The effect of temperature on growth performance and aerobic metabolic scope of juvenile Arctic charr, *Salvelinus alpinus* (L.).

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Abstract

In recent years, Arctic char populations in Iceland have declined and the objective of this experiment was to elucidate these changes by examining the effect of temperature (5, 9, 13, 17 and 21 °C) on the survival, growth rate, metabolism, and physiological indices of juvenile Arctic char (initial mean body mass 4.02±0.8 g). During the 100-day study, mortality was 60% in fish reared at 21 °C, while at lower temperatures it was below 5%. However, Arctic char populations in Iceland are declining in locations where the ambient temperature is lower, suggesting that other factors may be more important in determining the abundance of the species. The $T_{\text{opt}}$ for growth was near at 14 °C. Growth rate was progressively reduced at supra-optimum temperatures with almost no growth at 21 °C. Indicators of energy reserves: condition factor, relative intestinal mass and hepatosomatic index are all consistent with reduced feed intake at supra-optimum temperatures. The standard and maximum metabolic rate (SMR; MMR), as well as the aerobic scope for activity (AS), were at maximum at 13 °C. The routine metabolic rate (RMR) increased exponentially with temperature and, at T21, it was equal to the MMR suggesting, that the RMR was limited by the MMR. Moreover, increased heart- and gill mass at 21 °C are consistent with increased stress on the cardiovascular system. These findings are in keeping with the OCLTT hypothesis that the thermal tolerance of fish is limited by the capacity of the cardiovascular system to deliver oxygen and support metabolism.

Taken together, the results of this experiment suggest that growth rate is reduced at supra-optimum temperatures because of reduced energy intake, increased metabolic demand, and limitations in the capacity of the cardiovascular system to support metabolic rate at high temperatures.

At lower temperatures, growth does not appear to be limited by the AS.
Introduction

Arctic charr has the northernmost distribution of all anadromous and freshwater fishes (Klemetsen et al., 2003). It is found in the Arctic, subarctic, boreal, and temperate regions, with natural populations as far south as the British Isles and in the Alps (Klemetsen et al., 2003). There is evidence that the distribution of the species is shifting due to climate change. Models predicting the future distribution of Arctic charr suggest that the southern distribution of Arctic charr will be pushed further north with global warming (Chu et al., 2005; Hein et al., 2012; Svenning et al., 2016).

Moreover, at least 11% of Arctic charr populations in Britain and Ireland are now extirpated (Maitland et al., 2007). The decline in Arctic charr populations appears to be occurring simultaneously in different parts of the distribution range and under different physical and ecological conditions since parallel decreases in the catches of Arctic charr have been observed in the UK (Winfield et al., 2010), Norway (Svenning et al., 2012) and Iceland (Winfield et al., 2010; Svenning et al., 2012) over the last 20 to 30 years. The reasons for this decline in Arctic charr and other salmonid populations are not entirely clear although they are likely linked to a progressive increase in ambient temperature (Elliott and Elliott, 2010; Jonsson and Jonsson, 2009), although it is difficult to establish causative links (Winfield et al., 2010).

The hypothesis of oxygen- and capacity-limited thermal tolerance (OCLTT) (Clarke and Pörtner, 2010; Pörtner and Farrell, 2008; Pörtner and Knust, 2007) was proposed to explain the effects of climate on the distribution of fish. It suggests that the upper thermal tolerance of fish is limited by oxygen supply, primarily due to the failure of the cardiovascular system to deliver oxygen from gills to tissues (Eliason et al., 2013; Farrell, 2009). The OCLTT draws on Fry’s paradigm (Fry, 1971; Fry, 1947), which suggests that temperature limits processes or activities, such as growth, through its effect on metabolic rate. The metabolic scope for activity or aerobic scope (AS) is the difference between the maximum
aerobic metabolic rate (MMR) and standard metabolic rate (SMR: The metabolic rate of resting unfed animals) (Fry, 1971) and reflects the capacity of the animal to sustain aerobic metabolic activity over and above the SMR. The AS increases up to a maximum (or a plateau) at the temperature optimum ($T_{opt}$) for aerobic scope and then decreases at supra-optimal temperatures in much the same way as the growth rate does (Brett and Groves, 1979; Clark et al., 2008; Eliason and Farrell, 2015; Farrell, 2009; Lee et al., 2003; MacNutt et al., 2006). The OCLTT suggests that reduced survival of fish at increased temperature is linked to reduced capacity to support the AS and, as a result, necessary metabolic functions (Pörtner and Farrell, 2008). However, the concept has been widely debated (Clark et al., 2013; Norin et al., 2014; Lefevre, 2016; Jutfelt et al., 2018).

The heat increment or the specific dynamic action (SDA) is the metabolic cost of digestion, absorption, and assimilation of nutrients and results in a near doubling of the SMR at peak levels (Beamish and Trippel, 1990; Jobling, 1981; Thorarensen and Farrell, 2006) following a meal. The metabolic cost of the SDA may limit the capacity of fish to perform other activities such as swimming (Alsop and Wood, 1997; Thorarensen and Farrell, 2006b). However, for long-term survival and growth, the AS must be large enough to accommodate the metabolic demands of the SDA. There appears to be a good correlation between the AS and growth rate of Atlantic cod at different oxygen levels (Claireaux et al., 2000) and in sea bass (*Dicentrarchus labrax*) at different temperatures (Claireaux and Lefrançois, 2007). Moreover, in many fish species, the $T_{opt}$ for AS coincides with the $T_{opt}$ for growth (Brett, 1976; Khan et al., 2014; Lefrançois and Claireaux, 2003) or feed intake (Brett, 1976; Khan et al., 2014; Mallekh and Lagardere, 2002). These results are consistent with Fry’s paradigm and may suggest that the growth rate of fish at different
temperatures may be limited through effects on the AS. However, correlations do not necessarily suggest causation and the shape of the curve for AS at different temperatures may resemble the effect of temperature on growth without any causative link.

Several studies have been conducted on the effect temperature on the growth and survival of Arctic charr. The $T_{\text{opt}}$ for growth of Arctic charr appears to lie between 12 and 17 °C (Imsland, Gunnarsson and Thorarensen, 2020; Elliott and Elliott, 2010; Gunnarsson et al., 2011; Jobling, 1983; Larsson and Berglund, 1998, 2005; Larsson et al., 2005; Lyytikainen and Jobling, 1998; Swift, 1964) depending, in part, on the size of the fish (M. Jobling, 1983; Joblin et al., 1993). Projections from a study of juvenile fish from 11 populations of Arctic charr acclimatized to 5-20 °C (Larsson et al., 2005) suggest that there is no growth at or above 21-22 °C and this is consistent with the finding of Thyrel et al., 1999) that Arctic charr juveniles acclimatized to 20-23 °C will cease feeding above 21-22 °C. The upper incipient temperature (the temperature at which 50% of fish can survive for extended periods) for juveniles is 22-24°C and the lethal temperature is 24-26 °C (Baroudy and Elliott, 1994; Elliott and Klemetsen, 2002; Lyytikäinen et al., 1997b). Arctic charr is more tolerant to low temperatures than other salmonids (Elliott and Elliott, 2010) and the lower incipient temperature for Arctic charr is less than 1°C (Siikavuopio et al., 2010). Arctic charr will grow at temperatures even as low as 0.3 °C (Brännäs and Linnér, 2000; Brännäs and Wiklund, 1992; Siikavuopio et al., 2010; Wandsvik and Jobling, 1982).

The relationship between temperature and metabolism has been studied in many fish species (e.g Claireaux et al., 2000; Lefrançois & Claireaux, 2003; Tirsgaard, Behrens & Steffensen, 2015a) including salmonids (Brett and Glass, 1973; Brett, 1976; Atkins and Benfey, 2008; Hvas et al., 2017). However, there is limited information available about the relationship between metabolic rate and temperature in Arctic charr. Huuskonen
et al. (2003) studied the effect of temperature on SMR at 2°C and 7°C in newly hatched Arctic charr. The metabolic rate and heart rate of Arctic charr have been measured in acute thermal challenge tests where the temperature is increased at a rate of about 2 °C-hour⁻¹ (Gilbert et al., 2020; Penney et al., 2014) The results of Gilbert et al. (2020) suggest that the aerobic scope of Arctic charr is constant between 4°C and 16°C although MMR increases linearly and the minimum metabolic rate increases exponentially in this temperature range. Furthermore, heart rate during thermal challenge peaks near 20 °C and, at higher temperatures, heart rate is reduced (Gilbert et al., 2020; Penney et al., 2014). This suggests that the cardiovascular system of Arctic charr may have problems coping with temperatures above 20 °C, at least when acutely increased.

Lyytikäinen and Jobling (1999) estimated the metabolic budget of juvenile Arctic charr at three constant temperatures (11°C, 14.4°C, and 17.7°C) and their results suggest that in this range the RMR of Arctic charr increases exponentially with increasing temperature. However, at the highest temperature, feed intake was reduced while the metabolic rate was increased and, as a result, the growth rate was reduced. Lyytikäinen and Jobling (1999) did not estimate the AS. The present study was undertaken to examine the effect of temperature on the relationship between survival, growth, and AS in Arctic charr and specifically to test the hypothesis that the growth performance of Arctic charr at different temperatures may be a function of aerobic scope.

Material and methods

Experimental fish

Juvenile Arctic charr (4.02±0.8g, mean body mass±S.D.) from an aquaculture strain were obtained from Holalax fish farm and brought to Verið research station in Sauðárkrókur, Iceland, where the experiments were performed. Fish were distributed at random among 25 circular tanks (17L),
40 - 46 fish in each tank. The water temperature in the tanks was 10 °C and the fish were given 15 days to acclimate to these conditions. After that, the temperature was changed to the target temperature of the treatments over three days. At the end of the experiment, fish were euthanized with an overdose of anesthesia (ethyleneglycomophenylether, 0.3mL·L-1, Ásgeir Sigurðsson ehf., Iceland). The experiment was conducted in compliance with the regulations of the Icelandic commission for animal welfare.

Control of oxygen saturation, temperature, and feeding

During the experiment, the fish were reared at five different temperatures for 96 days. The target temperatures were 5°C, 9°C, 13°C, 17°C and 21°C (T5, T9, T13, T17, T21) covering a large part of the temperature range for Arctic charr (Elliott and Elliott, 2010). The mean temperatures during the experiment were (mean±SD) 5±0.3°C, 9.2±0.3°C, 13.1±0.5°C, 17.4±0.4°C, and 21.3±0.5°C. Each treatment was tested in five replicates.

The fish were reared in freshwater, maintaining oxygen saturation close to 100%. The oxygen saturation was measured daily with a handheld oxygen meter (YSI 550A) in the tanks each morning before the fish were fed and adjusted by increasing the water flow rate if required. The total water flow into each tank was 1.5-3 L·min⁻¹.

The fish were fed extruded feed (1.0mm, 1.2mm, and 1.5mm) from Biomar, Denmark, and 1.8mm and 2mm from LAXÁ feed mill, Iceland adjusting pellet size as the fish grew. The feed contained 52% crude protein, 19% crude fat, and 9% crude ash. Feed was presented with automatic feeders that delivered continuously feed in excess (as indicated by the presence of feed remains on the bottom of the tanks) except on Sundays when the fish were not fed.
Growth

Before measurements, the fish were netted out of the rearing tanks and anesthetized. All fish were individually weighed and measured (fork length) to the nearest 0.01 g and 0.1 cm at the beginning of the experiment and then at four-week intervals until the end of the experiment after 96 days. The specific growth rate (SGR) was calculated as (%·day⁻¹):

\[
SGR = 100 \times \frac{\ln w_2 - \ln w_1}{t_2 - t_1}
\]

Where \(w_1\) and \(w_2\) are the body mass at the beginning \((t_1)\) and end \((t_2)\) of the growth period respectively.

The Fulton’s condition factor \((K)\) was calculated as:

\[
K = \frac{W \times 100}{L^3}
\]

Where, \(W=\)body mass of fish (g), \(L=\)Length of fish (cm).

The body-mass length relationship was calculated according to the formula:

\[
W = a \times L^b
\]

The hepatosomatic index (HSI) and relative intestinal mass (RIM) were calculated as indicators of the amount of energy reserves in the fish.

Furthermore, as indicators of stress on the cardiorespiratory system, the relative ventricular (RVM) and gill (RGM) masses were calculated as a percentage of the body mass from a sample of 20 fish at each temperature treatment:
Where organ and body mass are in g.

**Measurements of oxygen consumption**

All measurements of metabolic rate were performed during the last five weeks of the experiment alternating the measurements between treatments to reduce any potential effects of changes during time.

The standard metabolic rate (SMR) was measured in 9 fish with an automated intermittent flow respirometer (Svendsen et al., 2016). Food was withheld for 48h before the measurement. Three fish were netted at random out of the rearing tanks and placed in each of three horizontal chambers (ID 33-80 mm, Loligo System®) at their rearing temperature. The bacterial oxygen consumption was measured simultaneously in an identical chamber without any fish during the same period. The respirometers were cleaned after each measurement session. The oxygen consumption was measured by automatically closing the water inflow into the chambers for 30 minutes while simultaneously measuring oxygen concentration with OXY-4 mini oxygen meter probes (Loligo System). A side branch, continuously circulated water from the chambers over the probes, mixing the water in the tubes at the same time. During the time the water inflow was closed the oxygen saturation in the chambers was reduced from 100% down to 60-83% saturation. Such a decrease is harmless for Arctic charr (Beuvard et al., in preparation). After each measurement, the inflow was opened again to restore the oxygen levels. The oxygen concentration was recorded automatically with OXY4v2_11FB (Loligo Systems). Oxygen consumption was calculated from the slope of the curve of oxygen concentration (mg·L⁻¹) over time:
\[ \dot{M}O_2 = \frac{\Delta [O_2]_T/\Delta t - \Delta [O_2]_B/\Delta t}{\text{body mass of fish}} \times V \]

Where \( V \) is the net volume of water in the chamber, hoses, and pump (1.7L) excluding the volume of fish. The \( \dot{M}O_2 \) measurements were corrected by subtracting the background oxygen consumption \( \Delta [O_2]_B/\Delta t \) measured in the empty chamber from the \( \Delta [O_2]/\Delta t \) from the total oxygen consumption \( \Delta [O_2]_T/\Delta t \) in the chambers with fish.

When measuring SMR, the fish were placed in the chambers at 8 am before the measurements. The SMR was measured during the following night when the fish had settled in the chambers. The \( \dot{M}O_2 \) was measured once every hour for seven hours starting at 10 pm (Fig. 1). The mean \( \dot{M}O_2 \) was stable during the time of measurements in groups T5, T9, and T21, while in T13 it decreased towards the end of the period and in T17 it decreased progressively over the night (Fig. 1). For each fish, the three lowest values for \( \dot{M}O_2 \) with the highest R-squared (R\( \geq 0.99 \)) were used to estimate the SMR. Routine Metabolic Rate (RMR) and Maximum Metabolic Rate (MMR) were measured on groups of fish in the rearing tanks to reflect as well a possible the \( \dot{M}O_2 \) of undisturbed and fed continuously fish. Probes were placed in four tanks at each temperature. The measurements of RMR and MMR were performed one week before SMR measurements. The total number of fish measured was 195 fish for T5 and T9, 191 for T13, 179 for T17, and 82 for T21. The \( \dot{M}O_2 \) was measured by closing off the inflow into the tank and calculated from the drop in oxygen concentration as described above. This procedure was repeated three times for each tank with 24 hours intervals between measurements.
Since the tanks were not closed to air at the surface, tests were performed to estimate the amount of oxygen entering the water while $\dot{M}O_2$ was measured. First, the oxygen saturation was reduced to near zero by bubbling nitrogen gas through the water. Then the rise in oxygen concentration was measured over six hours. The diffusion of gasses from air to water is determined by Fick’s law: $V'_{\text{gas}} = A \times D \times \Delta P / T$, where $V'_{\text{gas}}$ is the rate of diffusion of gas across a permeable membrane, $D$ is the diffusion coefficient for the gas, $A$ is the surface area of the membrane, and $T$ is the thickness of the membrane and $\Delta P$ is the difference in partial pressure across the membrane. Other factors will also affect the $\dot{M}O_2$ measurements, such as the convective mixing of water in the tanks. The tests were performed under the same temperature conditions as the measurements of oxygen consumption and with the same placements of the probes. Therefore, all factors in the Fick’s equation, apart from $\Delta P$, were assumed to be equal during the tests and the actual measurements of $\dot{M}O_2$. The mean increase in $[O_2]$ during the six hours while the tests were performed was 0.25 mg·L$^{-1}$ (4 mmHg). The mean $\Delta P$ between air and water during the tests was 153 mmHg while the mean $\Delta P$ during the measurements of oxygen consumption was 30 mmHg. These calculations suggest that oxygen entering from the air would change the measurements of oxygen consumption by less than 4%.

The maximum metabolic rate (MMR) was similarly measured in the rearing tanks with all fish present, fed, and active. The fish were chased to exhaustion with a pipe for 5 minutes and at this point, most or all of the fish ceased to respond to prodding. The metabolic rate was measured immediately after that.
Statistical analyses

All statistical analyses were performed by using R version 3.2.0 (R Core Team, 2014). The initial and final mean body mass, length, and condition factor of the fish were compared with a nested one-way ANOVA (tanks nested as random factors within treatments) using the lme function of the nlme package in R (Pinheiro et al., 2017). The assumptions of normality and homoscedasticity of the data were confirmed with Q-Q-plots of residuals and by comparing the variances within treatments with Levene’s tests. The data for body mass was square-root transformed to conform to the assumptions of normality. The $T_{\text{opt}}$ for growth were estimated by fitting third order polynomials to the data for the mean increase in body-mass and length in different tanks. Third-order polynomials gave a significantly better fit ($p<0.0001$) than second-order polynomials (F-test) while fourth-order polynomials did not give a significant ($p=0.3$) better fit than the former. Furthermore, the third-order polynomial gave the lowest AIC values. The $T_{\text{opt}}$ for growth and the 95% confidence intervals (95%CI) were determined with non-parametric bootstrap methods using the Boot package in R (Angelo and Ripley, 2016). The relative organ masses and $MO_2$ were compared using a one-way ANOVA and the SGR was compared with a one-way ANOVA on the mean values for each tank. A Tukey’s HSD test was used for post hoc comparison of different treatments. Mortality was compared with a Kruskal-Wallis test and pairwise comparisons using Wilcoxon rank sum test with Benjamini and Hochberg adjustment for multiple comparisons.
Results

Mortality

The mortality was highest in the T21 group (Fig. 2) and from day 21, the mortality in this group was significantly higher than in other groups. (Kruskal-Wallis chi-squared = 14.2-16.5, df = 4, p-value = 0.002-0.005) (Fig. 2). At the end of the study, the mortality was 60% in T21 and less than 5% in all other groups. Most of the mortality in T21 occurred between days 45 and 70 (Fig 2).

Growth

Temperature significantly affected the growth of the fish (Fig 3). The final body-mass was highest in T13 and significantly higher than in all other groups (Table 1) similarly, the increase in body mass and SGR were highest in T13 and T17 (Table 1; Fig. 2a).

The mean increases in body-mass in T5 and T21 were 70% and 96% lower respectively than in T13 (Fig 3a). The growth in T9 was intermediate. The estimated optimum temperature for growth in body mass (Fig. 3a) was 14.3 °C (95%CI 13.7-14.9).

The mean increase in length was also highest in T13 (Fig. 3b). The increase in length in T5 and T21 was 46% and 8% of T13 respectively (Fig 3b). The estimated optimum temperature for growth in length (Fig. 2b) was 13.8 °C (95%CI 13.2-14.4).

The Fulton’s condition factor (K) increased progressively, albeit not significantly (p=0.2551, F=1.3623), from 5 to 17 °C while the lowest condition factor was in T21 (Table 1). The allometric relationship between body mass and length was estimated for all groups during the entire experiment (Table 1) and was not significantly different among groups T5, T9, and T13. However, the higher exponents (b) at T17 and T21 than
at lower temperatures indicate that the growth in mass relative to length was comparatively higher at the higher temperatures. Furthermore, the constants (a) for T17 and T21 were significantly lower than in the other groups (Table 1).

Temperature affected both indicators of cardio-respiratory stress, RVM, and RGM. The RVM was significantly different at different temperatures (p<0.0001, F=70.107). The mean RVM was highest in T21 (0.204%) and 48% higher than at 13 °C (0.138%) (Fig 4a). Furthermore, there was a significant positive correlation (R: 0.33; p=0.003) between temperature and RVM at temperatures between 5 to 17 °C although mean values were not significantly different (Fig. 4a). The RGM was also significantly affected by the temperature (p=0.1179, F=2.4873), decreasing progressively from 5 to 17 °C (by 20%) and then increasing by 24% from 17 to 21 °C (Fig. 4b).

The indicators of energy reserves, the hepatosomatic (HSI) index, and the relative intestinal mass (RIM) both suggested reduced energy reserves at higher temperatures. The HSI decreased significantly (p<0.0001, F=72.937) from 5 to 13 °C (Fig 4c), and similarly the RIM was reduced at elevated temperatures (p<0.0001, F=10.475), being significantly lower at either 17 or 21 °C than at 5, 9, and 13 °C (Fig 4d).

Table 1. The growth fish during the experiment, condition factor (K), and coefficients for the relationship body-mass = a × length\(^b\) of Arctic charr. Values shown in parentheses are standard errors of the means. Values labeled with different superscripts are significantly different.
<table>
<thead>
<tr>
<th>T°C</th>
<th>n</th>
<th>Initial body-mass</th>
<th>Final body-mass</th>
<th>Initial fork length</th>
<th>Final fork length</th>
<th>SGR</th>
<th>K</th>
<th>a</th>
<th>b</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>195</td>
<td>3.83(±0.04)b</td>
<td>9.87(±0.24)d</td>
<td>7.81(±0.03)c</td>
<td>10.15(±0.07)c</td>
<td>0.98(±0.05)c</td>
<td>0.886(±0.006)d</td>
<td>0.0035(±0.0002)a</td>
<td>3.42(±0.02)a</td>
</tr>
<tr>
<td>9</td>
<td>195</td>
<td>4.07(±0.05)a</td>
<td>17.06(±0.51)c</td>
<td>7.96(±0.03)a</td>
<td>11.86(±0.10)b</td>
<td>1.49(±0.03)b</td>
<td>0.908(±0.009)a</td>
<td>0.0035(±0.0002)a</td>
<td>3.42(±0.01)a</td>
</tr>
<tr>
<td>13</td>
<td>191</td>
<td>4.24(±0.06)a</td>
<td>23.95(±0.64)a</td>
<td>8.00(±0.03)a</td>
<td>13.12(±0.10)a</td>
<td>1.80(±0.03)a</td>
<td>0.94(±0.009)b</td>
<td>0.0033(±0.0002)a</td>
<td>3.45(±0.01)a</td>
</tr>
<tr>
<td>17</td>
<td>179</td>
<td>4.22(±0.06)a</td>
<td>20.71(±0.68)b</td>
<td>7.93(±0.04)b</td>
<td>12.18(±0.13)b</td>
<td>1.66(±0.05)b</td>
<td>0.963(±0.009)b</td>
<td>0.0027(±0.0002)b</td>
<td>3.55(±0.01)b</td>
</tr>
<tr>
<td>21</td>
<td>82</td>
<td>3.73(±0.05)b</td>
<td>4.69(±0.10)d</td>
<td>7.64(±0.03)c</td>
<td>7.96(±0.06)d</td>
<td>0.29(±0.06)d</td>
<td>0.837(±0.009)c</td>
<td>0.0017(±0.0002)c</td>
<td>3.78(±0.05)c</td>
</tr>
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</table>

**Oxygen consumption**

Temperature significantly (p<0.0001) affected SMR (p=0.004058, F=9.2191), MMR (p=0.03126; F=5.8267) and RMR (p<0.001, F=30.763) (Fig. 5). The SMR was highest at 13 °C and significantly higher than in either T5 or T9. The SMR in T17 and T21 was intermediate (Fig. 5). The MMR was also highest at 13 °C and significantly higher than at all other temperatures (Fig. 5). The MMR was significantly lower in T5 than at all other temperatures. The AS was also the largest in T13 and smallest in T5 (Fig. 5). The AS in T17 and T21 were not significantly different. The RMR increased exponentially with increasing temperature and in T21, the RMR was equal to the MMR and consumed the entire AS.

**Discussion**

*Effect of temperature on mortality*
This is the first study to measure simultaneously survival, growth, metabolic rate, and scope for activity of juvenile Arctic charr at different temperatures and provides novel insights into the capacity of the species to tolerate higher temperatures. High mortality was found only at 21°C, where 60% of the fish died during the experiment while mortality at temperatures of 17 °C or lower was under 5% (Fig. 2). These results are consistent with the findings of other studies showing that the upper incipient lethal temperature for juvenile Arctic charr is 19-21 °C, depending on the life stage and acclimation temperature (Baroudy and Elliott, 1994; Elliott and Klemetsen, 2002; Thyrel et al., 1999a). A similar pattern of protracted mortality was observed in Atlantic salmon reared at 23°C (Hvas et al., 2017). The thermal tolerance of juvenile Arctic charr appears to be similar for populations at the southern distribution range of the species in Lake Windermere, England (54°22’ N) (Baroudy and Elliott, 1994), at high latitudes (69–70° N) in Lake Inari, Finland (Lyytikäinen et al., 1997) and Fjellfrøsvatn, Norway (Elliott and Klemetsen, 2002) as well as in populations found between 63 and 68 °N in Sweden (Thyrel et al., 1999) and Iceland (present study). The population used in the present study comes from an aquaculture breeding program but still has similar thermal tolerance as other populations of Arctic charr throughout the distribution range. These findings suggest that populations from the entire distribution range of Arctic charr show little or no specific adaptions of the traits tested for temperature tolerance. The protracted mortality over time and the fact that some of the fish survived the experiment suggests that there is a significant individual variation in thermal tolerance of juveniles Arctic charr. However, the similarities in thermal tolerance of populations at the northern and southern borders of distribution suggest that the phenotypic plasticity of the Arctic charr allows them to
adequately cope with the range of temperatures to which it is exposed in its natural distribution range. In contrast, different populations of sockeye salmon (*Oncorhynchus nerka*) appear to show adaptations to their thermal environment (Eliason et al., 2011).

It is of interest to consider the decline in Arctic charr populations in Iceland and other Nordic countries (Malmquist et al., 2009; Reist et al., 2006; Svenning et al., 2016; Svenning et al., 2012) in light of these results. Detailed, long term temperature records exist from the shallow lake Elliðavatn (64°05’16”N 21°46’37”W), which has comparatively high summer temperatures for Iceland. These records show that while the lake temperature has increased over the past 30 years, it rarely reaches 18-21 °C and then only for few consecutive days each summer (Malmquist et al., 2009).

The findings of the present and other studies (Baroudy and Elliott, 1994; Elliott and Klemetsen, 2002; Lyytikäinen et al., 1997b; Thyrel et al., 1999a) suggest that Arctic charr should be able to tolerate these temperatures for short periods without significant mortality. Nonetheless, the catches of Arctic charr in the lake and the abundance of juveniles have decreased by 70% since 1987 (Malmquist et al., 2009). The same trend is observed in several other anadromous and resident charr populations in Iceland (Guðbergsson, 2017; Thordardottir and Guðbergsson, 2017) living at even lower summer temperatures. This suggests that the temperature-dependent mortality observed in the present study is not contributing to the decline in Arctic charr populations in Iceland. However, thermal tolerance at other life stages may be important, such as the thermal limits for egg and embryonic development, which are lower than the limits for growth or survival of juvenile and adult fish (Gillet, 1991; Jobling, 1996; Van Der Kraak and Pankhurst, 2008; Olk et al., 2019). Egg survival of Arctic charr is negatively correlated with summer and autumn temperatures (Jeuthe et al., 2015; Jeuthe et al., 2013), and similarly, temperatures above 5-8 °C during the last weeks before spawning can reduce
Egg quality (Gillet, 1991). Furthermore, the disease and parasite tolerance of Arctic charr at higher temperatures may also contribute to the decline in charr populations (Bruneaux et al., 2017; Karvonen et al., 2010). Interestingly, there is no evidence of food shortage causing the reduction in the Arctic charr population in Elliðavatn since the k and stomach content of the Arctic charr have not changed in recent years (Malmquist et al., 2009).

**Effect of temperature on growth**

The optimum temperatures for growth in body mass and length were both near 14°C (Fig. 3) and this is in accord with results from previous studies on the effect of temperature on Arctic charr that have reported optimum temperature for growth between 13°C and 17°C (Elliott and Elliott, 2010; Gunnarsson et al., 2011; Jobling, 1983; Jobling et al., 2010; Larsson and Berglund, 1998, 2005; Larsson et al., 2005; Lyytikäinen et al., 1997a; Siikavuopio et al., 2013; Swift, 1964; Thyrel et al., 1999b). As for the temperature tolerance discussed above, these findings suggest that few if any specific adaptations with regards to growth at different temperatures in Arctic charr populations from different latitudes. Results from a comprehensive study of the growth of 11 Arctic charr populations from watercourses between 54 and 70° N suggests no major differences in the shape of temperature growth curves of the populations and no evidence of significant counter gradient in growth potential depending on latitude (Larsson et al., 2005). The only indication found of adaptive variation in growth potential was related to life-history characteristics and diet (Larsson et al., 2005).
At supra-optimum temperatures, the growth rate fell precipitously with increasing temperature, and, at 21 °C, there was little or no growth (Fig. 3). Feed intake could not be directly measured in the present experiment, however, results from other studies suggest that feed intake of Arctic charr decreases progressively as temperature increases above the optimum and near 21-22 °C they do not feed at all (Larsson and Berglund, 2005; Lyytikäinen and Jobling, 1999; Lyytikäinen et al., 1997a; Thyrel et al., 1999a). The lack of growth in T21, as well as indications of lower energy reserves (k, RIM, and HIS; Table 1; Fig. 4c, d), are all consistent with feed intake being progressively reduced above \(T_{opt}\).

**Oxygen consumption**

The measurements of oxygen consumption provide an interesting insight into the energy budgets of the fish. Here, we first discuss the validity of the methods used. The SMR was measured using common techniques for respirometry (Chabot et al., 2016). However, the measurements of RMR and MMR were performed in open tanks and this method may be prone to errors due to the diffusion of oxygen from the air to water. However, the tests we performed under identical conditions without any fish present suggested that this error is minimal (<4%). A second factor that could have introduced an error is insufficient mixing of the water in the tanks while the inflow was turned off (Rodgers et al., 2016). Insufficient mixing can affect the outcome of the measurements by decreasing the apparent volume of the tanks if all the fish are aggregated in a certain area. As a result, the position of the probe and the distribution of the fish can cause errors in measurements that either increase or decrease the apparent RMR and MMR. If the fish were evenly distributed through all parts of the tanks, the error would have been minimal. The density of the fish in the tanks, when the metabolic rate was measured, was rather high (17-59 kg·m\(^{-3}\)) and observations of the tanks showed that
the fish were fairly evenly distributed. Besides, the spontaneous activity of the fish facilitates mixing (Rasmussen et al., 2005). Therefore, we suggest that any errors introduced into the measurements of RMR and MMR due to lack of mixing were minimal. The measurements of the MMR were performed by chasing all the fish and then measuring the MMR in the tanks after most or all of the fish had ceased to respond to prodding. A more common method to estimate MMR is to measure each fish individually either in a swim tunnel or in a chamber after chasing (Norin and Clark, 2016). Here we measured MMR in the tanks with spontaneously active fish without the added stress of removing them from the tanks or exposing them to an alien environment in a swim tunnel or metabolic chamber. In a separate study (Nelson and Thorarensen, 2018), conducted on similar size Arctic charr from the same source, the MMR was measured in swim tunnels following a constant acceleration test (CAT). The MMR (5.6 mg·min⁻¹·kg⁻¹) at 5 °C was 28% higher than measured in the present study (4.36 mg·min⁻¹·kg⁻¹) while, at 13 °C, the MMR was 48% lower (6.7 mg·kg⁻¹·L⁻¹) than estimated in the present study (12.6 mg·min⁻¹·kg⁻¹). This comparison suggests that the MMR estimated in the present study did not introduce a systematic error that either under- or overestimated the variables and we suggest that they provide a valid estimate of RMR and MMR. Moreover, the measurements of RMR and MMR at different temperatures are comparable since all were performed under identical conditions.

The values for metabolic rate obtained in this study correspond well with those of other studies on Arctic charr and salmonids. The SMR increased progressively with temperature although a small peak was evident at 13 °C (Fig. 5). Similar increases in SMR with temperature have been observed in studies on other fish species (Brett, 1976; Brett, 1971; Claireaux and Lagardère, 1999; Khan et al., 2014; Lefrançois and Claireaux, 2003; Mallekh
Studying larger (747 g), seawater-acclimated Arctic charr in a swim tunnel at 10.5 °C, Penney et al. (2014) found a lower SMR (1.4 mg·min⁻¹·kg⁻¹) than the present experiment (2.3 mg·min⁻¹·kg⁻¹ for 17 g fish), however, these differences are likely primarily due to scaling effects on metabolic rate and adjusting the values with a mass exponent of 0.8-0.85 (Clarke and Johnston, 1999; Killen et al., 2007) yields similar SMR. The rise in RMR with temperature was much more pronounced than the rise in SMR (Fig. 5). Similar increases in RMR with temperature have previously been reported in other salmonid species (Brett, 1971, 1979; Hvas et al., 2017) and Arctic charr at higher temperatures (Lyytikainen and Jobling, 1999). The RMR measured in the present experiment was similar to that reported in other studies on slightly larger (90-105 g) Arctic charr (Christiansen et al., 1991; Jorgensen et al., 1993; Lyytikäinen and Jobling, 1999) at similar temperatures when corrected with a mass exponent of 0.8-0.9 (Killen et al., 2010; Killen et al., 2007; Norin and Gamperl, 2018). Lyytikäinen and Jobling (1999) measured the RMR of Arctic charr of similar size at 11, 14.4, and 17.7 °C. At 11 and 14.4 °C, the RMR measured in the present study (extrapolating values between temperatures) was 27% and 36% higher than that reported by Lyytikäinen and Jobling (1999) while at 17 °C, the difference was less than 6%. Furthermore, the proportional increases in RMR between temperatures were similar in both studies. The similarities between the metabolic values reported in the present and other studies on Arctic charr lends further credence to the results of the present study.

Metabolic rate and growth
The results of this study shed an interesting light on the potential relationship between RMR, MMR, AS, and the growth of fish. Previous studies have indicated that the growth of Atlantic cod and seabass may be limited by the AS (Chabot and Claireaux, 2008; Claireaux and Lefrançois, 2007). Consistent with this proposition, the growth, MMR, and AS all peaked at 13 °C (Fig. 3a,5), and from 5 to 13 °C, the growth rate was positively correlated with AS ($R: 0.93$). However, a significant correlation does of course not necessarily indicate that the growth was limited by the AS. For the AS to limit growth, it would have to limit and/or protract the maximum SDA in postprandial fish. Digestion and absorption are a process that may take hours after a meal and, in aquaculture fish, it may be nearly continuous. Therefore, the SDA may be limited by the ability to sustain high metabolic rates over long periods in contrast to the MMR which is a measure of short-term (minutes) capacity (Norin and Clark, 2016). The sustainable metabolic output of fish is lower than the MMR (Beamish, 1978). For example, Hvas and Oppedal (2017) found that while Atlantic salmon post-smolts could only maintain $U_{\text{crit}}$ for less than 30 minutes, they were able to swim for over 4 hours at 80% of $U_{\text{crit}}$. This suggests that the MMR (and AS) could be reduced without limiting the sustainable energy output. For example, the results of Beuvard et al. (in prep.) show that at 9 °C, the AS of Arctic charr is reduced when oxygen saturation is reduced from 100% to 60% without any effect on growth. In the present study, we found that the metabolic demands of the RMR between 5 and 13 °C only consumed 8-50% of the AS suggesting that growth is unlikely limited by the AS at temperatures of 13 °C and below.

The growth of the charr was reduced by 93% when the temperature was increased from 17 to 21 °C (Fig. 3a) while the AS was unchanged (Fig. 5). However, the RMR increased exponentially with temperature and more so than the SMR (Fig. 5). A similar increase in RMR or maintenance
ration with temperature has been observed in other studies on salmonids (Brett, 1976; Hvas et al. 2017). At 21 °C, the RMR consumed all the AS and was equal to the MMR. This may suggest that, at 21 °C, the RMR is limited by the MMR. Furthermore, this may also suggest that growth was limited by the MMR (or AS) at this temperature if the maximum SDA was reduced and/or protracted. It is not clear why the RMR increases proportionately more than the SMR, but Brett (1979, 1976) suggested that this could be due to reduced efficiency of metabolic processes or feed processing as temperature increases.

The concept of scope for growth “the difference between the energy of the food an animal consumes and all other energy utilization and losses” was originally coined by (Warren and Davis, 1967) and later elaborated by Brett (1976, 1979). It describes the energy available for anabolic processes as the difference in energy intake and energy consumption (and losses). Therefore, it is better suited to predict growth than the concept of scope for activity and Fry’s paradigm, which only addresses catabolic processes. It is interesting to consider the results of the present study in the context of scope for growth. They and results of other studies on Arctic charr (Lyytikäinen and Jobling, 1999) suggest that as temperature increases above $T_{opt}$, feed intake is reduced and this would reduce the scope for growth. At the same time, the RMR increased exponentially which would reduce the scope of growth even further as the energy consumption increases. Therefore, the reduced growth at supra-optimum temperatures is consistent with the concept of scope for growth. At 21 °C, there was no growth suggesting that the fish were eating no more than maintenance rations. One of the factors contributing to the high mortalities at 21 °C may have been that some of the fish were not even able to acquire enough energy through feed and/or sustain the metabolic rate required to support maintenance functions. In
summary, the results of the present study may identify three factors that all may contribute to the reduced growth at supra-optimum temperatures: (1) Increased metabolic demands of the fish (RMR), (2) Reduced feed (energy) intake (3) Inability of the cardio-respiratory system to support the increasing energy demands of the fish. Of course, these factors may be interrelated and are not mutually exclusive. For example, reduced feed intake may be important to limit the high RMR when the temperature increases. As mentioned above, Arctic charr appears to require the full capacity of the MMR (or AS) to support aerobic metabolism at 21 °C. Results from thermal challenge tests on Arctic charr (Gilbert et al., 2020; Penney et al., 2014) show arrhythmias and reduced heart rate above 20 °C which is also consistent with the cardiovascular system being challenged to its maximum. The MMR of salmonids depends on the capacity of the cardiorespiratory system to deliver oxygen (Gallaugher et al., 2001; P. Gallaugher et al., 1995a) and the results of the present study suggest that there was added stress on the cardio-respiratory system at 21 °C. The increased RVM at 21 °C (82% larger than in T5) indicates physiological adaptations to increase oxygen delivery to allow higher maximum cardiac output (Gamperl and Farrell, 2004). The RVM was also positively correlated with temperature below 21 °C and this is consistent with our previous results on Arctic charr (Ruiz and Thorarensen, 2001). This is uncommon among salmonids where RVM tends to decrease as temperature increases (Gamperl and Farrell, 2004; Anttila et al., 2015). The difference between Arctic charr and other salmonid species may relate to the fact that it is adapted to survive and grow at lower temperatures than other salmonids and, therefore, the increased temperature may pose a comparatively greater challenge to the cardiovascular system of Arctic charr. The RGM was reduced as temperature increased from 5 to 17 °C. However, there was a significant increase in RGM from 17 to 21
°C (Fig. 4b). This suggests that the charr acclimated to a high temperature by increasing the gill area to facilitate trans-branchial diffusion. Therefore, both the changes in heart and gill mass may reflect physiological changes aimed at supporting the increased RMR at high temperatures. These findings are consistent with the prediction of the OCLTT hypothesis (Pörtner and Farrell, 2008) that the upper incipient temperature for charr is limited by the capacity of the cardiorespiratory system to deliver oxygen to tissues. However, as described above, this limitation does not appear to be a factor contributing to the decline of Arctic charr populations in Iceland which is occurring at lower temperatures.

Conclusion

The results of the present study provide an interesting insight into the effect of increased ambient temperatures on the survival and growth of juvenile Arctic charr. The results suggest that up to 17 °C, the temperature has a limited effect on the survival of Arctic charr juveniles. The optimum temperature for the growth of Arctic charr juveniles in body mass and length is near 14°C. The results of the present and other studies suggest that temperature tolerance and the optimum temperature for the growth of Arctic charr throughout the distribution range of the species is similar. Arctic charr populations in Iceland, and other countries, have declined recently. However, current ambient temperatures in Icelandic freshwater systems are unlikely to cause mortalities in juvenile fish. As the temperature is increased above supra-optimum levels, the growth of Arctic charr is reduced. The results of this study suggest that three factors could contribute to the reduced growth as temperature increases: (1) Reduced feed intake, (2) increased metabolic rate, and (3) the capacity of the cardiorespiratory system to sustain metabolism.
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4. References


