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Muscle fibre number varies with haemoglobin phenotype in Atlantic cod as predicted by the optimal fibre number hypothesis

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Atlantic cod (*Gadus morhua* L.) with the HbI-(2/2) haemoglobin phenotype have a higher blood oxygen affinity at low temperatures and a lower routine metabolic rate than individuals with the HbI-(1/1) phenotype. In the present study, muscle structure was found to be related to haemoglobin phenotype in a coastal population of Atlantic cod from the Saltenfjord region of Northern Norway. The maximum number of fast muscle fibres (FN_{max}) was reached at approximately 39 cm fork length and was 15% greater in the HbI-(1/1) than in the HbI-(2/2) phenotypes whereas the average fibre diameter for fish of the same fork length was significantly lower. Theoretically, the higher oxygen affinity of the HbI-(2/2) phenotype in the cold water of northern latitudes could have resulted in a relaxation of diffusional constraints at the level of individual muscle fibres, permitting the observed increase in fibre diameter. The results support the optimal fibre number hypothesis which envisages a trade-off between diffusional constraints and the energy cost of maintaining ionic homeostasis with fewer larger diameter muscle fibres in the HbI-(2/2) phenotype contributing to a lower routine metabolic rate.

1. INTRODUCTION

Haemoglobin from Atlantic cod *Gadus morhua* occurs as two homozygous [HbI-(1/1), HbI-(2/2)] and one heterozygous phenotype [HbI-(1/2)] based on agar gel-electrophoresis (Sick 1961). In the North Atlantic, HbI-(2/2) is present at higher frequencies in the north whereas HbI-(1/1) is dominant in the south (Sick 1965). Haemoglobin phenotypes comprise combinations of four alpha and beta globin genes (Verde & diPrisco 2004). The HbI-(2/2) phenotype has a higher oxygen binding affinity than the HbI-(1/1) phenotype below 10 °C (Brix *et al.* 1998). At temperatures above 14 °C, and at some blood pHs, the HbI-(1/1) phenotype has a higher oxygen affinity than the HbI-(2/2) phenotype with intermediate values found in the

HbI-1/2 phenotype (Karpov & Novikov 1981). Preferred body temperature is significantly lower for HbI-(2/2; 8.2 °C) than HbI-(1/1; 15.4 °C) phenotypes (Petersen & Steffensen 2003). At 6 °C, competitive feeding behaviour was higher for the HbI-(2/2) than HbI-(1/1) phenotypes (Salvanes & Hart 2000) and was correlated with a higher growth rate and age at first spawning (Mork *et al.* 1984; Jørstad & Nævdal 1994). In contrast, the aerobic scope for activity and maximum swimming speed were significantly higher for the HbI-(1/1) than the HbI-(2/2) phenotypes (Petersen & Stefensen 2003). Thus at northern latitudes Atlantic cod with the HbI-(1/1) phenotype appears to live more active life-styles than cod with the HbI-(2/2) phenotype, but mature later and grow more slowly.

The maximum number (FN_{max}) of fast fibres is positively correlated with ultimate body size (Johnston *et al.* 2003a). Dwarfism in landlocked populations of Arctic charr (*Salvelinus alpinus*; Johnston *et al.* 2004) and Atlantic salmon (*Salmo salar*; Johnston *et al.* 2005) has led to a rapid loss of muscle fibres. The radiation of Antarctic notothenioid fishes resulted in a relaxation of diffusional constraints due to climatic cooling, and phylogenetic analyses demonstrated a dramatic reduction in FN_{max} relative to the ancestral condition (Johnston *et al.* 2003a). This led us to advance the optimal fibre number hypothesis, which provides a physiological explanation for changes in FN_{max} with body size and environment (Johnston *et al.* 2003a, 2004, 2005). The energy cost of maintaining ionic homeostasis is thought to represent 20–40% of routine metabolic rate (Jobling 1994). Theoretically, as the surface to volume ratio of muscle sarcolemma decreases so does the passive leak of ions across the membrane and hence the cost of maintaining ionic homeostasis involving ATP-dependent pumps. According to our hypothesis there is an optimal fibre number for a given body size and environment which minimizes the energy costs of maintaining ionic homeostasis across the surface area of the muscle sarcolemma. Fibres that exceed the optimum diameter will incur the penalty of diffusional limitation for oxygen and other metabolites while smaller diameter fibres have an unnecessarily high routine metabolic rate, limiting behavioural flexibility. In the case of Atlantic cod living in the same northern environment the optimal fibre number hypothesis predicts that the HbI-(1/1) phenotype with a faster lifestyle would have smaller diameter fibres and a higher FN_{max} than the HbI-(2/2) phenotype. The aim of the present study was to test this prediction for a cod population from the Saltenfjord region of northern Norway.

2. MATERIAL AND METHODS

Atlantic cod (*G. morhua* L.; 29.0–101.4 cm fork-length (FL, length from the tip of the snout with closed mouth to the centre fork of the tail), 250–10 415 g $n=49$] were caught between June and August 2005 in the Saltenfjord region of Northern Norway (latitude 67°17′ N) using a beam trawl and line fishing. Fishes were killed by a blow to the head and pithing. Haemoglobin phenotypes were determined by agar gel electrophoresis (Fyhn *et al.* 1994). The *PanI* locus was used a genetic marker to distinguish between coastal and Arctic cod because these populations might also differ in muscle fibre number. Genetic divergence at the pantophysin (*PanI*) locus was determined using a PCR-based assay to score polymorphisms (Fevolden & Pogson 1997).

Frozen sections (7 µm) were prepared from 6 to 12 muscle blocks/fish at 0.7 FL, sampling all the fast muscle on one side of the trunk. Fast and slow fibres were distinguished using the S-58 myosin antibody (Johnston *et al.* 2004). One hundred randomly

selected fast muscle fibres were digitized per block and fibre number and diameter estimated as previously described (Johnston *et al*. 1999). Non-parametric statistical techniques were used to fit smoothed probability density functions (pdfs) to the measured diameters using a kernel function (Bowman & Azzalini 1997; smoothing coefficient $h = 0.105$). The non-parametric Kolmogorov–Smirnov two-sample test statistic was used to test the null hypothesis that pdfs of fibre diameter for HbI-(1/1) and HbI-(2/2) phenotypes were equal over all diameters. FN_{max} was analysed in a general linear model ANOVA with haemoglobin phenotype and *PanI* genotypes as fixed factors. For average fibre diameter, fork length was included as a covariate.

3. RESULTS

Muscle fibre recruitment ceased at around 39 cm fork length. The two smallest fish sampled had not stopped recruiting fast muscle fibres and were excluded from further analysis. There were 12 HbI-(1/1), 19 HbI-(1/2) and 18 HbI-(2/2) haemoglobin phenotypes. Forty three fish were the *PanI*-(A/A) genotype and six fish the *PanI*-(A/B) genotype (figure 1b). FN_{max} was different between haemoglobin phenotypes ($F_{2,46} = 3.3$, $p = 0.046$), but not *PanI* genotypes. FN_{max} was 15% greater for the HbI-(1/1) than the HbI-(2/2) phenotype (figure 1a, $p < 0.01$; Tukey's *post hoc* test). The maximum fibre diameter reached 500 μm in the largest fish, a HbI-(2/2) phenotype (data not shown). Fish with the HbI-(1/1) phenotype had smaller average diameters than for the HbI-(2/2) phenotype for a given fork length (figure 1b; $F_{2,49} = 3.41$, $p = 0.042$). Fibre diameter was not different between *PanI* genotypes. Figure 2 illustrates the pdf of fibre diameter for five fish from each of the HbI-(1/1) and HbI-(2/2) phenotypes matched for fork length (see legend). The right-hand side pdf for the HbI-(1/1) phenotype (solid line) was shifted to the left relative to the HbI-(2/2) phenotype (dashed line) whereas the right-hand side of the distribution showed a shift to the right for the HbI-(2/2) relative to the HbI-(1/1) phenotype ($p < 0.02$; Kolmogorov–Smirnoff test).

4. DISCUSSION

Numerous stocks of Atlantic cod (*G. morhua* L) occur in the Northeast Atlantic and Baltic Sea with different life-history characteristics and migration patterns (Brander 1994). In northern Norway, two main groups can be distinguished, coastal cod and northeast Arctic cod. The latter migrate from feeding areas in the Barents Sea and near Svalbard to spawning areas on the Norwegian coast, most importantly the Lofoten Islands (Brander 1994). The temperature experienced during early life stages can influence the number of fast muscle fibres recruited (Johnston *et al*. 2003b). To interpret our results it is important to consider whether the fish studied are likely to come from the same spawning stock. Pantophysin is a useful genetic marker for stock discrimination with the *PanI*[A] and *PanI*[B] alleles occurring at higher frequencies in costal and northeast Arctic cod, respectively (Fevolden & Pogson 1997; Case *et al*. 2005). Our fish were caught after the northern migration of post-spawning NE Arctic cod and were genotyped *PanI*-(A/A) or *PanI*-(A/B) suggesting they come from a coastal spawning population. Although some admixing of populations spawning in different
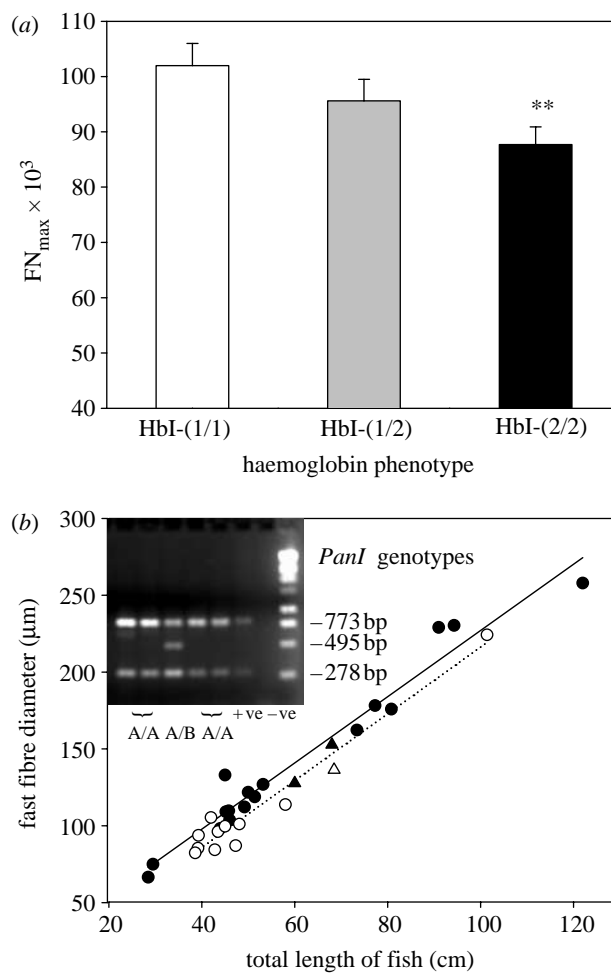

Figure 1. (a) Maximum number and (b) average diameter of fast muscle fibres in Atlantic cod (*Gadus morhua*) with different haemoglobin phenotypes. In (a) mean+s.e., 12 HbI-(1/1), 19 HbI-(1/2) and 18 HbI-(2/2) phenotypes, [**]indicates significant difference $p < 0.01$. In (b) HbI-(1/1) phenotypes (open symbols), HbI-(2/2) phenotypes (closed symbols), *PanI*-(A/A) genotypes (circles), *PanI*-(A/B) genotypes (triangles). First order regression lines were fitted: HbI-2/2 (solid line), HbI-1/1 (dotted line). Insert shows a typical RT-PCR gel for *PanI* genotypes with positive and negative controls.

local environments may have occurred, it is highly unlikely that developmental plasticity could explain our results (figure 1a,b).

Mitochondria are present in the central core of fast muscle fibres indicating a requirement for adequate oxygen diffusion during recovery metabolism (Archer & Johnston 1991; Johnston *et al*. 2004). Factors that affect tissue perfusion such as haemoglobin properties, blood flow and the number and distribution of capillaries will act to determine the limits for diffusion at the level of individual muscle fibres (Egginton *et al*. 2002). Theoretically, the appearance of a haemoglobin phenotype with a higher oxygen affinity could have allowed a relaxation of diffusional constraints due to a higher partial pressure of oxygen at the fibre surface (Hill 1965). In the present study, there is clear evidence that fibre diameters are larger for the HbI-(2/2) than the HbI-(1/1) phenotype (figures 1b and 2), which would theoretically reduce the energetic costs of ionic
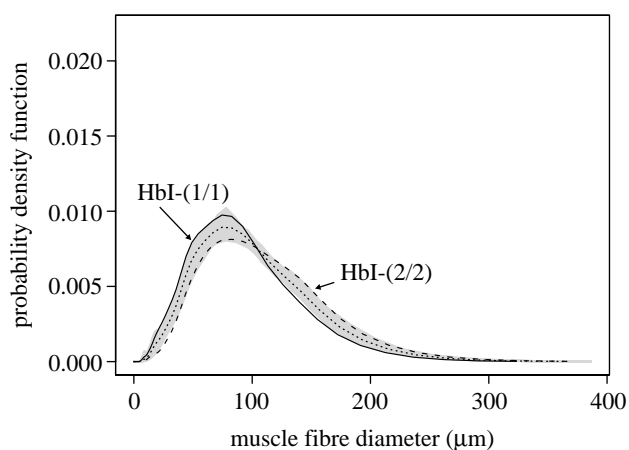
Figure 2. Probability density functions of fibre diameter for five HbI-(1/1) (solid line; 45.3 ± 1.0 cm fork length) and five HbI-(2/2) (dashed line; 45.8 ± 0.9 fork length) haemoglobin phenotypes (mean $\pm$ s.e.). The dotted line shows the average pdf of fibre diameters across all genotypes and the shaded area 100 bootstrap values of the combined population of diameters.

homeostasis by decreasing the surface to volume ratio of the sarcolemmal membrane and contribute to the observed reduction in routine metabolic rate. The increase in fibre diameter allows a decrease in FN_{max} as now fewer muscle fibres are required to reach the maximum body size; representing the trade-off inherent in the optimal fibre number hypothesis. It appears mutations in a few globin-encoding genes have led to changes in tissue structure, physiology, behaviour and life-history strategies with profound consequences for the biogeographic patterning of the species.

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