

## RESEARCH ARTICLE

# The upper temperature and hypoxia limits of Atlantic salmon (*Salmo salar*) depend greatly on the method utilized

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## ABSTRACT

In this study, Atlantic salmon were: (i) implanted with heart rate ( $f_H$ ) data storage tags (DSTs), pharmacologically stimulated to maximum  $f_H$ , and warmed at  $10^\circ\text{C h}^{-1}$  (i.e. tested using a 'rapid screening protocol'); (ii) fitted with Doppler® flow probes, recovered in respirometers and given a critical thermal maximum ( $\text{CT}_{\text{max}}$ ) test at  $2^\circ\text{C h}^{-1}$ ; and (iii) implanted with  $f_H$  DSTs, recovered in a tank with conspecifics for 4 weeks, and had their  $\text{CT}_{\text{max}}$  determined at  $2^\circ\text{C h}^{-1}$ . Fish in respirometers and those free-swimming were also exposed to a stepwise decrease in water oxygen level (100% to 30% air saturation) to determine the oxygen level at which bradycardia occurred. Resting  $f_H$  was much lower in free-swimming fish than in those in respirometers (~49 versus 69 beats  $\text{min}^{-1}$ ) and this was reflected in their scope for  $f_H$  (~104 versus 71 beats  $\text{min}^{-1}$ ) and  $\text{CT}_{\text{max}}$  (27.7 versus 25.9°C). Further, the Arrhenius breakpoint temperature and temperature at peak  $f_H$  for free-swimming fish were considerably greater than for those tested in the respirometers and given a rapid screening protocol (18.4, 18.1 and 14.6°C; and 26.5, 23.2 and 20.2°C, respectively). Finally, the oxygen level at which bradycardia occurred was significantly higher in free-swimming salmon than in those in respirometers (~62% versus 53% air saturation). These results highlight the limitations of some lab-based methods of determining  $f_H$  parameters and thermal tolerance in fishes; and suggest that scope for  $f_H$  may be a more reliable and predictive measure of a fish's upper thermal tolerance than their peak  $f_H$ .

**KEY WORDS:**  $\text{CT}_{\text{max}}$ , Thermal tolerance, Heart rate, Hypoxia tolerance, Data loggers, Bradycardia

## INTRODUCTION

Global sea surface temperatures are expected to rise by  $\sim 1.5^\circ\text{C}$  in the next two decades (IPCC, 2022), and this will have a significant impact on many marine ectotherms, including fishes (Gamperl et al., 2020; Genin et al., 2020; Laufkötter et al., 2020; Pinsky et al., 2019; Reid et al., 2019; Viglione, 2021). In addition, an increase in the frequency and severity of storms and weather events (Bender et al., 2010) will increase temperature variability (Frölicher et al., 2018; Solomon, 2007; Szekeres et al., 2016; Viglione, 2021), and these temperature fluctuations present challenges for both wild and farmed fish.

Temperature has been described as the 'abiotic master factor' for fishes (Brett, 1971) as it controls/limits behaviour and physiological functions (Fry, 1947). Given that fish are ectotherms, and that their body temperature is largely dependent on that of the water they live in, changes in temperature impact many aspects of their biology, health and welfare (Alfonso et al., 2021; Brett, 1971, 1979; Gamperl et al., 2020; Oppedal et al., 2011; Pörtner and Farrell, 2008; Tromp et al., 2018; Wade et al., 2019). Fish have a preferred thermal range at which growth and performance are maximum, and large deviations from this range have detrimental impacts including decreased appetite (and thereby growth), reproductive fitness, increased stress, and temperature-related mortality (Alfonso et al., 2021; Burke et al., 2020; Dahlke et al., 2020; Gamperl et al., 2020; Reid et al., 2019).

Limitations in performance beyond a species-specific thermal optimum ( $T_{\text{opt}}$ ) have been linked to constraints in the capacity of aquatic organisms to meet their oxygen demands, as depicted in the widely recognized, but also controversial (Clark et al., 2013a,b; Jutfelt et al., 2014; Lefevre, 2016; Norin et al., 2014), oxygen- and capacity-limited thermal tolerance (OCLTT) theory originally described by Pörtner (Pörtner, 2002, 2010; Pörtner et al., 2017) which is based on Fry (1947). With regards to meeting the demands of increased temperature and determining a fish's upper thermal tolerance, an increase in heart rate ( $f_H$ ) plays a key role in increasing oxygen delivery to the tissues (Farrell et al., 2009; Wang and Overgaard, 2007) as: increases in cardiac output ( $\dot{Q}$ ; the amount of blood pumped by the heart per minute) with temperature are achieved solely by increases in  $f_H$  [i.e. stroke volume ( $S_V$ ), the amount of blood pumped per heart beat, is largely unchanged; Clark et al., 2008; Farrell and Smith, 2017; Gollock et al., 2006; Mendonça and Gamperl, 2010; Steinhausen et al., 2008; Stevens et al., 1972]; and recent studies suggest that increases in  $f_H$  are critical to the survival of fish at high temperatures when water oxygen levels are decreased (i.e. when experiencing hypoxia) (Gamperl et al., 2021; Leeuwis et al., 2021). Indeed, it has been suggested that cardiac ( $f_H$ ) failure is functionally, and more ecologically, relevant than the temperature at which the fish loses equilibrium (i.e. reaches its critical thermal maximum,  $\text{CT}_{\text{max}}$ ) (Sidhu et al., 2014).

Given concerns about the effects of increasing ocean temperatures and heat waves on fish populations (Lefevre et al., 2021; Little et al., 2020; Pörtner and Knust, 2007; Pörtner et al., 2017; Sinclair et al., 2016; van der Walt et al., 2021), the scientific community has been determining the upper thermal tolerance of numerous fish species, and examining how this parameter relates to oxygen consumption and cardiac function (Anttila et al., 2014; Casselman et al., 2012; Hansen et al., 2017; Leeuwis et al., 2019; Mignucci et al., 2021; Motyka et al., 2017; Muller et al., 2020). The most widely used method to determine the acute upper thermal tolerance of fishes is the  $\text{CT}_{\text{max}}$  test (Becker and Genoway, 1979). This test/protocol involves increasing water temperature at a constant rate ( $^\circ\text{C h}^{-1}$ ) from the fish's acclimation temperature until loss of equilibrium (LOE), defined as the loss of a fish's ability

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**List of abbreviations**

ABT	Arrhenius breakpoint temperature
CT <sub>max</sub>	critical thermal maximum
DST	data storage tag
ECG	electrocardiogram
$f_H$	heart rate
$f_{H,ABT}$	heart rate at Arrhenius breakpoint temperature
$f_{H,crit}$	oxygen level at the onset of bradycardia
$f_{H,max}$	maximum heart rate
$f_{H,peak}$	maximum heart rate during thermal challenge
$f_{H,scope}$	heart rate range from 10°C to highest (peak) value
LOE	loss of equilibrium
OCLTT	oxygen- and capacity-limited thermal tolerance
Q <sub>10</sub>	temperature coefficient
Q <sub>10,preABT</sub>	temperature coefficient from 10°C up to the Arrhenius breakpoint temperature
RVM	relative ventricular mass
$T_{arr}$	temperature at which heart rate arrhythmias begin
$T_{f_{H,peak}}$	temperature of peak heart rate during a thermal challenge
$T_{opt}$	optimal temperature
TMS	tricaine methanesulfonate

to maintain normal dorsal–ventral orientation (Brauner and Richards, 2020). The rate of temperature increase varies greatly in the literature, with values ranging from 18°C h<sup>-1</sup> (Anttila et al., 2013; Åsheim et al., 2020; Becker and Genoway, 1979; Gallant et al., 2017) to ~1–2°C h<sup>-1</sup> (Blasco et al., 2020; Motyka et al., 2017; Leeuwis et al., 2019; Penney et al., 2014; Zanuzzo et al., 2019); the latter is the maximum that would be considered ecologically relevant under most conditions (Caissie et al., 2012; Desforges et al., 2023; Richards, 2011; Rodnick et al., 2004). Such tests have often been performed in respirometers so that oxygen consumption can be measured, and heart function can be monitored by implanting flow probes around the ventral aorta (Gamperl et al., 2011; Gollock et al., 2006; Keen and Gamperl, 2012; Mendonça and Gamperl, 2010). However, more recently, a ‘rapid screening protocol’ originally developed by Casselman et al. (2012) has been increasingly used to determine the thermal tolerance of fishes. In this protocol, fish are anaesthetized and placed supine in a water bath, injected with atropine (to block cholinergic tone on the heart) and isoproterenol (to ensure maximum adrenergic cardiac stimulation), and  $f_H$  is measured using subdermal electrocardiogram (ECG) electrodes as water temperature is increased rapidly (at 10–18°C h<sup>-1</sup>). However, there are concerns about how accurately  $f_H$ –temperature relationships and indices used to estimate a species’ thermal tolerance (e.g. Arrhenius breakpoint temperature, ABT; temperature at peak heart rate,  $f_{H,peak}$ ; temperature of cardiac arrhythmias,  $T_{arr}$ ) under these highly controlled/manipulated and unnatural conditions reflect those in free-swimming fishes (Motyka et al., 2017; also see references below).

Data storage tags (DSTs; also called data loggers) have recently been used to determine breakpoints in cardiac function (i.e. in  $f_H$ ) in anaesthetized Roman sea bream (*Chrysoblephus laticeps*; Skeeles et al., 2020) and white sea bream (*Diplodus capansis*; van der Walt et al., 2021) following the protocol developed by Casselman et al. (2012) and the authors acknowledge that there are limitations in using such manipulated conditions for estimating cardiac parameters. Further, Mignucci et al. (2021) recently compared the  $f_H$  of gilthead sea bream (*Sparus aurata*) that were implanted with DSTs while free-swimming in a tank versus recovered in a respirometer. These authors reported that fish in the respirometers

had a higher resting  $f_H$  and suggested that biologging provides more reliable insights into the cardiac and behavioural responses of fish to environmental stressors. Clearly, more research needs to be conducted in this area so that we can understand the constraints of highly manipulated measurement conditions on fish cardiac function, and how they relate to measures of cardiac function (and thus thermal tolerance) in various species under ‘real world’ conditions. Such information is critical to ensuring that conservation and management policies implemented to protect fish species in the wild, or strategies to safeguard fish held in aquaculture operations, are based on accurate and reliable information about their thermal biology.

Therefore, the objective of this study was to compare measures of upper thermal (CT<sub>max</sub>) and hypoxia (the oxygen level at which bradycardia is initiated) tolerance determined using  $f_H$  DSTs in free-swimming fish (after 4 weeks of post-surgical recovery) with that of fish: (i) exposed to the rapid screening protocol of Casselman et al. (2012); and (ii) fitted with blood flow probes in a respirometer. These experiments were performed on Atlantic salmon because it is a eurythermal fish whose environmental tolerances have been reported in several studies (e.g. Anttila et al., 2013, 2014; Gallant et al., 2017; Hvas et al., 2017; Leeuwis et al., 2019; Penney et al., 2014), global populations of this species are declining (Dadswell et al., 2021; Chaput, 2012; Nicola et al., 2018; Mills et al., 2013), and it is an important aquaculture species that is experiencing elevated temperatures and hypoxic conditions at cage-sites in several regions (Burke et al., 2020; Burt et al., 2012; Gamperl et al., 2021; Oldham et al., 2017; Oppedal et al., 2011; Stehfest et al., 2017).

**MATERIALS AND METHODS**

This research was approved by the Institutional Animal Care Committee of Memorial University of Newfoundland and Labrador (protocol #21-05-KG) and performed in accordance with the Canadian Council on Animal Care Guidelines on the ‘Care and Use of Fish in Research, Teaching and Testing’ (<https://ccac.ca/Documents/Standards/Guidelines/Fish.pdf>).

**Fish husbandry**

The Atlantic salmon (*Salmo salar*) used in these studies were age ≥1 year, of Saint John River origin, and originally supplied by Cooke Aquaculture Inc. (Ormocto, NB, Canada) as pre-smolts. These fish were smolted at the Dr Joe Brown Aquatic Research Building (Ocean Sciences Centre, Memorial University of Newfoundland and Labrador), and held there for ~9 months, prior to being moved to the Laboratory for Atlantic Salmon and Climate Change Research (LASCCR, Ocean Sciences Centre). At the LASCCR, the fish were held in 2.2 m<sup>3</sup> tanks supplied with seawater at 10°C and at ≥100% air saturation and exposed to a 14 h light:10 h dark photoperiod. During this period, they were fed 4 mm Signature Salmon Ration-PW (Northeast Nutrition Inc., Truro, NS, Canada) at a ration of 1% body mass ( $M_b$ ) day<sup>-1</sup>. In all experiments mass (g), fork length (cm) and ventricular mass (g) were recorded. The fish used in these experiments averaged 838.2±13.4 g in mass and 41.6±0.3 cm in length (means±s.e.m.).

**Experimental design****Group 1: rapid screening using anaesthetized fish****Surgery**

Prior to implantation, micro-HRT DSTs (8.3 mm×25.4 mm, 3.3 g; Star Oddi, Garðabær, Iceland) were inserted into Star Oddi’s communication interface (COM-BOX), and Mercury (v.6.02) software running on a computer was used to program them. The

start date and time, sampling intervals and frequencies were all set using this software. The DSTs were set to record  $f_H$  (at 100 Hz for 6 s) and temperature ( $5\text{--}45^\circ\text{C}$ ;  $\pm 0.2^\circ\text{C}$ ) every 15 s for 4 recordings, followed by 1 recording where the ECG was saved, and this continued for the duration of the experiment. Raw ECGs were saved to validate the data collected (i.e. determine R–R intervals and  $f_H$ ).

Each fish was netted from their tank and anaesthetized in oxygenated seawater containing tricaine methanesulfonate (Syncaïne TMS;  $0.2\text{ g l}^{-1}$ ; Syndel Laboratories Canada, Nanaimo, BC, Canada) until ventilatory movements ceased. The fish were then placed supine on a wetted surgical sponge, and their gills were continuously supplied with  $\sim 10^\circ\text{C}$  oxygenated seawater containing a maintenance dose of TMS ( $0.05\text{ g l}^{-1}$ ). A small mid-ventral incision ( $1.1\pm 0.03\text{ cm}$ ), at the posterior limit of the base of the pectoral fins, was made in the fish's body wall using a scalpel. A micro-HRT DST was then inserted into the abdominal cavity (blunt-end first) towards the posterior of the fish, and then pulled forward using a suture (2-0) pre-tied at the middle of the DST to within  $0.5\text{ cm}$  of the pericardium. A  $\frac{1}{2}$  circle,  $28\text{ mm}$ , cutting edge needle (SE-MH 28, Mani Surgical Needles, Utsunomiya, Japan) was then used to pass the suture through the body wall and to start to close the incision. Finally, the incision was closed using 3-0 silk sutures: 2–4 interrupted stitches depending on incision length. Vaseline was applied to the incision to prevent water from entering the wound. DST implantation took approximately 5 min; thereafter, the fish was placed supine on a V-board and moved to a shallow water table (50 l) filled with  $10^\circ\text{C}$  oxygenated seawater containing a maintenance dose of TMS ( $0.05\text{ g l}^{-1}$ ). The fish were completely submerged in the seawater, and aerated seawater from the bath was constantly pumped over the fish's gills. After  $f_H$  and temperature data had been recorded for 1 h, the fish were given sequential injections of atropine sulfate ( $1.2\text{ mg kg}^{-1}$ ) and isoproterenol ( $4\text{ }\mu\text{g kg}^{-1}$ ) (Sigma-Aldrich Canada Co., Oakville, ON, Canada) via the caudal blood vessels to block vagal tone and to maximally stimulate cardiac adrenergic  $\beta$ -adrenoreceptors, respectively (Casselmann et al., 2012). Both drugs were dissolved in a 0.9% sodium chloride (NaCl) solution, and frozen at  $-80^\circ\text{C}$ , prior to use.

#### Temperature challenge

Thirty minutes following the drug injections, water temperature was increased from the fish's acclimation temperature ( $10^\circ\text{C}$ ) to  $28^\circ\text{C}$  at  $10^\circ\text{C h}^{-1}$  (Casselmann et al., 2012) using a circulating water bath connected to a titanium coil submerged in the seawater. Water temperature was recorded using a PT 100 probe ( $-30\text{--}150^\circ\text{C}$ ; resolution:  $0.02^\circ\text{C}$ , accuracy:  $\pm 0.5^\circ\text{C}$ ) connected to a Firesting  $\text{O}_2$  meter and a computer running Pyro Oxygen Logger software (PyroScience GmbH, Aachen, Germany).  $f_H$  and the fish's internal temperature were measured using the DSTs. In addition, in a subset of experiments ( $n=3$ ), a calibrated thermocouple (Model: HHC201, Type K Thermocouple,  $-100\text{--}1372^\circ\text{C}$ , accuracy: 0.1%; Omega Engineering Inc., Norwalk, CT, USA) was placed directly under the liver for the duration of the experiment to validate the DST's values of internal body temperature. Thereafter, the fish were euthanized in  $0.4\text{ g l}^{-1}$  TMS. Following euthanasia, the heart was removed from the fish, and the bulbous arteriosus and atrium were separated from the ventricle. The ventricle was then blotted dry on Kimwipes and weighed. Finally, the DST was retrieved, and the data downloaded.

#### Hypoxia challenge

No hypoxia challenge was performed on this group. Atropine sulfate blocks vagal tone and, thus, the fish are unable to initiate bradycardia/slow their  $f_H$  when exposed to hypoxic water.

## Group 2: traditional respirometry

### Surgery

Each fish was netted from their tank and anaesthetized in oxygenated seawater containing TMS ( $0.2\text{ g l}^{-1}$ ) until ventilatory movements ceased. The fish were placed on a wet foam pad on a surgical table while their gills were irrigated continuously with oxygenated seawater containing a maintenance dose of TMS ( $0.1\text{ g l}^{-1}$ ). Then, the salmon were placed on their right side, and umbilical tape was passed under the gill arches and secured to the surgical table to allow access to the opercular cavity. A small puncture was then made in the skin just below the junction of the second and third gill arches with a pair of sharp-pointed forceps, and the ventral aorta was carefully located by expanding the hole using blunt dissection. Once identified, the ventral aorta was freed from the surrounding tissue using a pair of curved forceps without damaging the pericardium, and a Doppler® flow probe (Model ES Cuff-type Transducer, 20 MHz, Iowa Doppler Products, Iowa City, IA, USA), 1.3 mm in diameter, was fitted around the ventral aorta. Finally, the flow probe lead was connected to a directional pulsed Doppler® flow meter (Model 545C-4; Bioengineering, University of Iowa, Ames, IA, USA) interfaced with a MP100 data acquisition system connected to a laptop computer running Acknowledge (v.3.8.2, BioPac Systems Inc., Goleta, CA, USA) to ensure that the signal was of high quality, and the probe lead was secured to the fish at three locations using 3-0 silk suture: one suture immediately ventral to the pectoral fin, one just below the lateral line and one in front of the dorsal fin.

Upon completion of surgery, individual fish were placed in a  $\sim 20\text{ l}$  cylindrical respirometer ( $20\text{ cm diameter}\times 54.6\text{ cm length}$ ) submerged in a shallow ( $25\text{ cm deep}$ ) water table containing fully aerated seawater at  $10^\circ\text{C}$ . The respirometer received a constant flow of water at a rate of  $10\text{ l min}^{-1}$  from a submersible pump (model 1048; EHEIM GmbH & Co., Deizisau, Germany). Water in the experimental water table was supplied from a large ( $\sim 300\text{ l}$ ) reservoir whose temperature was controlled by a custom-designed heater/chiller (Technical Services, Memorial University of Newfoundland and Labrador). The fish were allowed to recover/acclimate inside the respirometers for  $\sim 20\text{ h}$  (i.e. until the first morning prior to the hypoxia challenge).

### Hypoxia challenge

Approximately 20 h following surgery and acclimation to the respirometers, water oxygen levels were decreased step-wise every 60 min until the  $\text{O}_2$  level of 30% air saturation was reached (Fig. S1). Water air saturation was lowered by bubbling nitrogen gas into the seawater reservoir as controlled using a computer running WitroxCTRL software (Loligo Systems, Viborg, Denmark) that was interfaced with a fibre optic  $\text{O}_2$  meter and an  $\text{O}_2$  and temperature probe (Loligo Systems). This system controlled two solenoid valves which released air or nitrogen gas into the reservoir as required. Measurements of  $f_H$  were taken at every 5% decrease.  $f_H$  was recorded using the Doppler® flow probe and recording system, as previously described, and  $f_H$  (beats  $\text{min}^{-1}$ ) was determined by averaging three sections of 15 consecutive systolic peaks.

After the measurements at 30% air saturation were taken,  $\text{O}_2$  in the respirometers was increased to 100% over  $\sim 30\text{ min}$ . The fish were left undisturbed in the respirometers for  $\sim 36\text{ h}$  at their acclimation temperature ( $10^\circ\text{C}$ ) to recover.

### Temperature challenge

An acute upper thermal challenge to the fish's  $\text{CT}_{\text{max}}$  was used to determine the salmon's upper thermal tolerance. This is a standard



protocol in the Gamperl lab (see Norin et al., 2019; Zanuzzo et al., 2019; Leeuwis et al., 2019; Motyka et al., 2017; Ern et al., 2016), in which water temperature is increased by  $2^{\circ}\text{C h}^{-1}$  until the fish loses equilibrium (LOE) (Fig. S1).  $f_{\text{H}}$  was recorded at each  $1^{\circ}\text{C}$  increment. The temperature at which LOE occurred was used as the  $\text{CT}_{\text{max}}$  value. Thereafter, the fish were euthanized with  $0.4 \text{ g l}^{-1}$  TMS, the heart was removed, and the bulbous arteriosus and atrium were separated from the ventricle. The ventricle was then blotted dry on Kimwipes and weighed. For the hypoxia experiment,  $f_{\text{H,crit}}$  was determined as the point at which bradycardia occurred, and ABT,  $f_{\text{H,ABT}}$ ,  $Q_{10,\text{preABT}}$ ,  $f_{\text{H,scope}}$  and  $f_{\text{H,peak}}$  were determined as described below.

### Group 3: free-swimming fish with DSTs

#### Surgery

Prior to implantation, the DSTs (micro-HRT; Star Oddi) were set to record  $f_{\text{H}}$  (at 100 Hz for 6 s) and temperature ( $5\text{--}45^{\circ}\text{C}$ ;  $\pm 0.2^{\circ}\text{C}$ ) every 2 h for 24 h prior to the hypoxia challenge and between the hypoxia challenge and thermal challenge, and every 10 min during the hypoxia and thermal challenges. Raw ECGs were saved with every recording to determine R–R intervals ( $f_{\text{H}}$ ).

The micro-HRT tags were implanted in the fish as described above. The fish were then placed into a  $2.2 \text{ m}^3$  round tank with  $\sim 40$  conspecifics (stocking density  $18 \text{ kg m}^{-3}$ ), that was supplied with seawater at  $10^{\circ}\text{C}$  and with  $\geq 100\%$  air saturation in the LASCCR facility for 3 weeks. This duration of recovery was used as Zrini and Gamperl (2021) showed that this post-surgical period is required for resting  $f_{\text{H}}$  to return to stable levels. During this period, the fish were hand fed  $1 \times$  daily at  $1\% M_{\text{b}} \text{ day}^{-1}$  and maintained on a 14 h light:10 h dark photoperiod. Seven days prior to the hypoxia challenge, tagged fish ( $n=6$  per experiment, see below) were moved into a  $0.5 \text{ m}^3$  tank and remained in this tank for a week before the hypoxia and thermal challenges.

#### Hypoxia challenge

The oxygen level was decreased by bubbling nitrogen in the header tank which supplied the tank with  $5 \text{ l min}^{-1}$  of seawater. A step-wise decrease in oxygen every 60 min was achieved, with steps of 100%, 80%, 70%, 60%, 50%, 40% and 30% air saturation as described above (Fig. S1). Temperature and oxygen were continuously monitored using a YSI 5500D MultiDO Optical Monitoring and Control Instrument (Yellow Springs Instruments, Yellow Springs, OH, USA), and a GoPro<sup>®</sup> camera mounted above the tank was used to monitor the fish in real-time and record behaviour throughout the experiment. Following the last step, oxygen was increased over  $\sim 30$  min to  $\sim 100\%$  air saturation, and the fish were left undisturbed for 36 h.

#### Temperature challenge

A  $\text{CT}_{\text{max}}$  test was used to determine the fish's upper thermal tolerance. Water temperature was increased by  $2^{\circ}\text{C h}^{-1}$  by increasing temperature in the header tank that supplied the tank with seawater, while maintaining water oxygen levels at  $\sim 100\%$  air saturation (Fig. S1). As in the hypoxia experiment, a YSI 5500D MultiDO Optical Monitoring and Control Instrument (Yellow Springs Instruments) and a GoPro<sup>®</sup> camera, were used to monitor the water and fish in real-time, respectively. The temperature at which LOE occurred was recorded as the  $\text{CT}_{\text{max}}$  value. Following LOE, the fish were euthanized with  $0.4 \text{ g l}^{-1}$  TMS, the heart was removed and the ventricle was weighed. Then, the DSTs were recovered. Two experiments were conducted, and all fish were exposed to the hypoxia challenge prior to the temperature challenge ( $n=6$  per experiment,  $n=12$  fish total).

The data from the DSTs were downloaded using the COM-BOX and analysed using Mercury software running on a computer. For the first experiment,  $f_{\text{H,crit}}$  was determined as the point at which bradycardia occurred, and ABT,  $f_{\text{H,ABT}}$ ,  $Q_{10,\text{preABT}}$ ,  $f_{\text{H,scope}}$  and  $f_{\text{H,peak}}$  were determined as described below.

#### Data analysis

In these experiments, relative ventricular mass (RVM) was calculated as:

$$\text{RVM} = 100 \times \left[ \frac{M_{\text{v}}}{M_{\text{b}}} \right], \quad (1)$$

where  $M_{\text{v}}$  is ventricle mass and  $M_{\text{b}}$  is body mass (both measured in grams). The oxygen at which bradycardia occurred ( $f_{\text{H,crit}}$ ) was determined by plotting  $f_{\text{H}}$  against air saturation (%) to find the change in slope for each individual. This was determined for each individual using the segmented package (v.1.4-1) in R, which uses a piecewise regression to determine the breakpoint in the relationship. However,  $f_{\text{H,crit}}$  could not be determined for 2 fish tested in the respirometers ( $n=8$ ). The normal exponential increase in  $f_{\text{H}}$  with temperature becomes discontinuous prior to arrhythmia (Casselman et al., 2012; Somero, 2011). The temperature at which this transition occurs is termed the ABT. ABT and the  $f_{\text{H}}$  at the ABT ( $f_{\text{H,ABT}}$ ) were determined for each fish by plotting the natural logarithm of  $f_{\text{H}}$  against the inverse of temperature (in Kelvin) and finding the point of change in slope using the segmented package (v.1.4-1) in R (Casselman et al., 2012) (e.g. Fig. S2). However, ABT could not be accurately determined for 3 fish tested in the respirometers and 5 fish free-swimming in the tank ( $n=7$  for both groups). The  $Q_{10}$  for  $f_{\text{H}}$  was calculated for each individual across the temperature range from  $10^{\circ}\text{C}$  to a temperature just below the ABT ( $Q_{10,\text{preABT}}$ ) using the formula:

$$Q_{10} = \left( \frac{f_{\text{H},2}}{f_{\text{H},1}} \right)^{10/(T_2 - T_1)}, \quad (2)$$

where  $f_{\text{H},1}$  and  $f_{\text{H},2}$  are the heart rate at the first ( $T_1$ ) and second ( $T_2$ ) temperatures, respectively. The scope for  $f_{\text{H}}$  ( $f_{\text{H,scope}}$ ) was calculated as the difference between  $f_{\text{H}}$  at  $10^{\circ}\text{C}$  and the highest recorded  $f_{\text{H}}$  ( $f_{\text{H,peak}}$ ), regardless of temperature. Graphs were created using Prism 9 ([www.graphpad.com](http://www.graphpad.com)) and statistical analyses was completed using R (<http://www.R-project.org/>). Assumptions of normality and homogeneity of variance were performed by visual inspection of  $Q$ – $Q$  plots and histograms of residuals. Resting (or 'undisturbed') values for  $f_{\text{H}}$  were compared between groups using a one-way ANOVA using values at the beginning of sedation or resting measurements (at  $10^{\circ}\text{C}$  and  $100\%$  air saturation). Differences in morphometric measurements (RVM, mass, length),  $\text{CT}_{\text{max}}$ , ABT,  $f_{\text{H,ABT}}$ ,  $Q_{10,\text{preABT}}$ ,  $f_{\text{H,scope}}$  and  $f_{\text{H,peak}}$  were compared between the experimental groups using one-way ANOVAs (Table S1). Unpaired  $t$ -tests were used to compare  $\text{CT}_{\text{max}}$ ,  $f_{\text{H,crit}}$  and  $f_{\text{H}}$  at  $100\%$  and  $30\%$  air saturation for fish tested in the respirometers and free-swimming in the tank (Table S1). Linear mixed-effects models (LME) with temperature/oxygen and treatment (i.e. group) as fixed effects, an interaction term for the two, and fish as a random factor, were used to assess changes in  $f_{\text{H}}$ . A linear regression was used to plot the relationship between  $f_{\text{H,scope}}$  and the temperature at  $f_{\text{H,peak}}$  ( $T_{f_{\text{H,peak}}}$ ) for all groups. A linear regression was fitted to the  $f_{\text{H}}$  versus temperature relationship of free-swimming fish from  $10$  to  $26^{\circ}\text{C}$  during the  $\text{CT}_{\text{max}}$  test. The level of statistical significance used was  $P < 0.05$ , and all values in the text, tables and figures are means  $\pm 1$  s.e.m.

## RESULTS

## Body and cardiac morphometrics

There were no differences in fish size (mass or length) between the experiments ( $P>0.05$ ) (Table 1). However, the RVM of fish tested using traditional respirometry was significantly higher than that of fish assessed using the rapid screening protocol ( $0.090\pm 0.004$  versus  $0.077\pm 0.003$ ,  $P<0.05$ ) (Table 1). The RVM of free-swimming fish with implanted DSTs was not significantly different from that of the other treatments ( $P>0.05$ ) (Table 1).

Resting  $f_H$ 

Resting  $f_H$  values at  $10^\circ\text{C}$  were significantly lower in the free-swimming fish with DSTs ( $48.9\pm 1.9$  beats  $\text{min}^{-1}$ ,  $P<0.05$ ; Table 1, Fig. 1) than in fish assessed using the rapid screening protocol and traditional respirometry ( $69.9\pm 1.1$  and  $68.7\pm 1.7$  beats  $\text{min}^{-1}$ , respectively; Table 1, Fig. 1).

## Acute hypoxia challenge

The  $f_H$  values for free-swimming fish with DSTs and fish tested in the respirometers remained consistent with values at 100% air saturation until the initiation of bradycardia. The initiation of bradycardia ( $f_{H,\text{crit}}$ ) was significantly different between groups and occurred at  $62.1\pm 2.4\%$  air saturation in free-swimming fish and  $52.6\pm 2.6\%$  air saturation in fish tested in the respirometers ( $P<0.05$ ) (Table 1, Fig. 2). The mean  $f_H$  in both groups was significantly lower by 45% air saturation than the initial  $f_H$  (at 100% air saturation).  $f_H$  for fish in the respirometers continued to decline linearly from the initiation of bradycardia until 30% air saturation whereas the  $f_H$  of free-swimming fish reached a plateau at  $\sim 45\%$  air saturation and remained between 24 and 28 beats  $\text{min}^{-1}$ ,  $\sim 40\%$  below initial values. Reduced swimming/movement of free-swimming fish was observed as  $\text{O}_2$  level was decreased and many ( $\sim 50\%$  of fish) struggled to maintain equilibrium at 30% air saturation, whereas there were no observable changes in behaviour for fish tested in respirometers. At 30% air saturation,  $f_H$  was significantly lower in free-swimming fish ( $25.2\pm 2.2$  beats  $\text{min}^{-1}$ ) than in fish in the respirometers ( $36.9\pm 11.2$  beats  $\text{min}^{-1}$ ) (Table 1, Fig. 2). The average  $f_H$  decreased by  $\sim 40\%$  in the free-swimming fish and  $\sim 50\%$  for fish in respirometers (by  $\sim 20$  and 37 beats  $\text{min}^{-1}$ , respectively) at 30% air saturation as compared with normoxic values.

## Temperature challenge

The ABT was significantly lower ( $14.6\pm 0.2^\circ\text{C}$ ) using the rapid screening protocol than when fish were tested in the respirometers and in free-swimming fish ( $18.1\pm 1.6$  and  $18.4\pm 1.4^\circ\text{C}$ , respectively) (Table 1).  $f_{H,\text{peak}}$  was also significantly lower using the rapid screening protocol compared with that of free-swimming fish ( $126.0\pm 4.5$  beats  $\text{min}^{-1}$  versus  $153.1\pm 4.7$  beats  $\text{min}^{-1}$ ,  $P<0.05$ ) (Table 1). In contrast, the temperature at which  $f_{H,\text{peak}}$  was reached was significantly different between all groups, with the lowest temperature measured in the rapid screening protocol ( $20.2\pm 0.4^\circ\text{C}$ ) followed by the fish in respirometers ( $23.2\pm 0.7^\circ\text{C}$ ) and, finally, free-swimming fish ( $26.5\pm 0.4^\circ\text{C}$ ) (Table 1). The free-swimming fish also had a significantly higher  $f_{H,\text{scope}}$  ( $104.2\pm 4.6$  beats  $\text{min}^{-1}$ ) as compared with the fish in respirometers and fish assessed using the rapid screening protocol ( $70.8\pm 4.0$  and  $57.8\pm 3.8$  beats  $\text{min}^{-1}$ , respectively) (Table 1). The  $\text{CT}_{\text{max}}$  of free-swimming fish was  $27.7\pm 0.4^\circ\text{C}$ , and this value was significantly higher than that of fish tested in the respirometers ( $25.9\pm 0.6^\circ\text{C}$ ) (Table 1, Fig. 1). Both fish in respirometers and free-swimming fish in the tank displayed an increase in activity/movement near their  $\text{CT}_{\text{max}}$ .

The internal (core) body temperature recorded by the DST and the thermocouple was consistently lower than the water temperature during the rapid screening protocol (temperature increase:  $10^\circ\text{C h}^{-1}$ ), and this difference was  $2.5^\circ\text{C}$  at  $28^\circ\text{C}$  (Fig. 3). In contrast, DST temperature in the free-swimming fish was always consistent with that measured in the water (Fig. 3).

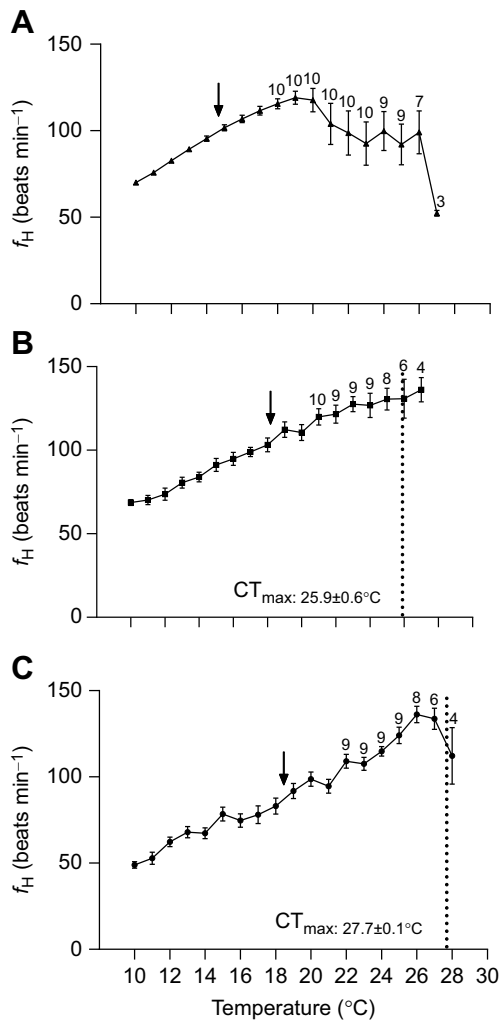
## DISCUSSION

To predict (and implement conservation and management strategies to mitigate) the potential impacts of climate change-related environmental challenges on marine fish populations, it is critical that we have accurate data on their sub-lethal and lethal tolerances. It has been suggested that  $f_{H,\text{max}}$  and its associated rate transition temperature can be used to determine the thermal tolerance of fishes, and that rapid measurements of  $f_{H,\text{max}}$  offer functional and ecological insights into the acute upper thermal limits of this taxon (e.g. their  $\text{CT}_{\text{max}}$ ) (Ferreira et al., 2014; van der Walt et al., 2021). Thus, the goal of this study was to compare two commonly used methods of determining a fish's  $f_H$  response to acute warming and their  $\text{CT}_{\text{max}}$  with that measured in free-swimming individuals. Further, this study examined the salmon's  $f_H$  response to hypoxia and the oxygen level at which bradycardia was initiated in

Table 1. Comparison of morphometric, heart rate and tolerance metrics between the three treatment groups

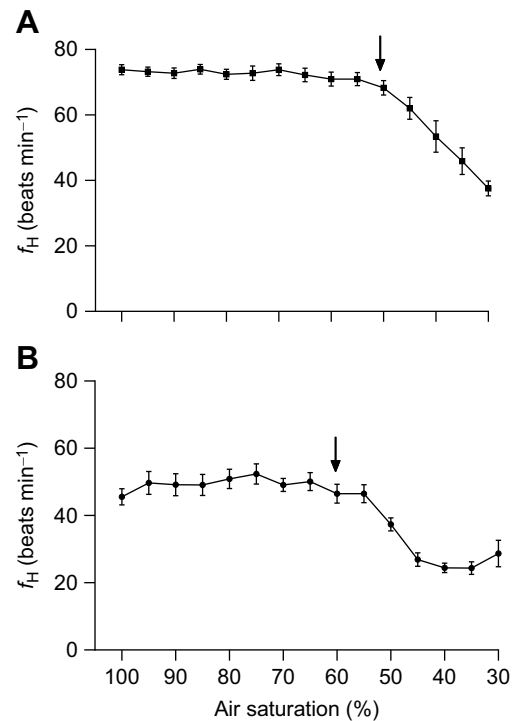
	Group 1 'Rapid screening protocol' ( $n=11$ )	Group 2 Traditional respirometry ( $n=10$ )	Group 3 Free-swimming with DSTs ( $n=12$ )
Mass (g)	$851.7\pm 18.8^a$	$855.8\pm 36.3^a$	$811.2\pm 12.0^a$
Fork length (cm)	$41.0\pm 0.4^a$	$42.1\pm 0.9^a$	$41.7\pm 0.3^a$
RVM (%)	$0.077\pm 0.003^a$	$0.090\pm 0.004^b$	$0.087\pm 0.003^{a,b}$
$\text{CT}_{\text{max}}$ ( $^\circ\text{C}$ )	–	$25.9\pm 0.6^a$	$27.7\pm 0.1^b$
ABT ( $^\circ\text{C}$ )	$14.6\pm 0.2^a$	$18.1\pm 1.6^{a,b}$	$18.4\pm 1.4^b$
Resting $f_H$ at $10^\circ\text{C}$ (beats $\text{min}^{-1}$ )	$69.9\pm 1.1^a$	$68.7\pm 1.7^a$	$48.9\pm 1.9^b$
$f_{H,\text{peak}}$ (beats $\text{min}^{-1}$ )	$126.0\pm 4.5^a$	$138.4\pm 4.7^{a,b}$	$153.1\pm 4.7^b$
$T_{f_{H,\text{peak}}}$ ( $^\circ\text{C}$ )	$20.2\pm 0.4^a$	$23.2\pm 0.7^b$	$26.5\pm 0.4^c$
$f_{H,\text{scope}}$ (beats $\text{min}^{-1}$ )	$57.8\pm 3.8^a$	$70.8\pm 4.0^a$	$104.2\pm 4.6^b$
$Q_{10,\text{preABT}}$	$2.21\pm 0.05^a$	$1.64\pm 0.20^b$	$1.94\pm 0.10^{a,b}$
$f_H$ at 100% air saturation (beats $\text{min}^{-1}$ )	–	$73.8\pm 17.2^a$	$45.6\pm 2.4^b$
$f_H$ at 30% air saturation (beats $\text{min}^{-1}$ )	–	$36.9\pm 11.2^a$	$25.2\pm 2.2^b$
$\text{O}_2$ level at bradycardia (%)	–	$52.6\pm 2.6^a$	$62.1\pm 2.4^b$

RVM, relative ventricular mass;  $\text{CT}_{\text{max}}$ , critical thermal maximum; ABT, Arrhenius breakpoint temperature;  $f_H$ , heart rate;  $f_{H,\text{peak}}$ , peak heart rate;  $T_{f_{H,\text{peak}}}$ , temperature at peak heart rate;  $f_{H,\text{scope}}$ , heart rate range from  $10^\circ\text{C}$  to peak;  $Q_{10,\text{preABT}}$ , temperature coefficient from  $10^\circ\text{C}$  to the Arrhenius breakpoint temperature. Dissimilar letters indicate a significant difference ( $P<0.05$ ) between groups. All values are means  $\pm$  s.e.m.



**Fig. 1. Comparison of changes in the heart rate ( $f_H$ ) of Atlantic salmon during a temperature challenge.**  $f_H$  is shown for (A) fish implanted with data storage tags (DSTs) and given a 'rapid screening protocol' ( $n=11$ ), (B) fish tested using traditional respirometry and implanted with Doppler<sup>®</sup> flow probes ( $n=10$ ) and (C) free-swimming fish held with conspecifics and implanted with DSTs ( $n=12$ ). Numbers above the symbols indicate decreased sample size at a specific temperature. The average critical thermal maximum ( $\text{CT}_{\text{max}}$ ) value (mean $\pm$ s.e.m.) is shown using a dotted line in B and C. Arrows indicate the Arrhenius breakpoint temperature (ABT). Temperature was increased at  $10^{\circ}\text{C h}^{-1}$  in A and at  $2^{\circ}\text{C h}^{-1}$  in B and C.

free-swimming fish versus those post-surgery in a respirometer. Overall, the data reveal that the rapid screening protocol and measuring  $f_H$  responses in a respirometer do not provide values for these important parameters that are quantitatively similar/comparable to those in free-swimming fishes. For example: the ABT as determined by the rapid screening protocol was  $\sim 4^{\circ}\text{C}$  lower in Atlantic salmon than that determined in a respirometer and in free-swimming fish; the temperature at  $f_{H,\text{peak}}$  differed considerably between the three methods ( $\sim 20$ ,  $\sim 23$  and  $\sim 26.5^{\circ}\text{C}$ , respectively); the  $\text{CT}_{\text{max}}$  of free swimming fish was  $\sim 1.8^{\circ}\text{C}$  higher than that measured for fish in a respirometer fitted with a Doppler<sup>®</sup> flow probe; and the  $f_{H,\text{crit}}$  and  $f_H$  at 30% air saturation were very different in these two groups. These differences are likely due, in part, to the much higher starting  $f_H$  in the rapid screening protocol and respirometer studies, and the reduced scope for  $f_H$  that this affords. However, the rate of heating/warming, and potential differences

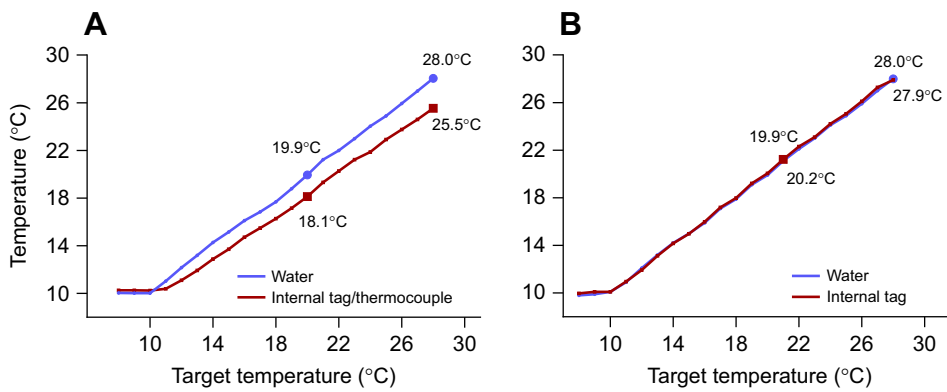


**Fig. 2. Comparison of changes in  $f_H$  as water oxygen level was lowered from 100% to 30% air saturation.** Average  $f_H$  values at each 5% decrease in air saturation are plotted for (A) fish fitted with Doppler<sup>®</sup> flow probes and tested using traditional respirometry ( $n=10$ ) and (B) free-swimming fish in a tank with conspecifics and implanted with DSTs ( $n=12$ ). The arrows indicate the average ABT as determined using individual fish.

between water temperature and the fish's core temperature, must also be considered as potential sources of error/variation.

### Resting $f_H$

Most commonly, measurements to assess the environmental tolerances of fishes have been performed within a laboratory setting because of the practicality of manipulating conditions in a controlled manner (e.g. changing temperature or dissolved oxygen) and the requirement of being in close proximity to a data acquisition system and computer to monitor physiological variables. More recently, advancements in bio-logging (DSTs) have enabled the continuous monitoring of several parameters in free-swimming fish, with many users recommending a minimum of 1–2 weeks of post-surgical recovery before starting measurements (Bjarnason et al., 2019; Brijs et al., 2018, 2019; Ekström et al., 2018; Hvas et al., 2020). More specifically, studies have noted that while initial stabilization of  $f_H$  takes a minimum of 2–4 days post-surgery, there is a further and more gradual decline in  $f_H$  that lasts for up to 3 weeks (Føre et al., 2021; Hvas et al., 2020; Yousaf et al., 2022; Zrini and Gamperl, 2021). Given the short recovery period of fish fitted with Doppler<sup>®</sup> flow probes, it is not surprising that the  $f_H$  for salmon at rest was elevated (by  $\sim 20$  beats  $\text{min}^{-1}$ , 38%) as compared with fish free-swimming in tanks after 4 weeks of recovery. Assessing the contribution of confinement versus surgery to the reported elevation in the  $f_H$  of fish in the respirometers was beyond the scope of this study. However, Altimiras and Larsen (2000) compared the resting  $f_H$  of rainbow trout (*Oncorhynchus mykiss*) measured in a swim tunnel using biopotentials in the water (i.e. a non-invasive technique) with that of previous studies that used surgical techniques to record this parameter, and reported that  $f_H$  was



**Fig. 3. Comparison of water temperature and internal tag (DST) temperature during the temperature challenge for anaesthetized and free-swimming Atlantic salmon.** The rate of temperature increase was (A)  $10^{\circ}\text{C h}^{-1}$  for anaesthetized fish versus (B)  $2^{\circ}\text{C h}^{-1}$  for free-swimming fish. In three fish, core body temperature was also measured with a calibrated thermocouple. This gave identical data to those recorded by the DST.

much lower using the former method. This latter study suggests that stress associated with short post-surgical recovery (18–66 h) was a major contributor to the much higher  $f_{\text{H}}$  in the respirometer-confined salmon in this study. Indeed, Porter et al. (2022) recently measured the plasma cortisol levels of salmon at 24 h post-surgery in a respirometer at  $8^{\circ}\text{C}$ , and these values (mean  $47.9\text{ ng ml}^{-1}$ ) were much greater than those measured in the same population of fish when quickly sampled from their holding tanks ( $5\text{--}10\text{ ng ml}^{-1}$ ; Vadboncoeur et al., 2023). However, the effects of confinement itself cannot be overlooked. For example, Mignucci et al. (2021) recently reported that gilthead seabream (*Sparus aurata*) implanted with DSTs had a higher  $f_{\text{H}}$  than free-swimming fish ( $\sim 75$  versus  $105\text{ beats min}^{-1}$ , respectively) independent of time post-surgery. Clearly, confined and instrumented fish have substantially elevated  $f_{\text{H}}$  and, thus, are questionable to use in estimating resting  $f_{\text{H}}$  in free-swimming fishes in the wild or in aquaculture.

### Hypoxia response

The slowing  $f_{\text{H}}$  in response to decreased/low dissolved oxygen levels, known as hypoxic bradycardia, is an important physiological response and potentially aids in the survival of fish that encounter low levels of oxygen (Farrell, 2007; Stecyk, 2017). This reflex response occurs as a result of increased cholinergic nervous tone within the cardiac branch of the vagus nerve of fishes as described by Stecyk (2017). While the significance of hypoxic bradycardia is not fully understood, several cardiac benefits resulting from this reflex have been proposed, including increased time for diffusion of oxygen into the myocardium, reduced cardiac oxygen demands, increased coronary blood flow during the prolonged diastolic period, and improved cardiac contractility (Farrell, 2007; Joyce et al., 2016; McKenzie et al., 2009).

This response of  $f_{\text{H}}$  to decreasing dissolved oxygen levels has been studied in other salmonids at similar temperatures ( $10\text{--}12^{\circ}\text{C}$ ; e.g. *Oncorhynchus mykiss*; Holeyton and Randall, 1967; Marvin and Heath, 1968; Randall and Smith, 1967), and the onset of bradycardia for this species ( $\sim 50\text{--}65\%$  air saturation) is very similar to what we report for Atlantic salmon in respirometers post-surgery and when free-swimming. Also consistent with this literature is the linear decrease in  $f_{\text{H}}$  reported for salmon in the respirometers. The  $f_{\text{H}}$  for fish in the respirometers decreased by 50% ( $30\text{ beats min}^{-1}$ ) from the initiation of bradycardia at  $52.6 \pm 2.6\%$  to 30% air saturation. The response to hypoxia in free-swimming salmon with DSTs differed in two ways as compared with these fish. First, the  $f_{\text{H,crit}}$  in the latter group occurred at  $62.1 \pm 2.4\%$  air saturation, at  $\sim 10\%$  higher air saturation than for fish in the respirometers. While there are limited data with which to compare this finding, Mignucci et al. (2021) reported similar results in

gilthead seabream (*Sparus aurata*). Mignucci et al. (2021) did not mathematically calculate the breakpoint in the oxygen- $f_{\text{H}}$  response ( $f_{\text{H,crit}}$ ); however, based on a visual interpretation of fig. 2 in their paper, it appears that there was also an earlier breakpoint (i.e. decrease in  $f_{\text{H}}$ ) in free-swimming fish as compared with those in respirometers. Second, the decrease in  $f_{\text{H}}$  between the oxygen level at the initiation of bradycardia ( $f_{\text{H,crit}}$ ) and 30% air saturation was not linear in the free-swimming salmon. It decreased by  $\sim 24\text{ beats min}^{-1}$  (50%) from 55% to 45% air saturation but did not decrease further. This is unlikely to be a result of the activity level of the free-swimming fish. Although their activity was not quantified in this study, it decreased with the seawater oxygen level, and fish were inactive and just maintaining their position in the water column at the lowest oxygen levels used in this study. This decrease in activity is not a novel finding. For example, Schurmann and Steffensen (1994) reported that Atlantic cod (*Gadus morhua*) decreased their swimming activity threefold between  $\sim 60\%$  and 20% air saturation.

Instead, we propose that this different  $f_{\text{H}}$  response between the two groups may be related to the extent of cholinergic tone on the heart of resting fish in respirometers versus free-swimming fish. Cholinergic tone is a major determinant of  $f_{\text{H}}$  in fish, initiates bradycardia, and has been shown to be affected by several factors (Sandblom and Axelsson, 2011; Wood et al., 1979; Wood and Shelton, 1980). In instrumented Atlantic salmon (confined to a respirometer) at  $8^{\circ}\text{C}$ , cholinergic tone is only 12.4%, and this low tone likely contributed to their higher resting  $f_{\text{H}}$  values ( $59.8 \pm 2.6\text{ beats min}^{-1}$ ; Porter et al., 2022). It is quite possible that the low  $f_{\text{H}}$  in the free-swimming fish in this experiment was mediated, at least partially, by higher cholinergic tone on the heart and, thus, these fish likely had limited scope to decrease  $f_{\text{H}}$  as the oxygen level in the water decreased. The hypothesis that the reduction in  $f_{\text{H}}$  induced by bradycardia is dependent on resting  $f_{\text{H}}$ /cholinergic tone is clearly worth further investigation.

### Acute warming responses

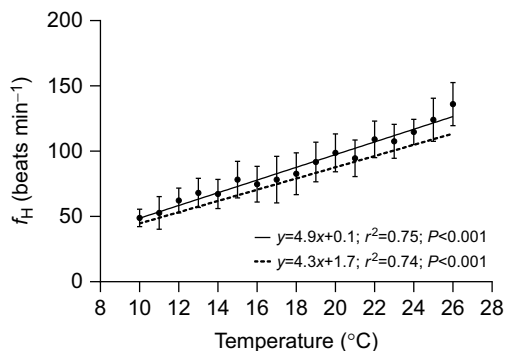
While  $f_{\text{H}}$  increased linearly with temperature in all fish, the ABT was substantially lower for fish exposed to the rapid screening protocol ( $14.6 \pm 0.2^{\circ}\text{C}$ ) in comparison to the  $\sim 18^{\circ}\text{C}$  measured in both free-swimming fish and those in respirometers. This is a large (and important) difference, and would have been even greater if, as for most previous studies using the Casselman et al. (2012) protocol, we had only measured water temperature. In this study data loggers simultaneously recorded  $f_{\text{H}}$  and internal (core) temperature during the rapid screening protocol, and this temperature differed from that of the water (Fig. 3), whereas this difference was not seen in the free-swimming fish at a warming rate of  $2^{\circ}\text{C h}^{-1}$ . While the size of the



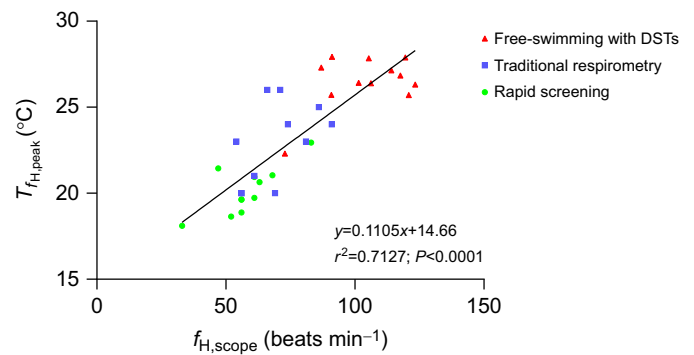
salmon exposed to the rapid screening protocol (~800 g) likely contributed to the large difference in core versus water temperature that we report, this overall result/finding is not surprising. A difference of 1–2°C has been reported for small Atlantic cod (*Gadus morhua*; ~100 g) and a 0.2°C discrepancy has even been reported in zebrafish (*Danio rerio*) (Jutfelt et al., 2019; Morgan et al., 2019). In addition, Sandblom et al. (2016) identified this issue in preliminary studies with perch (*Perca fluviatilis* L.) and reduced the rate of heating in their rapid screening protocol to 3°C h<sup>-1</sup> to avoid this issue. These above issues question the use of the rapid screening protocol (especially at high rates of heating) for determining the ABT of  $f_{H,i}$  and, thus, its accuracy when estimating the  $T_{opt}$  of fishes.

With respect to estimating/determining maximum cardiac values related to upper thermal tolerance, the rapid screening protocol also gives very different values when compared with those of free-swimming fish implanted with DSTs and given a long (appropriate) period of surgical recovery. In fact,  $T_{f_{H,peak}}$  was >6°C lower with the rapid screening protocol (20.15±0.4 versus 26.49±0.4°C), and this value was ~7.5°C lower than the  $CT_{max}$  of the free-swimming fish implanted with DSTs. This is the first study to directly compare these methods for determining thermal tolerance-related parameters in fishes and raises significant concerns about the use of this protocol for determining peak  $f_{H,i}$  values (and thus upper temperature tolerance) in fishes, and the suitability of using values derived from this method for predicting the impact of marine heatwaves on the distribution and survival of fishes. This is particularly true given the confidence we have in the temperature-related data we obtained in the free-swimming fish. The temperature– $f_{H,i}$  relationship obtained for these fish is extremely similar to that reported by Gamperl et al. (2021) for salmon implanted with DSTs in a commercial sea-cage during a marine heat wave (see Fig. 4). In comparison to the rapid screening protocol and the free-swimming fish with DSTs, the data for fish in the respirometers was intermediate. The ABT and  $f_{H,i}$  were comparable to values for the free-swimming fish, whereas  $T_{f_{H,peak}}$  was ~3.5°C lower.

With regard to what is potentially mediating the difference in  $f_{H,peak}$  (and thus the estimates of upper thermal tolerance that would be derived from them) between these experiments, the data are quite revealing. In the rapid screening protocol, resting  $f_{H,i}$  following injections of atropine and isoproterenol was artificially elevated (69.9±1.1 beats min<sup>-1</sup>), and this in addition to a lower  $f_{H,scope}$  (126±4.5 beats min<sup>-1</sup>) limited the fish's  $f_{H,scope}$



**Fig. 4. Comparison of the average  $f_{H,i}$  of free-swimming Atlantic salmon implanted with DSTs during a temperature challenge at 2°C h<sup>-1</sup>.** The solid line represents the linear regression for this relationship in fish ( $n=12$ ) in this study (with the symbols indicating means±s.e.m.). The dashed line is the daytime linear regression reported in Gamperl et al. (2021) for free-swimming fish ( $n=5$ ) in a commercial sea-cage implanted with  $f_{H,i}$  DSTs during a summer heat wave in Newfoundland in 2019.



**Fig. 5. Relationship between the scope for heart rate ( $f_{H,scope}$ ) and the temperature of peak  $f_{H,i}$  ( $T_{f_{H,peak}}$ ).** The symbols represent each treatment group: circles, fish anaesthetized and implanted with DSTs ( $n=11$ ); squares, fish implanted with Doppler® flow probes and tested using traditional respirometry ( $n=10$ ); and triangles, free-swimming fish held with conspecifics and implanted with DSTs ( $n=12$ ).

(57.8±3.8 beats min<sup>-1</sup>) as compared with free-swimming conspecifics with DSTs (104.2±4.6 beats min<sup>-1</sup>). Indeed, if you plot the  $T_{f_{H,peak}}$  for all three groups versus their  $f_{H,scope}$  (Fig. 5), there is a very strong positive relationship ( $r^2=0.713$ ) between these two parameters. Collectively, these data highlight the importance of the available scope for  $f_{H,i}$  in determining a fish's upper thermal tolerance, and of eliminating or reducing stress (and therefore resting  $f_{H,i}$ ) in fish used in protocols designed to determine their upper thermal tolerance. Further, they emphasize how stressors in the natural environment or in aquaculture operations could result in these animals being more susceptible to acute increases in temperature.

We were not able to measure  $CT_{max}$  for fish given the rapid screening protocol as the salmon were anaesthetized throughout, or confidently determine their  $T_{arr}$  (the temperature at which arrhythmias first occur) from the ECG recordings in fish with DSTs as the measurement period in the tags used was only 6 s. However, we did obtain values for  $T_{f_{H,peak}}$  and  $CT_{max}$  of the free-swimming salmon and those tested in respirometers. Despite showing no significant difference in  $f_{H,peak}$ , the free-swimming fish had values for  $T_{f_{H,peak}}$  and  $CT_{max}$  that were ~3.3 and 1.8°C higher, respectively, than those of fish in the respirometers. That the  $CT_{max}$  of these two groups was quite similar/comparable is not surprising. Based on a number of studies that have compared  $CT_{max}$  between different fish populations/families (Anttila et al., 2013; Bartlett et al., 2022; Ignatz et al., 2023), those acclimated to temperature differences of <10°C (Beitinger et al., 2000; Anttila et al., 2015; Morgan et al., 2019), or exposed to suboptimal conditions (e.g. hypoxia; Anttila et al., 2015; Ern et al., 2016), it appears that  $CT_{max}$  displays limited plasticity/variability (~1.5–3.0°C) when values are compared at the same rate of heating. This is likely due the specific mechanisms that determine when a fish loses equilibrium (this parameter defining when a fish has reached its  $CT_{max}$ ) (Ern et al., 2023).

### Summary and perspectives

There is accumulating evidence that the geographical distribution of aquatic ectotherms can be predicted based on their thermal limits (Sunday et al., 2012; Stuart-Smith et al., 2017; Payne et al., 2021), and that increases in the frequency and severity of temperature extremes (i.e. heat waves and 'cold shocks'/'cold stress') will be important determinants of the survival and population strength



(biomass) of fish species in the future (Cheung and Frölicher, 2020; Cheung et al., 2021; Genin et al., 2020; Perry et al., 2005; Szekeres et al., 2016; Reid et al., 2022). Thus, obtaining meaningful and accurate values for the thermal limits of various species-specific biological processes will be key to implementing conservation and management strategies (McKenzie et al., 2016; Ørsted et al., 2022; Desforges et al., 2023; Bates and Morley, 2020). This may be particularly true with regard to the impacts of heat waves as recent analyses suggest that the heat failure (mortality) rate of ectothermic species, including fishes, doubles (on average) for every 1°C increase within the stressful temperature range (Jørgensen et al., 2022; Ørsted et al., 2022). While  $CT_{max}$  (loss of equilibrium) has been used by a number of authors to estimate the upper thermal tolerance of fishes, the rates of heating used are often faster than ecologically relevant (i.e.  $>5^{\circ}C\ h^{-1}$ ; Caissie et al., 2012; Desforges et al., 2023; Richards, 2011; Rodnick et al., 2004; Gilbert and Farrell, 2021), and others have suggested that  $CT_{max}$  is not the most ecologically relevant measure of a fish's thermal tolerance (Bartlett et al., 2022; Ignatz et al., 2023). For example, high rates of heating do not allow for the balance in damaging and regenerative (or 'plastic') responses that ultimately define critical boundary temperatures (Ørsted et al., 2022). Thus, other protocols and methodologies need to be developed, and evaluated/validated, with regard to determining the thermal tolerance of fish to short-term (acute) warming events.

In this first of its kind study, we compared the temperature-dependent  $f_H$  responses of Atlantic salmon when exposed to temperatures approaching their upper thermal limit using three different protocols/experimental approaches. This study clearly shows that there are major differences in temperature-dependent  $f_H$  parameters obtained using the three methods, and that the rapid screening protocol greatly underestimated the thermal limits/tolerance of free-swimming Atlantic salmon. This finding is troubling given the number of researchers using this protocol to assess the upper thermal tolerance of fishes. Further, it highlights the rapid screening protocol's limitations with respect to determining the upper thermal tolerance of fish in their natural environment and suggests that this protocol should not be used to predict the vulnerability of wild fishes to heat waves (e.g. van der Walt et al., 2021). However, it is not surprising as this protocol (Casselman et al., 2012) was originally designed to examine whether ABT could be used to estimate a species'  $T_{opt}$  for aerobic scope, not a species' upper thermal tolerance. Also, the injection of atropine and isoproterenol artificially elevates the fish's initial (resting)  $f_H$  and, thus, reduces the available scope to increase  $f_H$  – a parameter which our results suggest may be a key factor in determining a fish's environmental tolerances/limits (e.g. see Fig. 5).

Instead, our results support the conclusion of Mignucci et al. (2021) that biologging on free-swimming fishes provides reliable, ecologically relevant, insights into the cardiac and behavioural responses of fish to environmental stressors and suggest that the  $T_{f_H,peak}$  of free-swimming fishes is a good/accurate predictor of the upper thermal tolerance of this taxa. This is based on the extremely similar relationship between  $f_H$  and temperatures from 10 to 20°C in this lab-based study and that determined for Atlantic salmon in a large sea-cage recorded during a heat wave in Newfoundland (Gamperl et al., 2021; Fig. 4), and that the  $T_{f_H,peak}$  and  $CT_{max}$  of salmon in this study were within 1.8°C. In addition, there are several features of DSTs that make them a valuable tool for assessing fish thermal biology going forward. First, the cost of heart rate DSTs is decreasing, and there are DSTs that are capable of simultaneously recording  $f_H$  and activity (3D acceleration). These aspects will allow

DSTs to be used by a broader range of scientists, and to account for changes in behaviour (e.g. swimming speed: Zrini and Gamperl, 2021; Warren-Myers et al., 2023) when using them to examine aspects of the thermal biology and bioenergetics of fishes. There are also DSTs now available that are capable of recording ECGs for intervals up to 15 s at 100 Hz (as opposed to the 6 s recording limit in the DSTs used in this study) which will make the accurate determination of  $T_{arr}$  (although we believe  $T_{f_H,max}$  is a more ecologically relevant measure of a fish's thermal tolerance) possible. Finally, the environmental tolerances of several individuals can be measured simultaneously. The latter point is important as this allows for data on a large number of fish to be collected in a relatively short period of time, and this was one of the arguments for developing the rapid screening protocol.

This, however, is not to say that there are no drawbacks to using this experimental approach. First, DSTs have a limited battery life, they cannot presently be implanted into very small fish, and measuring  $f_H$  in free-swimming fishes requires that you have an appropriate place to hold the fish post-implantation. Second, measuring  $f_H$  in free-swimming fishes does not allow one to examine the mechanistic basis/es for differences in environment-dependent  $f_H$  responses and tolerances between species, populations or as impacted by specific conditions. In this regard, our results suggest that protocols used to measure heart function in fishes, and that allow for the measurement of other physiological parameters (e.g. oxygen consumption) and blood sampling/agent administration, could be modified so that they provide more accurate measures of thermal tolerance. The salmon implanted with Doppler® flow probes had equivalent values for the ABT for  $f_H$ , a value for  $f_{H,peak}$  that was not significantly different from that of free-swimming fishes, and a  $CT_{max}$  that was only 1.8°C lower as compared with the latter group. It is possible that longer surgical recovery times, and approaches to lower confinement stress (e.g. Altimiras and Larsen, 2000), could lower the fish's resting  $f_H$  and, thus, allow the fish to have a more realistic  $f_H$  scope available to meet environmental and other challenges.

Finally, our results highlight the potential interaction between the degree of stress experienced by fishes, and how this influences their capacity to respond to other environmental challenges. With regards to  $f_H$ , it has already been shown in sablefish (*Anoplopoma fimbria*) (Leeuwis et al., 2021) and Atlantic salmon (A.K.G., J. J. H. Nati, K. A. Clow, R.M.S., L. Gerber, E. F. C. Peroni and E. S. Porter, unpublished data) that the cholinergic-mediated bradycardia induced by hypoxia prevents fish from being able to increase  $f_H$  during an acute thermal challenge and reduces their thermal tolerance ( $CT_{max}$ ). In addition, in this study, we suggest that: the low  $f_H$  in truly resting (unstressed) fish limits their capacity to reduce  $f_H$  when exposed to oxygen-limited conditions (i.e. due to an already high cholinergic tone on the heart); and that stress-induced increases in  $f_H$  limit the  $f_{H,scope}$  available to deal with rising temperatures and, thus, result in a reduction in upper thermal tolerance. Both of these observations/hypotheses require validation via additional experiments, but potentially add greatly to our understanding of fish cardiorespiratory physiology and the mechanistic basis of environmental tolerances.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: R.M.S., A.K.G.; Methodology: R.M.S., A.K.G.; Validation: R.M.S.; Formal analysis: R.M.S.; Investigation: R.M.S.; Resources: A.K.G.; Data curation: A.K.G.; Writing - original draft: R.M.S.; Writing - review & editing: R.M.S.,

A.K.G.; Supervision: A.K.G.; Project administration: A.K.G.; Funding acquisition: A.K.G.

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### Data availability

All relevant data can be found within the article and its [supplementary information](#).

### References

- Alfonso, S., Gesto, M. and Sadoul, B. (2021). Temperature increase and its effects on fish stress physiology in the context of global warming. *J. Fish. Biol.* **98**, 1496–1508. doi:10.1111/jfb.14599
- Altimiras, J. and Larsen, E. (2000). Non-invasive recording of heart rate and ventilation rate in rainbow trout during rest and swimming. Fish go wireless!. *J. Fish. Biol.* **57**, 197–209. doi:10.1111/j.1095-8649.2000.tb00786.x
- Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A., Wolters, W. R. and Schulte, P. M. (2013). Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *J. Exp. Biol.* **216**, 1183–1190. doi:10.1242/jeb.080556
- Anttila, K., Couturier, C. S., Overlie, O., Johnsen, A., Marthinsen, G., Nilsson, G. E. and Farrell, A. P. (2014). Atlantic salmon show capacity for cardiac acclimation to warm temperatures. *Nat. Commun.* **5**, 4252. doi:10.1038/ncomms5252
- Anttila, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppänen, E., Kolari, I. and Nikinmaa, M. (2015). Warm acclimation and oxygen depletion induce species-specific responses in salmonids. *J. Exp. Biol.* **218**, 1471–1477. doi:10.1242/jeb.119115
- Åsheim, E., Andreassen, A., Morgan, R. and Jutfelt, F. (2020). Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish. *J. Exp. Biol.* **223**, jeb229195. doi:10.1242/jeb.229195
- Bartlett, C. B., Garber, A. F., Gonen, S. and Benfey, T. J. (2022). Acute critical thermal maximum does not predict chronic incremental thermal maximum in Atlantic salmon (*Salmo salar*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **266**, 111143. doi:10.1016/j.cbpa.2022.111143
- Bates, A. E. and Morley, S. A. (2020). Interpreting empirical estimates of experimentally derived physiological and biological thermal limits in ectotherms. *Can. J. Zool.* **98**, 237–244. doi:10.1139/cjz-2018-0276
- Becker, C. D. and Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environ. Biol. Fishes* **4**, 245–256. doi:10.1007/BF00005481
- Beitinger, T. L., Bennett, W. A. and McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ. Biol. Fishes* **58**, 237–275. doi:10.1023/A:1007676325825
- Bender, M. A., Knutson, T. R., Tuleya, R. E., Sirutis, J. J., Vecchi, G. A., Garner, S. T. and Held, I. M. (2010). Modeled impact of anthropogenic warming on the frequency of intense Atlantic hurricanes. *Science* **327**, 454–458. doi:10.1126/science.1180568
- Brauner, C. J. and Richards, J. G. (2020). Physiological performance in aquaculture: using physiology to help define optimal conditions for growth and environmental tolerance. *Fish Physiol.* **38**, 83–121. doi:10.1016/bs.fp.2020.10.001
- Bjarnason, Á., Gunnarsson, A., Árnason, T., Oddgeirsson, M., Sigmarsson, A. B. and Gunnarsson, Á. (2019). Validation of ECG-derived heart rate recordings in Atlantic cod (*Gadus morhua* L.) with an implantable data logging system. *Anim. Biotelemetry* **7**, 13. doi:10.1186/s40317-019-0176-4
- Blasco, F. R., Esbaugh, A. J., Killen, S. S., Rantin, F. T., Taylor, E. W. and McKenzie, D. J. (2020). Using aerobic exercise to evaluate sub-lethal tolerance of acute warming in fishes. *J. Exp. Biol.* **223**, jeb218602. doi:10.1242/jeb.218602
- Brett, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool.* **11**, 99–113. doi:10.1093/icb/11.1.99
- Brett, J. R. (1979). Factors affecting fish growth. In *Fish Physiology*, Vol. 8 (ed. W.S. Hoar, D.J. Randall and J.R. Brett), pp. 599–675. New York: Academic Press.
- Brijs, J., Sandblom, E., Axelsson, M., Sundell, K., Sundh, H., Huyben, D., Broström, R., Kiessling, A., Berg, C. and Gräns, A. (2018). The final countdown: Continuous physiological welfare evaluation of farmed fish during common aquaculture practices before and during harvest. *Aquaculture* **495**, 903–911. doi:10.1016/j.aquaculture.2018.06.081
- Brijs, J., Sandblom, E., Rosengren, M., Sundell, K., Berg, C., Axelsson, M. and Gräns, A. (2019). Prospects and pitfalls of using heart rate bio-loggers to assess the welfare of rainbow trout (*Oncorhynchus mykiss*) in aquaculture. *Aquaculture* **509**, 188–197. doi:10.1016/j.aquaculture.2019.05.007
- Burke, H., Gardener, I. and Farrell, A. P. (2020). A review of the 2019 Newfoundland and Labrador South Coast cultured Atlantic salmon mortality event. Department of Fisheries and Land Resources, Government of Newfoundland and Labrador, Special Studies and Reports. Retrieved from <http://www.gov.nl.ca/ffa/files/publications-pdf-2019-salmon-review-final-report.pdf>.
- Burt, K., Hamoutene, D., Mabrouk, G., Lang, G., Lang, C., Puestow, T., Drover, D., Losier, R. and Page, F. (2012). Environmental conditions and occurrence of hypoxia within production cages of Atlantic salmon on the south coast of Newfoundland. *Aquac. Res.* **43**, 607–620. doi:10.1111/j.1365-2109.2011.02867.x
- Caissie, D., Breau, C., Hayward, J. and Cameron, P. (2012). Water temperature characteristics within the Miramichi and Restigouche rivers. *DFO Can. Sci. Advis. Sec. Sci. Advis. Rep.* **165**, 1–31.
- Casselmann, M. T., Anttila, K. and Farrell, A. P. (2012). Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. *J. Biol.* **80**, 358–377. doi:10.1111/j.1095-8649.2011.03182.x
- Chaput, G. (2012). Overview of the status of Atlantic salmon (*Salmo salar*) in the North Atlantic and trends in marine mortality. *ICES Mar. Sci.* **69**, 1538–1548. doi:10.1093/icesjms/fss013
- Cheung, W. W. W. and Frölicher, T. L. (2020). Marine heatwaves exacerbate climate change impacts for fisheries in the northeast Pacific. *Sci. Rep.* **10**, Article 1. doi:10.1038/s41598-020-63650-z
- Cheung, W. W. L., Frölicher, T. L., Lam, V. W. Y., Oyiniola, M. A., Reygondeau, G., Sumaila, U. R., Tai, T. C., Teh, L. C. L. and Wabnitz, C. C. C. (2021). Marine high temperature extremes amplify the impacts of climate change on fish and fisheries. *Sci. Adv.* **7**, eabh0895. doi:10.1126/sciadv.abh0895
- Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G. and Farrell, A. P. (2008). Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *Am. J. Physiol.* **295**, R1631–R1639. doi:10.1152/ajpregu.90461.2008
- Clark, T. D., Sandblom, E. and Jutfelt, F. (2013a). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771–2782. doi:10.1242/jeb.084251
- Clark, T. D., Sandblom, E. and Jutfelt, F. (2013b). Response to Farrell and to Pörtner and Giomi. *J. Exp. Biol.* **216**, 4495–4497. doi:10.1242/jeb.096313
- Dadswell, M., Spares, A., Reader, J., McLean, M., McDermott, T., Samways, K. and Lilly, J. (2021). The decline and impending collapse of the Atlantic salmon (*Salmo salar*) population in the North Atlantic Ocean: a review of possible causes. *Rev. Fish. Sci.* **30**, 215–258. doi:10.1080/23308249.2021.1937044
- Dahlke, F. T., Wohlrab, S., Butzin, M. and Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science* **369**, 65–70. doi:10.1126/science.aaz3658
- Desforges, J. E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K. M., Eliason, E. J., Dressler, T. L., McKenzie, D. J., Bates, A. E., Lawrence, M. J., Fanguie, N. et al. (2023). The ecological relevance of critical thermal maxima methodology (CTM) for fishes. *J. Fish Biol.* **102**, 1000–1016. doi:10.1111/jfb.15368
- Ekström, A., Axelsson, M., Gräns, A., Brijs, J. and Sandblom, E. (2018). Importance of the coronary circulation for cardiac and metabolic performance in rainbow trout (*Oncorhynchus mykiss*). *Biol. Lett.* **14**, 20180063. doi:10.1098/rsbl.2018.0063
- Ern, R., Norin, T., Gamperl, A. K. and Esbaugh, A. J. (2016). Oxygen dependence of upper thermal limits in fishes. *J. Exp. Biol.* **219**, 3376–3383. doi:10.1242/jeb.143495
- Ern, R., Andreassen, A. H. and Jutfelt, F. (2023). Physiological mechanisms of acute upper thermal tolerance in fish. *Physiology (Bethesda)* **38**, 141–158. doi:10.1152/physiol.00027.2022
- Farrell, A. P. (2007). Tribute to P. L. Lutz: a message from the heart – why hypoxic bradycardia in fishes? *J. Exp. Biol.* **210**, 1715–1725. doi:10.1242/jeb.02781
- Farrell, A. P. and Smith, F. M. (2017). Cardiac form, function and physiology. In *Fish Physiology*, Vol. 36 (ed. A. K. Gamperl, T. E. Gillis, A. P. Farrell and C. J. Brauner), pp. 155–264. New York, NY: Academic Press.
- Farrell, A. P., Eliason, E. J., Sandblom, E. and Clark, T. D. (2009). Fish cardiorespiratory physiology in an era of climate change. *Can. J. Zool.* **87**, 835–851. doi:10.1139/Z09-092
- Ferreira, E. O., Anttila, K. and Farrell, A. P. (2014). Thermal optima and tolerance in the eurythermic goldfish (*Carassius auratus*): relationships between whole-animal aerobic capacity and maximum heart rate. *Physiol. Biochem. Zool.* **87**, 599–611. doi:10.1086/677317
- Føre, M., Svendsen, E., Økland, F., Gräns, A., Alfredsen, J. A., Finstad, B. and Hedger, R. D. (2021). Heart rate and swimming activity as indicators of post-surgical recovery time of Atlantic salmon (*Salmo salar*). *Anim. Biotelemetry* **9**, 1–13. doi:10.1186/s40317-020-00226-8
- Frölicher, T. L., Fischer, E. M. and Gruber, N. (2018). Marine heatwaves under global warming. *Nature* **560**, 360–364. doi:10.1038/s41586-018-0383-9
- Fry, F. E. (1947). Effects of the environment on animal activity. *Publications of the Ontario Fisheries Research Laboratory* **68**, 1–62.
- Gallant, M. J., LeBlanc, S., MacCormack, T. J. and Currie, S. (2017). Physiological responses to a short-term, environmentally realistic, acute heat



- stress in Atlantic salmon, *Salmo salar*. *FACETS* 2, 330-341. doi:10.1139/facets-2016-0053
- Gamperl, A. K., Swafford, B. L. and Rodnick, K. J. (2011). Elevated temperature, per se, does not limit the ability of rainbow trout to increase stroke volume. *J. Therm. Biol.* 36, 7-14. doi:10.1016/j.jtherbio.2010.08.007
- Gamperl, A. K., Ajiboye, O. O., Zanuzzo, F. S., Sandrelli, R. M., Peroni, E. F. and Beemelmanns, A. (2020). The impacts of increasing temperature and moderate hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic salmon (*Salmo salar*). *Aquaculture* 519, 734874. doi:10.1016/j.aquaculture.2019.734874
- Gamperl, A. K., Zrini, Z. A. and Sandrelli, R. M. (2021). Atlantic salmon (*Salmo salar*) cage-site distribution, behaviour, and physiology during a Newfoundland heat wave. *Front. Physiol.* 12, 719594. doi:10.3389/fphys.2021.719594
- Genin, A., Levy, L., Sharon, G., Raitso, D. E. and Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish. *Proc. Natl. Acad. Sci. U.S.A.* 117, 25378-25385. doi:10.1073/pnas.2009748117
- Gilbert, M. J. H. and Farrell, A. P. (2021). The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic char (*Salvelinus alpinus*), a northern cold-water specialist. *J. Therm. Biol.* 95, 102816. doi:10.1016/j.jtherbio.2020.102816
- Gollock, M. J., Currie, S., Petersen, L. H. and Gamperl, A. K. (2006). Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. *J. Exp. Biol.* 209, 2961-2970. doi:10.1242/jeb.02319
- Ignatz, E. H., Sandrelli, R. M., Vadboncoeur, É., Zanuzzo, F. S., Perry, G. M. L., Rise, M. L. and Gamperl, A. K. (2023). The Atlantic salmon's (*Salmo salar*) incremental thermal maximum is a more relevant and sensitive indicator of family-based differences in upper temperature tolerance than its critical thermal maximum. *Aquaculture* 574, 739628. doi:10.1016/j.aquaculture.2023.739628
- IPCC (2022). Summary for Policymakers. In *Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. H.-O. Pörtner, D. C. Roberts, E. S. Poloczanska, K. Mintenbeck, M. Tignor, A. Alegría, M. Craig, S. Langsdorf, S. Lösschke, V. Möller and A. Okem), pp. 3-33. Cambridge University Press. doi:10.1017/9781009325844.001
- Hansen, A. K., Byriell, D. B., Jensen, M. R., Steffensen, J. F. and Svendsen, M. S. (2017). Optimum temperature of a northern population of Arctic charr (*Salvelinus alpinus*) using heart rate Arrhenius breakpoint analysis. *Polar Biol.* 40, 1063-1070. doi:10.1007/s00300-016-2033-8
- Holeton, G. F. and Randall, D. J. (1967). Changes in blood pressure in the rainbow trout during hypoxia. *J. Exp. Biol.* 46, 297-305. doi:10.1242/jeb.46.2.297
- Hvas, M., Folkedal, O., Imsland, A. and Oppedal, F. (2017). The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. *J. Exp. Biol.* 220, 2757-2764. doi:10.1242/jeb.154021
- Hvas, M., Folkedal, O. and Oppedal, F. (2020). Fish welfare in offshore salmon aquaculture. *Rev. Aquac.* 13, 836-852. doi:10.1111/raq.12501
- Jørgensen, L. B., Ørsted, M., Malte, H., Wang, T. and Overgaard, J. (2022). Extreme escalation of heat failure rates in ectotherms with global warming. *Nature* 611, 93-98. doi:10.1038/s41586-022-05334-4
- Joyce, W., Ozolina, K., Mauduit, F., Ollivier, H., Claireaux, G. and Shiels, H. A. (2016). Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. *Biol. Lett.* 12, 20150708. doi:10.1098/rsbl.2015.0708
- Jutfelt, J., Gråns, A., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Sundell, K., Axelsson, M. (2014). Response to 'How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope – remarks on the article by Gråns et al.'. *J. Exp. Biol.* 217, 4433-4435. doi:10.1242/jeb.115410
- Jutfelt, F., Roche, D. G., Clark, T. D., Norin, T., Binning, S. A., Speers-Roesch, B., Amcoff, M., Morgan, R., Andreassen, A. H. and Sundin, J. (2019). Brain cooling marginally increases acute upper thermal tolerance in Atlantic cod. *J. Exp. Biol.* 222, jeb208249. doi:10.1242/jeb.208249
- Keen, A. N. and Gamperl, A. K. (2012). Blood oxygenation and cardiorespiratory function in steelhead trout (*Oncorhynchus mykiss*) challenged with an acute temperature increase and zatebradine-induced bradycardia. *J. Therm. Biol.* 37, 201-210. doi:10.1016/j.jtherbio.2012.01.002
- Laufkötter, C., Zscheischler, J. and Frölicher, T. L. (2020). High-impact marine heatwaves attributable to human-induced global warming. *Science* 369, 1621-1625. doi:10.1126/science.aba0690
- Leeuwis, R. H. J., Nash, G. W., Sandrelli, R. M., Zanuzzo, F. S. and Gamperl, A. K. (2019). The environmental tolerances and metabolic physiology of sablefish (*Anoplopoma fimbria*). *Comp. Biochem. Physiol. A* 231, 140-148. doi:10.1016/j.cbpa.2019.02.004
- Leeuwis, R. H. J., Zanuzzo, F. S., Peroni, E. F. C. and Gamperl, A. K. (2021). Sablefish (*Anoplopoma fimbria*) research suggests that limited capacity to increase heart function may leave hypoxic fish susceptible to heat waves. *Proc. Roy. Soc. B* 288, 20202340. doi:10.1098/rspb.2020.2340
- Lefevre, S. (2016). Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO<sub>2</sub> and their interaction. *Conserv. Physiol.* 4, cow009. doi:10.1093/conphys/cow009
- Lefevre, S., Wang, T. and McKenzie, D. J. (2021). The role of mechanistic physiology in investigating impacts of global warming on fishes. *J. Exp. Biol.* 224, jeb238840. doi:10.1242/jeb.238840
- Little, A. G., Loughland, I. and Seebacher, F. (2020). What do warming waters mean for fish physiology and fisheries? *J. Fish Biol.* 97, 328-340. doi:10.1111/jfb.14402
- Marvin, D. E. and Heath, A. G. (1968). Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of fresh-water fish. *Comp. Biochem. Physiol.* 27, 349-355. doi:10.1016/0010-406X(68)90777-9
- McKenzie, D. J., Skov, P. V., Taylor, E. W. T., Wang, T. and Steffensen, J. F. (2009). Abolition of reflex bradycardia by cardiac vagotomy has no effect on the regulation of oxygen uptake by Atlantic cod in progressive hypoxia. *Comp. Biochem. Physiol. Part A: Mol. Int. Physiol.* 153, 332-338. doi:10.1016/j.cbpa.2009.03.009
- McKenzie, D. J., Axelsson, M., Chabot, D., Claireaux, G., Cooke, S. J., Corner, R. A., De Boeck, G., Domenici, P., Guerreiro, P. M., Hamer, B. et al. (2016). Conservation physiology of marine fishes: State of the art and prospects for policy. *Conserv. Physiol.* 4, cow046. doi:10.1093/conphys/cow046
- Mendonça, P. C. and Gamperl, A. K. (2010). The effect of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (*Pseudopleuronectes americanus*). *Comp. Biochem. Physiol. A* 155, 245-252. doi:10.1016/j.cbpa.2009.11.006
- Mignucci, A., Bourjea, J., Forget, F., Allal, H., Dutto, G., Gasset, E. and McKenzie, D. J. (2021). Cardiac and behavioural responses to hypoxia and warming in free-swimming gilthead seabream, *Sparus aurata*. *J. Exp. Biol.* 224, jeb242397. doi:10.1242/jeb.242397
- Mills, K. E., Pershing, A. J., Sheehan, T. F. and Mountain, D. (2013). Climate and ecosystem linkages explain widespread declines in North American Atlantic salmon populations. *Glob. Chang. Biol.* 19, 3046-3061. doi:10.1111/gcb.12298
- Morgan, R., Sundin, J., Finnøen, M. H., Dresler, G., Vendrell, M. M., Dey, A., Sarkar, K. and Jutfelt, F. (2019). Are model organisms representative for climate change research? Testing thermal tolerance in wild and laboratory zebrafish populations. *Conserv. Physiol.* 7, coz036. doi:10.1093/conphys/coz036
- Motyka, R., Norin, T., Petersen, L. H., Huggett, D. B. and Gamperl, A. K. (2017). Long-term hypoxia exposure alters the cardiorespiratory physiology of steelhead trout (*Oncorhynchus mykiss*) but does not affect their upper thermal tolerance. *J. Therm. Biol.* 68, 149-161. doi:10.1016/j.jtherbio.2016.03.007
- Muller, C., Childs, A.-R., Duncan, M. I., Skeeles, M. R., James, N. C., van der Walt, K.-E., Winkler, A. C. and Potts, W. M. (2020). Implantation, orientation and validation of a commercially produced heart-rate logger for use in a perciform teleost fish. *Conserv. Physiol.* 8, coaa035. doi:10.1093/conphys/coaa035
- Nicola, G. G., Elvira, B., Jonsson, B., Ayllón, D. and Almodóvar, A. (2018). Local and global climatic drivers of Atlantic salmon decline in southern Europe. *Fish. Res.* 198, 78-85. doi:10.1016/j.fishres.2017.10.012
- Norin, T., Malte, H. and Clark, T. D. (2014). Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *J. Exp. Biol.* 217, 244-251. doi:10.1242/jeb.089755
- Norin, T., Canada, P., Bailey, J. A. and Gamperl, A. K. (2019). Thermal biology and swimming performance of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). *Peer J.* 7, e7784. doi:10.7717/peerj.7784
- Oldham, T., Dempster, T., Foss, J. and Oppedal, F. (2017). Oxygen gradients affect behaviour of caged Atlantic salmon (*Salmo salar*). *Aquac. Environ. Int.* 9, 145-153. doi:10.3354/aei00219
- Oppedal, F., Dempster, T. and Stien, L. H. (2011). Environmental drivers of Atlantic salmon behaviour in sea cages: a review. *Aquaculture* 311, 1-18. doi:10.1016/j.aquaculture.2010.11.020
- Ørsted, M., Jørgensen, L. B. and Overgaard, J. (2022). Finding the right thermal limit: A framework to reconcile ecological, physiological and methodological aspects of CT<sub>max</sub> in ectotherms. *J. Exp. Biol.* 225, jeb244514. doi:10.1242/jeb.244514
- Payne, N. L., Morley, S. A., Halsey, L. G., Smith, J. A., Stuart-Smith, R., Waldock, C. and Bates, A. E. (2021). Fish heating tolerance scales similarly across individual physiological and populations. *Commun. Biol.* 4, 264. doi:10.1038/s42003-021-01773-3
- Penney, C. M., Nash, G. W. and Gamperl, A. K. (2014). Cardiorespiratory responses of seawater-acclimated adult Arctic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) to an acute temperature increase. *Can. J. Fish. Aquat. Sci.* 71, 1096-1105. doi:10.1139/cjfas-2013-0569
- Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science* 308, 1912-1915. doi:10.1126/science.1111322
- Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L. and Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569, 108-111. doi:10.1038/s41586-019-1132-4
- Porter, E. S., Clow, K. A., Sandrelli, R. M. and Gamperl, A. K. (2022). Acute and chronic cold exposure differentially affect cardiac control, but not cardiorespiratory function, in resting Atlantic salmon (*Salmo salar*). *Cur. Res. Physiol.* 5, 158-170. doi:10.1016/j.crphys.2022.03.002



- Pörtner, H.-O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Bioch. Physiol. A* **132**, 739-761. doi:10.1016/S1095-6433(02)00045-4
- Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **213**, 881-893. doi:10.1242/jeb.037523
- Pörtner, H. O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95. doi:10.1126/science.1135471
- Pörtner, H. O. and Farrell, A. P. (2008). Physiology and climate change. *Science* **322**, 690-692. doi:10.1126/science.1163156
- Pörtner, H.-O., Bock, C. and Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *J. Exp. Biol.* **1**, 2685-2696. doi:10.1242/jeb.134585
- Randall, D. J. and Smith, L. S. (1967). The effect of environmental factors on circulation and respiration in teleost fish. *Hydrobiol* **19**, 113-124. doi:10.1007/BF00142057
- Reid, G. K., Gurney-Smith, H. K., Marcogliese, D. J., Knowler, D., Benfey, T. and Garber, A. F. (2019). Climate change and aquaculture. *Aquat. Environ. Int.* **11**, 569-602.
- Reid, C. H., Patrick, P. H., Rytwinski, T., Taylor, J. J., Willmore, W. G., Reesor, B. and Cooke, S. J. (2022). An updated review of cold shock and cold stress in fish. *J. Fish Biol.* **100**, 1102-1137. doi:10.1111/jfb.15037
- Richards, J. G. (2011). Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.* **214**, 191-199. doi:10.1242/jeb.047951
- Rodnick, K. J., Gamperl, A. K., Lizars, K. R., Bennett, M. T., Rausch, R. N. and Keeley, E. R. (2004). Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *J. Fish Biol.* **64**, 310-335. doi:10.1111/j.0022-1112.2004.00292.x
- Sandblom, E. and Axelsson, M. (2011). Autonomic control of circulation in fish: a comparative view. *Auton. Neurosci.* **165**, 127-139. doi:10.1016/j.autneu.2011.08.006
- Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijis, J., Sundström, L. F., Odelström, A., Adill, A., Aho, T. and Jutfelt, F. (2016). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nat. Commun.* **7**, 11447. doi:10.1038/ncomms11447
- Schurmann, H. and Steffensen, J. F. (1994). Spontaneous swimming activity of Atlantic cod *Gadus morhua* exposed to graded hypoxia at three temperatures. *J. Exp. Biol.* **197**, 129-142. doi:10.1242/jeb.197.1.129
- Sidhu, R., Anttila, K. and Farrell, A. P. (2014). Upper thermal tolerance of closely related Danio species. *J. Fish Biol.* **84**, 982-995. doi:10.1111/jfb.12339
- Sinclair, B. J., Marshal, K. E., Sewell, M. A., Levesque, D. L., Willet, C. S., Slotsbo, S., Dong, Y., Harley, C. D. G., Marshall, D. J., Helsmuth, B. S. et al. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* **19**, 1372-1385. doi:10.1111/ele.12686
- Skeeles, M. R., Winkler, A. C., Duncan, M. I., James, N. C., van der Walt, K.-A. and Potts, W. M. (2020). The use of internal heart rate loggers in determining cardiac breakpoints of fish. *J. Therm. Biol.* **89**, 102524. doi:10.1016/j.jtherbio.2020.102524
- Soloman, S. (2007). Climate Change 2007: The physical science basis. Working Group I contribution to the fourth assessment report of the IPCC, pp. 1-56. Cambridge University Press.
- Somero, G. N. (2011). Comparative physiology: a "crystal ball" for predicting consequences of global change. *Am. J. Physiol.* **301**, R1-R14. doi:10.1152/ajpcell.00345.2010
- Stecyk, J. A. W. (2017). Cardiovascular responses to limiting oxygen levels. In *Fish Physiology*, Vol. 36, pp. 299-371. Elsevier.
- Stehfest, K. M., Carter, C. G., McAllister, J. D., Ross, J. D. and Semmens, J. M. (2017). Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors. *Sci. Rep.* **7**, 4545. doi:10.1038/s41598-017-04806-2
- Steinhausen, M. F., Sandblom, E., Eliason, E. J., Vehille, C. and Farrell, A. P. (2008). The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *J. Exp. Biol.* **211**, 3915-3926. doi:10.1242/jeb.019281
- Stevens, D. E., Bennion, G. R., Randall, D. J. and Shelton, G. (1972). Factors affecting arterial pressures and blood flow from the heart in intact, unrestrained lingcod, *Ophiodon elongatus*. *Comp. Biochem. Physiol. A* **43**, 681-695.
- Stuart-Smith, R. D., Edgar, G. J. and Bates, A. E. (2017). Thermal limits to the geographic distributions of shallow-water marine species. *Nat. Ecol. Evol.* **1**, Article 12. doi:10.1038/s41559-017-0353-x
- Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nat. Clim. Chang* **2**, 686-690. doi:10.1038/nclimate1539
- Szekerés, P., Eliason, E. J., Lapointe, D., Donaldson, M. R., Brownscombe, J. W. and Cooke, S. J. (2016). On the neglected cold side of climate change and what it means to fish. *Clim. Res.* **69**, 239-245. doi:10.3354/cr01404
- Tromp, J. J., Jones, P. L., Brown, M. S., Donald, J. A., Biro, P. A. and Afonso, L. O. (2018). Chronic exposure to increased water temperature reveals few impacts on stress physiology and growth responses in juvenile Atlantic salmon. *Aquaculture* **495**, 196204. doi:10.1016/j.aquaculture.2018.05.042
- Vadboncoeur, É., Nelson, C., Hall, J. R., Clow, K. A., Sandrelli, R. M., Brauner, C. J., Swanson, A. K. and Gamperl, A. K. (2023). Lowering temperature to 1°C results in physiological changes, stress and mortality in cultured Atlantic salmon (*Salmo salar*). *Aquaculture* **568**, 739313. doi:10.1016/j.aquaculture.2023.739313
- van der Walt, K.-A., Potts, W. M., Porri, F., Winkler, A. C., Duncan, M. I., Skeeles, M. R. and James, N. C. (2021). Marine heatwaves exceed cardiac thermal limits of adult spard fish (*Diplodus capensis*, Smith 1884). *Front. Mar. Sci.* **8**, 702463. doi:10.3389/fmars.2021.702463
- Viglion, G. (2021). FEVERS are plaguing the oceans—and climate change is making them worse. *Nature* **593**, 26-28. doi:10.1038/d41586-021-01142-4
- Wade, N. M., Clark, T. D., Maynard, B. T., Atherton, S., Wilkinson, R. J., Smullen, R. P. and Taylor, R. S. (2019). Effects of an unprecedented summer heatwave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (*Salmo salar*). *J. Therm. Biol.* **80**, 64-74. doi:10.1016/j.jtherbio.2018.12.021
- Wang, T. and Overgaard, J. (2007). The heartbreak of adapting to global warming. *Science* **315**, 49-50. doi:10.1126/science.1137359
- Warren-Myers, F., Svendsen, E., Føre, M., Folkedal, O., Oppedal, F. and Hvas, M. (2023). Novel tag-based method for measuring tailbeat frequency and variations in amplitude in fish. *Anim. Biotelemetry* **11**, 12. doi:10.1186/s40317-023-00324-3
- Wood, C. M. and Shelton, G. (1980). The reflex control of heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage, and systemic vasomotor tone. *J. Exp. Biol.* **87**, 271-284. doi:10.1242/jeb.87.1.271
- Wood, C. M., Pieprzak, P. and Trott, J. N. (1979). The influence of temperature and anaemia on the adrenergic and cholinergic mechanisms controlling heart rate in the rainbow trout. *Can. J. Zool.* **57**, 2440-2447. doi:10.1139/z79-316
- Yousaf, M. N., Røn, Ø., Hagen, P. P. and McGurk, C. (2022). Monitoring fish welfare using heart rate bio-loggers in farmed Atlantic salmon (*Salmo salar* L.): An insight into the surgical recovery. *Aquaculture* **555**, 738211. doi:10.1016/j.aquaculture.2022.738211
- Zanuzzo, F. S., Bailey, J. A. and Gamperl, A. K. (2019). The acute and incremental thermal tolerance of Atlantic cod (*Gadus morhua*) families under normoxia and mild hypoxia. *Comp. Biochem. Physiol.* **233**, 30-38. doi:10.1016/j.cbpa.2019.03.020
- Zrini, Z. A. and Gamperl, A. K. (2021). Validating heart rate and acceleration data storage tags for use in Atlantic salmon (*Salmo salar*). *Anim. Biotelemetry* **9**, 12. doi:10.1186/s40317-021-00235-1