

Compromised thermal tolerance of cardiovascular capacity in upstream migrating Arctic char and brown trout—are hot summers threatening migrating salmonids?

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†Author contribution: K.A. and T.K. conceived and designed the experiments. T.K. arranged the field sampling for the fish and he also caught the fish. G.M. and K.A. conducted the cardiovascular measurements. G.M. performed the molecular level analyses and she did the data analysis and drafted the manuscript. All authors read, contributed to, and approved the final manuscript.

Heat waves are threatening fish around the world, leading sometimes to mass mortality events. One crucial function of fish failing in high temperatures is oxygen delivery capacity, i.e. cardiovascular function. For anadromous salmonids, increased temperature could be especially detrimental during upstream migration since they need efficiently working oxygen delivery system in order to cross the river rapids to reach upstream areas. The migration also occurs during summer and early autumn exposing salmonids to peak water temperatures, and in shallow rivers there is little availability for thermal refuges as compared to thermally stratified coastal and lake habitats. In order to shed light on the mechanisms underpinning the capacity of migrating fish to face high environmental temperatures, we applied a physiological and molecular approach measuring cardiovascular capacities of migrating and resident Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in Northern Norway. The maximum cardiovascular capacity of migrating fish was significantly lower compared to the resident conspecifics. The onset of cardiac impairment started only 2°C higher than river temperature, meaning that even a small increase in water temperature may already compromise cardiac function. The migrating fish were also under significant cellular stress, expressing increased level of cardiac heat shock proteins. We consider these findings highly valuable when addressing climate change effect on migrating fish and encourage taking action in riverine habitat conservation policies. The significant differences in upper thermal tolerance of resident and migrating fish could also lead changes in population dynamics, which should be taken into account in future conservation plans.

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Introduction

Current trends in climate change are threatening animals all around the world, and the situation is especially alarming for ectothermic animals that cannot regulate their body temperature. Fish die-offs in freshwater habitats have, for example, already been associated with extreme summer conditions (Farrell, 2009; Till *et al.*, 2019). The situation could worsen in the future since climate variability is predicted to increase and actually pose a greater risk to species than directional climate change (Donelson *et al.*, 2018; Vasseur *et al.*, 2014). In order to act and assess conservation policies the upper thermal limits of species need to be known. The limits, however, are species specific (Beitinger *et al.*, 2000) and could also depend on the life stage of the animal (Pörtner & Farrell, 2008). At the individual level the limits are defined by functional capacities of different organ systems (Moyano *et al.*, 2020; Pörtner, 2002).

One of the functional capacities that temperature limits is circulatory oxygen delivery to tissues, i.e. function of cardiovascular system. For instance, modulation in heart rate (f_H) with temperature change happens rapidly in fish, making it well suited to measure when assessing the influence of temperature on cardiac function (Eliason and Anttila, 2017). In salmonids, increasing temperatures have, for example, been shown to lead to an impairment of the maximum heart rate (f_{Hmax}) when body temperature is above the optimum of the species and approaching its upper thermal limits (Anttila *et al.*, 2014; Eliason *et al.*, 2011; Steinhausen *et al.*, 2008). Impairment in f_{Hmax} will hinder the oxygen delivery to tissues and could eventually lead to failure in function of different organ systems. There are many physiological and biochemical interpretations on why the cardiac function fails beyond certain threshold (Haverinen & Vornanen, 2020; Iftikar *et al.*, 2015; Rodnick & Gesser, 2017; Vornanen *et al.*, 2014) but the mechanism becomes more complicated when contextualized in natural conditions where several biotic and abiotic factors are changing simultaneously. In salmonids, the threat from extreme summer temperatures could be especially high during the upstream migration (Eliason *et al.*, 2011). This life stage is energetically demanding because migrating fish need to swim upstream and through rapids and they are at limits of their aerobic performance already under normal summer temperatures (Farrell, 2009; Eliason *et al.*, 2011). Moreover, migration of salmonids occurs during summer and, therefore, entails entering to relatively warm water with little to no availability to thermal refuges in contrast with thermally stratified coastal and lake habitats (Bjornn and Reiser, 1991). Increased temperature may threaten the aerobic performance of the fish during this crucial life stage (Pörtner & Farrell, 2008) and lead to significant mortalities of migrating fish due to cardiac failure (Eliason *et al.*, 2011; Eliason *et al.*, 2013; Farrell, 2009).

Physiological failure given by sudden temperature increase is the consequence of equally compromised cellular function. It is well known that cell structure and activity are modified

as function of temperature (Somero, 2020). An exposure of organisms to a temperature several degrees higher than normal characteristically elicits the synthesis of a set of proteins termed heat shock proteins (HSPs) (Dietz and Somero, 1993). The main role of those chaperone family is to provide the cell protection by preventing aggregation or improper folding of proteins (Currie and Tufts, 1997), i.e. counteracting the potential detrimental effect of increasing temperature. However, their function might vary based on their molecular weight, and some of them are constitutively expressed during cellular homeostasis. Expression of HSPs is widely used to assess the level of cellular stress in thermal biology studies, therefore observing their pattern of expression in the cardiac cell might help to describe thermally related cardiac failure.

In this study we assessed the upper thermal limits, function of cardiovascular system and the cardiac cellular stress of two sympatric, partly anadromous, salmonid species, Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in Northern Norway (Fig. 1). The f_{Hmax} during temperature increase and temperature where fish get cardiac arrhythmias (T_{arr} , the upper thermal limit for normal cardiovascular performance) were analysed from wild char and trout at the beginning of their upriver migration and compared to their resident fresh-water conspecifics. Furthermore, we measured the levels of different HSPs to estimate the cellular stress these fish might be experiencing. The aim of the project was to evaluate the vulnerability of migrating and resident salmonids to extreme thermal episodes. For conservation purposes we are providing extremely important information since species and various life history strategies might have different capacities to respond to heat waves, leading to possible changes in ecosystem structure.

Methods

Sampling procedures and experimental set-up

Fish ($n=38$) sampling and testing were conducted during August 2017 from a river and lake system, in Botnvatnet water basin, Rognan, Norway (67.093, 15.514) (Fig. 1). Immature char ($n=19$) and brown trout ($n=19$) were caught either from the river Botnelva (67.09718, 15.47450; 67.09718, 15.47450) = *migrating fish* or lake Litlevatnet (67.097587, 15.484781) and Botnvatnet (67.097587, 15.484781) = *resident fish* using sport fishing gear (Fig 1.). Immediately after catching, the fish were transported in a 60-L bucket to measurement site at lakeshore and kept maximally 12 hours in a cage located nearby the lakeshore before performing measurements (Fig. 1). The experiment was approved (FOTS ID 13035) by the Norwegian Food Safety Authority under the regulation of the Research Animal Act (FOR-2015-06-18-761). The entry of fish from river to lake was closed with net and trap system starting from end of June 2017 (Davidsen *et al.*, 2019), thus, fish caught

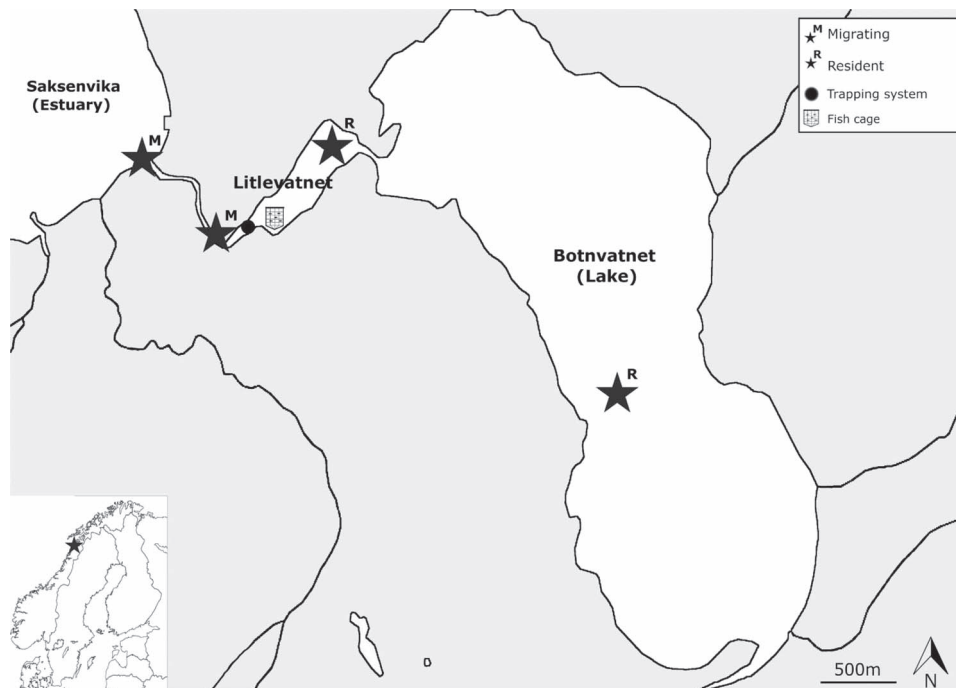


Figure 1: Botnvatnet water basin. Sampling area of migrating and resident Arctic char and brown trout. Black stars indicate the location where migrating (M) and resident (R) conspecific were caught.

from lake represented resident population that had been in lake the whole summer while fish caught from river were at early phases of migration (caught maximally 200 meters from fjord to upstream). Water temperature was monitored using temperature data loggers (DST milli-CT, www.star-oddi.com) in the lake during the sampling period. Data loggers in the lake were placed at 1 m depth. This environmental temperature, therefore, represent the maximal temperature where the fish were exposed to at lake habitat (maximal acclimatization temperature for resident fish). The temperature at the beginning of July was around 6°C and increased gradually to around 10°C at beginning of August when the experiments were conducted (Supplementary Figure 1; Monsen, 2019). The river temperature was 12.3°C at the time of the experiments (the river receives water just from the surface layer of the lake). The sea temperature (where the migrating fish were maximally acclimatized to) was reported by Davidsen *et al.*, (2019). Their loggers were also located at the 1 meter depth, thus, representing the maximal environmental temperatures the fish were exposed to before migration. According to Davidsen *et al.*, (2019) at the beginning of the July the sea temperature was around 10°C and increased gradually to 15°C at beginning of August (see Fig. 2 in Davidsen *et al.*, 2019 report, page 15).

Arrhythmia temperature (T_{arr}) and maximum heart rate (f_{Hmax}) was measured from 32 resident and migrating fish ($n=10$ migrating char, length: 29.8 ± 1.4 cm (mean \pm SD); $n=7$ resident char, length: 27.1 ± 2.8 cm; $n=7$ migrating

trout, length: 26 ± 3.1 cm; $n=8$ resident trout, length: 18.1 ± 1.8 cm) following method by Casselman *et al.*, (2012). Each fish was initially anesthetized with 100 ppm MS-222 (buffered with sodium bicarbonate, pH=7.0) and weighed before being placed in the experimental chamber with temperature-controlled water circulation. The water in the system was from the lake and it was kept at 10°C with Julabo chiller–heater (F32 ME, Julabo GmbH, Seelbach, Germany). The water was constantly aerated and contained 60 ppm buffered MS-222 keeping the fish under anaesthesia during the measurements. Water flow inside the chambers was directed over the gills of the fish. The electrocardiogram (ECG) recording was performed using silver electrodes touching the skin of the fish. A Grass 7 D amplifier (Astro-Med, Brossard, QC, Canada) amplified the ECG signal that was recorded with BioPac MP100 data acquisition system (BIOPAC System, Inc., Santa Barbara, California). The fish were allowed to recover from handling for 30 minutes. In order to achieve the maximum heart rate, fish received an intraperitoneal injection of atropine sulphate 1.2 mg kg^{-1} (Alfa Aesar™, Fisher Scientific Oy, Vantaa, Finland) and 15 minutes later isoproterenol $4 \text{ } \mu\text{g kg}^{-1}$ (Sigma-Aldrich, Darmstadt, Germany) injection. Fifteen minutes after the isoproterenol injection, water temperature was increased with 1°C steps every 6 minutes (i.e. 10°C h^{-1}) until first cardiac arrhythmia was observed (QRS complex or P wave was missing, Fig. 2), indicating arrhythmia temperature, T_{arr} . As shown by Casselman *et al.*, 2012, preliminary experiments with different heating rates (i.e. 2, 5 and 10°C h^{-1}) resulted



Fig. 2: ECG of a rhythmic vs arrhythmic heart rate.

in identical f_{Hmax} changes. Therefore, the $10^{\circ}\text{C h}^{-1}$ heating rate was adopted for the current experiment. Once this endpoint was reached, the fish were quickly removed from the experimental chamber and sacrificed with cranial percussion. Length and sex of the fish were recorded and ventricle was quickly removed and frozen with liquid nitrogen. Thereafter ventricles were kept in -80°C before further measurements. Six fish got immature arrhythmias after injections before heating procedure started and were removed from heart rate setup without cardiac measurements. The ventricles of these were also removed and processed as stated above.

HSP determination

Samples of trout and char ventricle (~ 15 mg) were taken and weighted, while the tissue was submerged in liquid nitrogen. Samples were homogenized in 6 volumes of lysis buffer (62.5 mM Tris-HCl, $1 \mu\text{g m}^{-1}$ leupeptin, $1 \mu\text{g m}^{-1}$ pepstatin, 1 mM PMSF, pH 6.8) using TissueLyser (Qiagen, Hilden, Germany) at 30 shakes s^{-1} for 2 min. Lysates were kept on ice and centrifuged at 5100 g for 10 min at 4°C . The supernatants were mixed with Laemmli buffer and denatured for 7 min at 95°C (Laemmli, 1970). To determine protein concentration, BCA Protein Assay was performed using serial dilution of bovine serum albumin ($1-10 \text{ mg ml}^{-1}$) as a standard (Thermo Scientific, Rockford, IL, USA). Spectrophotometric measurements were performed at 570 nm using a Wallac EnVision 2103 Multilabel Reader (Perkin Elmer, Turku, Finland). For Western blot, 20 μg of protein from each sample was loaded onto a TGX Stain-Free™ FastCast™ Acrylamide gels, 12% (BioRad, Cat#1610185). Proteins were separated by size at 200 V for 35–40 min. Thereafter the gels were scanned from total protein analyses with ChemiDoc MP Imaging System (Biorad, Hercules, CA, USA) (Fig. 3 g–i). From the gels the proteins were transferred to a Whatman nitrocellulose membrane, pore size $0.45 \mu\text{m}$ (Perkin Elmer, Boston, MA, USA), at 100 V for 1 h at $+4^{\circ}\text{C}$ and then incubated in Tris-buffered saline (TBS) blocking solution containing 5% non-fat powdered milk. Membranes were incubated overnight with rabbit polyclonal heat shock cognate 70 (HSC70) (ab137808) primary antibody (1:10000) (Abcam, Cambridge, UK), mouse monoclonal HSP90 beta (ab53497) primary antibody (1:1000) (Abcam, Cambridge, UK) or rabbit polyclonal anti-salmonid inducible HSP70 (AS05061A) primary antibody (1:10000) (Agrisera, Vännäs,

Sweden) in TBS-0.1% Tween-5% milk at $+4^{\circ}\text{C}$. Thereafter, membranes were incubated in TBS-0.1% Tween-5% milk with 1:5000 StarBright™ Blue 700 Goat Anti-Rabbit IgG (Biorad, Hercules, CA, USA) secondary antibody for the detection of HSC70 and HSP70, and 1:10000 of IRDye® 800CW Goat anti-Mouse IgG (Licor, Lincoln, NE, USA) secondary antibody to detect HSP90. After TBS-0.1% Tween membrane washing, the bands were visualized at 700 or 800 nm in ChemiDoc MP Imaging System (Biorad, Hercules, CA, USA) (Fig. 3 d–f). Densitometry was performed using ImageLab. Each gel contained gel loading control sample to account gel-to-gel variation in calculations. For estimating the relative protein levels of HSP70, HSP90 and HSC70, their band intensities were divided with total protein gel band intensities, i.e. giving HSP or HSC levels per total protein amount of samples.

Data and statistical analyses

Before analysing differences among populations and species the equal variances of measured parameters were tested with Brown–Forsythe test. Normality of residuals was tested with Shapiro–Wilk and Kolmogorov–Smirnov tests. Differences in arrhythmia temperatures, HSP and HSC levels among species and life history strategy (i.e. migrating or resident, M&R) were tested using two-way Analysis of Variance (ANCOVA) using length of the fish as covariate and followed with Tukey post hoc test if differences were noted. The length of the fish was used as covariate since it differed significantly among the group ($P < 0.001$). If ANCOVA assumptions were not satisfied, a Kruskal–Wallis one-way ANOVA on Ranks was performed and multiple comparisons were tested with Dunn’s method. Repeated measured of three-way ANCOVA was used to estimate differences in maximum heart rates among species and M&R in different temperatures. The statistical analyses were performed in temperatures between 10 to 16°C since after 16°C only 2 migrating char (out of 10) and 3 migrating trout (out of 7) were having rhythmic heart rate and rest of the fish were already removed from measuring setup. Meanwhile, for the study of HSPs, we considered also fish having a rhythmic heart rate after 16°C . Values are expressed as mean \pm SE. All the statistical analyses were performed using SigmaPlot14 (SyStat Software, San Jose, CA, USA) or IBM SPSS Statistics 24 (IBM corp. ©). Values showing a $P < 0.05$ were considered statistically significant.

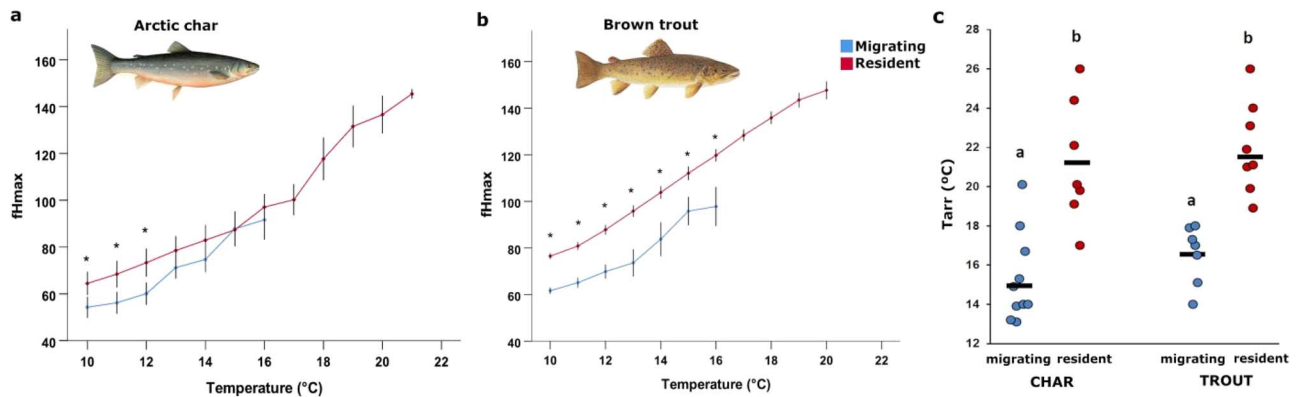


Fig. 3: Thermal capacity of the cardiovascular system of fish. Effect of temperature on maximum heart rate (f_{Hmax}) of migrating and resident (a) Arctic char and (b) brown trout. The values are expressed in beats per minute (bpm). * indicates statistically significant differences between resident and migrating fish. The f_{Hmax} values differed also significantly between species the char having lower heart rate ($F = 33.2$, $P < 0.001$). (c) Scatterplot of the arrhythmia temperature (T_{arr}) of the two species caught from different sites expressed in degree Celsius ($^{\circ}C$). Different letters indicate statistically significant differences between sites and species at $P < 0.05$. $n = 10$ for migrating char, $n = 7$ for resident char, $n = 7$ for migrating trout and $n = 8$ for resident trout. Values are shown as mean \pm SE.

Results

Physiological analyses showed that both species had extremely low upper thermal limits of their cardiovascular system at the beginning of return migration meanwhile the resident fish showed significantly higher cardiac capacity under high temperature (Fig. 3). The migrating char and trout got cardiac arrhythmias at $14.9 \pm 0.8^{\circ}C$ and $16.5 \pm 0.9^{\circ}C$, respectively (Fig. 3c, Table 1). The resident fish, on the other hand, reached arrhythmia at significantly higher temperatures, i.e. at $21.2 \pm 1.2^{\circ}C$ and $21.9 \pm 0.8^{\circ}C$ for char and trout, respectively (Fig. 3c, Table 1). Maximum heart rate in migrating char and trout between measuring temperatures of 10 – $16^{\circ}C$ was on average 9 and 19 beats per minute (bpm) lower compared to their resident conspecifics, respectively (Fig. 3a,b, Table 1). Furthermore, resident char showed on average ~ 18 bpm lower maximum heart rate than resident trout ($P < 0.001$) and migrating char had on average ~ 8 bpm lower f_{Hmax} than migrating trout ($P = 0.01$).

Regarding the cellular stress response, the HSP regulation differed among the groups analysed, and was observed to be differently expressed among life history strategies (M&R) and species. Specifically, when considering the HSP70s, differences between strategies were especially seen in char, where migrating ones showed higher expression of HSP70s compared to their resident conspecific (Fig 4a, Table 1). Meanwhile, species-specific expression pattern was observed merely in migrating fish, with the migrating char having 3.7-fold higher expression of HSP70s as compared to migrating trout ($P < 0.001$). There was not a statistically significant interaction between M&R and species ($P = 0.063$).

On the other hand, the expression pattern of HSP90 was found to be significantly upregulated in trout compared with char (Fig 4b, Table 1). The post hoc test revealed higher

expression of HSP90 in trout compared to char at migration stage ($P = 0.008$). However, no significant differences were seen between species in resident fish. There was not a statistically significant interaction between species and M&R in the expression of HSP90 ($P = 0.443$).

When considering the expression level of HSC70, we also found species-specific differences (Fig 4c, Table 1). Specifically, resident char showed higher level of HSC70 compared to resident trout ($P = 0.009$), meanwhile no differences were observed between the two species during migration ($P < 0.165$) or between migrating fish and resident ones ($P < 0.341$). No statistically significant interaction was observed between species and M&R in the expression of HSC70 ($P = 0.234$).

Discussions

In the current study both species had extremely low upper thermal limits of their cardiovascular system at the beginning of upstream migration when they would need efficiently working oxygen delivery system most to be able to complete the migration. River temperature was around $12.3^{\circ}C$ when the measurements were made and the majority of migrating fish got arrhythmias around 14 – $16^{\circ}C$, thus, only $+2$ – $4^{\circ}C$ increase of river temperature might be sufficient to initiate a mechanism of cardiac impairment. Interestingly, the resident fish had significantly higher cardiovascular performance and thermal tolerance (21 – $22^{\circ}C$) than migrating fish. These results were also reflected at cellular level since in general the migrating fish had much higher levels of HSPs indicating that migrating fish were under cellular stress. All of these results together indicate that climate warming and heat waves might result in higher detrimental effect on migrating fish,

Table 1: Temperature of arrhythmia (T_{arr}), maximum heart rate (f_{Hmax}) and HSPs (HSP70, 90 and HSC70) F and P -values across the different strategies (migration = M; resident = R) and species and their interaction. Since the size of the fish in groups differed significantly the length of the fish was used as covariate in all the analyses. All the F - and P -values presented in table are corrected for fish size as covariate. The F - and P -values of the fish size as covariate were $F = 52.8$ and $P < 0.001$. Statistically significant value are indicated in bold.

	M x R		CHAR x TROUT		Interaction	
	F	P	F	P	F	P
T_{arr} (°C)	33.3	<0.001	0.5	0.48	0.2	0.70
f_{Hmax}	33.2	<0.001	33.2	<0.001	5.7	0.02
HSP70	5.2	0.006	22.4	<0.001	3.7	0.06
HSP90	1.3	0.27	8.3	0.007	0.6	0.44
HSC70	0.9	0.34	9.3	0.004	1.5	0.23

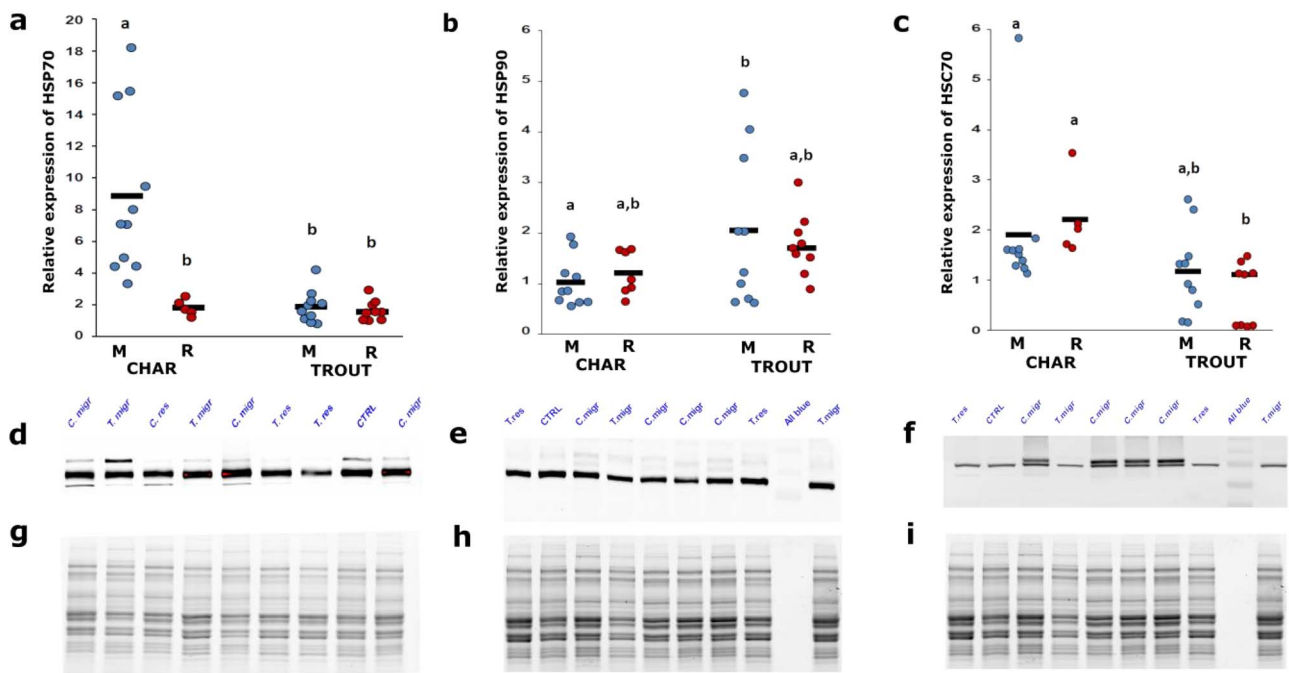


Fig. 4: Cellular stress in migrating and resident fish. Scatterplots show the relative expression of (a) HSP70, (b) HSP90 and (c) HSC70 in migrating Arctic char and brown trout caught from different sites. Different letters indicate statistically significant differences between sites and species at $P < 0.05$. (d–f) are representative examples of western blot membranes showing different densities of bands among species and sites. T. res = resident trout; T. migr = migrating trout; C. res = resident char; C. migr = migrating char; CTRL = gel control. All Blue = molecular weight standard. (g–i) are examples of stain free gels used for total protein normalization of the protein of interest. $n = 11$ for migrating char, $n = 7$ for resident char, $n = 10$ for migrating trout, $n = 9$ for resident trout.

while resident conspecifics are much more resilient against heat waves. If the climate continues warming as predicted and frequency and intensity of heat waves increase there might be a shift in life history strategies towards more resident freshwater population compared to migrating ones. Resident fish have been shown to grow slower because they are not utilizing the wealth of food sources that migrating fish have in sea (Nevoux *et al.*, 2019). This was seen also in the

current study since resident trout were smaller than migrating ones (no statistically significant differences between resident and migrating char). The change in life history strategies towards resident populations could, therefore, result less in productivity. This might have a negative impact not only at ecological level, but also on cultural aspects, as Arctic indigenous people's lifestyle has been based on river productivity of salmonids for several centuries. How fast the temperatures

will increase in future in rivers is, however, an open question and probably will depend, e.g. on the depth of the river, i.e. are there cool refuge areas available. The thermal limits of the different life history strategies need, however, to be taken into account when making predictions how climate warming will influence on these Northern salmonids.

The factors leading to cardiac failure during acute increase of the temperature are many and span from physiological to biochemical level (Steinhausen *et al.*, 2008; Moyes and Ballantyne, 2011; Vornanen, 2017). Generally, the onset of arrhythmia does not immediately correspond to a fatal episode for a fish, but if temperature increases any further the frequency and duration of arrhythmias increases which will become lethal (Gollock *et al.*, 2006). Moreover, arrhythmias are commonly observed near upper critical temperatures in fish (Heath and Hughes, 1973; Gollock *et al.*, 2006; Clark *et al.*, 2008; Eliason and Anttila, 2017). This has numerous implications at the ecological level because when fish reaches upper critical temperatures the aerobic performance declines rapidly, leading to decline in the aerobic swimming performance in salmonids (Eliason *et al.*, 2011; Eliason and Anttila, 2017). The results of the current study suggest that especially migrating char might be negatively influenced by even mild heat wave since they have (i) extremely low f_{Hmax} to support the aerobic swimming performance, (ii) extremely low upper thermal tolerance of cardiac performance and (iii) they were under severe cellular stress during migration. The f_{Hmax} values of the char in the current study were somewhat lower than the ones of the char from Canadian Arctic (Gilbert *et al.*, 2020) while the thermal tolerance of the char in Canada (cardiac arrhythmia at around 21.4°C) was close to thermal tolerance of the resident char in the current study (21.2°C). The char in Greenland, on the other hand, got cardiac arrhythmias between 11 and 18°C (Hansen *et al.*, 2017). The small differences between the studies could be due to, e.g. different heating rates or using anesthetized fish. Nevertheless, the current and previous studies suggest that char might be negatively influenced by even mild heat wave (Penney *et al.*, 2014; Hansen *et al.*, 2017; Gilbert *et al.*, 2020). Indeed, decline in char population densities has been already reported (Elliott and Elliott, 2010; Svenning *et al.*, 2016). Although in the current study the trout had also low thermal tolerance at beginning of migration and T_{arr} of species were not statistically different, the physiological and molecular results suggest that trout might have somewhat higher capacity to respond to heat waves. In the future, this could lead to changes in population structures in rivers and lakes that the species coinhabit and influence the whole ecosystem structures, which has been already shown (Svenning *et al.*, 2016). The results of the current study imply that greater emphasis should be given on species-specific mechanism of thermal response when addressing riverine conservation policies.

Interestingly, resident fish had much higher thermal tolerance having also higher heart rates. The reasons are currently unknown but could be due to change in the salinity gradient

in migrating fish during this energy demanding and crucial life stage. Migrating fish must adjust their physiology to a reversed osmotic gradient (Makino *et al.*, 2007; Groot, 2010; Evans *et al.*, 2011) during the upriver migration leading to a modification of the transcriptome (Evans *et al.*, 2011; Tseng & Hwang, 2008; Uchida *et al.*, 1997) which will consume energy. This could possibly influence negatively their cardiovascular capacities. Indeed, significant changes have been observed in function of cardiovascular system when fish have been moved from one salinity to other (Morgenroth *et al.*, 2019). Future studies should focus on whether migrating fish are suffering from osmoregulatory dysfunctions/modifications that adversely affect fish cardiovascular and swimming ability, and thus lead to premature mortality (Lapointe *et al.*, 2003). Moreover, possible increase of gene expression due salinity change (Evans *et al.*, 2011; Tseng & Hwang, 2008; Uchida *et al.*, 1997) might explain the higher HSPs level found in migrating fish, since this class of protein is produced not only under a thermal stress, but also for support the mechanism of correct folding for several other proteins during translation (Currie and Tufts, 1997).

Besides salinity change obviously some other factors could explain the differences between the groups. One of these could be the size differences of the fish. The resident trout were significantly smaller than migrating trout and similar trend was also observed in char. Previously, it has been shown that smaller fish have higher upper thermal tolerance as compared to their larger conspecifics (Underwood *et al.*, 2012). However, in the previous study the size differences have been hundreds of grams between individuals leading to 2°C differences in upper thermal tolerance, thus, size difference of few grams in the current study cannot solely explain ~6°C difference in upper thermal tolerance of resident and migrating fish. Third factor possibly influencing the results could be the thermal history of the fish as acclimatization will have significant influence on thermal tolerance of the fish (see review from Eliason & Anttila 2017). However, in the current study the fish in lake habitat, which had the high upper thermal tolerance, have been exposed to somewhat cooler temperature than fish at sea & river, thus, thermal history/acclimatization cannot possibly explain the differences between groups. However, since fish have capacity to acclimatize to high temperatures (Eliason & Anttila 2017) there might be a change for the migrating fish to increase their upper thermal tolerance if the temperature in the sea will rise in similar phase as the temperature in shallow rivers. Further studies are, therefore, warrant about both the acclimatization effects and how salinity could influence the upper thermal tolerance of migrating fish.

In the current study the HSPs profile was measured in order to assess a generalized stress response in migrating and resident fish and to our knowledge, this is the first study in which HSPs profile has been characterized in wild salmonid hearts in relation to cardiac performance during migration period. Our results revealed that both species were under severe cellular stress during upstream migration, and that

could reflect the impairments at the functional level. The presence of higher level of HSPs in migrating fish compared to the resident conspecific (HSP70 especially in char, while in migrating trout the HSP90 levels were high) might be due to higher stress in those fish. This is because HSPs synthesis is initiated following exposure to stress (Richter et al., 2010; van Oosten-Hawle et al., 2013). So far the HSP70 and HSP90 have been largely used to characterize heat stress responses (Lund et al., 2002; Metzger et al., 2016; Place & Hofmann, 2005) but other environmental stressors could induce their expression as well. For example in the study conducted by Evans et al., (2011) on wild migrating sockeye salmon, gene expression profile showed elevated induction of molecular response towards genes mostly related to temperature, salinity and pathogens exposure, with HSP70 being among the most expressed ones. Furthermore, osmotic stress has been shown to increase *hsp90* mRNA expression in Atlantic salmon (Palmisano et al., 2000), with this increase corresponding also in a coupling of transcription and translation of HSP90 (Pan et al., 2000). Thus, the high levels of HSPs in migrating fish could be due to high temperature that fish might encounter during the migration, but also the salinity gradient that those individuals have to face in this period. We cannot find any physiological reason why increased HSP90 response in trout was absent in char, but we hypothesize that the stress response, i.e. the pattern of expression of the HSPs, might be species-specific. Besides HSP70, migrating char had higher expression of constitutive for of HSC 70 (HSC70) than migrating trout and the polyclonal HSC70 antibody recognized two isoforms in char (Fig 3f). HSC70 acts as an important chaperone in non-stressed cells (Oksala et al., 2014). Its role in stressed-cell response is still nuanced (Fangue et al., 2006), but few studies had showed that its expression increased under higher temperatures in fish (Deane and Woo, 2005; Fangue et al., 2006; Oksala et al., 2014). Char having two isoforms and in general high HSC70s levels could possibly be related to their lower upper thermal tolerance (Penney et al., 2014) compared to brown trout. However, more studies are required to answer this question and to evaluate do the different isoforms have different functional roles in char.

The aim of the current study was to reveal if migrating and resident Arctic char and brown trout have similar cardiovascular capacities to respond to summer heat waves and does the species differ from each other. The research is urgent since frequency of heat waves are predicted to increase in future and mass mortality events of fish are already connected to high summer temperatures (Till et al., 2019). Our results show that early stages of upriver migration are one of the most sensitive stages in an anadromous salmonid lifespan, and that even slight increase of water temperature during this stage leads to a cardiac impairment which could result compromised swimming capacity and reduced probability for the char and trout to reach the lake which should be studied in future. Moreover, at the physiological and molecular level, thermal tolerance is impaired in migrating fish most probably because the fish are facing multiple energy requiring challenges simultaneously, i.e. migration and osmotic stress leading to cellular stress

response. However, further studies are warrant to reveal the molecular mechanisms why the fish using different migration strategies react differently to heat waves. Our results show that conservation plans should take account the low thermal tolerance of migrating salmonids and action could be taken by, e.g. building cool refuge areas. Furthermore, when making the conservation policies the physiology of the fish and differences between species and life-history strategies need to be addressed.

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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