Physiological effects of temperature on Greenland halibut Reinhardtius hippoglossoides shows high vulnerability of Arctic stenotherms to global warming

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Published in:
Journal of Fish Biology

DOI:
10.1111/jfb.15434

Publication date:
2023

Document version
Publisher's PDF, also known as Version of record

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Citation for published version (APA):
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Reinhardtius hippoglossoides shows high vulnerability of Arctic stenotherms to global warming

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Funding Information
Danmarks Frie Forskningsfond; Elisabeth and Knud Petersen Foundation; Marianne Rasmussen; Solar Fonden af 1978

Abstract

Global warming affects the metabolism of ectothermic aquatic breathers forcing them to migrate and undergo high-latitudinal distribution shifts to circumvent the temperature-induced mismatch between increased metabolic demand and reduced water oxygen availability. Here the authors examined the effects of temperature on oxygen consumption rates in an Arctic stenotherm, the Greenland halibut Reinhardtius hippoglossoides, and calculated the optimal temperature for maximum aerobic scope, \( T_{\text{opt,AS}} \), which was found to be 2.44°C. They also investigated cardiac performance as limiting the oxygen transport chain at high temperatures by measuring maximum heart rate \( f_{H_{\text{max}}} \) over acute temperature increases and found various metrics related to \( f_{H_{\text{max}}} \) to be at least 3.2°C higher than \( T_{\text{opt,AS}} \). The authors’ measured \( T_{\text{opt,AS}} \) closely reflected in situ temperature occurrences of Greenland halibut from long-term tagging studies, showing that AS of the species is adapted to its habitat temperature, and is thus a good proxy for the species’ sensitivity to environmental warming. The authors did not find a close connection between \( f_{H_{\text{max}}} \) and \( T_{\text{opt,AS}} \), suggesting that cardiac performance is not limiting for the oxygen transport chain at high temperatures in this particular Arctic stenotherm. The authors’ estimate of the thermal envelope for AS of Greenland halibut was from –1.89 to 8.07°C, which is exceptionally narrow compared to most other species of fish. As ocean temperatures increase most rapidly in the Arctic in response to climate change, and species in these areas have limited possibility for further poleward-range shifts, these results suggest potential severe effects of global warming on Arctic stenotherms, such as the Greenland halibut. The considerable economic importance of the species raises concerns for future fisheries and species conservation of Arctic stenotherms in the Northern Hemisphere.

Keywords

aerobic scope, Arrhenius breakpoint, ectotherm, heart rate, optimum temperature, thermal tolerance
1 | INTRODUCTION

Aquatic breathers will encounter physiological challenges during global warming (Perry et al., 2005). As average sea-surface temperatures are predicted to increase by 2.0–4.8°C by the end of the century (IPCC, 2013), it will drive up their metabolic oxygen demand (Pörtner, 2001; Pörtner, 2002) while simultaneously decreasing the oxygen concentration in the water (Verberk et al., 2011). Thermal tolerance and survivability have been the subjects of widespread investigations focusing on global change–related impacts on ecosystems, which is thought to influence Darwinian fitness, distribution and community composition (Drinkwater, 2005; Farrell, 2009; Pörtner, 2001; Pörtner, 2002; Pörtner & Farrell, 2008; Pörtner & Knust, 2007; Pörtner & Peck, 2010). From a global warming perspective, particularly in the realm of environmental influences on marine ecosystems, attention has been directed towards physiological responses in ectotherms relating to thermal tolerance and suitability of habitats (Farrell, 2009; Habary et al., 2017; McKenzie et al., 2016; Pörtner & Knust, 2007). Body temperature is a fundamental controlling factor of all activities in ectothermic animals (Angilletta et al., 2002; Angilletta Jr et al., 2006; Fry, 1971; Huey & Stevenson, 1979), and species have evolved their performances and tolerances to their habitats’ temperature regimes (Pörtner & Farrell, 2008). For example, species which have evolved in relatively thermally stable habitats typically have narrow thermal tolerance envelopes (Angilletta Jr et al., 2006; Habary et al., 2017; Huey & Kingsolver, 1993) and are therefore less resilient to temperature increases and thermal fluctuations, such as those caused by global warming (Angilletta Jr et al., 2006; Pörtner, 2001; Pörtner & Farrell, 2008). Unfortunately, there is a general lack of knowledge on the thermal performance of Arctic fishes and whether they will be Influenced as much as tropical fishes in future warmer waters (Nati et al., 2021). The development of eurythermy and stenothermy depends upon adaption to specific climate zones (Pörtner & Farrell, 2008) and is fundamentally driven by the metabolic maintenance costs associated with the inhabited temperature regimes (Pörtner, 2001). In aquatic animals, whole animal oxygen consumption (MO2) is usually used as a proxy for integrated aerobic metabolism (Nelson, 2016). Therefore, it provides a valuable tool for the evaluation of global warming effects on ectotherms (McKenzie et al., 2016). During acute warming, salmonids increase cardiac output exclusively through increased heart rate (fH) and not stroke volume (Casselman et al., 2012; Farrell, 2009). As a result, maximum heart rate (fHmax) has been shown to correlate closely with optimum temperature, Topt (Casselman et al., 2012; Fry, 1947) and has therefore been proposed as a proxy for thermal sensitivity of aerobic scope (AS) in fish (Drost et al., 2014; Farrell, 2009). The Greenland halibut (Reinhardtius hippoglossoides) is an Arctic stenothermal fish with significant socio-economic value in the North Atlantic (Boje et al., 2014; Stortini et al., 2017) and will likely be subject to considerable environmental disturbance as a result of global warming (Dupont-Prinet et al., 2013; Ghinter et al., 2021; Pilet et al., 2016). Knowledge of the thermal sensitivity and physiological response to temperature change would thus be valuable to help predict future scenarios for this species (McKenzie et al., 2016) and add to the limited body of literature on responses to climate warming on stenothermal Arctic fishes.

The aims of the present study were to establish AS through conventional respirometry methods and derive Topt as a baseline metric for the thermal optimum for Greenland halibut. Further, the authors evaluated cardiac performance (fHmax) as a proxy to determine Topt by applying the Arrhenius breakpoint analysis as outlined by Casselman et al. (2012).

2 | MATERIALS AND METHODS

All animals were caught with permission from the Government of Greenland, fish-holding followed national guidelines and all experiments were approved by the Ministry of Fisheries, Hunting and Agriculture with permit reference 2970185.

2.1 | Husbandry

Fish for the metabolic rate experiments (MO2) [n = 28, body mass = 1052 ± 241 g (mean ± s.d.)] were caught by local fishermen at depths of 250–650 m on longlines baited with Capelin, Mallotus villosus, in Godthåbsfjord, Greenland (Nuuk, Greenland: 64° 10’ 53.076” N, 51° 41’ 38.896” W) from 27 April to 8 June 2016 (average water temperature at catch: 1.6°C, range: 1.1–2.1°C). Healthy animals apparently unjured by capture were kept at the shore-base in insulated fish tanks (1000 l) at 35°C for 24 h dark-regime. After the fish were transferred from the holding tank on the boat to the shore-based holding tanks, they were acclimated to 0, 2, 4 and 6°C for 5, 6, 7 and 13 days (average values), respectively, where water temperature was maintained at ±1°C by titanium aquarium heaters (AquaMedic GmbH, Bissendorf, Germany), controlled by Trixie Digital thermostats (Trixie, Tarp, Germany; www.trixie.de). The fish were not fed during the acclimation to ensure that all animals were in a postprandial state (Chabot et al., 2016). Fish were used in the respirometry trials as soon after acclimation as possible. The sample size for each temperature was n = 7.

Fish for the maximum heart rate experiment [n = 10, body mass = 1268 ± 300 g (mean ± s.d., length = 53.1 ± 4.8 cm)] were caught in Uummannaq Fjord (70° 40’ 35.967” N, 52° 7’ 52.538” W) in gillnets at depths ranging from 400 to 600 m [average water temperature at catch: 1.6°C (range: 1.1–2.1°C)]. Once removed from the gillnets, the trial fish were transferred to acclimation tanks onboard the scientific research vessel R/V SANNA (100 l per aerated seawater fish) maintained at 0°C. Fish were acclimated for at least 24 h before the experiment and not fed during this time.

2.2 | Oxygen consumption rates

Oxygen consumption (MO2) was measured on individual fish using intermittent flow respirometry (Steffensen, 1989; Svendsen, Bushnell,
Christensen, & Steffensen, 2016) in a rectangular PVC respirometer (25 × 60 × 9 cm) with a volume of 11.7 l (including the re-circulation loop) and a respirometer to fish volume ratio of c. 10, a preferable ratio due to the low expected MO2 and high O2 solubility of water at low temperatures (Svendsen, Bushnell, & Steffensen, 2016). The respirometer was immersed in a 70 l tank connected to a 350 l aerated, temperature-controlled water reservoir. Experimental temperatures were controlled by a programmable relay (PR-5714D, PR Electronics, Rønde, Denmark, ±0.1° C). Cooling was ensured by a HETO CBN –30 series cooling unit (Heto-Holten, Birkerød, Denmark; heto-holten.com), containing diluted ethylene glycol cooling liquid (1:4), controlled by a Conrad Universal thermostat (Universal Thermostat UT 200, ±0.1° C) (Conradaelectronics, Hirschau, Germany). The dissolved oxygen saturation level (% DO) inside the respirometer was measured every second using a fibre-optic oxygen sensor (Fibox 3, Precision Sensing GmbH, Regensburg, Germany) placed in the re-circulation loop. Zero calibration of the oxygen probe was performed in a 1:1 mixture of borax (sodium borate) and sodium thiosulfate, and 100% calibration was performed in air-saturated water.

MO2 experiments were automated using software (AquaResp 3.0; www.aquaresp.com, University of Copenhagen) with flush time set to 260 s, ensuring at least 95% water exchange in the respirometer, and a wait time of 80 s to ensure proper water mixing in the closed period before measurements commenced. The measuring time was set to 900 s to produce measurable, but not severe, declines in oxygen content within the respirometer during the measurement cycle (Svendsen, Bushnell, Christensen, & Steffensen, 2016). For example, at maximum metabolic rates oxygen content decreased by approximately 12%. Standard metabolic rate measurements typically produced a 4% decline.

To elicit a measure of maximum metabolic rate (MMR), the highest achievable metabolic rate under the given environmental circumstances, each fish was manually exercised (chased) to exhaustion (Reidy et al., 1995). The chase protocol ended after 1–2 min when the fish no longer reacted to tail pinches. It was immediately followed by a 5 min air exposure, after which the fish was quickly transferred to the respirometer to measure MMR, which was taken to be the first three measurements after introduction into the metabolic chamber. The fish was then left undisturbed in the respirometer to measure the standard metabolic rate (SMR). SMR in this context is considered the lowest amount of energy required to sustain homeostasis in a non-moving, post-absorptive fish (Beamish, & Henry, and P. S. Mookherjii, 1964; Chabot et al., 2016; Fry, 1971; Krogh, 1914).

Although there was almost no spontaneous activity noted, as can be very prevalent in other species, SMR was still determined using the double Gaussian method (Chabot et al., 2016). Metabolic rates fell to consistent, minimal, values c. 10–15 h post-introduction into the chamber. Nonetheless, SMR estimations were based on 20–50 h range data sets. SMR in this context considered the lowest amount of energy required to sustain homeostasis in a non-moving, post-absorptive fish (Beamish, & Henry, and P. S. Mookherjii, 1964, Chabot et al., 2016, Fry, 1971, Krogh, 1914).

### 2.3 Maximum heart rate

Maximum heart rate measurements were carried out in a polyethylene box (62 × 51 × 30 cm) filled with 20 l of aerated sea water. The temperature was maintained by an aquarium heater (300 W) and Cold finger chiller (Hetofrig, Birkerød, Denmark) controlled by a programmable relay (PR-5714D, PR Electronics). The core temperature of the trial fish was measured by inserting a thermometer probe rectally (PR-5714D) and logging the analogue output every second (Acqknowledge, version 4.1.1, Biopac Systems, Inc., Goleta, CA, USA; www.biopac.com). An electrocardiogram (ECG) was recorded by inserting two insulated steel wires (A-M Systems, Sequim, WA, USA) near the heart and amplifying the signal using a UIM 100EC amplifier (Biopac Systems, Inc.; www.biopac.com) connected to a Biopac MP-150 unit. Signals were amplified (5000×) and digitally filtered (50 Hz line filter band stop; low pass: 5 Hz; high pass: 7 Hz) and recorded at 200 Hz using Acqknowledge, version 4.1.1.

Before the experimental trials, the fish were anaesthetized in 5 l seawater-holding tanks containing 100 mg l⁻¹ of MS-222 (Sigma-Aldrich, St. Louis, MO, USA) for 3–4 min. Anaesthesia was confirmed when the fish became unresponsive to light tap pinches and could not sustain ventilatory movements. At that point the fish was immediately transferred to the experimental tank and placed blindside up. Ventilation was artificially maintained with a subsurface aquarium pump (EHEIM Universal 1005; Eheim, Deizisau, Germany; www.eheim.com), with the water being introduced into the oral cavity through a soft tube (gill irrigation: 4.5 l min⁻¹). The re-circulated sea water, initially at 0°C, contained 50 mg l⁻¹ of MS-222 for maintaining the anaesthetic state.

Once situated in the experimental setup and surgical implantation of thermal probe and ECG wires was completed, the heart rate of the fish was monitored for at least 1 h until it stabilized, after which the fish was given an intraperitoneal injection of 1.2 mg kg⁻¹ of atropine sulphate (Sigma-Aldrich) to block any vagal tone. After 15 min in equilibrium phase, 8 μg kg⁻¹ of isoproterenol (Sigma-Aldrich) was delivered intraperitoneally to stimulate cardiac adrenergic β-receptors and elicit maximum heart rate. All drugs were prepared in isotonic solutions (9% NaCl). Atropine was prepared daily, and isoproterenol was prepared immediately before use, as described by Casselman et al. (2012). Sufficient stimulatory doses used to establish fmax were determined from preliminary trials (n = 5). Heart rate did not increase further than 6% on average after drug administration.

Beginning at a water temperature of 0°C, acute warming of the fish was carried out by incrementally increasing the temperature every 15 min, as described by Casselman et al. (2012). The temperature increments were as follows: 0–5°C; 0.5°C increase; 5–10°C, 1°C increase; and 10–22°C, 2°C increase. The size range of the temperature intervals was based on the thermal optima of the Greenland halibut according to Vihtakari et al. (2021) and Boje et al. (2014), allowing the highest-temperature interval resolution near the predicted thermal optimum. For each incremental set point, an additional aquarium heater (100 W) was initially used for elevating water temperature
0.2–1°C. Variations in the rate of change in core temperature of a fish corresponded to an exponential exchange rate of heat between the mass of the organism (\(m_{\text{organism}}\)) and surroundings (Stevens & Sutterlin, 1976). Consequently, slightly overheating the experimental water tank (c. 0.5°C) initially resulted in a much-faster stabilization of the fish core temperature, as the mass of the fish decreased the temperature in the ambient water. Heart rate measurements were recorded when the core temperature of the trial fish was stabilized after c. 15 min. The experiment was terminated when bradycardia or cardiac arrhythmia occurred. At the end of the trial, the animal was euthanized by a blow to the head and by cutting the neural cord and dorsal aorta.

### 2.4 Analyses and statistics

Mass-specific oxygen consumption rates were calculated using the following equation:

\[
\text{MO}_2 = \frac{V_{\text{resp}} \Delta DO_2}{m_{\text{fish}} \Delta t}
\]

where \(\beta\) is the oxygen solubility (mg l\(^{-1}\)) at the given conditions (\(\beta\); based on Green & Carritt, 1967), \(m_{\text{fish}}\) is the mass of the fish (g), \(V_{\text{resp}}\) is the volume of the respirometer after the fish volume was subtracted and \(\Delta DO_2/\Delta t\) is the change in oxygen during the measurement period calculated from the slope of a linear regression based on the change in %DO\(_2\) over time.

SMR was determined by plotting MO\(_2\) measurements in a histogram and fitting them to a double Gaussian distribution, as described by Steffensen et al. (1994) and Chabot et al. (2016). MMR was determined as the highest MO\(_2\) reading within the first three measurement cycles after manually exercising the fish. AS was calculated as the difference between MMR and SMR (Bushnell et al., 1994).

The relationship between MO\(_2\) and temperature (\(T\)) was fitted with an exponential function for SMR and a quadratic function for MMR:

\[
\text{SMR}(T) = b \cdot e^{aT}
\]

\[
\text{MMR}(T) = a \cdot T^2 + b \cdot T + C
\]

The intersection between MMR and SMR was determined based on the fitted regressions: Equations 2 and 3.

AS in relation to temperature was fitted to a quadratic regression:

\[
\text{AS}(T) = a \cdot T^2 + b \cdot T + C
\]

The theoretical ideal temperature for AS, \(T_{\text{opt,AS}}\), was determined by finding the vertex from the quadratic fitted to AS in relation to temperature (Boje et al., 2014):

\[
T_{\text{opt,AS}} = -\frac{b}{2a}
\]

\[Q_{10}\] values for SMR (between the highest and lowest temperatures) and \(Q_{10}\) breakpoint temperature, \(T_{\text{AB}}\), were calculated from the equation, as described by van’t Hoff:

\[
Q_{10} = \frac{R_2}{R_1} \left( \frac{T_2}{T_1} \right)
\]

where \(R_1\) and \(R_2\) are equal to MO\(_2\) at the two following time series and \(T_1\) and \(T_2\) are equal to the given temperature between two temperature series.

Quadratic regressions, Equation (4), were fitted to AS of various eurythermal and fishes showing the comparison of AS as a function of increasing temperature (Figure 1c).

\(f_{\text{Hmax}}\) (bpm) was determined from manual identification of the inverse R–R wave peak interval, from the mean of a 10 heartbeat series, after equilibrium was established at the individual thermal intervals (Figure 2a). To determine \(T_{\text{opt}}\) for \(f_{\text{Hmax}}\), as defined by Casselman et al. (2012), the Arrhenius breakpoint temperature (\(T_{AB}\)), where \(f_{\text{Hmax}}\) ceases to increase exponentially (Ferreira et al., 2014, Hansen et al., 2017), was calculated by plotting log-transformed (ln) heart rates (bpm) against the reciprocal of absolute temperature (1000 K\(^{-1}\)). \(T_{AB}\) was determined by fitting two linear regressions to individual \(f_{\text{Hmax}}\) determinations, with the intersection representing the transition temperature for the exponential increase in \(f_H\) and a decrease beyond \(T_{\text{opt}}\) (Figure 2b) (Yeager & Uttsch, 1989). The best-fit regression for \(T_{AB}\) and all figures was determined using Python 2.7. Thermal influence on \(f_{\text{Hmax}}\) was determined at each temperature increment by calculating a \(Q_{10}\)-value (Equation 6).

Statistical significance was determined using one-way ANOVA and then a Bonferroni post-test for means of significance between groups (denoted letters in Figure 1a). Level of significance is \(\alpha = 0.05\). Statistical analysis was carried out using IBM SPSS Statistics, version 24 (Armonk, New York, USA; http://www.ibm.com/analytics/us/en/technology/spss/). A Student’s t-test was carried out for comparison of means between heart metrics; that is, Arrhenius breakpoint temperature (\(T_{AB}\)), the temperature at highest \(f_{\text{Hmax}}\) (\(T_{\text{max}}\)) and \(Q_{10}\) breakpoint temperature (\(T_{\text{Q10}}\)). Level of significance is \(\alpha = 0.05\).

### 3 RESULTS

All results are shown as mean ± s.d. unless otherwise noted.

#### 3.1 Oxygen consumption rates

The effect of temperature and MO\(_2\) shown in Figure 1a is summarized in Table 1. SMR increased with temperature over the measured
at 0°C to 56.4 ± 9.7 mg O₂ kg⁻¹ h⁻¹ at 2°C, increasing to 60.8 ± 9.8 mg O₂ kg⁻¹ h⁻¹ at 4°C and 51.4 ± 5.2 mg O₂ kg⁻¹ h⁻¹ at 6°C, significantly changing with temperature (P < 0.05) (one-way ANOVA, df = 27, F = 17.62), but there was no significant effect of temperature on MMR (one-way ANOVA, df = 27, F = 0.108; Figure 1a). The curve fit for MMR was MMR(T) = −1.07997² + 7.1305T + 47.973, with an r² of 1.

There was an overall significant effect of temperature on AS (one-way ANOVA, df = 27; P < 0.05; Figure 1b) between 2 and 6°C. AS(T) could be described by AS(T) = −1.2886T² + 6.2908T + 33.118, with an r² of 0.95. T_{opt}(AS_{max}) was determined as 2.44°C.

3.2 | Cardiac performance

All fish increased f_{Hmax} steadily from 9.9 ± 0.9 bpm at 0°C to 25.8 ± 2.2 bpm at 9°C, at which point several individuals showed signs of cardiac arrest (Figure 2c: connecting lines between data marks from 0 to 12°C). f_{Hmax} showed variations from 12°C up to the highest point at 22°C. The highest f_{Hmax} for each trial occurred before cardiac arrest, which was determined when individual fish lost heart rate equilibrium, that is, rapid decrease in f_{Hmax}. T_{AB} was found to be 5.6 ± 0.3°C and was calculated for each fish with an average of 19.4 ± 1.7 bpm, T_{EA} = 7.9 ± 1.3°C and T_{EB} = 14.5 ± 1.1°C (Figure 2d), all statistically different (df = 18, P < 0.05).

4 | DISCUSSION

T_{opt,AS} in Greenland halibut from two fjords in western Greenland was found to be 2.44°C. Whereas some species do not show a distinct T_{opt,AS}, for example, by a plateau in AS towards the critical thermal maximum (Christensen et al., 2021; Gräns et al., 2014), the T_{opt,AS} of Greenland halibut was clearly defined by a distinct decrease in AS towards higher and lower temperatures. These findings are in line with multi-variate kernel density modelling from pan-Arctic observations, as Vihtakari et al. (2021) present presence-only data, showing the spatial distribution of Greenland halibut from various depth strata (50–2000 m) across temperature regimes from −2 to 8°C. The size-based data set reveals that smaller specimens (<60 cm) were distributed on average (median values) <2°C, and larger fish (>60 cm) primarily >2°C, with substantial decreasing presence between 6 and 8°C, throughout West-, North- and East Atlantic and the Bering Sea (Vihtakari et al., 2021). Migration to various temperature regimes may reflect life stage and body mass changes (Christensen et al., 2020). Similarly, growth rates of juvenile Greenland halibut in Disko Bay are known to be enhanced by temperature, favouring growth optima at c. 2.5°C (Sünksen et al., 2010; Wheeland & Joanne Morgan, 2020). Moreover, average in situ occurrence temperature from temperature-logging tagging studies (Boje et al., 2014) on wild Greenland halibut shows a weighted daily temperature average of 2.46°C, based on 288 days in Disko Bay and 1062 days in Ilulissat Icefjord, with observed temperature occurrences between 1.15 and 2.85°C.

FIGURE 1 The aerobic scope of acclimated Greenland halibut. (a) MMR (maximum metabolic rate) (□) and SMR (standard metabolic rate) (■) in relation to temperature (n = 7, mean ± s.d.). Line fits are extrapolated between the estimated minimum and maximum possible acclimation temperatures. Letters denote significance when different (P < 0.05). (b) AS (●) in relation to temperature and the fitted curve between the estimated minimum and maximum possible acclimation temperatures. T_{opt}(AS_{max}) was determined as 2.44°C and is indicated by a solid vertical line. (c) Blue line: Greenland halibut (current study), purple line: Atlantic cod Gadus morhua (Tirsgaard et al., 2015), turquoise line: Pikeperch Sander lucioperca (Frisk et al., 2012). Black line: rainbow trout Oncorhynchus mykiss (Chen et al., 2015), red line: Green Chromis Chromis viridis (Habary et al., 2017)
The overlap between the authors’ estimated $T_{opt,AS}$ (2.44°C) and the in situ temperatures strongly indicates that $T_{opt,AS}$ closely evolved around the local habitat's temperature regime in Greenland halibut (Pörtner & Farrell, 2008). Therefore, the effects of temperature on AS can be assumed to be a reasonable proxy for the thermal sensitivity of the species.

The $f_{Hmax}$ metric derived in the present study, $T_{AB}$, for Greenland halibut was more than 3.2°C higher than $T_{opt,AS}$, which corresponds to about 30% reduction in AS compared to $T_{opt,AS}$. Farrell (2009) suggests the heart becomes the weak link for the cardiorespiratory oxygen cascade when salmonid species approach $T_{opt}$, based on the loss of cardiac output predominantly because of the effects on heart rate. This notion was supported by Casselman et al. (2012), who applied the $f_{Hmax}$ Arrhenius breakpoint analysis on salmonids and suggested it as a potential high-throughput technique for determining $T_{opt}$ in teleosts. Nonetheless, as the authors did not find a close link between $T_{AB}$ and $T_{opt,AS}$ in Greenland halibut, a stenothermic species, it would appear to challenge the generalization of using $T_{AB}$ as a reliable surrogate for thermal optima determination in all teleosts (Figure 2).

### Table 1

Aerobic metabolic parameters as a function of environmental temperatures: MO2SMR, MO2MMR and AS (means ± s.d.)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mean mass (g)</th>
<th>MO2SMR (mg O2 kg⁻¹ h⁻¹)</th>
<th>MO2MMR (mg O2 kg⁻¹ h⁻¹)</th>
<th>AS (mg O2 kg⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°C</td>
<td>950 ± 391</td>
<td>15.2 ± 16.3 (B)</td>
<td>56.4 ± 9.7 (A)</td>
<td>33.3 ± 8</td>
</tr>
<tr>
<td>2°C</td>
<td>1164 ± 132</td>
<td>16.3 ± 1.3 (B)</td>
<td>60.8 ± 9.8 (A)</td>
<td>40.1 ± 10.7</td>
</tr>
<tr>
<td>4°C</td>
<td>1066 ± 199</td>
<td>22.6 ± 4.6 (C)</td>
<td>60.8 ± 9.8 (A)</td>
<td>38.1 ± 12.8</td>
</tr>
<tr>
<td>6°C</td>
<td>1027 ± 182</td>
<td>27.1 ± 1.7 (C)</td>
<td>51.4 ± 5.2 (A)</td>
<td>24.3 ± 6.9</td>
</tr>
</tbody>
</table>

Note: Letters denote statistical significance ($P < 0.05$).

Abbreviations: AS, aerobic scope; MMR, maximum metabolic rate; SMR, standard metabolic rate.
The estimated absolute temperature range for AS of Greenland halibut was from $-1.89$ to $8.07\, ^\circ C$. Although acclimation to $8\, ^\circ C$ was not possible due to high mortality rates, the temperature limits where $AS = 0$ correspond to the onset of observed cardiac arrest during the $f_{\text{Hmax}}$ measurements starting to emerge at $8-9\, ^\circ C$ (red area in Figure 2c) and $T_{\text{opt}}$ at $7.9 \pm 1.3\, ^\circ C$, indicating the upper $T_{\text{crit}}$ to be c. $8\, ^\circ C$. Therefore, $AS = 0$ may represent a proxy for the critical thermal limits (Payne & Smith, 2017; Sunday et al., 2011) as the maximum substrate affinity of enzymes reflects the acclimation temperature of the organism, that is, the temperature of the environment (Hochachka & Somero, 1968; Somero & Hochachka, 1968). It is essential to recognize that the findings of this research are specific to the context of the acclimation process used. Acclimation plays a crucial role in preparing the fish for experimentation and ensuring that the results obtained are as accurate and reliable as possible. Nonetheless, there are inherent disadvantages and challenges associated with the acclimation processes. First, the duration of the acclimation period can significantly influence the fish’s physiological responses. A shorter acclimation time might not allow fish to fully adjust to the new environment, leading to potential stress responses that could affect the experimental results. On the contrary, longer acclimation periods are likely to introduce other confounding factors, such as stress from prolonged confinement, changes in social interactions and alterations in feeding behaviour. In this study, metabolic rate measurements were performed on temperature-acclimated fish, which allowed to assess their metabolic performance under controlled conditions. Nonetheless, heart rate measurements were conducted on acclimated fish exposed to an acute thermal challenge. The acute thermal challenge could provoke stress responses or other physiological changes that might not be observed under stable acclimation conditions.

Stenothermy arises as an adaptation to environmental stability (Pörtner, 2001). Due to the hydrological properties resulting from low temperatures, Arctic water has high oxygen content in comparison to temperate and tropical regions (Steffensen et al., 1994; Verberk et al., 2011). Reversible temperature acclimation characterizes the norm of temperate and tropical species; these species are distinctly adaptable and responsive to a seasonal shift in the thermal envelop. Therefore, true stenothermy may well reflect only the nature of cold-adapted species, due to the highly adapted metabolic scope characteristics (Pörtner, 2001).

Capacity for acclimation of tropical reef stenotherms is, similarly, likely to be limited. When exposed to temperature oscillations, the pomacentrid, Chromis virdis (Figure 1c), exhibits little or no plasticity of the thermal envelope and may exclusively act upon poleward population migration when relocating to new refuges (Habary et al., 2017).

The thermal range for AS of Greenland halibut is narrow compared to eurythermic teleosts, such as Pikeperch Sander lucioperca, Atlantic cod Gadus morhua and Rainbow trout Oncorhynchus mykiss (Figure 1c), demonstrating that Greenland halibut is a true Artic stenotherm likely making the species very susceptible to climate change (Nati et al., 2021).

Nonetheless, for Arctic stenotherms, range shifting in response to climate-driven increase in water temperature is severely limited as they already inhabit the edge of the poles themselves, with the southern populations already impacted and on the decline, for example, in the St. Lawrence (Ghinter et al., 2021). Given that climate change-related environmental warming is happening four times faster in the Arctic than in the tropics, true Artic stenotherms may be even more vulnerable to global warming than tropical stenotherms (Rantanen et al., 2022).

In Nuup Kangerlua (Godthåbsfjord), Greenland halibut occupy a relatively stable habitat where temperatures typically range between 1 and 3°C at depths greater than 300 m during the seasons (Mortensen et al., 2018). Nonetheless, the distribution of water masses in Davis Strait, where the presumed spawning grounds and the known important nursery areas for juvenile Greenland halibut are located, is influenced by two current systems: the West Greenland Current (WGC) and Baffin Island Current (BIC). The WGC contains warm and saline subpolar mode water in the deeper layers with temperature 2-4°C and salinity close to 35 ppt, also referred to as Atlantic water or Irminger water (Rysgaard et al., 2020). The BIC is colder (−1.8°C) and slightly fresher (34 ppt), containing water formed by winter convection events. An increase in temperature along West Greenland and in the Disko Bay around 1997 has been linked to variation in water masses (Mortensen et al., 2022). Species-specific physiological effects are causally linked to migratory behaviour observed in Greenland halibut (Morgan et al., 2013, Wheeland & Joanne Morgan, 2020), and such geographical displacement may squeeze habitat vacancy and reduce stock size. This study implies that variability in the ocean temperature could in turn have a major impact on fishery for Greenland halibut carried out locally from small open boats and dog sledges to large trawlers and longliners operating offshore. Indeed the Greenland halibut is an important socio-economic resource for local communities and constitutes a vital part of the economy in Greenland.

In summary, this study shows a close relationship between $T_{\text{opt,AS}}$ and in situ temperature occurrence of the Greenland halibut, showing that AS is a relevant, valuable metric for evaluating and predicting the future dispersal range of the species with environmental warming. Various analyses of $f_{\text{Hmax}}$ with increasing temperature did not reflect $T_{\text{opt,AS}}$, suggesting that cardiac performance is not the limiting factor during acute temperature increase for this ecototherm, as otherwise demonstrated for salmonid fish (Casselman et al., 2012; Farrell, 2009). Future research is certainly warranted to test for general and phylogenetical patterns of $f_{\text{Hmax}}$ as a proxy for $T_{\text{opt,AS}}$; similarly, investigations on an alternative limiting factor for the oxygen transport chain in fishes are needed.

AUTHOR CONTRIBUTIONS

A.R. directed the fieldwork and primary research in Nuuk, Greenland, and was responsible for writing the manuscript. M.B.S.S. carried out programming, prepared the figures and wrote the manuscript. R.N. contributed to planning and execution of the field- and laboratory work. E.A.F.C. was involved in writing the manuscript. P.G.B. contributed to laboratory work with A.R. on R/V SANNA and was involved in writing the manuscript. J.F.S. was involved in
planning and performing the laboratory work as well as writing the manuscript.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help and logistic support from our local fisherman Apollo in Nuuk, as well as the crew of R/V Sanna. We also express our gratitude for financial support from Marianne Rasmussen, Elisabeth and Knud Petersen Foundation, Solar Fondens at 1978 and Independent Research Fund Denmark to author J.F.S.

CONFLICT OF INTEREST

The authors have no competing interest to declare.

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