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Condition, prolonged swimming performance and muscle metabolic capacities of cod *Gadus morhua*

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Summary

This study evaluated the link between swimming endurance and condition of Atlantic cod *Gadus morhua* that had been fed or starved during the 16 weeks preceding the tests, and assessed whether muscle metabolic capacities explain such links. The condition factor [(somatic mass × fork length^{-3})×100] of starved cod was 0.54±0.1 whereas that of fed cod was 0.81±0.1. In white and red muscle, we measured four glycolytic enzymes: phosphofructokinase (PFK), pyruvate kinase (PK), creatine kinase (CK) and lactate dehydrogenase (LDH), two mitochondrial enzymes: cytochrome *c* oxidase (CCO) and citrate synthase (CS), a biosynthetic enzyme, nucleoside diphosphate kinase (NDPK), glycogen and protein levels and water content. Muscle samples were taken at three positions along the length of the fish; starvation affected the metabolic capacities of white muscle more than those of red muscle. The levels of glycolytic enzymes and glycogen changed more in white than red muscle during starvation. Both in fed and starved cod, muscle metabolic capacities varied with position along the fish; starvation reduced this longitudinal variation more in white than red muscle. In white muscle of fed cod, the glycolytic enzyme levels increased from head to tail, while in starved cod this longitudinal variation disappeared. In red muscle mitochondrial enzyme levels were highest in the caudal sample, but fewer differences were found for glycolytic enzymes. Swimming endurance was markedly affected by fish condition, with starved fish swimming only 30% of the time (and distance) of fed fish. This endurance was closely linked with the number of burst–coast movements during the test and the activity of CCO and LDH in white muscle. The number of burst–coast movements was significantly linked with condition factor and PFK activity in caudal red muscle and gill arch mass. Our data indicated that cod use both glycolytic and oxidative capacities to support endurance swimming. Furthermore, swimming endurance is linked with the metabolic capacities of red and white muscle.

Key words: Atlantic cod, *Gadus morhua*, white muscle, red muscle, aerobic metabolism, anaerobic metabolism, longitudinal variation, prolonged swimming.

Introduction

As swimming speed and endurance directly affect prey capture, escape from predators, migratory capacity and hence the selection of favourable environmental conditions, swimming capacity is a central determinant of the fitness of fish. Swimming performance in fishes is generally classified according to the time a specific speed can be maintained: (i) sustained swimming (at least 200 min); (ii) prolonged swimming (between sustained and sprint swimming) and (iii) sprint swimming (less than 15–20 s) (Webb, 1994). Whereas some fishes are specialised for high speed sustained swimming (i.e. skipjack tuna *Katsuwonus pelamis*) or acceleration (pike *Esox lucius*), others, such as Atlantic cod *Gadus morhua*, are generalists (Webb, 1984).

Vertebrate muscle can power movements covering a wide range of speeds and durations (Jayne and Lauder, 1994), mainly due to the existence of fibre types with different contractile and metabolic properties. The body musculature of fish is principally composed of red and white fibres, although intermediate pink fibres exist (Johnston, 1981; Patterson et al., 1974; Akster, 1985; Akster et al., 1985). The bulk of fish muscle is composed of fast-twitch glycolytic (white) fibres (Bone et al., 1978; Webb, 1978). The slow-twitch oxidative (red) fibres are confined to a small section of the skeletal musculature beneath the lateral line, the relative importance of which increases towards the tail (Bone, 1966; Bone et al., 1978; Videler, 1993). Pink fibres lie between white and red fibres and have intermediate metabolic capacities (Coughlin and Rome, 1996; Coughlin et al., 1996). Although sustained swimming principally relies upon red fibres, which are resistant to fatigue and have a slow shortening speed (Bone et

al., 1978), the pink fibres may be important in sustained swimming due to their intermediate characteristics (faster rate of relaxation and maximum velocity of shortening) (Coughlin et al., 1996). The relative proportion of fibre types reflects the locomotory style of the species (Bone, 1966). Accordingly, fish species that are specialised for high-speed, sustained swimming have a greater proportion of red fibres in their musculature. When the swimming speed increases, the tail-beat frequency rises and white fibres are increasingly recruited (Bone et al., 1978; Johnston and Moon, 1980). Short powerful sprints of activity are primarily supported by the contraction of white glycolytic fibres. White fibres may also be recruited during sustained swimming (Burgetz et al., 1998).

Recruitment of muscle fibres during undulatory swimming changes along the fish body, with individual species differing in their recruitment patterns (van Leeuwen et al., 1990; Rome 1992; Wardle and Videler, 1993; Jayne and Lauder, 1995; Thys et al., 2001). Gadoid fishes, such as cod, use subcarangiform swimming, where swimming is powered by the myotomal musculature and the caudal fin. Subcarangiform swimming leads to the inclusion of a complete propulsive wave within the body length; however, major increases in amplitude are restricted to the posterior half to third of the body (Webb, 1978). The power produced by white fibres in the more rostral sections is transmitted caudally, stretching the caudal fibres during their activation (Altringham et al., 1993; Davies et al., 1995; Johnston et al., 1995). The requirements of tension development during transmission of force to the tail may explain the higher catabolic capacities of white muscle from the caudal region of Atlantic cod (Martínez et al., 2000).

Although much is known about how fish use their different muscle fibres during swimming, little is known about the impact of energetic status upon swimming performance or about the physiological determinants of swimming capacity. A fish's energetic condition has a pronounced impact upon the metabolic and contractile capacities of muscle and upon the availability of fuels for swimming (Moon and Johnston, 1980; Sullivan and Somero, 1983; Black and Love, 1986; Kiessling et al., 1990; Lambert and Dutil, 1997a). Starvation decreases the activities of muscle enzymes and the concentrations of contractile proteins (Beardall and Johnston, 1983; Houlihan et al., 1988). The responses to starvation are more pronounced in white than red fibres (Beardall and Johnston, 1983, 1985; Lowery and Somero, 1990), suggesting that endurance at sustained swimming speeds would be spared during decreases in energetic status. On the other hand, virtually all protocols measuring sustained and prolonged swimming lead to recruitment of white muscle (Burgetz et al., 1998; McDonald et al., 1998; Reidy et al., 2000). Thus, we reasoned that simultaneous study of swimming endurance at prolonged speeds and its potential biochemical determinants would allow us to determine whether endurance is best predicted by oxidative or glycolytic capacities.

In the Northwest Atlantic Ocean, cod can experience long periods (8 months) with low food availability (Lilly, 1994; Kulka et al., 1995). These lead to seasonal reductions in their energetic status (condition) and muscle metabolic capacities (Guderley et al., 1996; von Herbing and Boutilier, 1996; Lambert and Dutil, 1997b; Martínez et al., 1999), which result in increased natural mortality, particularly during the spawning and migration periods (Dutil and Lambert, 2000). If reductions in condition diminish swimming performance, they may well increase the vulnerability of cod to predation and capture by mobile fishing gears. Given the economic importance of the cod, knowledge of the link between condition and swimming performance is of both practical and fundamental interest.

This paper describes the effects of condition on the swimming endurance of cod from Newfoundland waters. We established two groups of fish: (i) cod that were fed twice a week for 16 weeks and (ii) cod that were starved for 16 weeks. We then compared the swimming endurance of the fed and starved cod. We measured endurance at speeds greater than the maximum sustained swimming speeds (U_{max}) published for this species (Beamish, 1978; He, 1991), to assure the participation of red and white muscle. We assessed the metabolic capacities of white and red muscle sampled at three locations along the body (behind the head, at the middle of the body and at the caudal peduncle), by measuring the activity of four glycolytic enzymes: phosphofructokinase (PFK), pyruvate kinase (PK), creatine kinase (CK) and lactate dehydrogenase (LDH), two mitochondrial enzymes: cytochrome *c* oxidase (CCO) and citrate synthase (CS), and a biosynthetic enzyme, nucleoside diphosphate kinase (NDPK). We also measured the concentrations of water, glycogen and protein, in red and white muscle sampled at these locations. Lactate content was determined on white muscle biopsies taken at the middle of the body immediately after the endurance test. Growth rates were calculated from changes in mass and length over the 16 weeks. During each swimming test, we noted the swimming behaviour, including the number of burst–coast movements made by the fish. These data were used to examine the links between condition, enzyme levels in white and red muscle along the body and endurance swimming of cod.

Materials and methods

Experimental animals

Atlantic cod *Gadus morhua* L. were captured by bottom trawl in Trinity Bay, Newfoundland, in late March 1997 and transported to the Northwest Atlantic Fisheries Centre in St John's. All fish were habituated to laboratory conditions for 2 months before the treatment groups were established. After this period, fish were anaesthetised with 2-phenoxyethanol at a concentration of $0.125\,ml\,l^{-1}$ in seawater at ambient temperature, and tagged with spaghetti tags (Floy Tag Inc., Washington, USA). Then, healthy fish were randomly allocated to either the fed or starved groups (final N=19 and N=14, respectively; Table 1). Each group was placed in a circular fibreglass tank (diameter 1.7 m and depth 1.5 m) at ambient seawater temperatures and salinity for 16 weeks. Temperatures were low (<4°C) for much of this time and gradually rose to attain 8.8°C towards the end of the feeding treatment. One

Table 1. *General characteristics of Atlantic cod subjected to two different feeding regimes for 16 weeks at ambient seawater temperatures (1–8.8°C)*

	Feeding regime	
	Starved	Fed
Initial mass (g)	1429±442	1185±352
Initial length (cm)	54.4±5.6	52.1±6.1
(Initial mass × fork length^{-3})×100	0.86±0.1	0.80±0.1
Final mass (g)	928±302	1200±384
Final length (cm)	54.9±5.8	52.5±6.2
Final CF	0.54±0.1	0.81±0.1
Growth rate (mass% day^{-1}) over 16 weeks	–0.35±0.1	0.07±0.05
Hepatosomatic index	1.1±0.4	3.9±2.0
Gonadosomatic index	0.7±0.4	1.0±0.7
Hematocrit (%)	29.8±7.8	32.7±6.4
Gill arch mass (g)	29.4±9.0	29.7±9.4
Heart mass (g)	1.9±0.5	2.1±0.8
Ratio male/female	5/9	7/12

Values are means ± S.D.; N=19 for the fed group and 14 for the starved group.

group was fed to satiation with squid twice a week during the 16 weeks, while the other group received nothing. When the fish were ready to be tested, they were moved, in groups of five, and placed in rectangular tanks (1.3 m × 1.3 m × 1.3 m) at Memorial University of Newfoundland's Ocean Sciences Centre in Logy Bay. We allowed 24 h for recuperation from the stress of transport before carrying out the swim tests. Fish were weighed (to ±0.1 g) and measured for standard length *SL* (to ±0.1 cm) at the beginning and at the end of the 16 week treatment. Of the 33 fish, there were 21 females (9 and 12 in starved and fed groups, respectively) and 12 males (5 and 7 in starved and fed groups, respectively).

Endurance test

Endurance was tested using a swimming flume, following the methods of Winger et al. (2000). Individual cod underwent a training routine before beginning the endurance test. This routine began with a minimum 10 min habituation in the flume at a speed of 0.25 body lengths per second (*BL* s^{-1}), followed by a 10 min orientational swim at 0.5 *BL* s^{-1}. The swimming speed was gradually increased from 0.5 to 1.0 *BL* s^{-1} over 5 min. The fish were allowed 5 min of swimming at 1.0 *BL* s^{-1} before gradually increasing the swimming speed to the target speed of 1.8 *BL* s^{-1}, a speed greater than the U_{crit} of cod at these temperatures (He, 1991; Winger et al., 2000). Swimming was encouraged using pairs of electrodes in the downstream end of the swimming flume. A pulsing stimulus (2 Hz) with peak voltage of 15 V was applied to encourage the fish to swim against the flow until exhaustion. A fish was considered exhausted when it was unable to lift off the downstream electric net after 10 s. When individual endurance exceeded 200 min, the tests were terminated. Water temperature during the swimming trials ranged from 10–14°C; no significant impact of water temperature on swimming endurance or any of the performance parameters was apparent. Swimming trials were filmed using a Sony® CCD-TR500 video camera (30 frames s^{-1}). Video footage of the swimming fish was analysed using a Panasonic® time-lapse video recorder (model AG-6730) to determine the number of burst–coast movements made by the fish during the swimming test, as well as to examine the overall swimming behaviour.

Tissue sampling

Immediately after the swim test, we took a biopsy of white muscle below the first dorsal fin, according to Mair (1989). The cod were allowed to recuperate in a holding tank for 72 h before being anaesthetised with 2-phenoxyethanol at a concentration of 0.125 ml l^{-1} in seawater at ambient temperature. The cod were killed by a blow to the head, measured and weighed. White and red muscle (3–5 g for white, 1–2 g for red) were sampled at three sites along the length of the fish body: (1) just behind the head, (2) at the middle of the second dorsal fin of the fish and (3) in the caudal region. All samples were weighed (to ±0.1 g). White muscle was sampled above the lateral line, while red muscle was taken at the level of the lateral line (Fig. 1). To avoid contamination of the white muscle samples by red or pink fibres, white muscle samples were dissected deep in the trunk. The rostral red muscle may have been contaminated by pink fibres, however, given the thinness of the red muscle layer and the difficulty of identifying pink fibres. Samples of white and red muscle were immediately frozen in liquid nitrogen, then stored at –70°C before transport on dry ice to Université Laval where they were again stored at –70°C until biochemical assays were done.

Tissue extraction

Tissue extracts were prepared by homogenising samples in 10 volumes of 50 mmol l^{-1} imidazole HCl, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} ethylene diamine tetraacetic acid (EDTA), 1 mmol l^{-1} reduced glutathione and 0.1% Triton X-100, pH 7.5, using a Polytron homogeniser (Brinkmann Instruments, Switzerland) for three 20 s periods. The samples were

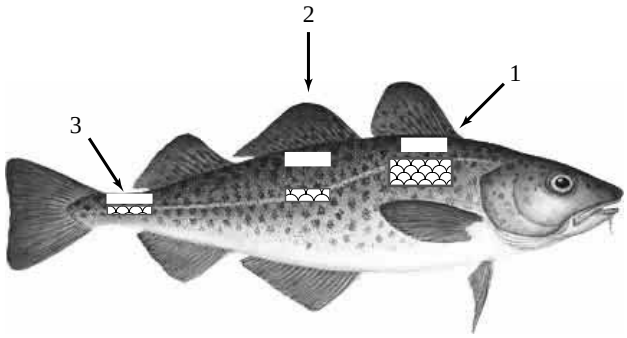

Fig. 1. White and red muscle were sampled along the length of the cod body (1) behind the head, (2) in the middle of the body and (3) at the caudal peduncle.

maintained on ice during and between periods of homogenisation.

Enzyme activity assays

All enzyme activities, except for creatine kinase, were measured according to Couture et al. (1998). For creatine kinase (E.C.2.7.3.2CK), the reaction mixture contained: 75 mmol l^{-1} Tris-HCl, 5 mmol l^{-1} $MgCl_2.6H_2O$, 4 mmol l^{-1} glucose, 0.75 mmol l^{-1} ADP, 5 mmol l^{-1} AMP, 0.3 mmol l^{-1} NADP, 24 mmol l^{-1} phosphocreatine (omitted in control), hexokinase and glucose-6-phosphate dehydrogenase in excess, pH 7.6. Activities are expressed in international units (i.u.; μmol substrate transformed to product min^{-1} g^{-1} tissue wet mass. Muscle enzyme activities were measured in the order: PFK, PK, CK, LDH, CS, CCO and NDPK. All assays were run in duplicate.

Concentration of muscle protein, glycogen, lactate and muscle water content

Protein concentration in muscle extracts was measured using bicinchoninic acid (Smith et al., 1985). Muscle water content was determined after drying 2 g of muscle for 48 h at 60°C. Glycogen content was measured in muscle samples taken at the time of death (72 h after the end of the swimming tests). Lactate content was determined on white muscle biopsies taken immediately after the endurance test. Glycogen and lactate content were measured using the method of Keppler and Decker (1974).

Hematocrit

Hematocrit was determined on blood sampled immediately after death. Blood was taken directly from the heart cavity with a heparinised microhematocrit tube and centrifuged for 10 min using an Adams Autocrit minicentrifuge (Clay-Adams Inc., New York, USA).

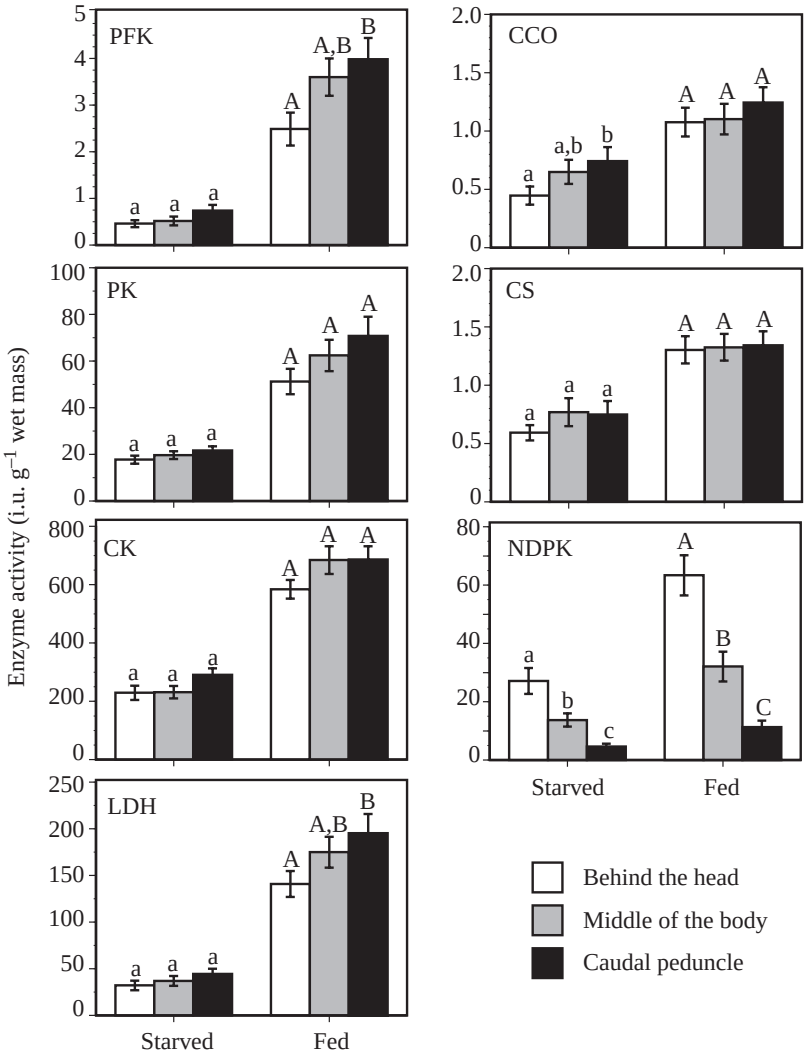

Fig. 2. Activities of glycolytic, mitochondrial and biosynthetic enzymes in white muscle, sampled at three sites along the length of the body (behind the head, in the middle of the body and at the caudal peduncle) in starved and fed cod. Values are means ± S.E.M. Letters indicate statistical differences among the sampling positions in each treatment (starved and fed); two columns with the same letter do not differ (*a posteriori* comparisons, $P<0.05$). PFK, phosphofructokinase; PK, pyruvate kinase; CK, creatine kinase; LDH, lactate dehydrogenase; CCO, cytochrome *c* oxidase; CS, citrate synthase; NDPK, nucleoside diphosphate kinase.

Calculations and statistical analysis

Length and total mass were used to calculate a condition factor [CF = (mass × fork length^{-3})×100, where somatic mass is in g and fork length in cm]. Somatic mass was calculated by subtracting gonad mass and stomach contents from the total mass of the fish. Liver and gonads were weighed to calculate the hepatosomatic index HI (liver mass / somatic mass) and the gonadosomatic index GI (gonad mass / somatic mass) (Table 1). Individual growth rates (% day^{-1}) were calculated using the following equation:

$$\text{Growth rate} = \left(\frac{\log_e W_2 - \log_e W_1}{t_1 - t_2} \right) \times 100 , \qquad (1)$$

where W_1 represents the initial mass in g or fork length in cm, W_2 the final mass in g or fork length in cm and t the number of days.

We used JMP 3.2.1 (SAS Institute Inc., Duxbury Press, USA, 1996) to examine the effect of feeding treatment and site of sampling (with repeated measures on individual fish) on enzyme activity, protein, glycogen and water contents. In these models, feeding treatment was a factor, sampling site was nested within fish and fish was nested within feeding treatment. An interaction term between feeding treatment and sampling site was included. Enzyme activity, protein, glycogen and water contents were the variables. These models were run separately for red and white muscle. Differences

were considered significant at P=0.05. The *a posteriori* 'add contrasts' function was used to establish differences due to feeding treatment at each sampling site. Longitudinal differences in enzyme activity were examined within each feeding group, using models in which site of sampling was the factor and *a posteriori* comparisons established specific differences.

Failure time analysis was used to assess the effect of the variables on swimming endurance (Winger et al., 1999, 2000). Given that cod swam for a minimum of 20 min before reaching the target speed (1.8 *BL* s^{-1}), we used the time swum by fish from 0.5 *BL* s^{-1} until exhaustion as the dependent variable. This allowed us to include the six starved cod that did not attain the target speed. Time swum was analysed using the Cox proportional hazards model (Cox and Oakes, 1984; Winger et al., 1999). Due to the large number of variables, as well as their probable colinearity, we pre-selected variables before their inclusion. First, failure time analyses were performed for each variable to identify those that showed a significant (P<0.15) relationship with the endurance hazard rate. A stepwise failure time analysis was then performed on these selected variables. The criterion for entry and removal of a variable into the model was the significance of the χ^2 score statistic (P<0.05 to stay in the model). Once the model was built, we checked the colinearity between the remaining variables, removing variables with marked colinearity (statistical condition index >30). Models were built using the PHREG procedure and colinearity was analysed using the REG procedure in SAS system 6.12 (SAS institute Inc., Cary, NC, USA, 1996–1999).

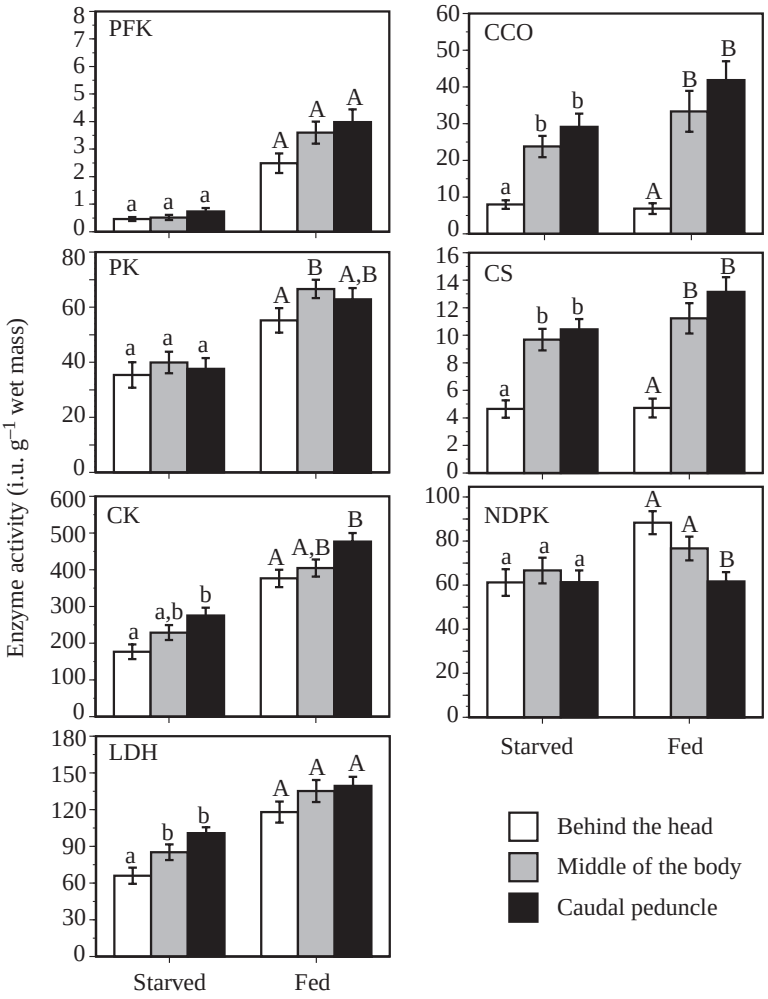

Fig. 3. Activities of glycolytic, mitochondrial and biosynthetic enzymes in red muscle, sampled at three sites along the length of the body (behind the head, in the middle of the body and at the caudal peduncle) in starved and fed cod. Values are means ± S.E.M. Letters by the error bars indicate statistical differences among the sampling positions in each treatment (starved and fed); two columns with the same letter do not differ (*a posteriori* comparisons, P<0.05). For enzyme abbreviations, see Fig. 2.

Results

After 16 weeks, the condition factor of the fed and starved cod differed greatly: 0.54±0.1 (starved) and 0.81±0.1 (fed) (P<0.0001). Growth rates based on total mass of fish showed that starved cod lost mass at a rate of –0.27±0.08% day^{-1}, while fed cod did not grow (0.08±0.05% day^{-1}) (P<0.0001). The hepatosomatic index was significantly lower in starved than fed cod (1.1±0.4 and 3.9±2.0, respectively) (P<0.0001), while the gonadosomatic index, hematocrit, heart mass and gill arch mass were similar (P=0.31, P=0.24, P=0.54 and P=0.92, respectively) (Table 1).

Effects of feeding regime on biochemical parameters

The impact of starvation upon enzyme levels was more pronounced in white than in red muscle (Figs 2, 3). Starvation significantly decreased the activity of all enzymes measured in white muscle (P<0.0001). This effect was greater for the glycolytic than the mitochondrial enzymes (58–86% reductions for the former *versus* 40–59% decreases for the latter). The decrease in NDPK activity was intermediate. The site of sampling affected the activity of several enzymes (P<0.05), particularly in fed cod; PFK and LDH were higher in the last third of the body while NDPK showed the opposite pattern. As starvation attenuated the effect of position, an interaction between feeding regime and site of sampling in white muscle was observed for PFK, PK, CK, LDH (P<0.05) and NDPK (P=0.0001). CCO activities in white muscle increased from head to tail in starved cod. Starvation significantly reduced the activity of the enzymes measured in red muscle (Fig. 3) (PFK, PK, CK and LDH: P<0.0001; CS, P=0.0103; CCO, P=0.0241; NDPK, P=0.0013), but the changes were smaller than in white muscle. Again decreases were more pronounced for glycolytic than mitochondrial enzymes (28–67% for glycolytic enzymes *versus* 1.5–30% for mitochondrial enzymes) and NDPK was intermediate (0.6–30%). In red muscle, strong longitudinal differences

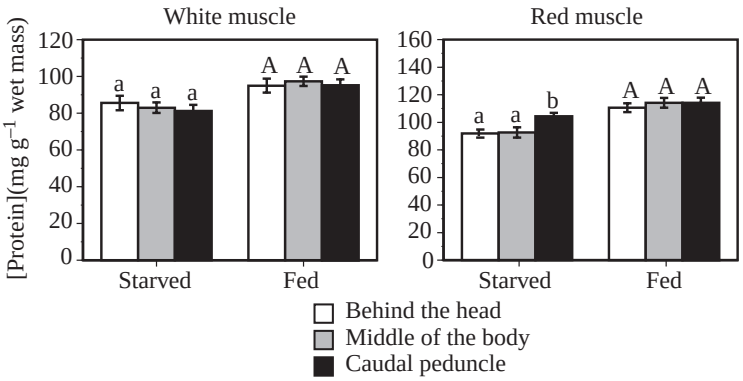

Fig. 4. Concentration of total protein, in red and white muscle, sampled at three sites along the length of the body (behind the head, in the middle of the body and at the caudal peduncle) in starved and fed cod. Values are means ± S.E.M. Letters indicate statistical differences among the sampling positions in each treatment (starved and fed); two columns with the same letter do not differ (*a posteriori* comparisons, $P<0.05$).

were found for the mitochondrial enzymes, while the longitudinal differences for glycolytic enzymes were less pronounced. For both starved and fed cod, the activity of CCO, CS and CK differed with the site of sampling ($P<0.01$). For PK and NDPK an effect of position was only apparent in fed cod (interaction between site and feeding treatment, $P<0.05$), whereas for LDH the effect was only apparent in starved cod. In red muscle sampled behind the head and at the middle of the body, the activity of the mitochondrial enzymes was not reduced by starvation. Red muscle NDPK levels were only affected by starvation in the sample taken behind the head.

Starvation decreased protein concentrations by up to 15% in white muscle ($P=0.0003$) and up to 19% in red muscle ($P<0.0001$). The site of sampling only affected protein concentration in red muscle of starved cod (Fig. 4).

In both red and white muscle and at each site of sampling, glycogen and water content changed with feeding ($P<0.0001$) (Fig. 5). Glycogen concentrations were higher in red than white muscle ($P<0.0001$). Glycogen content in white muscle was up to 12 times higher in fed than starved cod, whereas in red muscle this difference was sixfold. In red and white muscle, decreases in glycogen content were similar at the three sampling sites and glycogen levels did not differ with sampling site. On the other hand, water content changed with site of sampling in both muscles, particularly in fed cod. Significant interactions between feeding regime and the site of sampling were only observed for water content in red muscle ($P=0.006$).

White muscle lactate concentration at the end of the endurance tests was 1.6 times higher in fed than in starved cod ($P=0.036$) (Fig. 6). A similar result was found for the burst–coast movements during the tests (2.5 times; $P<0.0001$). However, burst–coast movements were not correlated with lactate concentration in either group ($P=0.16$). The time and distance swum were 3.4- and 4.7-fold higher in fed than starved cod (time swum, $P=0.022$; distance swum, $P=0.019$) (Fig. 7).

Determinants of swimming performance

Of 33 cod, 6 did not achieve the target speed, 24 became exhausted during the test and 3 swam to the end. Although we had more females than males, and although females were slower than males, sex did not affect the time swum ($P>0.05$). Of all the variables included in the failure time analyses, only three were significant determinants of the time cod swam and explained 87% of the variance: (i) the number of burst–coast

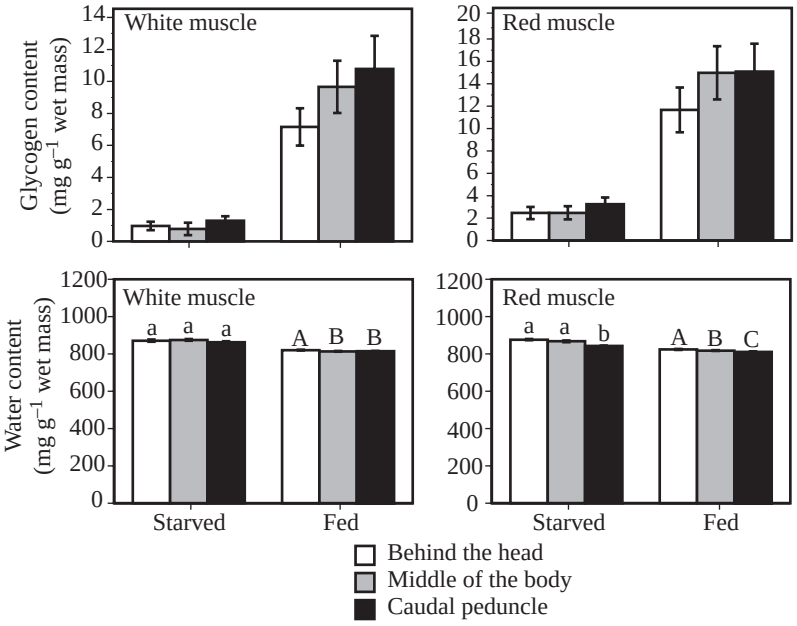

Fig. 5. Glycogen and water content in white and red muscle, sampled at three sites along the length of the body (behind the head, in the middle of the body and at the caudal peduncle) in starved and fed cod. Values are means ± S.E.M. Glycogen content was significantly higher in fed than in starved fish in both types of muscle ($P<0.0001$). For water content, letters indicate statistical differences among the sampling positions in each treatment (starved and fed); two columns with the same letter do not differ (*a posteriori* comparisons, $P<0.05$).

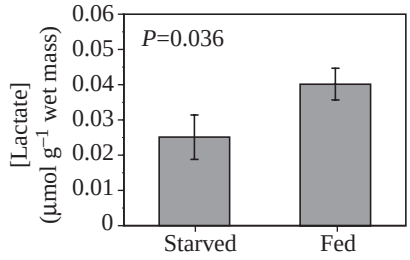

Fig. 6. Lactate concentration in white muscle, sampled after the swim test in starved and fed cod. Statistical differences (*P* value) between groups are indicated.

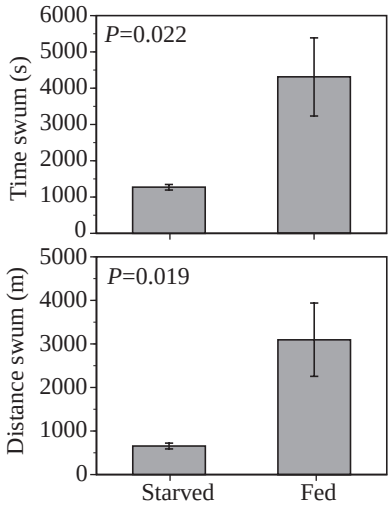

Fig. 7. Time (s) and distance (m) swum by starved and fed cod. Statistical differences (*P* value) between groups are indicated.

movements, (ii) LDH activity in white muscle at the caudal peduncle, and (iii) CCO activity in white muscle at the caudal peduncle. Of these variables, the number of burst–coast movements was the most important ($P<0.0001$, $r^2=0.81$) and positively influenced the time swum. LDH in the caudal white muscle samples was next in importance ($P=0.0002$) and the mitochondrial enzyme, CCO, in caudal white muscle was third ($P=0.02$) (Table 2). Because we were interested in identifying the biochemical or morphological variables that explain the length of time that cod were able to swim, we constructed a model without burst–coast movements. This model showed that condition factor and heart mass explained 60% of the variability of the length of time swum by cod. Condition factor was positively related ($P<0.0001$, $r^2=0.41$), while heart mass was negatively related to time swum ($P=0.0014$, $r^2=0.18$) (Table 3).

Table 2. *Failure time analysis of the time swum by Atlantic cod, using the Cox proportional hazards model, including all the variables measured*

Variables in the model	r^2	χ^2	d.f.*	*P* value
Burst–coast	0.81	55.4	1	<0.0001
LDH activity in white muscle caudal sample	0.04	7.6	1	0.0002
CCO activity in white muscle caudal sample	0.02	4.9	1	0.0217
	Model r^2	Model χ^2	d.f.	*P* value
	0.87	67.9	3	<0.0001

LDH, lactate dehydrogenase; CCO, cytochrome *c* oxidase.

*, degrees of freedom.

Variables were entered into the model using forward stepwise selection.

Burst–coast indicates the total number of burst–coast movements made by fish during the swim test.

Table 3. *Failure time analysis of the time swum by Atlantic cod, using the Cox proportional hazards model, including all the variables measured except the burst–coast movements*

Variables in the model	r^2	χ^2	d.f.*	*P* value
Condition factor	0.41	17.48	1	<0.0001
Heart mass	0.19	12.62	1	0.0014
	Model r^2	Model χ^2	d.f.	*P* value
	0.60	30.10	2	<0.0001

*, degrees of freedom.

Variables were entered into the model using forward stepwise selection.

Given that the number of burst–coast movements was a major determinant of cod endurance, we constructed a model to examine which biochemical or morphological variables could explain the number of burst–coasts made by the fish during prolonged swimming. This model showed that the condition factor ($P<0.0001$, $r^2=0.54$), gill arches ($P=0.004$, $r^2=0.10$) and PFK activity in caudal samples of red muscle ($P=0.03$, $r^2=0.05$) explained 69% of the variability of this swimming pattern. The condition factor and PFK activity positively influenced burst–coast movements whereas the gill arches were negatively linked with these movements (Table 4).

Discussion

Swimming endurance expressed as the time or distance swum by cod was markedly affected by condition. The endurance of starved cod was approximately 30% that of fed cod and several of the starved cod were unable to reach the target speed of $1.8\,BL\,s^{-1}$. Starvation reduces routine locomotor activity in herring, plaice and three cyprinids (Blaxter and Ehrlich, 1974; Wieser et al., 1992). Such reductions in routine activity may constitute a strategy to save energy as well as reflecting a decreased swimming capacity. As expected,

Table 4. *Failure time analysis of the burst–coast movements made by Atlantic cod during the swimming test, using the Cox proportional hazards model*

Variables in the model	r^2	χ^2	d.f.*	*P* value
Condition factor	0.5	26.14	1	<0.0001
Gill arch mass (g)	0.10	7.89	1	0.0039
PFK activity in red muscle caudal sample	0.05	4.50	1	0.0285
	Model r^2	Model χ^2	d.f.	*P* value
	0.69	38.53	3	<0.0001

*, degrees of freedom.

PFK, phosphofructokinase.

Variables were entered into the model using forward stepwise selection.

condition affected the metabolic properties of glycolytic fibres more than those of oxidative fibres (see Greer-Walker, 1971; Beardall and Johnston, 1983), but even though the metabolic capacities of red muscle were spared during starvation, endurance at prolonged speeds was markedly reduced.

Starvation had a greater impact upon glycolytic than mitochondrial enzymes, particularly in white muscle. Our results confirm those reported for plaice and cod (Moon and Johnston, 1980; Martínez et al., 2002). In the present study, we extend this pattern to red muscle of cod, in which starvation led to marked reductions in glycolytic enzyme activities while leaving mitochondrial activities virtually unchanged. Further, starvation led to the virtual disappearance of the longitudinal variation of glycolytic enzyme activities in white muscle but only slightly attenuated positional differences in mitochondrial enzyme activities in red muscle.

The pronounced impact of energetic condition upon muscle metabolic capacities allowed us to demonstrate that capacities of white fibres are central determinants of prolonged swimming performance. The endurance of cod was most closely linked with the number of burst–coast movements during the swimming tests. Burst–coast swimming is considered an intermittent (unsteady) swimming behaviour, powered by white fibres. Webb (1994) suggests that it may be used as a gait during swimming over a portion of an individual's performance range or as a method to conserve energy at prolonged swimming speeds. Theoretical modelling suggests that burst–coast swimming is up to 2.5 times less costly than steady swimming (Videler and Weihs, 1982). This swimming behaviour is observed in cod during U_{crit} protocols (Nelson et al., 1994, 1996; Reidy et al., 2000). Our results confirm that during aerobic exercise (i.e. sustained swimming), fish are able to recruit white fibres (Rome, 1992; Burgetz et al., 1998). Further, we found that swimming endurance was significantly related to the activity of CCO and LDH in caudal samples of white muscle. As most of the cod's muscle mass is composed of white fibres, their use during endurance swimming would appear to be advantageous.

Given the central role of burst–coast movements in endurance swimming, we examined the determinants of this swimming behaviour. Cod with higher condition factors and higher red muscle PFK activity performed more burst–coast movements, whereas those with larger gill arches performed fewer burst–coast movements. Heart mass, which was closely related to gill arch mass, was also negatively linked with burst–coast movements. These relationships suggest a compromise between aerobic performance and anaerobic burst–coast swimming such as found by Reidy et al. (2000). As starvation only had a limited impact on gill arch and heart size, but markedly reduced condition factor and muscle enzyme activities, the trade-off between aerobic and anaerobic swimming capacities should be independent of feeding status. In agreement with this, negative relationships between burst–coast movements and gill arch mass (or heart mass) were observed in both treatment groups, which leads to the apparent paradox that cod with better capacity for oxygen uptake and distribution actually showed decreased prolonged swimming capacity, due to reduced reliance upon burst–coast movements.

Muscle activity changes from front to rear during swimming (Jayne and Lauder, 1995; Thys, 1997). Rostral muscle fibres generate power while shortening, whereas caudally active fibres resist stretching (negative work). The duration of the activity of the rostral fibres is longer than that of caudal fibres, but the time of relaxation of caudal white muscle fibres is slower than that of rostral ones. On the other hand, the rate at which caudal muscle relaxes is critical, because while ensuring force transmission during stretching, this rate determines the overall thrust produced by the tail region, which ultimately affects the performance of the whole organism (Swank et al., 1997). Thus, maximum tail beat frequency depends largely on the properties of the caudal fibres. Our results show that caudal muscle fibres have a high capacity for power generation. Given the decrease in fish muscle mass approaching the tail as well as the central role of these fibres in force transmission, it is crucial to maintain a high capacity for caudal power development. This may explain why enzyme activities in the caudal samples of white and red muscle were correlated with swimming endurance.

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