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The effect of temperature and body size on metabolic scope of activity in juvenile Atlantic cod *Gadus morhua* L.

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ABSTRACT

Changes in ambient temperature affect the physiology and metabolism and thus the distribution of fish. In this study we used intermittent flow respirometry to determine the effect of temperature (2, 5, 10, 15 and 20 °C) and wet body mass (BM) (~30–460 g) on standard metabolic rate (SMR, mg O_2 h^{-1}), maximum metabolic rate (MMR, mg O_2 h^{-1}) and metabolic scope (MS, mg O_2 h^{-1}) of juvenile Atlantic cod. SMR increased with BM irrespectively of temperature, resulting in an average scaling exponent of 0.87 (0.82–0.92). Q_{10} values were 1.8–2.1 at temperatures between 5 and 15 °C but higher (2.6–4.3) between 2 and 5 °C and lower (1.6–1.4) between 15 and 20 °C in 200 and 450 g cod. MMR increased with temperature in the smallest cod (50 g) but in the larger cod MMR plateaued between 10, 15 and 20 °C. This resulted in a negative correlation between the optimal temperature for MS (T_{opt}) and BM, T_{opt} being respectively 14.5, 11.8 and 10.9 °C in a 50, 200 and 450 g cod. Irrespective of BM cold water temperatures resulted in a reduction (30–35%) of MS whereas the reduction of MS at warm temperatures was only evident for larger fish (200 and 450 g), caused by plateauing of MMR at 10 °C and above. Warm temperatures thus seem favourable for smaller (50 g) juvenile cod, but not for larger conspecifics (200 and 450 g).

1. Introduction

Temperature influences all physiological and biochemical processes in animals, and climate change/global warming will therefore influence their biology and distribution. In accordance with this prediction, many marine and terrestrial species already exhibit shifts in distribution area, tracking the temperature range observed in their original environments (Rose, 2004; Drinkwater, 2005; Perry et al., 2005; Parmesan, 2006; Nye et al., 2009). Acknowledging that Atlantic cod as a species have a large geographic distribution area and will tolerate a broad thermal niche (−1.5 to 19 °C for North Atlantic stocks; (Righton et al., 2010)) a change in water temperature might have opposite effects on Atlantic cod populations on the northern and southern edges of their distribution (Pörtner, 2001; Sirabella et al., 2001; Pörtner et al., 2008; Kjesbu et al., 2014). Furthermore, within populations, some size groups/ontogenetic stages may be more vulnerable to temperature changes than others, affecting their survival and habitat selection; field observations have e.g. shown a temperature related shift in spawning habitats of Arcto-Norwegian cod *Gadus morhua*, choosing northern spawning areas in warm periods and more southern in colder periods (Sundby and Nakken, 2008), and a decrease in the Atlantic cod recruitment in the North Sea has been associated with higher-than-average water temperatures during the last decade (O'Brien et al., 2000). Moreover, there is a narrowing of the thermal range for North Atlantic cod stocks during the spawning season (Righton et al., 2010). Together this suggests that early life stages are the more sensitive to the thermal environment. Considering the great economic importance of Atlantic cod we nevertheless know surprisingly little about how temperature changes affect the metabolism of different size groups. Ultimately, such insight can be used to assess how a future temperature increase may impact growth performance and distribution of cod populations.

Numerous recent studies have linked fitness and growth potential of fish populations to their oxygen transport capacity (Pörtner and Knust, 2007; Farrell et al., 2008; Neuheimer et al., 2011). The conceptual background for this link is based on the aerobic metabolic scope (MS; the difference between the standard metabolic rate, SMR, and the maximal metabolic rate, MMR) which reflects the capacity of the cardio-respiratory systems to provide oxygen for all activities beyond SMR. In some studies the term active metabolic rate (AMR) is used to describe the maximal metabolic rate (Fry, 1971; Cutts et al., 2002). Thus, activities such as foraging and digestion or somatic and gonadal growth all require energy that must be supported by increased oxygen consumption over that used for basic homeostatic maintenance (Fry, 1971; Pörtner and Farrell, 2008; Pörtner, 2010; Pörtner and Peck, 2010). At increasing temperatures both SMR and MMR increase, MMR increases however at a faster rate than SMR, and MMR even declines

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as it approaches the upper thermal tolerance (Pörtner, 2002; Pörtner and Farrell, 2008). Thus, the aerobic scope is maximized at some 'intermediate' temperature and diminishes at temperatures above and below the so-called 'pejus' (getting worse) temperatures (Frederich and Pörtner, 2000; Pörtner, 2001). Beyond the pejus temperature the decrease in MS affects all higher functions (muscular activity, behaviour, growth and reproduction) and might thereby shape the long-term fate of species (Pörtner and Knust, 2007). When temperature becomes unfavourable and MS limited, fish may migrate to more suitable habitats to optimize fitness, changing their biogeographically distribution.

To understand how thermal changes affect the physiology, behaviour and biogeographic distribution of Atlantic cod a more detailed description of how SMR, MMR, and MS change with body size and temperature, covering not only the preferred temperature range but also the more extreme temperatures, is therefore required. The aim of the present study was to contribute to the understanding of the ecological consequences of increasing temperature on the metabolism of different sized Atlantic cod, information which can be used in bio-energetic models estimating predation and growth rates and predicting stock dynamics (Hansson et al., 1996; Essington et al., 2001; Jørgensen et al., 2012). Atlantic cod ranging in size from approximately 30 to 460 g were chosen to extend the previous determinations from young-of-the-year juveniles (Peck et al., 2003) to the onset of maturity. SMR and MMR were determined at 2, 5, 10, 15 and 20 °C, temperatures covering the annual thermal range encountered by Atlantic cod. Power functions were used to identify the correlation between body mass (BM) and SMR and MMR at each tested temperature. Furthermore, multiple functions were generated to determine the combined effect of BM and temperature on SMR and MMR, respectively, and to deduce the optimal temperature for MS at different body sizes.

2. Material and methods

2.1. Fish and maintenance

A total of 301 Atlantic cod *G. morhua* L. (5–460 g) were caught by trawling or fish traps in the northern part of Øresund (~56° 02′ N and 12° 64′ E), Denmark, from February 2012 and throughout the subsequent year. Following catch fish were transported to the Marine Biological Section, University of Copenhagen, and held for one week in 0.7–1.5 m^3 holding tanks (10 °C, 31‰ salinity) where after they were separated into five groups, ensuring a broad range of BM represented in each group. The fish were randomly acclimated in small groups to one of the test temperatures of 2, 5, 10, 15 and 20 °C (±0.3 °C) by modifying the water temperature by 1 °C day^{-1}. When the desired temperature was reached fish were held at that temperature for two to three weeks. The average BM range and sample size were respectively; 2 °C (199 g, 30–464 g N = 50), 5 °C (185 g, 28–450 g, N = 66), 10 °C (210 g, 5–469 g, N = 74), 15 °C (201 g, 34–456 g, N = 60) and 20 °C (197 g, 29–478 g, N = 51). During the whole period fish were fed with herring (*Clupea harengus*) three times a week. The average food consumption per tank decreased at cold and warm temperatures and increased mortality was observed amongst the larger individuals at 20 °C.

2.2. Respirometry

The respirometry setup consisted of two 50-L tanks, one containing four static respirometers and one serving as a sump for aeration, water changes and temperature control. An EHEIM pump (EHEIM, Deizisau, Germany) ensured continuous water exchange between the two tanks. An inline bio filter and UV disinfection unit (Tetra pond UV1000, Melle, Germany) maintained water quality, while temperature was kept at the test temperature ± 0.1 °C via a programmable digital indicator (PR5714, PR electronics 2704, Rønde, Denmark) and a Hetotherm thermostat bath (Heto, Denmark).

Measurements of oxygen consumption rate (MO_2, mg O_2 h^{-1}) were carried out using computerized intermittent flow respirometry allowing long term (>48 h) repeated measurements (Steffensen, 1989). For MO_2 measurements a fish was placed in a 0.5–7 L (depending on fish size, ensuring water to fish ratio of 10–20) cylindrical Plexiglas respirometer with two tubes at each end for a flushing loop and recirculation loop (0.5–5 L min^{-1}). Oxygen tension (pO_2) was continuously measured in the recirculation loop using a fiber optic oxygen sensor (PreSens, Regensburg, Germany or Firesting, PyroScience, Aachen, Germany) at a frequency of 1 Hz. AutoResp software (version 2.1.3; Loligo Systems, Tjele, Denmark) and AquaResp software (www.aquaresp.com) controlled the respirometer flushing times and calculated the MO_2 online (Steffensen et al., 1984).

MO_2 was measured continuously in loops of 10 min, which were composed of a flush period (4–7 min), wait period (1 min) and measuring period (3–6 min) (Schurmann and Steffensen, 1997). The duration of the measuring period was increased with decreasing temperature and increasing water volume vs. fish volume ratio.

MO_2 was calculated from a linear regression describing the decline in respirometer pO_2 during the measuring period and calculated according to:

$$MO_2 = V * \left(\frac{d(pO_2)}{dt}\right) * \alpha \quad (1)$$

where V is the respirometer volume (L) minus fish volume, $d(pO_2)/dt$ is the linear regression slope (mm Hg h^{-1}) and α is the water oxygen solubility (mg O_2 mm Hg^{-1} L^{-1}).

Preliminary testing demonstrated that the set-up ensured a coefficient of determination (r^2) associated with each MO_2 measurement that was always >0.95.

2.3. Experimental protocol

Fish were fasted prior to the experiment, the period for fasting at specific temperatures was determined by a concurrent SDA experiments where cod (173.9 ± 5.8 g, average ± SEM) digested a herring fillet corresponding to 5% wet BM in 10 days at 2 °C and 5 °C, 5 days at 10 °C, and 7 days at 15 °C and 20 °C respectively (own unpublished observations). Following the acclimation and starvation period fish were weighed and exercised intensively to exhaustion by continuous chasing for 5 to 7 min in a circular 50-L tank, including frequent short-term periods of air exposure. At 20 °C the chase period was shortened to 3 min because the fish became exhausted faster and some mortality was observed following a five to seven minute chase. Immediately after chasing fish were transferred to the respirometer and after a one minute wait period MO_2 measurements were started. MO_2 was measured in 90 second periods (without flushing) to generate high frequency MO_2 measurements. When respirometer pO_2 had declined to 70% flushing was initiated. The 90 second measuring periods were continued until a significant decline in MO_2 was observed (usually within 10–20 min) where after MO_2 was measured in 10 min loops for 48 h to determine SMR. If a fish following the stress test (only observed at 20 °C) turned belly up or MO_2 rapidly decreased below the expected SMR, the experiment was terminated. All experiments were carried out according to the animal welfare regulation of the University of Copenhagen and EU directive 2010/63/EU for animal experiments.

2.4. Data handling and analysis

MMR was determined as the highest MO_2 measured in the experiment (48 h). SMR was determined in resting fish by a double normal distribution according to (Steffensen et al., 1994) of MO_2 measured in the last 12 h of the experiment. Linear regressions describing SMR and MMR in correlation to BM at 2, 5, 10, 15 and 20 °C were calculated on

log transformed data (Sigmaplot 11, Systat Software Inc.) according to the equation

$$\text{Log } MO_2 = Y_0 + a \times \log(\text{mass}). \quad (2)$$

The change in metabolic scope (MS) with BM at the five temperatures was calculated as the difference between the MMR and the SMR obtained from the equations in Table 1 (shown in Fig. 4).

To describe the combined effects of temperature and BM on SMR and MMR multiple functions for SMR and MMR were made. The multiple functions were based on Eq. 2 but to include the effect of temperature, Y_0 was substituted with an exponential regression, describing the correlation between the Y_0 values and temperature. Furthermore, the a value in Eq. 2 was substituted with a second order polynomial function ($a_1 \times T^2 + b_1 \times T + c_1$) describing the correlation between the scaling exponents (a value in Table 1). Rewritten, the multiple functions of SMR and MMR were described by the formula

$$MO_2 = a \times e^{(b \times T)} \times \text{mass}^{\left(a_1 \times T^2 + b_1 \times T + c_1\right)}. \quad (3)$$

where T is the temperature and wet body mass = BM (g).

The optimal temperature for MS (T_{opt}) of a 50, 200 and 450 g cod, respectively, was determined as the temperature giving the maximal MS based on SMR and MMR calculated from Eqs. 4 and 5, respectively.

Q_{10} values were calculated according to Schurmann and Steffensen (1997).

3. Results

For juvenile Atlantic cod ranging in size between approximately 30 and 460 g SMR increased with temperature and BM according to a power function (Fig. 1). The average scaling exponent was 0.87 and ranged from 0.82 to 0.92, with the lowest values at the extreme temperatures (Table 1). The increase in SMR with temperature, calculated as Q_{10}, was similar within the range of 5 to 15 °C (1.8–2.1) calculated for a 50, 200 and 450 g Atlantic cod (Fig. 2). An increase in temperature from 2 to 5 °C resulted in augmented Q_{10} values (2.6–4.3), with the highest values found in the larger cod. On the contrary, a temperature increase from 15 to 20 °C resulted in lower Q_{10}, being 1.6 and 1.4 for 200 and 450 g cod respectively, whereas no significant change was observed for 50 g fish (Fig. 2).

MMR increased with BM at all temperatures according to a power function with an average scaling exponent of 0.87 (0.79–0.93) (Table 1). For the smallest cod, MMR increased throughout the temperature range. This was however not the case for larger cod where MMR was comparable at 10, 15 and 20 °C.

The combined effect of temperature and BM on SMR and MMR were described by multiple regressions:

$$\text{SMR} = \left(0.0687 \times e^{(0.0596 \times T)}\right) \times \text{mass}^{\left(-0.000892 \times T^2 + 0.0211 \times T + 0.788\right)} \quad (4)$$

Table 1
Summary of the linear equations (Log $MO_2 = Y_0 + a^*\log(\text{mass})$) in Figs. 1 and 2 describing respectively the standard (SMR) and maximum metabolic rate (MMR) in relation to wet body masses (BM) of cod at 2, 5, 10, 15 and 20 °C. Brackets are SE values.

Temperature	MO_2	Y_0	A	R^2	N	p
2	SMR	−1.064 (0.08)	0.82 (0.04)	0.92	49	<0.0001
2	MMR	−0.534 (0.07)	0.871 (0.03)	0.96	38	<0.0001
5	SMR	−1.059 (0.07)	0.889 (0.03)	0.94	59	<0.0001
5	MMR	−0.596 (0.05)	0.931 (0.03)	0.97	50	<0.0001
10	SMR	−0.913 (0.04)	0.884 (0.02)	0.97	71	<0.0001
10	MMR	−0.407 (0.05)	0.904 (0.02)	0.96	66	<0.0001
15	SMR	−0.856 (0.08)	0.922 (0.04)	0.92	58	<0.0001
15	MMR	−0.263 (0.07)	0.843 (0.03)	0.96	37	<0.0001
20	SMR	−0.58 (0.06)	0.847 (0.03)	0.96	38	<0.0001
20	MMR	−0.141 (0.08)	0.797 (0.04)	0.93	38	<0.0001

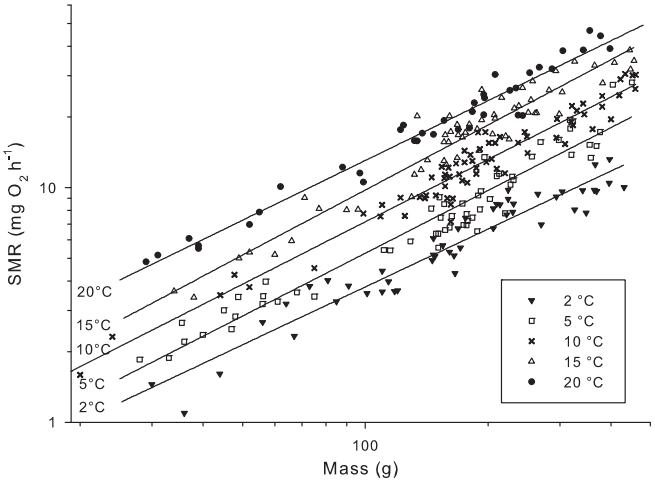

Fig. 1. Standard metabolic rate (SMR) of Atlantic cod with different wet body masses (BM) at 2, 5, 10, 15 and 20 °C, respectively. The solid lines are linear regressions describing the log transformed data (Log $MO_2 = Y_0 + a^*\log(\text{mass})$). For regression details see Table 1.

$$\text{SMR} = \left(0.226 \times e^{(0.0572 \times T)}\right) \times \text{mass}^{\left(-0.00074 \times T^2 + 0.0106 \times T + 0.87\right)} \quad (5)$$

where SMR and MMR are in mg O_2 h^{-1}, T = temperature (°C) and mass = wet BM (g). The exponential equation part describes the temperature correlation and the polynomial equation part the equation between the scaling exponents in Table 1 (see Material and methods for more details).

Irrespective of body size, MS increased with temperature up to 10 °C, to reach its maximum at that temperature (Fig. 4. calculated based on equations in Table. 1). For the smallest cod, MS was maintained at 15 °C, whereas a 5% reduction in MS was evident at 20 °C. On the contrary, temperatures above 10 °C negatively impacted MS in medium sized and large cod,, resulting in a 24 and 33% reduction of MS at 20 °C, respectively. At 2 °C, the reduction in MS (30–35%) was size independent. The change in MS with temperature and BM resulted in an optimal temperature for MS (T_{opt}) of 14.5, 11.8 and 10.9 °C in a 50, 200 and 450 g cod respective (Fig. 4).

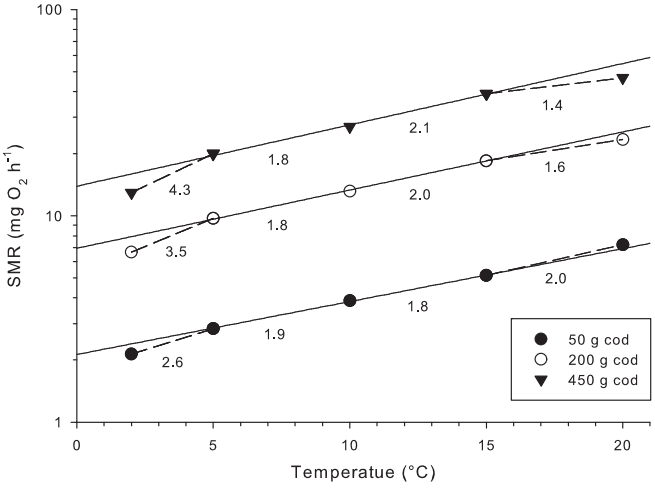

Fig. 2. The effects of temperature on standard metabolic rate (SMR) in a 50, 200 and 450 g Atlantic cod. The solid lines represent the calculated exponential increase in SMR with temperature, based on measurements at 5, 10 and 15 °C, whereas the dashed lines represent the change between 2 and 5 °C, and 15 and 20 °C. The numbers below the lines are the calculated Q_{10} for each temperature increment (i.e, for 2 to 5 °C, for 5 to 10 °C etc.).

4. Discussion

Changes in ambient water temperature affect the physiology and metabolism and thus the distribution of fish. Exact estimates of fish physiological tolerances are therefore needed to improve our understanding of potential impacts of climate-driven global warming. We here show, that between 2 and 20 °C SMR for Atlantic cod increased with BM and temperature (Fig. 1). The same was evident for MMR in juvenile cod (50 g), whereas MMR in the larger cod (200 and 450 g) levelled off at 10 °C and remained at comparable levels at 15 and 20 °C, indicating increasing temperature-dependent limitation of the performance of cardio-respiratory system with BM (Fig. 3). Furthermore, the optimal temperature for MS (T_{opt}) as determined by multiple functions decreased with BM, being respectively 14.5, 11.8 and 10.9 °C in a 50, 200 and 450 g cod (Fig. 4).

4.1. Metabolic scope, SMR and MMR

As ectothermic animals' vital rates of metabolism are affected by ambient temperatures, e.g. through changes in oxygen diffusion and enzyme activity. Thus, also SMR and MMR are temperature-dependent. The ongoing climatic change with increasing water temperatures has caused a pole ward shift in the distribution area for many fish species (Drinkwater, 2005; Perry et al., 2005; Parmesan, 2006). This movement from warm towards colder areas could be driven by reduced MS at increasing temperatures. Opposite, population of fish living at the northern limit of their distribution area like e.g. North Sea anchovies, may benefit from warming waters to increase their MS and hence growth potential (Petitgas et al., 2012). Optimal use of the available thermal habitat may also occur at much shorter time scales (e.g. hours or days) through short-distance horizontal and vertical movements (Neverman and Wurtsbaugh, 1994; Righton et al., 2010).

During acclimation to cold temperatures fish may increase mitochondrial density and aerobic capacity to maintain a high MS (Guderley, 1998; Johnston et al., 1998; Pörtner, 2001; Pörtner et al., 2005). This occurs at a metabolic cost as reflected in augmented SMR (Lannig et al., 2003). The present high Q_{10} (2.6–4.3) found for all cod between 2 and 5 °C however contradicts these earlier findings. It instead suggests reduced SMR at 2 °C, which may be a strategy to counteract the reduced MMR and thus 'protect' MS at the lowest temperature, a response which has previously been observed in both the European eel (*Anguilla anguilla*) and Atlantic cod (Walsh et al., 1983; Egginton and Johnston, 1984; Claireaux et al., 2000; Methling, 2013). Though, despite

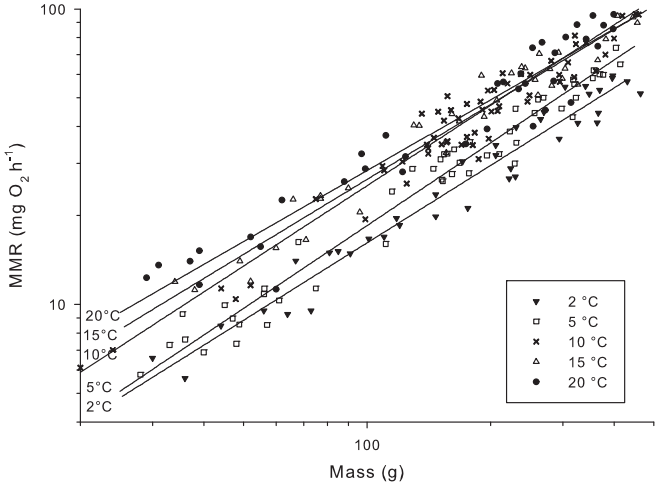

Fig. 3. Maximum metabolic rate (MMR) of Atlantic cod in relation to wet body masses (BM) at 2, 5, 10, 15 and 20 °C, respectively. The solid lines are linear regressions describing the log transformed data (Log $MO_2 = Y_0 + a^*\log(\text{mass})$). For regression details see Table 1.

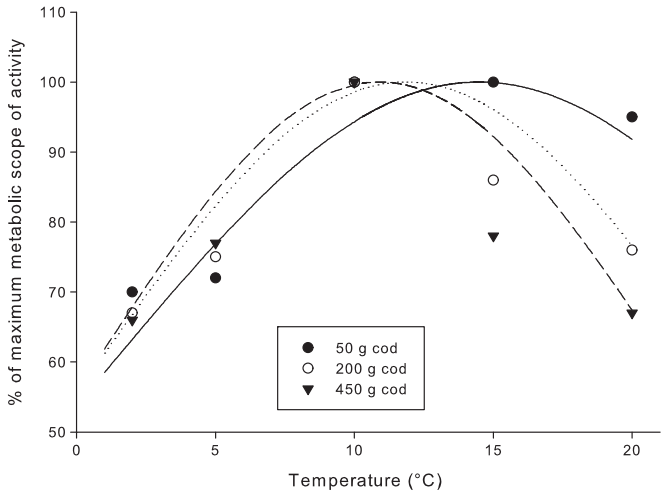

Fig. 4. The change in the metabolic scope of activity (MS) in relation to temperature in 50, 200 and 450 g Atlantic cod, described as % of the maximum MS measured. The data points are MS calculations based on the equations described in Table 1 and the lines are MS determined by the multiple regressions (Eqs. 4 and 5). MS was the highest at 10 °C for all BM and thus represents 100%. The full line represents MS of a 50 g cod, the dotted line of a 200 g cod and the dashed line of a 450 g cod.

this there was an overall reduction of MS by ~35% at 2 °C for all cod sizes. At warm temperatures, between 15 and 20 °C, the decrease in Q_{10} (1.6 and 1.4) in the 200 and 450 g cod may be linked to reduced mitochondrial density, with concurrent lowering of metabolic costs, which thus enable the fish to protect MS, as previously reported for various fish species (Johnston et al., 1998). On the contrary, SMR in small cod (50 g) increased unaffected at 20 °C (similar Q_{10}), which might be due to the continuously increase in MMR at warm temperatures and thus need for high mitochondrial density (Fig. 4).

Theory and some empirical evidence suggest that MMR in fish is linked to the maximum oxygen carrying capacity of the circulatory and ventilatory systems (Frederich and Pörtner, 2000; Pörtner, 2001). In sockeye salmon (*Oncorhynchus nerka*) heart rate peak around T_{opt} and the reduced performance above T_{opt} has thus been suggested to be due to cardiac insufficiency at warm temperatures (Steinhausen et al., 2008; Farrell, 2009). The plateauing of MMR seen here in the larger cod (but not smaller) indicates that in this species the circulatory and/or ventilatory system is more sensitive to warm temperature in larger conspecifics. At least in salmonids, small fish have relatively higher gill area, larger hearts and greater capacity for blood flow, suggesting a more efficient cardio-respiratory system as compared to larger conspecifics (Kolok and Farrell, 1994; Hidalgo et al., 1999). Furthermore, the present findings are supported by previous results where plateauing of MMR occurred between 10 and 15 °C in Atlantic cod ranging from 300 to 400 g (Schurmann and Steffensen, 1997). During July and August, the months where water temperatures approach yearly maxima, we found only small cod below approximately 50 g in shallow waters (3–4 m). A similar pattern has been observed for juvenile Atlantic cod at an inshore site off eastern Newfoundland. Here the smaller fish move from deeper waters towards the coast in spring, to initiate feeding when waters start heating up, whereas large mature cod remain at greater depths year-round. When waters cool at the onset of winter the juvenile fish seek the warmer, deeper waters (Methven and Bajdik, 1994). Overall, such size related behaviour where larger cod avoid waters of warm temperatures agrees well with the present negative correlation between T_{opt}, for MS and BM. For mature Scotian shelf cod (0.95–1.8 kg) T_{opt} has been suggested to be between 13 and 15 °C, based on MS determinations at 2, 5 and 10 °C (Claireaux et al., 2000). This is in the high end compared to the present T_{opt}, especially considering that T_{opt} decreased with BM. Notably however, several studies on cod growth maximization imply decreasing T_{opt} with size

and likewise for feed conversion efficiency (Pedersen and Jobling, 1989; Bjornsson et al., 2001; Imsland et al., 2005) i.e. supporting the present notion of an inverse relationship between T_{opt} and BM. Even though the cardiovascular system is directly affected by voluntary variations in temperature (Gräns et al., 2010) fish can obviously cope with short-term exposure to more extreme temperatures (Righton et al., 2010). However, long-term depression of MS, whether due to unfavourable temperatures or hypoxia exposure, may compromise vital body functions and thus reduce growth and survival rate. Cod in Øresund exhibit polymorphic haemoglobin variants and the present study animals were likely a mix of the two homozygous types, HbI-1/1 and HbI-2/2, and of the heterozygote (HbI-1/2) (Sick, 1965; Behrens et al., 2012). Laboratory studies have shown that temperature preference varies between haemoglobin types (Petersen and Steffensen, 2003; Behrens et al., 2012), but despite that haemoglobin polymorphism in cod has been investigated throughout the last 50 years there is no consensus on how genotype correlates with physiological traits such as e.g. growth (see (Ross et al., 2013) and references therein). Although purely speculative, it may also be that different cod populations comprised of several haemoglobin types during generations have adapted differently to optimize MS to the thermal conditions within their distribution area.

4.2. Scaling exponents

The vast majority of studies on fish in need of a scaling exponent to describe the relation between SMR and BM have used the average value of 0.79 derived by Clarke and Johnston (1999) from 69 teleost fish species in the post-larvae stage. Pronounced variations in scaling exponents inevitably occur between species, temperatures and life stages; notably, in Atlantic cod, scaling exponents range between 0.8 and 1.16 in larvae at 7–13 °C (Finn et al., 2002; Peck and Buckley, 2008), between 0.68 and 0.76 in young-of-the-year juveniles (0.02–5.4 g) at 4.5–15.5 °C (Peck et al., 2003), and is 0.82 in anesthetized fish at 12 °C (~30–1000 g) (Edwards et al., 1972) and between 0.79 and 0.89 in 0.15–7.1 kg specimens at 3–15 °C (Saunders, 1963). In comparison, the average scaling exponent derived in the present study was 0.87, ranging between 0.82 and 0.92, and with the two lowest values at the extreme temperatures, 2 and 20 °C. Clearly, knowledge on stage- and temperature specific scaling exponents is imperative to make robust extrapolations between fish sizes, and thus a prerequisite for sound parameterization of e.g. mechanistic individual-based models (IBMs) (Jørgensen et al., 2012).

4.3. Test method

Reviewing the literature it is evident that various experimental protocols have been used to determine MMR. The two most common approaches are to chase the fish to fatigue (Behrens and Steffensen, 2007; Jordan and Steffensen, 2007) or to use a critical swimming speed test (U_{crit} test; incrementally increase in swimming speed until fish fatigue; Behrens et al., 2006; Brett, 1964). The choice of test however needs to be considered carefully. A recent study on the coral reef fish bridled monocle bream *Scolopsis bilineata*, an excellent swimmer, found the U_{crit} protocol to be preferred over different chase protocols, as the former produced the 'maximal MMR' (Roche et al., 2013). On the contrary, Atlantic cod is a poor swimmer having difficulties maintaining a stationary position in swim flumes (Herbing and White, 2002; Fu et al., 2005), which has resulted in incongruent MMR determinations, with some studies measuring the highest MO_2 during an U_{crit} test (Tang et al., 1994; Schurmann and Steffensen, 1997) while others obtained MMR in the hours following an U_{crit} test (Soofiani and Priede, 1985; Bushnell et al., 1994). Continuous feeding may also elicit a substantial increase in MO_2, the so-called specific dynamic action (SDA), the energy expended on all activities of the body incidental to the ingestion, digestion, absorption, and assimilation of a meal (Secor, 2009). In cod however, MMR obtained by a chase protocol exceeds the maximum MO_2 observed in juveniles continuously feed to satiation (Soofiani and Hawkins, 1982). Own preliminary tests showed that only six of nineteen juvenile cod (28–61 g) were able to complete an U_{crit} test and reach an MMR comparable to the value obtained following handling and introduction to the swim tunnel. The remaining cod swam only for short periods of time in the swimming respirometer, in-between resting on the back grid. This suggests, in agreement with Reidy et al. (1995), that juvenile cod in general does not reach the highest MO_2 (i.e. the 'true' MMR) during an U_{crit} test, why we in the present study used – and will recommend for future studies on cod – a chase protocol including brief air exposure as the standard protocol for MMR determinations.

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