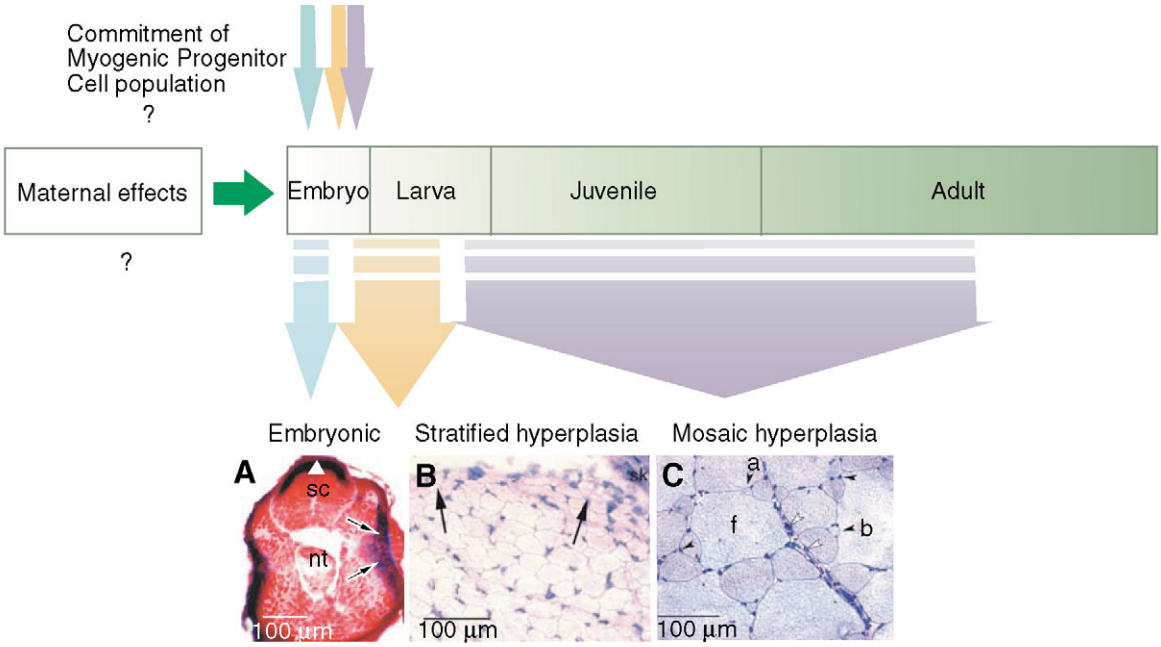

Fig. 3. The three phases of myogenesis in the fast myotomal muscle of the arctic charr *Salvelinus alpinus*: embryonic (blue arrow), stratified hyperplasia (orange arrow) and mosaic hyperplasia (mauve arrow) [based on (Johnston et al., 2004), and D. B. Sibthorpe and I.A.J., unpublished results]. Mosaic hyperplasia is quantitatively the most important phase of myogenesis. In teleosts maternal mRNA transcripts (maternal effects) drive development until the mid-blastula transition when zygotic transcription is initiated. The myoblasts that form the embryonic slow and fast muscle become committed to a myogenic progenitor cell population towards the end of gastrulation, which is much earlier than in amniotes. At least two further phases of myotube production can be distinguished in fast muscle, involving the production of muscle fibres within discrete germinal zones (stratified hyperplasia) and the widespread formation of fibres throughout the myotome (mosaic hyperplasia). (A) The rostral somites of an arctic charr embryo (large benthic morph) at the end of segmentation (751 h.p.f.) illustrating the embryonic phase of myogenesis. The arrows illustrate the intense staining for Pax 7 transcripts in the lateral margin of the myotome extending along the position of the major horizontal septum. The arrowhead shows intense staining in the dorsal region of the spinal cord. (B) Stratified hyperplasia (arrows) in the apical regions of the fast muscle layer of the myotome in an arctic charr juvenile, 4.5 cm fork length. (C) Past evidence of mosaic hyperplasia in the fast muscle of a piscivorous arctic charr morph 35.8 cm fork length. Mature fast fibres (f) are surrounded by daughter fibres at various stages of growth. The fibres labelled (a) and (b) are 14 and 18 µm diameter, respectively. Filled arrowheads represent myonuclei and unfilled arrowheads connective tissue nuclei. Abbreviations: nt, notochord; sc, spinal cord: sk, skin.

exhausted in the late larval or early juvenile stage, e.g. in sea bream *Sparus auratus* (Rowlerson et al., 1995), Atlantic herring *Clupea harengus* (Johnston, 1993; Johnston et al., 1998), Atlantic cod *Gadus morhua* (Galloway et al., 1999). Stratified hyperplasia is the only postembryonic mechanism of fast muscle myotube production in certain families (Harpagiferidae, Channichthyidae) of Antarctic notothenioids that show an associated dramatic reduction in the number of fast muscle fibres and a corresponding increase in fibre size (Johnston et al., 2003a). Myogenesis of intermediate muscle has been little studied but probably occurs by stratified hyperplasia from germinal zones adjacent to the slow muscle layer (Mascarello et al., 1995; Rowlerson and Veggetti, 2001).

Mosaic hyperplasia

Mosaic hyperplasia is so-called because in this final phase of myogenesis myotubes form on the surface of fast muscle fibres throughout the myotomal cones, giving rise to a mosaic of fibre diameters. The immature fibres transiently express developmentally regulated isoforms of myosin heavy chains (Enion et al., 1995) and often have a higher content of glycogen and mitochondria than the more mature muscle fibres (Johnston et al., 1975b). Mosaic hyperplasia is important in species with a prolonged larval phase, such as Atlantic herring (Johnston et al., 1998a). It is the main mechanisms for expanding fast fibre number in the juvenile and adult stages of the vast majority of species, continuing until approximately 40% of the maximum fish length (Weatherley et al., 1988). Subsequent growth exclusively involves an increase in the length and diameter (hypertrophy) of the fibres. Thus postembryonic growth is achieved through an expansion in diameter of the immature muscle fibres. The maximum fibre diameter, on the other hand, is set by diffusional constraints that vary with body mass, activity patterns and metabolic demand (Johnston et al., 2003a; Johnston et al., 2004). All other things being equal, as body size increases and mass-specific metabolism declines as a function of body mass$^{-0.25}$, the relaxation of diffusional constraints allows the maximum fibre diameter (D_{max}) to increase. For sedentary species living at very low temperatures D_{max} continues to increase with body

length until the maximum body length, resulting in giant fibres of 500–600 μm diameter in some Antarctic teleosts (Johnston et al., 2003a). In warmer water and/or more active species D_{max} reaches a limiting value at much less than the maximum body length (Johnston et al., 2003b) (I.A.J., unpublished results).

Individual muscle fibres absorb additional myoblasts from the proliferating myogenic precursor cell (MPC) population as fibre diameter and length increase (Fig. 2). For salmonids the number of myonuclei increases as an approximately linear function of fibre diameter (Johnston et al., 2003b; Johnston et al., 2004). Very little is known about the genetic mechanisms regulating the formation of myotubes in fast muscle. Injury to the muscle can result in a new wave of myotube production to repair the damage even if muscle recruitment has stopped (Rowlerson et al., 1997). This suggests some mechanism for inhibiting myotube formation in undamaged muscle in fish that are greater than 40% of their maximum length. We have used suppression subtractive hybridisation (SSH) in the model species, the tiger pufferfish (*Takifugu rubripes*), to identify genes that were differentially expressed between myotube (+) and myotube (–) growth stages (Fernandes et al., 2005). The different patterns of myotube formation in slow and fast muscles, and gene expression patterns in non-muscle tissues, were used to distinguish between potential candidate genes involved in myotube formation and genes that changed with body size but were not related to myotube formation. Four strong candidates with appropriate tissue-specific expression patterns were identified that were 5–25-times upregulated concomitant with the inhibition of myotube formation in fast muscle (Fernandes et al., 2005). The expression of orthologues of these putative myotube inhibitory genes was investigated in the fast muscle of the zebrafish in relation to the cessation of muscle fibre recruitment (H. T. Lee and I. A. Johnston, manuscript submitted for publication). Two of the candidates were significantly upregulated at the body length where myotube production stopped, consistent with them having a role in the control of fibre recruitment. Candidate 1 (GenBank Accession No. CK829660 *Takifugu rubripes*) had nine exons predicted on Ensembl with conserved B302, fibronectin and SPRY receptor domains, suggesting it might be involved in the regulation of transcription and/or cell migration, differentiation and adhesion (Dickson et al., 1990). The Ensembl-predicted transcript structure for candidate 2 (GenBank Accession No. CK829660 *Takifugu rubripes*) comprised five exons that translated into a 483-residue protein with conserved POZ/BTB and BTB/kelch domains (Fernandes et al., 2005).

Plasticity of myogenesis in early life stages

Temperature

Prior to hatching there is no scope for fish embryos to select a new environment and thus they are at the mercy of ambient conditions of temperature, oxygen, salinity, pH, etc. Adverse environmental conditions in nature can be the cause of significant embryonic mortality (Laprise and Pepin, 1995). Sub-lethal environmental stress impacts on all aspects of development, including myogenesis, with potential consequences for the morphology, physiology, behaviour and survival of the hatched larvae.

At hatching, teleost myotomes usually have a single superficial layer of slow muscle fibres surrounding an inner core of rapidly differentiating embryonic fast muscle fibres (Fig. 4A,B). Both of these 'embryonic muscle fibre types' have a higher volume density of mitochondria (Vieira and Johnston, 1992) and a less well-developed anaerobic capacity than the corresponding muscle fibre types in juveniles (El-Fiky and Wieser, 1988). In Atlantic herring (*Clupea harengus*), the mitochondrial content in embryonic slow and fast muscle fibres increased with increasing rearing temperature to the hatching stage (Fig. 4C), whereas the myofibrillar volume density decreased (Vieira and Johnston, 1992). In contrast, cold acclimation in the juvenile stages of several species resulted in an expansion of the mitochondrial compartment (Johnston and Maitland, 1980; Egginton and Sidell, 1989), presumably to compensate for the effects of low temperature on diffusion and ATP synthesis rates. Embryonic temperature regime affects the relative timing of muscle differentiation in herring larvae, including the body lengths at which developmental-stage specific isoforms of the various myofibrillar isoforms and adult patterns of motor innervation first appeared (Vieira and Johnston, 1992; Johnston et al., 1997; Johnston et al., 1998b). Interestingly, the embryonic temperature continued to exert an effect on the relative timing of development even after temperature groups were transferred to a common temperature (Johnston et al., 2001). In herring, such heterochronies in the development of the paired and unpaired fins and associated fin ray musculature were found to affect escape swimming performance, and may influence larval mortality (Johnston et al., 2001).

Numerous studies have shown that temperature can affect the number and diameter of fast and slow myotomal muscle fibres in larval fish. Most published studies have taken eggs from individual or mixed families and incubated them at two or at the most three temperatures, and have either sampled the offspring at defined life history stages (e.g. hatching or first feeding) or at a range of body lengths post-hatch. The latter is preferable since fish reared at different temperatures do not necessarily hatch or start feeding at the same developmental stage. Various patterns of response of muscle cellularity to temperature have been described at hatch and the start of exogenous feeding. For example, at high- compared with low-egg incubation temperatures the following responses have been reported for fast muscle: either no change in muscle cellularity, e.g. sea bass *Dicentrachus labrax* (Ayala et al., 2000; Lopez-Albors et al., 2003), or fewer, larger diameter fibres, e.g. Atlantic salmon *Salmo salar* (Stickland et al., 1988), turbot *Scophthalmus maximus* (Calvo and Johnston, 1992), whitefish (Hanel et al., 1996) and plaice *Pleuronectes platessa* (Brookes and Johnston, 1993), or more fibres of smaller diameter, e.g. Atlantic herring *Clupea harengus* (Vieira and Johnston, 1992) and Atlantic cod *Gadus morhua* (Hall and Johnston, 2003). The number of slow muscle fibres at hatching is also sensitive to rearing temperature although

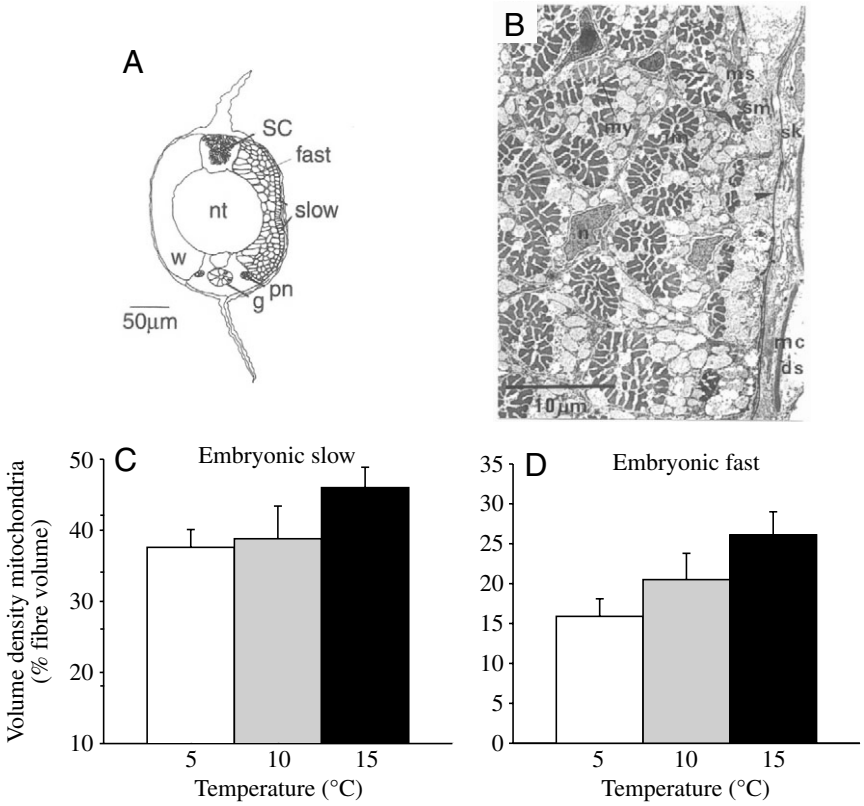

Fig. 4. Influence of temperature on the ultrastructure of myotomal muscle in larval Atlantic herring (*Clupea harengus*) (Vieira and Johnston, 1992). (A) Camera lucida drawing of a 1-day old larva sectioned immediately posterior to the yolk-sac. (B) Transverse electron micrograph of a 1-day old herring larva reared at 12°C. Abbreviations: ds, dermal scale; g, gut; im, embryonic fast muscle fibre; mc, mucocyte; ms, undifferentiated myoblast; mt, mitochondria; my, myofibril; n, myonucleus; nt, notochord; pn, pronephros; SC, spinal cord; sk, skin; sm, embryonic slow muscle fibre. (C,D) The volume density of mitochondria (% fibre volume) in (C) embryonic slow and (D) embryonic fast muscle of 1-day old larvae reared at 5, 10 or 15°C until hatching. Values represent means ± s.e.m., N=20 fibres from 5 larvae per temperature.

the response observed may differ from that in fast muscle (Stoiber et al., 2002; Hall and Johnston, 2003; Johnston et al., 2000). Population level variations in the responses have been documented for Atlantic herring spawning at different times of the year (Johnston et al., 1998a), reproductively isolated Atlantic salmon from different parts of a river catchment (Johnston et al., 2000), and between sea bass from the NE Atlantic and Mediterranean sea (Ayala et al., 2001). Clearly, the effects of temperature on the first two phases of myogenesis are complex and depend on the precise characteristics of the temperature regime (Stoiber et al., 2002).

The various patterns of muscle cellularity described cannot be interpreted without a detailed knowledge of how the norms of reaction for myotube formation change with respect to each phase of myogenesis. Fig. 5 illustrates how it is entirely possible to produce different patterns of muscle cellularity depending on the temperatures and time of sampling chosen for study. Thus such descriptive studies of myogenesis are of most value if the phenotypes are catalogued over the entire temperature range for development at multiple stages of ontogeny. The mechanisms whereby temperature can alter the number of muscle fibres is perhaps of more interest. Changes in the relative timing of the transcriptional networks, extracellular signalling molecules and/or intracellular growth factors regulating any of the steps in embryonic myogenesis and stratified hyperplasia have the potential to alter the number of muscle fibres present in embryos at hatch or first feeding. In general, factors that promote myoblast proliferation at the expense of cell cycle exit and differentiation would be expected to result in an increase in fibre number during the early phases of myogenesis. It is likely that heterochronies in numerous molecular species underlie changes in muscle cellularity with temperature. In Atlantic cod, closure of the blastopore occurred later with respect to segmentation at higher temperatures, at the 3-somite, 10-somite and 12-somite stages at 4, 7 and 10°C, respectively (Hall and Johnston, 2003). It was suggested that this change in the relative timing of epiboly might alter the time window for myoblast proliferation and/or their exposure to inductive signals (Hall and Johnston, 2003).

MyoG expression occurs following somite formation in a rostral to caudal wave (Weinberg et al., 1996; Temple et al., 2001). MyoG expression was retarded with respect to somite stage in rainbow trout embryos reared at 4°C compared to 12°C, consistent with a change in the relative timing of differentiation (Xie et al., 2001). At the eyed stage, northern analysis indicated greater myosin heavy chain expression, but lower MyoG expression in the caudal myotomes at high than low temperatures, consistent with a more advanced state of muscle differentiation. Examination of a broader range of temperatures established that there was an optimal temperature for both MRF and myosin heavy chain expression with respect to developmental stage (Wilkes et al., 2001). In contrast, in the model pufferfish *Takifugu rubripes* the relative timing of *myoG* expression was similar at 15, 18 and 21°C (Fig. 6). However, *myoG* were significantly higher at 21°C than 15°C (Fernandes et al., 2006). Changes in myoG and other factors have the potential to alter the timing of muscle differentiation and hence the time window for myoblast proliferation. However, given the redundancy of the transcriptional networks regulating myogenesis it is necessary to study the expression of many factors, preferably at the protein level, and to conduct functional (knockdown/over-expression) studies to establish the mechanisms.

Oxygen availability

Salmonids have large eggs, a protracted development time and hatch at an advanced stage of organogenesis. The chorion surrounding the embryo has been shown to represent a potential barrier to diffusion (Rombough, 1988). It has been suggested that at high temperatures during the later stages of embryogenesis the metabolic requirements of the developing embryo may not be matched by the supply of oxygen across the chorion, resulting in a physiological hypoxia (Matschak et al., 1995). The number of fast muscle fibres in Atlantic salmon (*Salmo salar*) embryos at hatch was found to be 15% higher at 6.5°C than 11°C in intact eggs, but was independent of temperature in dechorionated eggs (Matschak et al., 1995). The average fibre size was 30% greater at high temperatures in intact eggs, whereas average fibre size was greater at low than high temperatures in dechorionated eggs. In subsequent experiments, the effects of varying oxygen (50%, 100% and 150%) air saturation on fast fibre number, fibre size and myonuclear density was investigated in intact and dechorionated embryos (Matschak et al., 1997). Whilst lowering oxygen availability had some effects on the measured parameters, the results were not consistent with hypoxia being the main explanation for temperature effects on muscle cellularity, although it is probably a contributing factor. Hypoxia is also unlikely to be a factor in the development of small diameter pelagic eggs that hatch at a relatively early stage of organogenesis.

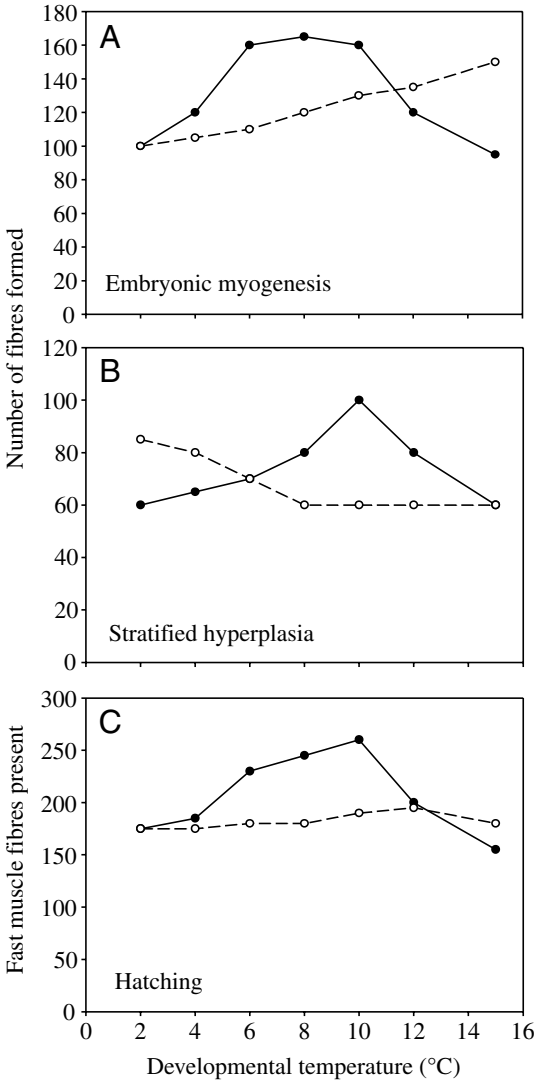

Fig. 5. Theoretical reaction norms for myotube production in fast muscle at different temperatures for (A) the embryonic and (B) the stratified hyperplasia stages of myogenesis prior to hatching. (C) The total number of fast muscle fibres per myotomal cross-section present at hatching at the different temperatures. Two reaction norms are illustrated (open and filled circles). In the situation illustrated by the open circles fast fibre number at hatching is apparently independent of temperature, but this is a consequences of the different reaction norms for embryonic and stratified hyperplasia. The norm of reaction illustrated by the filled symbols shows a temperature optimum for myotube production that differs somewhat for the two phases of myogenesis. Note in this example that studying fish reared at just 4 and 12°C and sampled at hatch would result in the erroneous conclusion that muscle fibre production was independent of temperature.

Persistent effects of embryonic temperature on post-embryonic growth

Embryonic myogenesis and stratified hyperplasia together only account for 30% of the total fast muscle fibres recruited in zebrafish (H. T. Lee and I. A. Johnston, manuscript submitted for publication), and less than 5% of the maximum fibre number (FN_{max}) in Atlantic salmon (Johnston et al., 2003b). Thus environmental plasticity in the first two phases of myogenesis may affect growth performance of the early life stages, but may not make much difference to FN_{max} in adult stages. Myogenic progenitor cells (MPCs), analogous to the satellite cells in adult mammalian muscle, are responsible for postembryonic growth in teleosts. MPCs in teleosts are not always found beneath the basal lamina of muscle fibres (Veggetti et al., 1990; Johnston et al., 2003b) and should not be called satellite cells. Recently, using chick–quail grafting experiments, the embryonic origin of the myogenic progenitor cells involved in postembryonic growth in amniotes was shown to be the dorsal compartment of the somite, the dermomyotome (Gros et al., 2005). Although the origin of myogenic progenitor cells in teleost embryos is unknown, changes in the number formed in the embryo have the potential to produce persistent effects on growth in adult stages.

Embryonic temperature was shown to produce long-lasting effects on myogenesis in the larvae of spring-spawning Clyde herring (*Clupea harengus*) (Fig. 7). This spawning stock deposit their eggs in dense mats on the seabed in March at an average temperature of 4.8°C to 10°C, depending on natural climatic variation (Jones and Jeffs, 1991). The transparent larvae remain in the plankton until late summer when they complete metamorphosis to the juvenile stage at 33–40 mm total length, by which time the sea temperature has risen to 12–16°C. The number of slow and fast muscle fibres recruited during the larval phases was found to differ with temperature

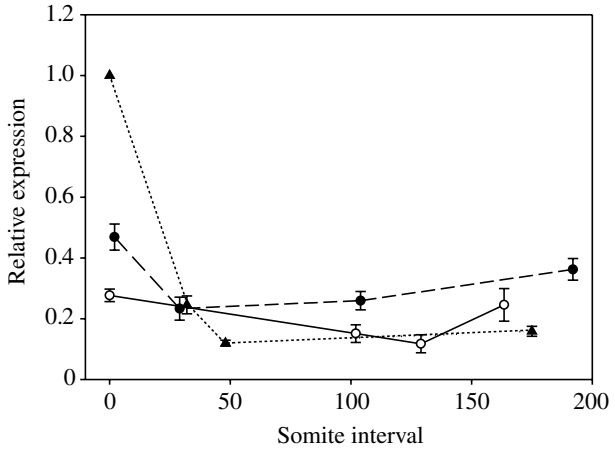

Fig. 6. Myogenin (*myog*) expression in embryos of the tiger pufferfish (*Takifugu rubripes*) reared at 15°C (open circles), 18°C (closed circles) and 21°C (closed triangles). The eggs were from a single cross. mRNA transcripts were measured by qPCR using 18S rRNA as an endogenous control. The results were normalised against the highest expression value (21°C, 40 h.p.f.) and plotted against somite interval (development time/divided by the time to form one somite pair) in an attempt to normalise developmental stage at the different temperatures. The results represent mean ± s.e.m. of 4 batches of embryos per temperature. From (Fernandes et al., 2006).

regime, more myotubes being produced for a given body length at higher temperatures (Fig. 7). In 1 day old larvae, the density of myoblasts per myotomal cross-section, as identified from electron micrographs, was around threefold higher at 8°C than 5°C, and was intermediate at 12°C (Johnston, 1993). In a subsequent study, eggs from the same population were incubated at either 5°C or 8°C until first feeding and then transferred to a common ambient temperature (Johnston et al., 1998a). The effects of embryonic temperature regime remained imprinted on myogenesis in later larval stages, resulting in a higher fibre number in the 5°C than 8°C groups.

Our working hypothesis to explain these results was that the temperature regime prior to first feeding affected the number of undifferentiated myoblasts and hence future growth potential. Manx herring spawn at higher sea temperatures than Clyde herring, but the larvae experience cooling rather than warming temperatures prior to metamorphosis the following spring. Larvae of Manx herring were shorter at first feeding than Clyde herring and had a higher temperature optimum for myogenesis and growth (Johnston et al., 1998a). Early thermal experience also affected the number of muscle fibres produced in relation to larval length in Manx herring (Johnston et al., 1998a). Differences in the number of slow and fast muscle fibres in relation to body length have also been reported for European pilchard larvae (*Sardina pilchardus*) sampled in the field during different seasons of the year (Catalán et al., 2004).

Atlantic salmon (*Salmo salar*) spends from just over a year to up to 5 years in freshwater prior to undergoing smoltification and migrating to the sea. The temperature regime during freshwater life was found to affect muscle fibre recruitment during the seawater stage of the life cycle (Fig. 8A). The eggs from of a minimum of 64 families from an inbred line of farmed salmon were incubated at either cool ambient temperatures or in water heated by 1–3°C relative to ambient. Fish were individually tagged and reared in the same replicated sea cages under identical environmental conditions and diet. Following smoltification and prior to transfer to sea cages, post-smolts were on average almost twice as heavy in the heated (61.6 g) than in the ambient (34.0 g) treatments, reflecting their greater growth opportunity: 4872 degree-days and 4281 degree-days, respectively. The seawater growth rate of the heated group was lower than the ambient group, such that both groups attained a mass of 3.7–3.9 kg after 450 days. The intensity of myotube production was higher in the ambient than in heated groups, resulting in 22.4% more fibres at the end of the fibre recruitment phase of growth (Fig. 8A). The density of myogenic

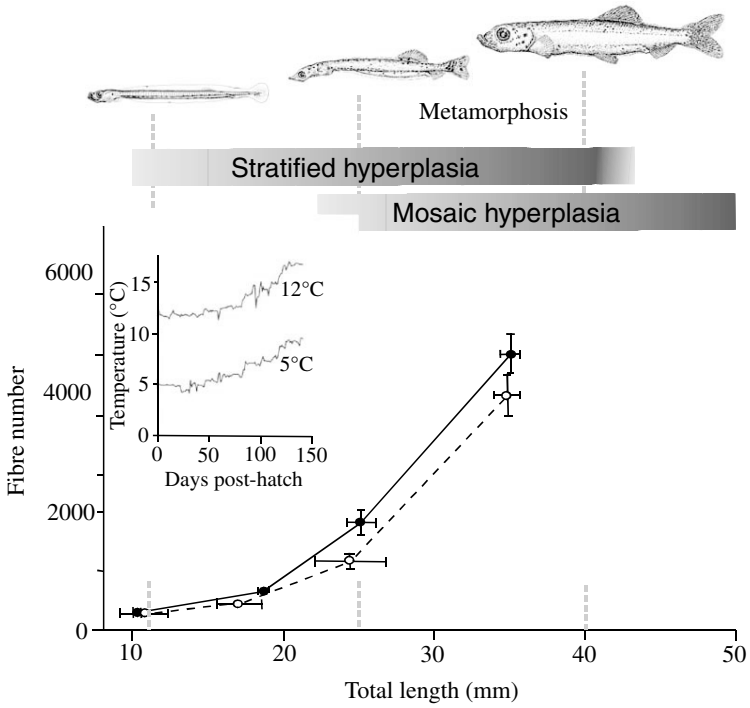

Fig. 7. The recruitment of fast muscle fibres at two temperature regimes in larval herring from hatching until the completion of metamorphosis. Adapted from (Johnston et al., 1998a). The temperature regimes used to rear the larvae are shown in the inset. The cool and warmer temperature regime started at 5°C and 12°C, respectively, and increased during the larval phase. The number of fast muscle fibres per myotomal cross-section for the cool (open circles) and warm (closed circles) are illustrated. The values represent mean ± s.e.m. for 12 larvae per stage/temperature for the first two stages and 6 larvae per temperature/stage for the remaining stages. The relative duration of stratified and mosaic hyperplasia and the morphology of the larvae at three stages is shown.

progenitor cells, identified using an antibody to c-met, was also higher in the ambient than heated treatments (Johnston et al., 2003b). Since the fish had been exposed to the same growth opportunity during seawater life the observed differences in myogenesis must have been imprinted from the freshwater stage, and may reflect an altered complement of myogenic progenitor cells. Interestingly, the myonuclei content of isolated fast fibres was also higher in the ambient than heated fish, e.g. 20.6% cm^{-1} higher in fibres of the maximum diameter (Fig. 8B). Thus a higher number of MPCs in the ambient than heated treatments may have resulted in a higher intensity of fibre recruitment and a higher content of myonuclei. These observations are consistent with a model in which there is a single population of MPCs providing the founder myoblasts for myotube formation and the myoblasts required for nuclear accretion, with their fate determined by local signalling.

Plasticity of adult growth

The signalling pathways regulating the proliferation and differentiation of myogenic progenitor cells are sensitive to environmental factors and feeding. In the sub-Antarctic plunderfish (*Harpagifer bispinis*), giving fasted individuals a satiating meal was shown to result in an increase in the number of cells in fast muscle expressing MyoD and PNCA (a cofactor to DNA polymerase δ), and this was followed by an increased abundance of c-met and myogenin expressing cells (Brodeur et al., 2003). This sequence of events is consistent with an initial activation of myogenic cell proliferation (expression of myoD/PCNA) followed by the production of new cells (increase in c-met positive cells) and the commitment of at least some of the progeny to differentiation (myogenin expression).

Photoperiod is probably the most important proximal cue regulating the seasonal timing of sexual maturation and somatic growth in salmonids, resulting in modified output of the neuroendocrine system (Hansen et al., 1992; Bromage et al., 1993). The rate of muscle fibre recruitment and hypertrophy in Atlantic salmon occurs at reduced rates during the winter months, when appetite is reduced due to low temperatures and short days (Johnston et al., 2003c). In salmon farming, artificial lighting is often used to boost growth during the winter months. We investigated myogenesis in the fast muscle of Atlantic salmon during their first sea-winter, comparing groups maintained under a natural photoperiod with fish subjected to continuous light (Johnston et al., 2003c). Lights were switched-on in duplicate sea cages on the 1st November, producing a small but significant increase in the number of MPCs (identified using an antibody to c-met) after 24–30 h relative to fish held in other sea cages under natural day-length. This increase in MPCs was transient and peaked at 70% of pre-light treatment levels after 40 days, corresponding to the period when the natural day-length was still shortening. Growth rate and the intensity of myotube production (Fig. 9A) were also significantly higher in the ambient than continuous light groups during the first 40 days. However, once day-length started to lengthen in the early spring the rate of muscle fibre recruitment was similar in both groups. It was suggested that short days inhibited the proliferation of MPCs and that this effect was counteracted by the continuous light treatment, causing an increase in the number of times the myogenic

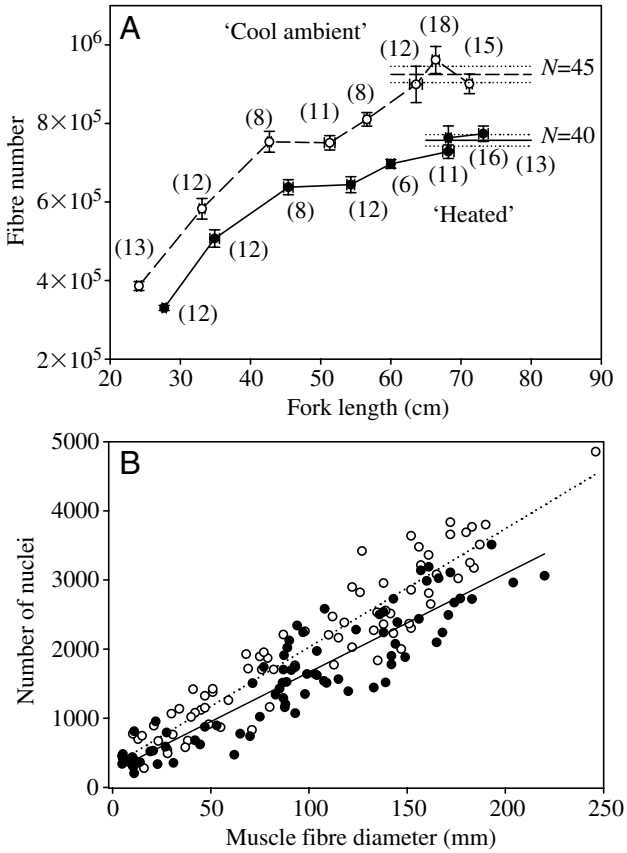

Fig. 8. Influence of freshwater temperature regime on the recruitment of fast muscle fibres during the seawater stages of Atlantic salmon (*Salmo salar* L). (A) The number of fast muscle fibres per myotomal cross-section (at the level of the first dorsal fin ray) in relation to fish fork length (cm) during seawater growth. Offspring from a large number of families were reared at cool ambient temperatures or in water heated by 1–3°C during the freshwater stages and then reared together in the same 5 m×5 m×5 m sea cages. The number of fish sampled at each fork length is shown in parentheses. The dotted lines (cool groups, N=45) and the solid lines (heated groups, N=40) represent fish in which fibre recruitment had ceased and these fibre number values represent the maximum (FN_{max}) for each freshwater treatment. (B) The myonuclei content of isolated fast muscle fibres in relation to fibre diameter (D) for seawater stages of Atlantic salmon. The open circles represent fibres from fish exposed to cool ambient temperatures and the closed circles fish exposed to water heated by 1–3°C during the freshwater stages. Myonuclei content was determined using single fibres stained with the fluorescent DNA stain Sytox Green™ (Molecular Probes, Leiden, Holland). The lines represent first order linear regressions fitted to the data. The regression equations were as follows: for ambient fish myonuclei=314+17.1D, and for heated fish myonuclei=237+14.3D. See the original publication (Johnston et al., 2003b) for further experimental details.

progenitor cells divided and/or a decrease in cell cycle time (Johnston et al., 2003c). Fibres isolated from fish from the continuous light cages also had a higher content of myonuclei than fish at ambient photoperiod, 27% more nuclei in 150 μm diameter fibres (Fig. 9B). In this experiment photoperiod manipulation had presumably altered the signalling pathways controlling myoblast proliferation, resulting in proportional increases in myotube production and the myonuclear content of fibres. Since this environmental intervention occurred just prior to the point that fibre recruitment terminated it had a permanent effect on FN_{max}, which was 23% higher in salmon from cages receiving continuous light (881 000) than in the ambient photoperiod fish (717 000) (Fig. 9A).

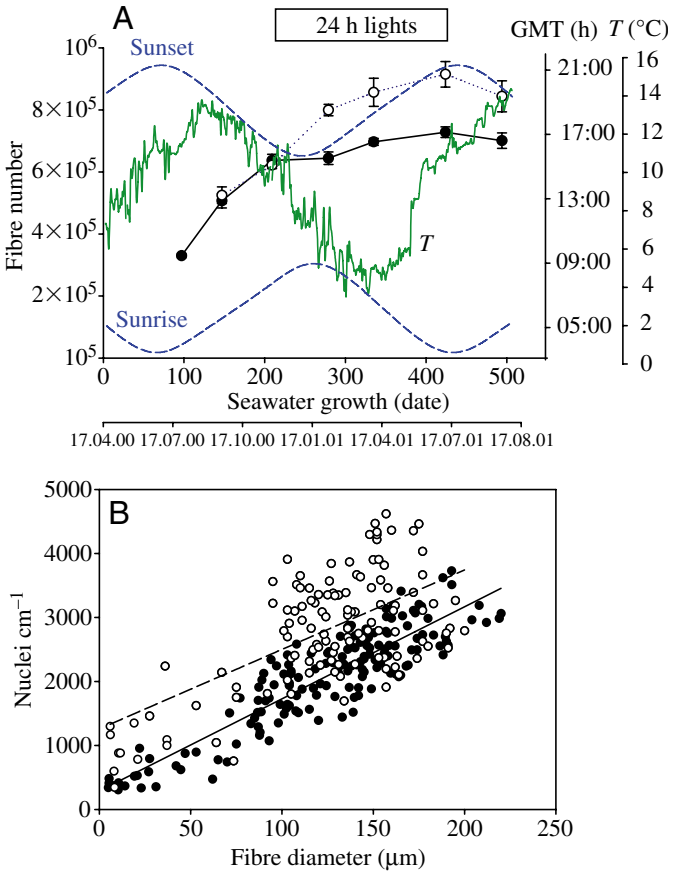

Fig. 9. The influence of photoperiodic regime on myogenesis in fast muscle of adult seawater stages of Atlantic salmon (*Salmo salar* L.). (A) The number of fast muscle fibres per myotomal cross-section in fish reared under 24 h continuous light (open circles) or at ambient photoperiod (closed circles). The broken blue line represents sunrise and sunset (Greenwich Mean Time) at Fort William, Scotland where the fish were reared and the green line illustrates daily recordings of sea temperature. The duration of 24 h lighting in the photoperiod manipulated cages is illustrated by the box. The values represent mean ± s.e.m. of 6–12 fish per sample, see original publication for details (Johnston et al., 2003c). (B) The myonuclei content of isolated fast muscle fibres in relation to fibre diameter (D; μm) for the 24 h light-treated fish (open circles) and the ambient day-length fish (closed circles). First-order linear regressions were fitted to the data. For the continuous light-treated fish myonuclei number=1258.1+12.4D (broken line) and for the natural day-length fish myonuclei number=288.6+14.4D (solid line). From (Johnston et al., 2003).

Myogenesis and exercise

Changing patterns of water flow that are maintained over long periods, e.g. between wet and dry seasons in freshwater rivers, may also result in altered patterns of swimming behaviour. The frequency and amplitude of tail-beats can potentially modify the characteristics of intracellular calcium transients and numerous downstream signalling pathways influencing muscle phenotype (Fig. 2). Several weeks of forced exercise training has been shown to result in a marked hypertrophy of slow and fast muscles in a number of teleost species relative to non-exercised groups, e.g. brook trout *Salvelinus fontinalis* (Johnston and Moon, 1980), Atlantic salmon (Totland et al., 1987), dace *Leuciscus cephalus* (Sänger, 1992), saithe *Pollachius virens* (Walker and Pull, 1973). In the common carp (*Cyprinus carpio*), forced swimming is also a powerful stimulus for nuclear accretion, and resulted in a higher myonuclear content in fibres from exercised than non-exercised fish (Martin and Johnston, 2006).

In mammals, the calcineurin signalling pathway is thought to play a role in regulating hypertrophic growth of skeletal muscle (Musaro et al., 2001). Prolonged increases in intracellular Ca^{2+} levels selectively activate calcineurin, resulting in the dephosphorylation of nuclear factor of T-cells 2 (NAFT2) and nuclear localisation of the calcineurin/NAFT2 complex (Dolmetsch et al., 1997). The latter event is thought to synergistically initiate a programme of gene expression leading to fibre hypertrophy (Musaro et al., 1999; Semsarian et al., 1999). However, exercise training leading to fast muscle hypertrophy in the rainbow trout only resulted in a minor increase in calcineurin localisation in the nucleus and total NFAT2 concentration decreased relative to tank-rested controls with no nuclear translocation (Martin and Johnston, 2005). These results indicate differences in the response of this signalling pathway to exercise stimuli between teleosts and mammals.

Potential ecological significance of environmental plasticity of myogenesis

Fast myotomal muscle comprises at least 60% of body mass in most teleosts and is therefore quantitatively an important tissue in determining the overall energy budget. Around 20–40% of routine energy consumption in teleosts is thought to be involved in maintaining ionic homeostasis (Jobling, 1994). Theoretically the cost of counteracting passive ion movements across the muscle sarcolemma would be expected to increase with the surface to volume ratio of the muscle fibres, i.e. to increase with increasing fibre number. We have proposed an optimal fibre number hypothesis, in which there is a trade-off between requirements to avoid diffusional constraints whilst maximising fibre diameter to minimise the

energy costs of ionic homeostasis (Johnston et al., 2003a). Thus the maximum diameter of single fibres (D_{max}) should be small enough to avoid anoxia in the centre, but as large as possible to reduce the maintenance costs of ion pumping across the membrane surface area, which is higher for small than large diameter fibres. As expected, the maximum fibre number is strongly correlated with body size for closely related species from the same habitat (Johnston et al., 2003a). Dwarfism is relatively common in land-locked salmonid populations and has occurred since the last Ice-age 9000 years ago. Ecological factors related to energetics and feeding are almost certainly responsible for establishing dwarfism, as was documented for Lake Whitefish populations (Trudel et al., 2001). However, once established we hypothesised that physiological factors related to scaling and the relatively high maintenance costs of supporting an excess number of fibres for the body size would have acted as a powerful selective force for reducing fibre number. In support of our hypothesis, dwarfism was shown to be associated with a 50% reduction in FN_{max} in the dwarf relative to large benthic Arctic charr morphs from Thingvallavatn, Iceland (Johnston et al., 2004) and a 75% reduction in FN_{max} in the landlocked Bleke salmon relative to migratory populations (Johnston et al., 2005). Temperature and oxygen availability and activity will also influence the 'ideal' fibre number for a given body size. An extreme example is provided by the core radiation of the Antarctic notothenioids. The relaxation of diffusional constraints due to the low temperature and sluggish lifestyle of these fishes resulted in a dramatic increase in maximum fibre diameter, and corresponding reduction in FN_{max} (Johnston et al., 2003a). The metabolic and fitness consequences of changes in muscle cellularity have not been addressed experimentally and this is an important area for future research.

Conclusions and future perspectives

There are considerable advantages to studying muscle plasticity in model fish species because of the extensive molecular and genetic resources available and the large community of scientists that has evolved around them. Four species have had their genomes sequenced to draft level (*Danio rerio*, *Takifugu rubripes*, *Tetraodon nigroviridis*, *Oryzius latipes*). The Japanese medaka (*O. latipes*) originates in a highly seasonal environment and is therefore likely to be particularly useful for investigating environment-induced muscle plasticity. Popular non-model species such as the common carp (*Cyprinus carpio*) and goldfish (*Carrasius auratus*) show enormous plasticity to environmental change. Focusing effort on a relatively few non-model freshwater and marine species that show significant environmental plasticity is probably the best way to increase understanding of the underlying mechanisms, because it will facilitate the establishment of a critical mass of molecular and genetic tools. In this regard, cDNA libraries, Expressed Sequence Tags and DNA microarrays are already available for the common carp, flounder (*Platichthys flesus*) and Atlantic salmon (*Salmo salar*) (Cossins and Crawford, 2005).

A good starting point for studies of muscle plasticity is the natural history of the species of interest. Salmon and trout embryos are subject to hypoxic conditions in the wild, which vary in severity in relation to water flow characteristics and the degree of infiltration of oxygen depleted groundwater. For example, in a study of 33 natural egg pockets of chum salmon *Oncorhyncus keta*, dissolved oxygen levels were found to vary from 2 to 10 mg l^{-1} at the time of spawning and generally declined during embryonic development (Peterson and Quinn, 1996). The interactive effects of temperature and oxygen on myogenesis during the development of salmonids *in situ* would be a fruitful area for future research. It is important to record as much information as possible about the genetic background and environmental history of the population being studied. Field observations of environmental parameters for the population under study can then be used to design laboratory experiments in which one or more parameters are systematically varied to investigate mechanisms.

Whether wild or captive populations are studied, there is considerable merit in adopting a systems biology approach in which environmental inputs, information from interacting physiological systems and muscle outputs are integrated and modelled with a quantitative framework. A systems approach may be equally useful for investigating the mechanisms and evolutionary significance of muscle plasticity. The acclimation of male mosquito fish to cool temperatures resulted in an increased swimming performance and mating success at cool relative to warm test temperatures (R. S. Wilson, E. Hammill and I. A. Johnston, manuscript submitted for publication). However, warm-acclimated fish were much more aggressive, presumably reflecting modified outputs of the neuroendocrine system. Under conditions of male-male competition, copulation success was highest for warm-acclimated individuals at all test temperatures (R. S. Wilson, E. Hammill and I. A. Johnston, manuscript submitted for publication). These experiments nicely illustrate that both the benefits of muscle plasticity are context-dependent and that the influence of the environment on other interacting physiological systems should also be considered.

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