J Comp Physiol B (2009) 179:623–633
DOI 10.1007/s00360-009-0344-6

ORIGINAL PAPER

Thermal acclimation to 4 or 10°C imparts minimal benefit on swimming performance in Atlantic cod (*Gadus morhua* L.)

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Received: 24 September 2008 / Revised: 21 January 2009 / Accepted: 24 January 2009 / Published online: 15 February 2009
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Abstract Thermal acclimation is frequently cited as a means by which ectothermic animals improve their Darwinian fitness, i.e. the beneficial acclimation hypothesis. As the critical swimming speed (U_{crit}) test is often used as a proxy measure of fitness, we acclimated Atlantic cod (*Gadus morhua*) to 4 and 10°C and then assessed their U_{crit} swimming performance at their respective acclimation temperatures and during acute temperature reversal. Because phenotypic differences exist between different populations of cod, we undertook these experiments in two different populations, North Sea cod and North East Arctic cod. Acclimation to 4 or 10°C had a minimal effect on swimming performance or U_{crit}, however test temperature did, with all groups having a 10–17% higher U_{crit} at 10°C. The swimming efficiency was significantly lower in all groups at 4°C arguably due to the compression of the muscle fibre recruitment order. This also led to a reduction in the duration of "kick and glide" swimming at 4°C. No significant differences were seen between the two populations in any of the measured parameters, due possibly to the extended acclimation period. Our data indicate that acclimation imparts little benefit on U_{crit} swimming test in Atlantic cod. Further efforts need to identify the functional consequences of the long-term thermal acclimation process.

Keywords *Gadus morhua* · Critical swimming speed · Beneficial acclimation hypothesis · Swimming efficiency · Oxygen consumption

Communicated by G. Heldmaier.

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Introduction

Temperature is a dominating physical factor that exerts significant influence over various biological traits. This phenonenon is most dramatically demonstrated in ectothermic animals such as fish. Most fishes exhibit some degree of reversible thermal plasticity, a process known as acclimatisation/acclimation (Johnston and Templeton 2002). A number of now classic studies published by Brett in the 1960s clearly demonstrated the existence of a thermal optimum for swimming performance (U_{crit}) in salmonids *Oncorhynchus* sp. Furthermore, he was able to show that this optimum could be shifted by long-term acclimation to cooler or warmer temperatures. This led to the development of a thermal tolerance window as outlined by Fry (1971). Essentially this states that a thermal window exists in which a given animal can function. A thermal optimum exists towards the upper end of the thermal window and is bound by the thermal tolerance limits. Acclimation to low or high temperatures typically extends these boundaries and improves measures of performance at lower and higher temperatures respectively.

While the physiological and cellular mechanisms responsible for acclimation are generally well understood, the adaptive significance of thermal acclimation is less well understood. It has long been held that acclimation to different temperatures enhances the Darwinian fitness of an

animal at the acclimation temperature, i.e. the beneficial acclimation hypothesis (BAH; Leroi and Bennett 1994). A number of studies have shown that not only is the BAH rejected when examined in "simple" biological systems such as *Escherichia coli* (Leroi and Bennett 1994), but also in more complex biological systems such as *Drosophila melanogaster* (Zamudio et al. 1995; Gibert et al. 2001). Indeed, recent work with mosquitofish *Gambusia holbrooki* has found the BAH does not reliably explain swimming performance in relation to mating success (Wilson et al. 2007). Nevertheless, swimming performance is often cited as an ecologically relevant proxy for Darwinian fitness in many other fish species. Indeed it may indirectly determine reproductive success in migratory salmonids (Farrell 1996), migratory eels *Anguilla anguilla* (van Ginneken et al. 2005), and migratory Atlantic cod *Gadus morhua* (Godø and Michalsen 2000). It also determines the ability of Atlantic cod to outrun a trawl (Winger et al. 2000).

Migratory or sustained swimming employs oxidative metabolic pathways and substrates while burst swimming, typically exhibited during the escape response (and presumably during mating attempts in mosquitofish) is anaerobic, i.e. glycolytically fuelled (Jones 1982; Hammer 1995; Nelson et al. 2002). The U_{crit} swimming test employs both aerobic and anaerobic components (Lurman et al. 2007). The Atlantic cod is a generalist swimmer, being specialised for neither endurance swimming nor burst swimming. As such, the U_{crit} sustained swimming speed test is often the test of choice employed to assess swimming performance in cod (Tytler 1978; Tang et al. 1994; Bushnell et al. 1994; Nelson et al. 1994, 1996; Schurmann and Steffensen 1997; Claireaux et al. 2000; Reidy et al. 2000; Martínez et al. 2004; Lapointe et al. 2006; Sylvestre et al. 2007). We hypothesised that acclimation of Atlantic cod to 10°C would result in a higher U_{crit} at 10°C compared to Atlantic cod acclimated to 4°C but swum at 10°C. We further hypothesised that a shift in thermal optimum would occur with thermal acclimation, such that a 4°C acclimated cod would have a higher U_{crit} when swum at 4°C than 10°C acclimated cod swum at 4°C.

Furthermore, a number of distinct cod populations exist (Nielsen et al. 2001) and have previously been shown to exhibit differences in various physiological parameters (Sick 1961; Karpov and Novikov 1980; Nelson et al. 1994; Pörtner et al. 2001; Gollock et al. 2006; Lucassen et al. 2006; Sylvestre et al. 2007). For example, a recent study found that growth rates of Atlantic cod from the North East Arctic/Barents Sea (NEAC) were much lower than in counterparts from the North Sea (NSC), and the NEAC also had a lower fecundity than the NSC (Pörtner et al. 2001). It appears that different cod populations are optimised for different thermal regimes. For this reason we also hypothesised that NEAC would swim faster at lower temperatures and NSC faster at higher temperatures.

Methods

Animals

A total of 24 NSC (*Gadus morhua*) were caught using traps during August 2004 from around Helgoland in the German Bight. Seawater temperature was 18°C. Fish were transported to the AWI in Bremerhaven aboard the RV Uthörn. Initially, the fish were maintained in a re-circulated 16°C aquarium for 2 weeks to recover from handling and transportation stress and then moved to a 10°C aquarium. After two additional weeks, half of the fish were moved to a 4°C aquarium. First generation cultured NEAC (*Gadus morhua*; a generous gift from M. Delghandi's at the IMR in Tromsø, Norway) were air freighted over a 24-h period to the AWI in September 2004. Upon arrival the fish were held at 4°C for a minimum of 2 weeks before half were moved to the 10°C aquarium. All fish were then kept at their respective acclimation temperatures until the experiments began in October 2005. The temperatures were chosen based on published data, where 4°C appears to be the average yearly temperature which the NEAC experience (Bergstad et al. 1987; Godø and Michalsen 2000), while 10°C is at the upper end of what NEAC experience during spawning at Lofoten, Norway. For the NSC, 4°C is at the lower extreme of their natural temperature range while 10°C is a mean temperature (German Federal Office for Shipping and Hydrography). Table 1 details the morphometric data of the Atlantic cod used in this study.

All fish were fed to satiation twice a week with a mixture of mussels (*Mytilus edulis*) and/or live common shrimp (*Crangon crangon*). Feeding was stopped 5–7 days before experimentation.

Table 1 Fish morphometrics

	4°C NEAC	10°C NEAC	4°C NSC	10°C NSC
N	6	6	6	6
Weight (kg)	0.71 ± 0.090	0.96 ± 0.064	0.90 ± 0.081	1.07 ± 0.160
Length (cm)	43.5 ± 0.98	49.3 ± 0.64	43.6 ± 1.55	47.0 ± 2.38
Condition factor	0.84 ± 0.046	0.80 ± 0.044	1.08 ± 0.037	0.99 ± 0.033

Experiments were carried out within German animal care legislature. Mortality during the entire holding period, i.e. from August 2004 to February 2006 was approximately 25%. No fish died during the course of the experiments, however, one died a week after experimentation.

Experimental set-up

The swim tunnel set-up has previously been described elsewhere (Lurman et al. 2007; Bock et al. 2008). Briefly, it consisted of three major parts. A Perspex pipe fed through a Bruker Biospec 47/40 NMR magnet. This pipe was attached to a seawater circulation system. When "closed," a 256-l volume was hermetically sealed to measure oxygen consumption. When "open," a supplemental 444-l volume of constantly aerated seawater was used to flush the system. Both circulations were temperature controlled to within ± 0.3°C. The gas-tightness of the closed system was checked periodically. A digital motion camera system was used for observing the fish. All swimming speeds were corrected for solid blocking effects using the procedure outlined by Nelson et al. (1994).

Surgical procedure

As part of an accompanying study using ^{31}P-NMR to examine metabolic status during swimming (Lurman et al. 2007), fish were initially anaesthetised with 0.05 mg/l MS-222 in seawater for 5 min. Fish were then transferred to an operating table where the gills were irrigated with 0.02 mg/l MS-222 in seawater. While anaesthetised, an inductive ^{31}P-NMR coil measuring 3 cm × 3 cm × 0.2 cm was sewn to the side of the body, 15 cm distal to the end of the caudal fin with two sutures on the leading edge. The trailing edge was left free to move. This took <15 min. Fish were then placed in a tubular shaped cage (15 cm diameter by 60 cm length) within the chamber. Here, the receive coil was positioned to allow optimal signal transduction while at the same time the fish was observed as it recovered from anaesthesia. Recovery usually took approximately 15 min. Before the fish had completely recovered, the cage was pulled by means of a cord into the centre of the magnet and the fish allowed to recover for at least another 2 h.

Exercise protocol

All fish were swum twice, once at the acclimation temperature and once at the non-acclimation temperature (Fig. 1 gives a visual representation of the protocol). Those fish swum at their non-acclimation temperature first were warmed or cooled after the surgery recovery period in two 3°C steps over a 4-h period to the required temperature, i.e. 4 or 10°C. Fish were then left overnight with minimal flow, i.e.

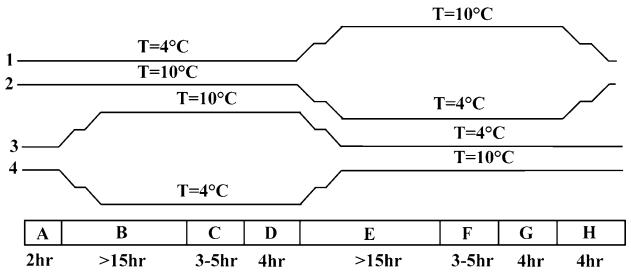

Fig. 1 Schematic representation of the experimental protocol showing temperature as a function of time. In *block A* the fish were first introduced into the chamber and allowed to recover from surgery for 2–3 h. In *block B and E* the temperature was changed, if necessary, in two 3°C steps lasting 4 h in total before the fish were left overnight at the required temperature. *Blocks C and F* represent the U_{crit} swim tests. *Blocks D and G* indicate recovery from the U_{crit} swim tests. In block H the temperature was changed, if necessary, back to the acclimation temperature before the fish were removed from the swim tunnel. *1* and *3* indicate 4°C acclimated cod, while *2* and *4* indicate 10°C acclimated cod. (See text for a full description)

3.3 m^3 h^{-1}. This typically equalled 0.15–0.19 BL s^{-1}. To avoid possible training effects biasing the groups, approximately half of the fish were swum at their acclimation temperature first and the other half at their non-acclimation temperature first. The routine metabolic rate was determined in fish swimming with minimal flow. The flow was then increased in 1 m^3 h^{-1} (approximately 0.05 BL s^{-1}) steps with each step lasting 30 min. At sufficiently high water flows, fish would rest on the rear grid of the cage. When two of these successive pauses lasted more than 20 s, a 9-V electric current was manually applied to a grid. U_{crit} was defined as the point when the fish was no longer able to move from the grid (as per Nelson et al. 1994), and calculated according to the formula given in Brett (1964).

$$U_{\text{crit}} = u_i + \left(\frac{t_i}{t_{ii} \times u_{ii}}\right)$$

where u_i is the highest velocity in BL s^{-1}, u_{ii} is the velocity increment, t_i is the time in minutes that the fish swam at the fatiguing velocity, and t_{ii} is the prescribed swimming period, i.e. 30 min. Following U_{crit}, the water flow was reduced to the minimum of 3.3 m^3 h^{-1} to allow recovery.

After 4-h of post exercise recovery at the swim temperature, fish were again either warmed or cooled in the same stepwise manner to the appropriate temperature and left at minimal flow overnight until they were swum again according to the same protocol outlined above on the third day. Fish were then allowed a further 4 h to recover at the swim temperature, and if necessary, warmed or cooled back to their acclimation temperature in the same stepwise manner outlined above, before they were taken out, the inductive coil removed and placed back in the aquarium. Water ammonium and nitrite contents were checked every 12 h and water was changed when necessary.

Tail-beat frequency measurement

Tail-beat frequency was measured manually by counting the number of tail beats in a 30-s period using the digital camera system. This was repeated eight times at each of the 30 min swimming stages. The mean of these eight was then taken as the tail-beat frequency. Eight 30 s sampling periods were not always possible at U_{crit}, so the mean was taken of as many sampling periods as were permitted, minimum 3. The time of the first kick was also recorded. The kick-and glide duration is the time taken from the first kick and glide burst until U_{crit}.

Oxygen consumption determination

Seawater oxygen content was measured constantly at a sampling rate of 0.5 Hz using Fibox optodes (Presens, Germany) with the temperature compensation entered manually. Optodes were zeroed chemically with sodium dithionite in seawater, and 100% was calibrated by placing the optode in the open swim-tunnel circulation. This was checked periodically against a MultiLine P4 CellOx 325 oxymeter (WTW, Germany).

Oxygen consumption was calculated from the slope of the drop in water oxygen content which was monitored over 20 min measurement periods at each speed. After the initial 20 min measurement period the circulation was opened for a 10 min flush/re-oxygenation. Water flow was maintained at the same speed during flushing. At the end of each experiment when the fish had been removed, a "blank" respiration run was performed to quantify any background microbial respiration. This was then subtracted from the fish's oxygen consumption rate. Oxygen consumption rates were corrected for possible minor allometric size effects using the mass exponent of 0.8 (Saunders 1963):

$$\dot{M}_{O_2} = \left(\frac{1}{M}\right)^{0.8} \times \dot{M}_{O_2}\mathrm{m}$$

where $\dot{M}o_2$ is the standardised oxygen consumption rate in $mgO_2\ kg^{-1}\ h^{-1}$, M is the mass of the fish in kilograms, and $\dot{M}o_2m$ is the measured oxygen consumption.

Data modelling and statistical analysis

Data modelling was performed using Graphpad Prism 4.0. For the determination of SMR, standardised oxygen consumption data were not transformed before analysis. Non-linear regression was used to fit a curve to the data on a plot of respiration as a function of swimming speed for each individual at each test temperature. The curve was described by the equation below:

$$\dot{M}o_2 = \mathrm{SMR} \times \exp^{(k \times U)}$$

where k is a constant and U is the speed in body lengths per second ($BL\ s^{-1}$). The best fitting curve allowed an estimation of the SMR at 0 swimming speed for each fish at each temperature. AMR was taken to be the maximal oxygen consumption rate measured during swimming. Swimming efficiency was assessed by first $\log_{10}$ transforming the standardised oxygen consumption data in Prism and then using the linear plot function to fit straight lines to the data. Tail-beat frequency was plotted as a function of the swimming speed and a non-linear plot function fit the following second order polynomial (quadratic) equation to the data:

$$\text{Tail-beat frequency} = \mathrm{A} + \mathrm{B} \times \text{speed} + \mathrm{C} \times \text{speed}^2$$

where A, B and C were unknown parameters estimated by best fit.

Statistical analysis was performed using SigmaStat 3.5. The fish total length was subjected to non-parametric Kruskal–Wallis tests for significance and post hoc Dunn's tests to distinguish between groups. Differences between mass and condition factor were tested using ANOVAs, while differences between SMR, AMR, net aerobic scope, U_{crit}, burst swimming duration, swimming efficiency parameters, and maximum tail-beat frequency, were tested using two-way repeated measures ANOVAs with Holm–Sidak's method to test for significance between individual groups and treatments. Data are presented throughout as mean values ± one SEM. Differences were considered significant when $P \leq 0.05$. An asterisk indicates a significant difference within the experimental group.

Results

Extrapolation of the oxygen consumption rate of cod at various speeds back to 0 speed made an estimation of the SMR for each experimental group possible. Although there was some variation in the SMR at a given test temperature, i.e. mean values between 28.8 and 51.0 mg $O_2\ h^{-1}\ kg^{-1}$ at 4°C and 60.4 and 103 mg $O_2\ h^{-1}\ kg^{-1}$ at 10°C, the repeated measures two-way ANOVA revealed a significant ($P = 0.025$) interaction between the acclimation and test temperatures. The test temperature had a significant effect on SMR ($P < 0.001$), with the estimated SMR being consistently higher at 10°C (Fig. 2). The Q_{10} values for SMR ranged between 3.2 and 4.4. Acclimation temperature alone had no significant overall effect ($P = 0.06$) on SMR. It did, however, significantly affect the SMR when estimated at 10°C only, where it was higher in the 4°C acclimated groups tested at 10°C. Population had no significant effect on SMR.

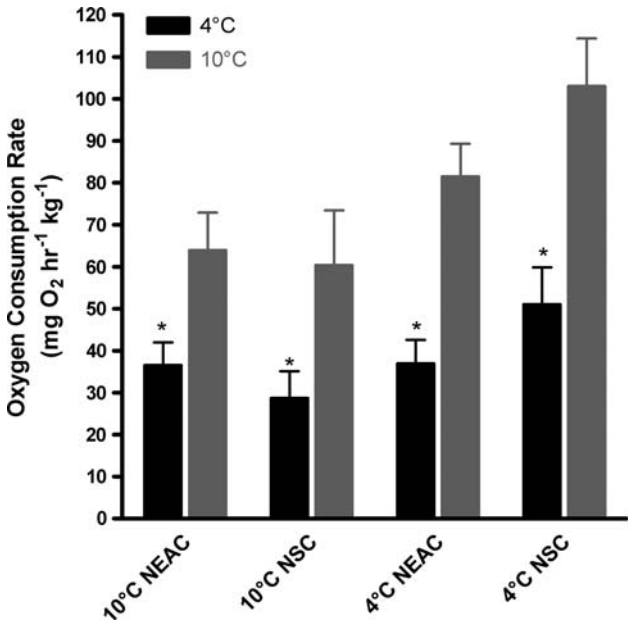

Fig. 2 Standard metabolic rate determined by extrapolation in NEAC and NSC. The temperature given on the *x*-axis indicates the acclimation temperature. The temperature indicated by tone on the *graph* indicates the actual test temperature. An *asterisk* indicates a significant difference between the 4 and 10°C test temperature within an experimental group

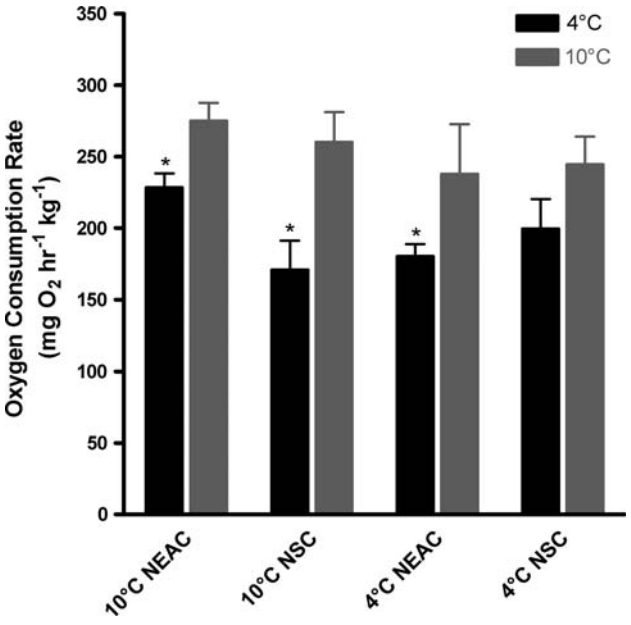

Fig. 3 Active metabolic rate in NEAC and NSC. The temperature given on the *x*-axis indicates the acclimation temperature. The temperature indicated by tone on the *graph* indicates the actual test temperature. An *asterisk* indicates a significant difference between the 4 and 10°C test temperature within an experimental group

A similar result was also seen for the measured active metabolic rate (Fig. 3). Here the effect of the test temperature was significant ($P < 0.001$), with the AMR being consistently higher when measured at the test temperature of 10°C. This was regardless of the acclimation temperature or population. No interactive effect was seen between acclimation and test temperatures. The combined effect of increased SMR and increased AMR resulted in an (albeit not statistically significant, i.e. $P = 0.09$) tendency for the net aerobic scope to be higher at 10°C (Table 2). In both the 4°C acclimated NEAC and NSC net aerobic scopes were approximately equal when tested at 4 or 10°C.

By plotting the log function of oxygen consumption rate as a function of different speeds (Fig. 4), it was evident that swimming efficiency, as indicated by the oxygen increment per speed increment (i.e. slope in Table 3), decreased significantly as the test temperature decreased ($P < 0.001$). Population had no significant effect. Linear fits had correlation coefficients between 0.85 and 0.57 (Table 3). The *y*-intercepts were consistently greater at a test temperature of 10°C, reflecting the higher SMR seen in Fig. 2.

The duration of burst swimming was also significantly longer when tested at 10°C ($P = 0.005$), except in the 10°C acclimated NEAC. This was due to greater inter-individual variation, however, the longer duration as a function of increased test temperature was similar to the other groups (Table 4). The mean burst duration lasted between 33.2 and 38.0 min at 4°C test temperature and 52.3 and 58.5 min at 10°C C-test temperature. Neither the acclimation temperature nor the population had any significant affect on the burst duration.

Acclimation and test temperature had a significant ($P = 0.02$) interactive affect on U_{crit}. Furthermore, a significant effect ($P < 0.001$) of test temperature alone was seen on U_{crit}, where U_{crit} was consistently higher by 10–17% at 10°C test temperature than at 4°C test temperature (Fig. 5). There was also a significant effect ($P = 0.03$) of the acclimation temperature on the critical swimming speeds attained when tested at 10°C, but not at 4°C, where the U_{crit}s for both 10°C acclimated groups were higher when tested at 10°C than those of the 4°C acclimated groups tested at 10°C.

With the exception of the 10°C acclimated NEAC at both test temperatures, the tail-beat frequency at a given speed was largely unaltered by either the acclimation temperature, test temperature or population (Fig. 6) (Table 6). Acclimation of NEAC to 10°C resulted in a significant right shift in tail-beat frequency meaning that the frequency was lower at a given speed. However, the effect of test temperature on the maximum tail-beat frequency achieved during swimming was significant ($P < 0.001$), with it being lower when tested at 4°C (Table 5). A significant interactive effect of acclimation temperature and test temperature was also seen ($P = 0.02$), yet acclimation alone had no significant effect.

Table 2 Net aerobic scope (NAS) in mg O_2 h^{-1} kg^{-1}

Test temperature	4°C NEAC	10°C NEAC	4°C NSC	10°C NSC
NAS at 10°C (mgO_2 h^{-1} kg^{-1})	180 ± 80.8	248 ± 35.3	142 ± 50.2	200 ± 50.9
NAS at 4°C (mgO_2 h^{-1} kg^{-1})	143 ± 23.4	192 ± 24.3	149 ± 45.1	142 ± 37.3

Population and acclimation temperature are indicated in the upper-most row. The left column indicates the test temperature

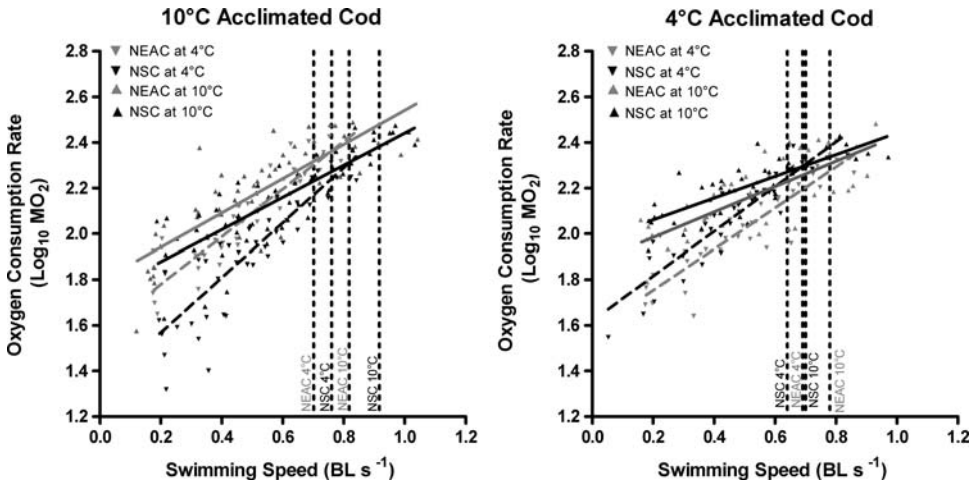

Fig. 4 Swimming efficiency for NEAC and NSC acclimated to 4 and 10°C (as indicated on the *graph*) as a function of relative swimming speed in body lengths per second). NEAC tested at 4°C are depicted with *grey downward pointing triangles* and a *dashed line*, while NEAC tested at 10°C are depicted with *grey upward pointing triangles* and a *grey solid line*. NSC tested at 4°C are depicted with *black downward pointing triangles* and a *dashed line*, while NSC tested at 10°C are depicted with *black upward pointing triangles* and a *grey solid line*. Critical swimming speeds are indicated by a *vertical dotted line*

Table 3 Swimming efficiency plot data

	Test temperature	4°C NEAC	10°C NEAC	4°C NSC	10°C NSC
Slope	10°C	0.56 ± 0.073*	0.90 ± 0.078*	0.47 ± 0.054*	0.703 ± 0.06*
	4°C	0.90 ± 0.077	1.04 ± 0.068	0.98 ± 0.074	1.20 ± 0.096
y-Intercept	10°C	1.87 ± 0.044	1.79 ± 0.041	1.97 ± 0.030	1.74 ± 0.037
	4°C	1.57 ± 0.041	1.57 ± 0.036	1.62 ± 0.037	1.33 ± 0.052
r^2	10°C	0.57	0.67	0.64	0.71
	4°C	0.81	0.85	0.84	0.77

* Significant difference between the 4 and 10°C test temperature within an experimental group

Table 4 Burst swimming duration in minutes

Test temperature	4°C NEAC	10°C NEAC	4°C NSC	10°C NSC
10°C	52.3 ± 11.7	53.8 ± 18.0	54.2 ± 13.0	58.5 ± 12.1
4°C	33.2 ± 4.9*	38.0 ± 5.9	33.8 ± 8.2*	36.6 ± 7.2*

Population and acclimation temperature are indicated in the upper-most row. The left column indicates the test temperature

* Significant difference between the 4 and 10°C test temperature within an experimental group

Discussion

The most salient feature of this study is that acclimation did not result in any major improvement in swimming performance at a given temperature, however the test temperature resulted in significant differences with fish swimming slower at 4°C test temperature. Similarly, the efficiency with which the fish swam was also largely unaffected by the acclimation temperature, but significantly affected by the acute test temperature. Biomechanically it was also evident that acclimation had no effect, with the maximal tail-beat frequency and burst swimming duration being consistently lower in all fish when swum at 4°C, regardless of acclimation temperature. As such, there was little support for the idea that acclimation was beneficial with respect to swimming performance.

The SMRs determined here through extrapolation (between 28.8 and 51.0 mg O_2 h^{-1} kg^{-1} at 4°C and 60.4

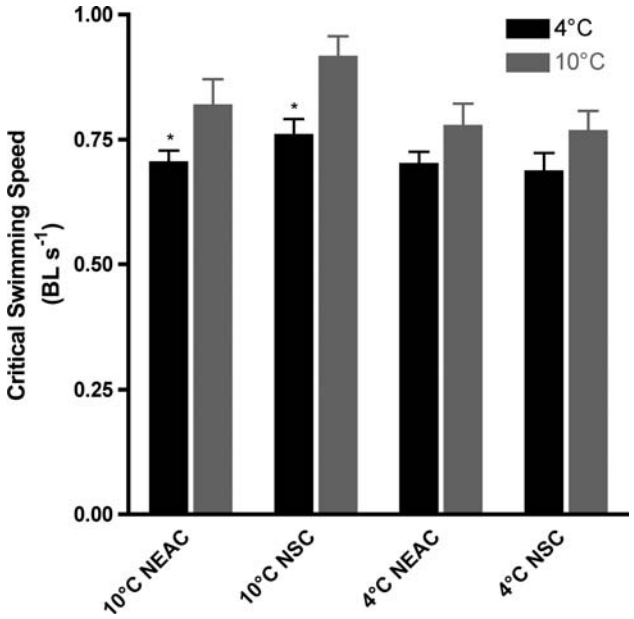

Fig. 5 Relative critical swimming speed in body lengths per second for NEAC and NSC. The temperature given on the *x*-axis indicates the acclimation temperature. The temperature indicated by tone on the *graph* indicates the actual test temperature. An *asterisk* indicates a significant difference between the 4 and 10°C test temperature within an experimental group

and 103 mg O_2 h^{-1} kg^{-1} at 10°C) are comparable with similarly sized Atlantic cod from Canadian waters at similar temperatures (Reidy et al. 2000; Webber et al. 1998; Nelson et al. 1994). This is a strong indicator that our set-up was no more stressful than those previously mentioned. This conclusion is further supported by the large aerobic scopes exhibited by the cod during swimming (Table 2).

Table 5 Maximum tail-beat frequency achieved during swimming

Test temperature	4°C NEAC	10°C NEAC	4°C NSC	10°C NSC
10°C	110.9 ± 2.8	98.5 ± 5.1	109.5 ± 5	114.2 ± 5.8
4°C	101.6 ± 2.8	76.4 ± 5.4	92.3 ± 4.5	91.3 ± 5.2*

* Significant difference between the 4 and 10°C test temperature within an experimental group

The influence of the acute test temperature was far greater than the acclimation temperature with SMR being consistently lower 4°C ($P < 0.001$).

Swimming efficiency was reduced at lower test temperatures meaning a larger amount of oxygen was required for each speed increment. The reduction in efficiency may be accounted for by (a) an increase in the water viscosity and consequent increase in drag (Webb 1975), although this would have been minimal given the small 6°C temperature change, and/or (b) compression of the recruitment order within the musculature. Previous work has looked at the contraction velocity of myofibres as a function of temperature, and a clear positive correlation was seen between temperature and contraction speed and power (Rome et al. 2000; Rome 1995). Consequently, more muscle fibres are required at lower temperatures, i.e. compression of recruitment order. The increased number of fibres recruited would lead to an increase in the amount of energy required, and ultimately oxygen needed to fuel their contraction, thus explaining the reduction in efficiency. We also hypothesised that as the test temperature was reduced there would also be a lowering of the muscular contraction speed and this would be evident in a lower tail-beat

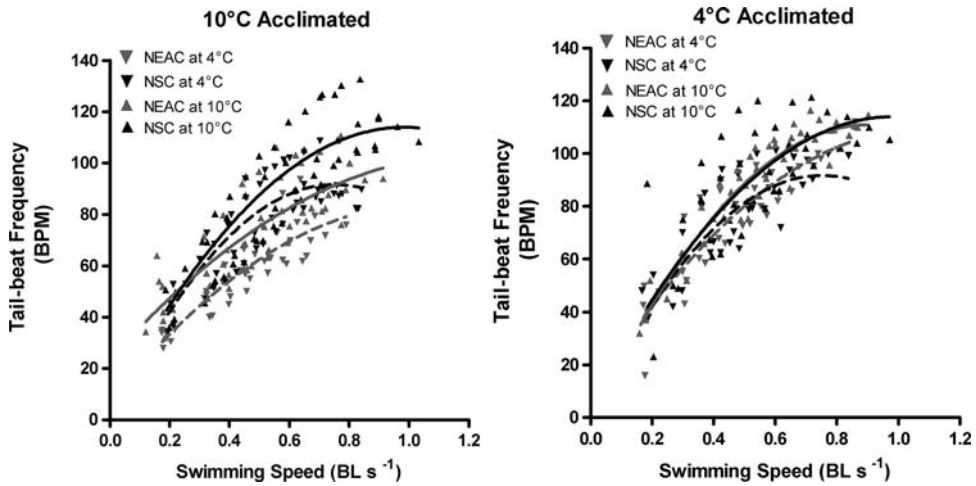

Fig. 6 Tail-beat frequency in beats per minute for NEAC and NSC acclimated to 4 and 10°C (as indicated on the *graph*) as a function of relative swimming speed in body lengths per second. NEAC tested at 4°C are depicted with *grey downward pointing triangles* and a *dashed line*, while NEAC tested at 10°C are depicted with *grey upward pointing triangles* and a *grey solid line*. NSC tested at 4°C are depicted with *black downward pointing triangles* and a *dashed line*, while NSC tested at 10°C are depicted with *black upward pointing triangles* and a *grey solid line*

Table 6 Tail-beat frequency non-linear regression best fit parameters

Population	NEAC	NSC	NEAC	NSC	NEAC	NSC	NEAC	NSC
Acclimation temperature	4	4	10	10	4	4	10	10
Test temperature	10	10	10	10	4	4	4	4
r^2	0.92	0.51	0.66	0.78	0.81	0.72	0.77	0.73
A	−1.80 ± 5.94	10.1 ± 11.61	23.4 ± 8.87	−1.18 ± 10.78	8.41 ± 10.4	15. 6 ± 18.1	7.03 ± 8.65	−9.24 ± 10.76
B	250 ± 24.1	204 ± 51.6	130 ± 38.9	228 ± 40.1	186 ± 45.4	206 ± 66.6	144 ± 40.1	275 ± 45.0
C	−139 ± 22.7	−120 ± 53.3	−53.2 ± 38.8	−109 ± 34.2	−86.2 ± 45.7	−114 ± 57.8	−67.8 ± 43.5	−190.5 ± 43.2

Mean ± SEM

frequency at a given speed. Yet from Fig. 6 it can be seen that this was not the case and that at a given speed, the tail-beat frequency was the same regardless of temperature, with the exception of the 10°C acclimated NEAC. On the other hand, we did see a reduction in the maximal tail-beat frequency. This also led to a shortening of the time that the fish spent using kick and glide propulsion (Table 4) indicating a reduced energy reserve and/or anaerobic scope.

Kick and glide swimming is anaerobically fuelled (Lurman et al. 2007), hence the shorter duration of kick and glide swimming also reflects a shift in the preferred metabolic pathway to aerobic metabolism with decreasing temperature. This shift has previously been observed in Norwegian coastal Atlantic cod in the form of reduced LDH activity at lower temperatures and taken to imply a reduction in the anaerobic capacity (Zakhartsev et al. 2004). Conversely, aerobic pathways were enhanced in cold acclimatised Newfoundland cod (Martínez et al. 1999) and Eastern Atlantic cod (Lannig et al. 2003). Indeed different aerobic and anaerobic capacities were documented in different populations of cod from Nova Scotia, where the authors concluded that despite the similar swimming performance, Bras d'Or cod were more reliant on anaerobic metabolism, as evidenced by a significantly greater build up of anaerobic end products, and greater ion disturbances during critical swimming than oceanic Nova Scotian cod (Nelson et al. 1994). A more recent comparison of two populations of Atlantic cod from the Gulf of St Lawrence and the Bay of Fundy found no difference in the swimming performance (Sylvestre et al. 2007). However, similar to Nelson et al. (1994), subtle differences were seen in the way swimming was fuelled with the relative aerobic capacity appearing greater in the Gulf of St Lawrence cod. This again reflects the shift towards more aerobic metabolic pathways as the result of cold adaptation, and it was also seen in the current study as a result of cold acclimation.

Yet in the current study no significant differences were seen between the two populations in any of the parameters measured. Previous studies in our lab have found differences in the fecundity, growth rate (Pörtner et al. 2001) and two aerobic metabolic marker enzymes (Lucassen et al. 2006; Lannig et al. 2003) suggesting that these two populations exhibited different metabolic phenotypes. The extended acclimation period in the current study, i.e. >1 year, may have extinguished any phenotypic differences between these two populations, indeed such long-term acclimation under artificial conditions is known to affect standard metabolic rate (Saint-Paul 1988). Furthermore, ontological conditions are known to have lasting impacts on phenotype (Lafrance et al. 2005; Gamperl and Farrell 2004; Johnston and Temple 2002; Claireaux et al. 2000; Jobling 1981) and the fact that our NEAC were first generation cultured cod while the NSC were wild caught may have played an important role.

That said, very subtle differences were seen in this study, the largest of which was a tendency for 4°C acclimated NSC to have a higher SMR and a lower AMR, which was most pronounced when measured at 10°C. As a consequence, both the net aerobic scope and swimming efficiency were lower in these cod. It appears that the 4°C acclimated NSC were still able to compensate their swimming performance to a reasonable degree. Although counter-intuitive given the ecological conditions experienced by these two populations, it may be that an acute increase from 4 to 10°C was more stressful for these cod than the NEACs and resulted in an artificially higher SMR.

In a number of now well-known experiments, Brett and colleagues were able to show that salmonids exhibit a thermal optimum, on either side of which, swimming performance is reduced (Brett et al. 1958; Brett 1965, 1967). Swimming performance has subsequently often been used as a proxy for Darwinian fitness and led to a large number of studies looking at swimming performance in response to a number of stimuli. While it is intuitive that swimming performance will influence the reproductive success of a fish species, particularly in migratory fishes (see Sect. "Introduction"), only one study has looked at this link directly. In the tropical mosquitofish, acclimation imparted a benefit on swimming performance in a U_{crit} swimming test which corresponded to an increase in the number of successful copulations under non-competitive conditions. However, when competition with other males was included, acclimation imparted no benefit on the number of successful copulations, and significantly more successful copulations were seen at the higher test temperature, i.e. 30°C (Wilson et al. 2007). This indicated that under certain, i.e. competitive conditions, U_{crit} had little impact on reproductive success, and as such, Wilson et al. concluded that simple improvements in swimming performance are not the only determinants of reproductive success.

In our study, we found a very clear affect of the acute test temperature on the swimming performance, with a reduction in the test temperature leading to reduced performance, as has been previously seen in Atlantic cod (Sylvestre et al. 2007). Acclimation, however, had only a minor impact on swimming performance at a given temperature, with 4°C acclimated cod performing similarly at 4 or at 10°C as 10°C acclimated cod. This begs the question, what is the purpose of acclimation, particularly if it comes at a metabolic cost? While a comparison of the two temperatures used here, i.e. 4 and 10°C, may not have revealed any major differences in performance as a result of acclimation, acclimation generally serves to extend or shift the functional range of the animal. For example, a 4°C acclimated cod would be hypothesised to exhibit significantly greater swimming performance at even lower temperatures, e.g. 1°C than cod acclimated to 10°C, while acclimation to higher temperatures will obviously have the reverse effect. As a corollary to this, we have also observed that in situ cardiac functioning was maintained in 4°C acclimated Newfoundland cod at 0–1°C, while hearts from 10°C acclimated Newfoundland cod failed (G.J. Lurman et al., unpublished data). Similarly, while acclimation of Danish Atlantic cod to 5 and 10°C revealed no difference in U_{crit} swimming performance, acclimation to 15°C resulted in a significantly higher U_{crit} (Schurmann and Steffensen 1997).

Ultimately however, our results suggests that over the temperature range examined in the current study, factors other than swimming performance may be of more relevance when examining Darwinian fitness in Atlantic cod. For example, thermal optima for other parameters such as growth and fecundity (Pörtner et al. 2001) appear to shift more readily with temperature.

It is necessary to mention that the critical swimming speed may have been compromised due to two factors, (a) drag induced by the NMR inductive coil and (b) the short working length of our swim tunnel (Farrell 2007). This would explain the continued increase in oxygen consumption previously seen in one population despite a change to kick and glide swimming (Lurman et al. 2007) and the fact that the U_{crit}s in this study are lower than those previously published for similarly sized cod (Sylvestre et al. 2007; Reidy et al. 2000; Nelson et al. 1994; see Lurman et al. 2007 for a more detailed discussion). However, this does not detract from the key finding that acclimation to 4 or 10°C had only a slight affect on swimming performance while the acute temperature was found to be far more important. This raises concerns about the validity of using swimming performance as a measure of Darwinian fitness.

Acknowledgments Erich Dunker and the AWI scientific workshop are gratefully thanked for constructing the swim tunnel and unerring support. Monkia Lange is also thank for support. Financial support for Glenn Lurman was provided by the MarCoPolI program of the Alfred Wegener Institute.

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