Proliferation of myogenic progenitor cells following feeding in the sub-antarctic notothenioid fish *Harpagifer bispinis*

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Summary

Feeding metabolism and the activation of myogenic progenitor cells were investigated in the fast myotomal muscle of the sub-Antarctic fish *Hapagifer bispinis* acclimatized to either simulated summer (10°C; 18h:6h light:dark) or simulated winter (5°C; 6h:18h light:dark) conditions. Ingestion of a single meal equivalent to 10% and 15% of body mass in simulated winter and summer groups, respectively, resulted in an average 2.6-fold and 3.6-fold increase in oxygen consumption, declining to 75% of peak values after 63h and 46h. In fasted individuals, the number of myogenic progenitor cells, identified by the expression of c-met, was not significantly different between simulated summer and winter fish, representing 6.6% and 5.8% of total myonuclei, respectively. However, the number of cells expressing myogenin was higher whereas the expression of MyoD was lower in winter than in summer groups. The ingestion of a single meal under winter and summer treatment regimes resulted in a significant increase in the number of cells expressing MyoD (51% and 111%) and PCNA (88% and 140%, respectively). This was followed by an increase in the abundance of c-met (74 and 85%) and myogenin (42 and 97%, respectively) positive cells, indicating the production of new myogenic progenitor cells and the commitment to differentiation of a number of them. These results show that the proliferation of myogenic progenitor cells can be induced by feeding in teleost fishes and that temperature and photoperiod influence the expression of myogenic regulatory factors.

Key words: myogenic progenitor cell, notothenioid fish, *Harpagifer bispinis*, temperature, photoperiod, satellite cells, specific dynamic action, oxygen consumption.

Introduction

Among all vertebrates, fish appear to occupy a unique position when growth patterns and strategies are considered (Mommsen, 2001). With very few exceptions, fish grow throughout their life, so that they become larger the longer they live (Jobling, 1994). Fish growth is also rather flexible, depending on temperature, food availability and developmental stage (Sumpter, 1992). This flexibility of growth allows the animals to compensate for negative environmental influences such as extended periods of starvation (Dobson and Holmes, 1984; Ali and Wootton, 2001) or short growing season (Conover and Present, 1990; Shultz et al., 1996).

Since the myotomal muscle constitutes the largest fraction of tissues in most fish species (Bone, 1978), plasticity of fish organismic growth implies a corresponding responsiveness in the growth dynamics of the muscle (Valente et al., 1999). Post-larval muscle growth in fish results from both an increase in diameter of already existing fibres (hypertrophy) and the recruitment of new fibres (hyperplasia) (Weatherley et al., 1988; Rowlerson and Veggeti, 2001). The skeletal muscle is a differentiated tissue and post-embryonic growth depends on the proliferation of a population of myogenic progenitor cells to provide a source of nuclei for both fibre recruitment and hypertrophy (Koumans and Akster, 1995). These progenitor cells are equivalent to the satellite cells described in mammals (Mauro, 1961) and can be identified by their expression of c-met, the receptor for hepatocyte growth factor (Cornelison and Wold, 1997), which is believed to be involved in their activation (Tatsumi et al., 1998).

Proliferation and differentiation of the myogenic progenitor cells is critically dependent on a family of four closely related muscle regulatory factors (MRFs) that share a common DNA-binding and dimerization motif referred to as the basic helix–loop–helix domain (Edmonston and Olson, 1993). This family consists of MyoD, myogenin, myf5 and MRF4 (Watabe, 2001; Rescan, 2001). The primary MRFs, MyoD and myf5, are required for myogenic determination, whereas the secondary MRFs, myogenin and MRF4, are required later to initiate and stabilize the differentiation program (Megeney and Rudnicki, 1995; Rudnicki and Jaenisch, 1995).

Although food intake is a major limiting factor of growth rate in fish (Brett, 1979; Wootton, 1998), the influence of individual feeding events on muscle growth dynamics remains largely unknown. In all animals, feeding produces an increase in metabolic rate, which is referred to as the specific dynamic action (SDA) (Jobling, 1994). This phenomenon is believed to result in large part from a stimulation of protein synthesis induced by the elevation of free amino acid concentrations (Brown and Cameron, 1991a,b; Houlihan et al., 1995). In marine ectotherms, the rate of oxygen consumption typically increases by 2–4 times pre-feeding levels within a few hours (Peck, 1998). The duration of the SDA, however, varies enormously with temperature and with the size and composition of the meal (Muir and Niimi, 1972; Jobling and Davies, 1980; Johnston and Battram, 1993).

Post-prandial elevations of free amino acid concentrations and protein synthesis have been previously noted in fish muscle (Lyndon et al., 1992; Carter et al., 2000), suggesting a stimulation of muscle tissue synthesis and turnover by feeding. In agreement with these observations, two recent studies also provided evidence that feeding status influences the cellular dynamics of muscle growth. In rainbow trout *Oncorhynchus mykiss*, primary myogenic cell lines isolated from fed animals were found to differ extensively in terms of their size, morphology and proliferation rate to those isolated from fasted animals (Fauconneau and Paboeuf, 2000). MyoD expression was also shown to increase significantly after a meal in the skeletal muscle of the Antarctic notothenioid fish *Notothenia coriiceps*, although observations were not continued long enough to determine whether this phenomenon was associated with a net increase in the number of myogenic cells (Brodeur et al., 2002).

The majority of Notothenioid fish, including *Harpagifer* species, have a low number of myotomal muscle fibres for their body size such that growth after the post-larval stages occurs entirely *via* fibre hypertrophy (Battram and Johnston, 1991; Fernandez et al., 2000; Johnston et al., in press). Such species therefore represent a simple model for studying fibre hypertrophy independently of fibre recruitment. *Harpagifer bispinis* is a demersal sit-and-wait predator that is relatively common in the inter-tidal zone of the Beagle Channel, Tierra del Fuego. It is subjected to large annual variations in temperature and day length and would be expected to exhibit highly seasonal growth patterns.

The aim of the present study was to investigate the influence of food intake on the activation and proliferation of myogenic progenitor cells by monitoring MRFs expression and total myogenic cell numbers following a single meal in *H. bispinis* acclimatized to either simulated summer (10°C; 18 h:6 h light:dark) or simulated winter (5°C; 6 h:18 h light:dark) conditions. We wished to test the hypothesis that feeding stimulated a net increase in myogenic progenitor cells and that the response was influenced by temperature and photoperiod, reflecting the seasonality of growth patterns. Preliminary experiments established the time-course of the SDA to provide an indirect estimate of the period over which a satiating meal stimulated protein synthesis and, potentially, muscle growth.

Materials and methods
Fish

Harpagifer bispinis (Forster 1801) were captured on the shore of the Beagle channel (Tierra del Fuego, Argentina) at low tide in December (austral summer) and transported to the Centro Austral de Investigaciones Cientificas (CADIC, Ushuaia, Tierra del Fuego, Argentina) where they were maintained in a recirculating seawater system (8–10°C, 18 h:6 h L:D) for 2–3 weeks before the experiment began. The fish were subsequently divided into two groups of 36 animals, which were simultaneously acclimatized for 1 month to either simulated summer (10°C, 18 h:6 h L:D) or winter (5°C, 6 h:18 h L:D) temperature and photoperiod. Fish were fed chopped hake fillets (*Merluccius hubbsi*) to satiation every 4 days throughout the acclimatization period. Average body mass and standard length of the fish used were 3.0±1.0 g and 52±6 mm (mean ± S.D., *N*=60), respectively.

Oxygen consumption

Oxygen consumption of the summer fish was measured in 300 ml respirometers (length×height×width: 12.5 cm×5 cm× 5 cm, but smaller 45 ml respirometers (length×height×width: 7.5 cm×2.5 cm×2.5 cm) were used for winter fish in order to detect accurately the smaller variations of dissolved oxygen concentrations generated by this group (due to lower oxygen consumption rates). Both types of respirometers were made of Perspex (60 mm thick) and possessed a port (1.5 cm diameter) covered with a rubber cap to allow water samples to be taken and food to be introduced without disturbing the fish. Black plastic sheets were fixed onto the sides and top of the chambers to reduce visual disturbance. Six respirometers were used in parallel for both groups of fish (1 fish per respirometer). The respirometers were immersed into two adjacent tanks of well-aerated seawater kept under either simulated winter or summer conditions of temperature and photoperiod. A variable speed pump continuously circulated water from the tank into the respirometers.

Oxygen consumption was estimated by sealing the respirometers and measuring the decrease in dissolved oxygen concentrations over a period of 60–180 min, depending on the metabolic state of the animal (fed or not). Water samples of 0.3 ml were taken through the rubber cap of the respirometers with a plastic syringe at the beginning and the end of the experimental period, and dissolved oxygen concentrations were immediately measured using a Clark-type polarographic electrode (model 5300, Yellow Springs Instruments, Yellow Springs, OH, USA). Fish were first fasted for 5 days before being placed in the respirometry chambers to remove the influence of digestive state on metabolic rate. After the animals were left to acclimatize to the chambers for 2 days without food, prefeeding levels of oxygen consumption were measured twice a day over the following 3 days. For the fish acclimatized

to simulated winter conditions, this meant that measurements were taken both in daylight and in the dark. Due to the longer photoperiod, all the measurements were taken in daylight for fish under simulated summer conditions.

Once prefeeding levels were established, the fish were fed a single satiating meal of live amphipods collected from the Beagle channel. In order to reproduce as closely as possible the influence of season on muscle growth, summer fish were fed a larger ration after preliminary observations showed that they had a greater appetite. Winter fish were fed an amount of amphipods corresponding to 10% of their body mass while summer fish ate an amount equivalent to 15% of their body mass. The fish consumed the prey readily in 15–20 min leaving only a few items, which indicates that the ration offered was close to satiation levels. The respirometers were sealed during feeding to prevent the prey from escaping, and uneaten food items were flushed out of the respirometer when water flow was reinstalled 30 min after food was offered. Postfeeding oxygen consumption was first measured 2 h after the meal was taken to ensure that the effect of prey capture on metabolic rate was minimal. Oxygen consumption was thereafter monitored regularly until it returned to prefeeding levels. The first few days after feeding, oxygen consumption was measured 2–3 times a day, but measurements were reduced to once per day as metabolic rate stabilised.

Myogenic progenitor cells

All 60 fish used were fasted during the last 12 days of the month's acclimatization period to simulated winter or summer conditions. After this initial period, each fish was weighed, individually placed in a cylindrical 250 ml plastic chamber (8.8 cm diameter) and left to acclimatize to the chamber for 2 days. The 60 chambers were immersed in tanks of well-aerated seawater kept under either simulated winter or summer conditions of temperature and photoperiod.

The bottom of the chambers was filled with small rocks, and the top end was covered with a piece of dark mesh fabric held in place with an elastic band to allow water exchange with the holding tank. At the end of the acclimatization period, six winter and six summer fish were sampled for muscle tissue (controls) while the remaining animals were individually fed a satiating meal of live amphipods collected from the Beagle channel (10% of body mass for winter fish and 15% of body mass for summer fish). Based on the data obtained relative to the time course of the SDA (see Fig. 1), muscle tissue was sampled in six animals at 6 h, 30 h, 72 h and 165 h after feeding for the winter fish, and 2 h, 8 h, 48 h and 120 h after feeding for the summer fish. These time points corresponding to the beginning, the peak, the decline and the end of the SDA, respectively.

Fish were killed by a sharp blow to the head. A transverse slice of the trunk (5 mm thick) was taken at a distance from the head equal to 0.7 of the fork length of the fish. The trunk slices were frozen on cork strips in isopentane cooled near its freezing point in liquid nitrogen, and stored at –20°C until sectioning. Frozen trunk slices (7 µm thick) were cut on a cryostat and mounted on glass slides coated with poly-L-lysine. The sections were air-dried and either processed for immunohistochemistry (see below) or stained with Sytox Green (Molecular Probes, Leiden, The Netherlands) for total myonuclei counts. When staining with Sytox Green, the sections were first washed twice in $2\times$ SSC (300 mmol l^{-1} NaCl, 30 mmol l^{-1} sodium citrate, pH 7.0), then incubated for 5 min in the dark in a solution of Sytox Green diluted 1:300 in $2\times$ SSC. Afterwards sections were washed four times in $2\times$ SSC and mounted under coverslips with fluorescence mounting medium. Total numbers of myonuclei per cross-sectional area (i.e. all nuclei present in the muscle) were determined from these sections using the image generated by combining a series of images taken at every µm throughout the whole thickness of the section using a Bio-Rad 2000 confocal microscope (*z*-series).

Immunohistochemistry

Two different staining procedures were used: a single staining against either MyoD or myogenin, and a double staining against both c-met and the proliferating cell nuclear antigen (PCNA). PCNA is a cofactor to DNA polymerase δ, whose levels correlate with DNA synthesis, reaching a maximum during the S-phase (Bravo et al., 1987; Baserga, 1991). Rabbit polyclonal antibodies obtained from Santa Cruz Biotechnology Inc were used against MyoD (M-318), myogenin (M-225) and c-met (m-met, SP260). The MyoD and myogenin antibodies react against a single band in western blots of fish muscle nuclear extracts (C. Martin and I. A. Johnston, unpublished data). A mouse monoclonal (clone PC10) antibody coupled to a dextran polymer molecule itself joined to a number of horseradish peroxidase molecules was obtained from Dako A/S (Dako EPOS™, cat. no. U7032) to stain for PCNA. For the single staining, the antibodies used against MyoD and myogenin were diluted 1:20 in a solution containing 1% (v/v) Triton X-100 and 1% (m/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS). For the double staining, the antibody used against c-met was diluted 1:20 in the Dako EPOS solution containing the antibody against PCNA.

Sections were fixed in acetone for 10 min and then placed in a solution containing 5% (v/v) normal goat's serum, 1% (v/v) Triton X-100 and 1% (m/v) BSA in PBS for 15 min to rehydrate them and block non-specific binding sites. Sections were subsequently washed $3\times$ for 2 min in PBS and incubated overnight at 4°C with the solutions containing either one (single staining) or two (double staining) primary antibody. After three washes in PBS, sections were incubated for 30 min with biotinylated goat anti-rabbit secondary antibody (Sigma Chemicals, Poole, UK) diluted 1:20 in a solution containing 1% (v/v) Triton X-100 and 1% (m/v) BSA in PBS. The sections were again washed $3\times$ 2 min in PBS and incubated for 30 min in a 1:20 dilution of extraAvidin peroxidase (single staining) or extraAvidin alkaline phosphatase (double staining; Sigma chemicals, Poole, UK) in 1% (v/v) Triton X-100 and 1% (m/v) BSA in PBS. For the double staining, alkaline phosphatase

activity was developed first using a solution containing Fast Blue BB, levamisole, naphtol-ASMX-phosphate and *N,N*-dimethylformamide in Tris buffer, which gives a blue end product (Van der Loos, 1999). For both the double and single staining, peroxidase activity was developed using 3-amino-9-ethylcarbazole, which gives a red end product. Counts of the number of immunoreactive cells were made from 30 fields of $0.01\,mm^2$ for each fish. For the double staining, it was noted whether the stained cells were red (PCNA positive), blue (c-met positive) or purple (c-met and PCNA positive).

Statistical analysis

For each molecular marker examined (MyoD, myogenin, PCNA, c-met), the number of immunoreactive cells observed before feeding and at the different sampling times after the meal were compared using a one-way analysis of variance (ANOVA), followed by a Tukey test for multiple comparisons when a significant difference was found between the groups. When normality and equal variance could not be achieved by transformation of the data, the various groups were compared by a non-parametric analysis of variance on ranks, followed by a Dunn test for multiple comparisons when a significant difference was found between the groups. The number of immunoreactive cells observed before feeding was also compared between winter and summer fish using a *t*-test or a Mann–Whitney Rank Sum test if normality and equal variance could not be achieved.

Results

Oxygen consumption

Average prefeeding metabolic rates were $55.8\pm2.9\,\mu g\,O_2\,h^{-1}\,fish^{-1}$ (mean $\pm$ S.E.M.) in fish at 5°C and $110\pm9\,\mu g\,O_2\,h^{-1}\,fish^{-1}$ (mean $\pm$ S.E.M.) in fish at 10°C, which represents a Q_{10} value of 3.9. Ingestion of a single meal equivalent to 10% and 15% of the fish body mass resulted in a 2.6- and 3.6-fold increase in oxygen consumption under simulated winter and summer conditions, respectively (Fig. 1). The time to reach the peak of oxygen consumption was more than 4 times longer in winter (27 h) than in summer (6 h) animals (Fig. 1). The 75% SDA (i.e. the time for the increase in oxygen consumption associated with feeding to decline to 25% of maximum value) was 63 and 46 h at 5 and 10°C, respectively, illustrating a faster rate of SDA progression at summer temperatures (Fig. 1).

Myogenic progenitor cells

Seasonal variations in MRFs expression

Total numbers of myonuclei did not significantly differ between fish acclimatized to simulated summer and winter conditions of temperature and photoperiod (winter: $887\pm30\,myonuclei\,mm^{-2}$, summer: $886\pm55\,myonuclei\,mm^{-2}$, mean $\pm$ S.E.M.). The density of myogenic progenitor cells per muscle cross-sectional area (as illustrated by the number of c-met positive cells) was also unaffected by acclimatization conditions in fasting individuals (5.8% and 6.6% of total
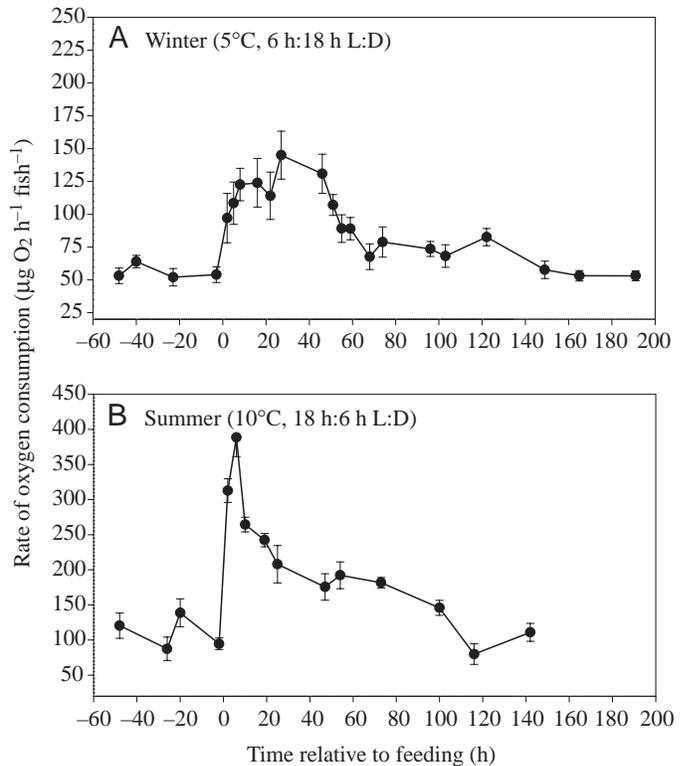

Fig. 1. Time-course of oxygen consumption following the ingestion of a single meal of amphipods in *Harpargifer bispinis* acclimatized to conditions of temperature and photoperiod simulating (A) winter and (B) summer. Values are means $\pm$ S.E.M. *N*=6 for both groups.

number of myonuclei, respectively). However, the percentage of c-met positive cells expressing MyoD was significantly ($P<0.05$) higher in summer (74%) than in winter (40%) fish whereas the proportion of myogenic cells expressing myogenin was greater in winter (71%) than in summer (33%) fish (Tables 1 and 2). The number of myogenic progenitor cells expressing PCNA was independent of acclimatization state (Tables 1 and 2).

Response to feeding

In fish acclimatized to simulated winter conditions, the number of cells immunopositive for PCNA and MyoD were significantly increased to 140% and 111% of pre-feeding values 30 h after the meal, i.e. when oxygen consumption was reaching its maximum (Table 1, Fig. 1A). This increase in PCNA and MyoD expression was followed by an increase in the number of c-met (75% and 85% of pre-feeding values) and myogenin (97% and 77% of pre-feeding values) positive cells 72 and 165 h after feeding, respectively, which denotes a net production of myogenic progenitor cells (Table 1). MyoD expression was back to prefeeding levels when the last samples were taken 165 h after feeding, but PCNA expression still remained elevated (Table 1).

As observed in winter fish, feeding also resulted in an increase in the number of cells expressing PCNA and MyoD in *H. bispinis* acclimatized to simulated summer conditions

Table 1. *Number of myogenic progenitor cells staining for c-met, PCNA, MyoD and myogenin per cross-sectional area of white muscle before and after a single meal of amphipods in* Harpagifer bispinis *acclimatized to simulated winter conditions of temperature and photoperiod*

Stained cells (number mm^{-2})	Before meal (N=6)	Time after meal (h)			
		6 (N=5)	30 (N=6)	72 (N=6)	165 (N=6)
c-met	51.7±3.8	48.8±4.4	74.8±6.9	90.2±6.4*	95.7±10*
PCNA	18.1±1.4	22.4±4.0	43.5±5.1*	55.6±5.2*	50.0±7.3*
MyoD	19.8±1.9	25.7±1.9	41.8±4.5*	39.6±4.0*	30.3±5.2
Myogenin	35.7±4.7	38.9±3.0	46.2±4.7	70.4±7.9*	63.4±4.8*

Values are means ± S.E.M.
*Significantly different from value before meal ($P<0.05$).

(Table 2). This elevation in the number of PCNA (88% of pre-feeding values) and MyoD (51% of pre-feeding values) positive cells was observed 48 h after the meal but was no longer detectable 120 h after feeding, when oxygen consumption was back to prefeeding levels (Table 2, Fig. 1B). As for winter animals, the number of c-met positive cells also increased towards the end of the SDA (74% of pre-feeding values, Table 2). However, in this case, no significant increase in the number of cells expressing myogenin was found, although slightly higher numbers were measured 120 h after the meal (Table 2).

Discussion

Under conditions of temperature and photoperiod simulating sub-Antarctic winter and summer, the ingestion of a single satiating meal by *H. bispinis* resulted first in an increase in the number of cells expressing MyoD and PCNA, followed by an increase in the abundance of c-met and myogenin positive cells (Tables 1 and 2). These results are evidence for a direct stimulation of myogenic cell proliferation by feeding, since this sequence of events is consistent with an initial activation of myogenic progenitor cell division (expression of MyoD and PCNA), followed by the production of new cells (increase in c-met positive cells) and the commitment to differentiation of a number of them (expression of myogenin). The close relationship observed between the time-courses of the SDA (Fig. 1) and the activation and proliferation of the myogenic

progenitor cells (Tables 1 and 2) furthermore suggest that this cell proliferation may contribute to the energetic expenditures constituting the SDA.

Although it is the first time, to our knowledge, that food intake has been directly linked with a stimulation of myogenic progenitor cell proliferation *in vivo*, this finding is not unexpected. Protein intake is known to increase growth hormone release in fish and growth hormone is a powerful stimulator of IGF-1 expression, which is, in turn, known to stimulate myogenic progenitor cells proliferation (Mommsen, 2001). In this context, the induction of myogenic cell proliferation by feeding may be seen as part of a wider regulatory system, orchestrated by the growth hormone, which aims at promoting growth when energy resources are available.

The number of myogenic cells generated in response to feeding did not appear to be directly related with temperature or the amount of food eaten since cell density approximately doubled in both groups of fish (Tables 1 and 2). Low temperatures could have been expected to extend the time needed for the cells to divide (Lloyd and Kippert, 1987; Vinogradov, 1999), but the low frequency of sampling used does not permit a conclusion to be drawn about the time course of the cell proliferation. The main difference between the responses to feeding of fish acclimatized to simulated winter and summer conditions resided in the expression of myogenin, which was much less pronounced in summer. This finding, along with the differences found in prefeeding levels of MyoD and myogenin between the two groups of fish (Tables 1 and

Table 2. *Number of myogenic progenitor cells staining for c-met, PCNA, MyoD and myogenin per cross-sectional area of white muscle before and after a single meal of amphipods in* Harpagifer bispinis *acclimatized to simulated summer conditions of temperature and photoperiod*

Stained cells (number mm^{-2})	Before meal (N=6)	Time after meal (h)			
		2 (N=6)	8 (N=5)	48 (N=6)	120 (N=6)
c-met	57.2±4.6	58.3±7.2	62.7±6.8	88.6±5.0*	99.6±8.8*
PCNA	22.0±4.3	19.3±2.8	27.7±5.2	41.3±3.5*	42.9±10.2
MyoD	41.8±3.6	45.1±3.0	50.2±2.2	63.3±4.4*	50.0±5.5
Myogenin	18.0±2.3	18.6±3.1	17.2±4.0	18.7±2.9	25.7±3.1

Values are means ± S.E.M.
*Significantly different from value before meal ($P<0.05$).

2), points to an influence of temperature and photoperiod in the control of cell differentiation.

Interestingly, the interval between the ingestion of the meal and the appearance of new c-met positive cells observed in the present study was shorter than the cell cycle durations previously estimated for the myogenic progenitors of unfed *H. bispinis* at both summer and winter temperatures. Indeed, whereas cell cycle durations of 150 and 81 h have been found for the myogenic progenitors of *H. bispinis* acclimatized to 5 and 10°C (J. C. Brodeur, J. Calvo, A. Clarke and I. A. Johnston, manuscript submitted for publication), the abundance of c-met positive cells was already significantly increased 72 and 48 h after the meal at the same two temperatures in the present study. This difference may either indicate that cell cycle progression rate is increased by feeding, or that a proportion of the activated cells were cells stopped at either one of the two cell-cycle checkpoints and that they therefore could divide faster since they had already progressed through part of the cell cycle (Walworth, 2000). This last possibility is in agreement with previous results on *Notothenia coriiceps*, which suggested that the myogenic cells activated by feeding were cells stopped at the G_1/S checkpoint of the cell cycle (Brodeur et al., 2002).

If all the cells produced after a single meal in the present experiment were incorporated into myofibres, the number of myonuclei would increase by approximately 5% after each meal, which is considerable. A proportion of these new cells may, however, be involved in nuclear turnover rather than fiber growth, since studies in the rat have shown that, in adult stages, 2% of the nuclei are replaced each week (Schmalbruch and Lewis, 2000). Programmed cell death (apoptosis) may also be involved in reducing the final number of myonuclei produced as it is a normal developmental event in proliferating myoblasts and postmitotic myofibres (Mampuru et al., 1996). Finally, it is also possible that the increase in myogenic cell number observed after feeding illustrates the re-establishment of an actively dividing population of myogenic progenitors that had been greatly reduced during fasting. Such an interpretation would be consistent with the observation of Fauconneau and Paboeuf (2000) that fasting suppresses initial proliferation of the myogenic cells *in vitro*.

There are now several lines of evidence suggesting the existence of distinct subpopulations of satellite cells in mammals (Schultz, 1996; Molnar et al., 1996). The picture emerging from the development of molecular markers for quiescent and activated satellite cells suggests the presence of at least three distinct subpopulations of cells with different levels of commitment (Hawke and Garry, 2001; Qu-Petersen et al., 2002). However, much less is known about the different types of myogenic progenitors present in fish. In salmon fry, 80% of the myogenic cells identified by c-met staining also expressed MyoD and/or myogenin, suggesting a late state of commitment (Johnston et al., 2000). A similarly high proportion of MyoD and/or myogenin positive cells was also found in the present study (70%, Tables 1 and 2), and the number of activated myogenic progenitors was furthermore

shown to vary with temperature, photoperiod and feeding. Although it is still an open question whether the myogenic population supporting hypertrophic growth is the same as that able to create new fibers, the results obtained in the present study can be considered specific of hypertrophic growth since *H. bispinis* of the size studied grows only by fibre hypertrophy.

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