



UNIVERSITY OF LEEDS

This is a repository copy of *Exploring nature's natural knockouts: In vivo cardiorespiratory performance of Antarctic fishes during acute warming.*

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/133549/>

Version: Published Version

Article:

Joyce, W, Egginton, S orcid.org/0000-0002-3084-9692, Farrell, AP et al. (3 more authors) (2018) Exploring nature's natural knockouts: In vivo cardiorespiratory performance of Antarctic fishes during acute warming. *The Journal of Experimental Biology*, 221 (15). 183160. ISSN 0022-0949

<https://doi.org/10.1242/jeb.183160>

© 2018. Published by The Company of Biologists Ltd. This is an article published in the *Journal of Experimental Biology*. Uploaded in accordance with the publisher's self-archiving policy.

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

RESEARCH ARTICLE

Exploring nature's natural knockouts: *in vivo* cardiorespiratory performance of Antarctic fishes during acute warming

William Joyce¹, Stuart Egginton², Anthony P. Farrell³, Elizabeth L. Crockett⁴, Kristin M. O'Brien⁵ and Michael Axelsson^{6,*}

ABSTRACT

We tested the hypothesis that blackfin icefish (*Chaenocephalus aceratus*), one of the six species in the family Channichthyidae (the icefishes) that do not express haemoglobin and myoglobin, lack regulatory cardiovascular flexibility during acute warming and activity. The experimental protocols were designed to optimize the surgical protocol and minimize stress. First, minimally invasive heart rate (f_H) measurements were made during a thermal ramp until cardiac failure in *C. aceratus* and compared with those from the closely related red-blooded black rockcod (*Notothenia coriiceps*). Then, integrative cardiovascular adjustments were more extensively studied using flow probes and intravascular catheters in *C. aceratus* during acute warming (from 0 to 8°C) at rest and after imposed activity. *Chaenocephalus aceratus* had a lower routine f_H than *N. coriiceps* (9 beats min^{-1} versus 14 beats min^{-1}) and a lower peak f_H during acute warming (38 beats min^{-1} versus 55 beats min^{-1}) with a similar cardiac breakpoint temperature (13 and 14°C, respectively). Routine cardiac output (\dot{Q}) for *C. aceratus* at ~0°C was much lower (26.6 ml min^{-1} kg^{-1}) than previously reported, probably because fish in the present study had a low f_H (12 beats min^{-1}) indicative of a high routine vagal tone and low stress. *Chaenocephalus aceratus* increased oxygen consumption during acute warming and with activity. Correspondingly, \dot{Q} increased considerably (maximally 86.3 ml min^{-1} kg^{-1}), as did vascular conductance (5-fold). Thus, unlike earlier suggestions, these data provide convincing evidence that icefish can mount a well-developed cardiovascular regulation of heart rate, cardiac output and vascular conductance, and this regulatory capacity provides flexibility during acute warming.

KEY WORDS: Cardiac output, Heart rate, Stroke volume, ECG, Warming, Sympathovagal balance

INTRODUCTION

The notothenioid suborder of fishes that are dominant in the frigid Southern Ocean include the remarkable haemoglobinless (Hb^-) Channichthyids (icefishes) (O'Brien and Crockett, 2013). The Hb^- state renders icefishes unique amongst adult vertebrates (Ruud, 1954) and a suite of compensatory cardiovascular modifications

accompanies the corresponding reduction in blood oxygen carrying capacity when compared with closely related red-blooded nototheniids (Axelsson, 2005), such as an enlarged heart (Holeton, 1970; Harrison et al., 1991) and greatly increased baseline vascular conductance (Hemmingsen and Douglas, 1977), which together permit a much greater cardiac output (\dot{Q}) than that of other fishes (Hemmingsen et al., 1972; Hemmingsen, 1991; Axelsson, 2005).

It is generally believed that icefishes can tolerate the Hb^- condition in part because of the extremely cold and stable temperature (~0°C) of the Southern Ocean, which not only increases the solubility of oxygen in seawater and the blood plasma (Ruud, 1954) but also results in a stable and low metabolic rate. Consequently, the fact that the area around the West Antarctic Peninsula, which is where many species of icefishes are found, is among the world's most rapidly warming biomes (Meredith and King, 2005; Clarke et al., 2007) is of considerable concern. In this region, average surface water temperatures have risen by approximately 1°C over the past 50 years, and this rate is expected to continue for the next century (IPCC, 2014), which could create a precarious situation for icefishes.

The lack of haemoglobin (and myoglobin) in icefishes is expected to be particularly problematic as the demand for oxygen transport increases during warming (Ruud, 1954; O'Brien and Crockett, 2013; Buckley et al., 2014; Beers and Jayasundara, 2015), but we do not understand how they are likely to respond. In fact, the only published study on oxygen consumption (\dot{M}_{O_2}) of *Chaenocephalus aceratus* discovered that \dot{M}_{O_2} increased only between 1 and 4°C, but not with additional warming to 10°C (Hemmingsen and Douglas, 1972). Icefishes also show a lower critical thermal maximum (CT_{max}) than sympatric Hb^+ nototheniids (Beers and Sidell, 2011). While these observations suggest that warming could create problems with internal oxygen transport, CT_{max} was insensitive to hyperoxia (Devor et al., 2016). This, together with the previous conflicting measurements of \dot{Q} , raises the question about the flexibility of cardiovascular function in icefishes.

For example, previous indirect estimates of routine \dot{Q} in *C. aceratus* (using the Fick principle) vary almost 3-fold from 60 ml min^{-1} kg^{-1} (Holeton, 1970) to 100–150 ml min^{-1} kg^{-1} (Hemmingsen et al., 1972). Furthermore, this range for routine \dot{Q} estimates could be even wider given the direct measurement of blood flow in the two cranial pairs of gill arches (10–15 ml min^{-1} kg^{-1}), which equates to routine \dot{Q} being 20–30 ml min^{-1} kg^{-1} by assuming equal blood flow in all branchial arteries (Hemmingsen et al., 1972). Despite this rather large range, the paradigm of a high routine \dot{Q} in icefishes (80–100 ml min^{-1} kg^{-1}) relative to Hb^+ species still persists in the literature (Hemmingsen, 1991; Tota et al., 1991; Zummo et al., 1995; Acierno, et al., 1997; Feller and Gerday, 1997; Sidell and

¹Department of Zoophysiology, Aarhus University, 8000 Aarhus C, Denmark.

²School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK.

³Department of Zoology, and Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC 45, Canada. ⁴Department of Biological Sciences, Ohio University, Athens, OH 45701, USA. ⁵Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775-7000, USA. ⁶Department of Biological and Environmental Sciences, University of Gothenburg, 4139 90 Gothenburg, Sweden.

*Author for correspondence (michael.axelsson@bioenv.gu.se)

 M.A., 0000-0002-7839-3233

O'Brien, 2006; Axelsson, 2005; Garofalo et al., 2009). Certainly, maximum \dot{Q} of icefishes determined *in vitro* with perfused heart preparations can be high (maximum \dot{Q} values between 100 and 300 ml min⁻¹ kg⁻¹ in *Chionodraco hamatus* at 3°C; Acierno et al., 1997; Tota et al., 1991). Nonetheless, our own recent work *in vitro* with perfused heart preparations found a lower maximum \dot{Q} in both *C. aceratus* (70 ml min⁻¹ kg⁻¹) and *Chionodraco rastrospinosus* at 1°C (126 ml min⁻¹ kg⁻¹) (S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished). Thus, if routine \dot{Q} for icefishes is close to maximum \dot{Q} , an obvious concern is that regulatory capacity is necessarily limited and there can be little cardiovascular flexibility during environmental challenges.

The aim of the present study was to test the hypothesis that *in vivo* cardiovascular function in *C. aceratus* lacks regulatory flexibility during acute warming. Comparisons could then be made with cardiorespiratory regulatory capacity of Hb⁺ nototheniids (e.g. Axelsson et al., 1992, 1994; Franklin et al., 2007; Campbell et al., 2009; Egginton and Campbell, 2016).

Previous studies have concluded that when Antarctic fishes are acutely warmed, they primarily adjust their heart rate (f_H) to regulate \dot{Q} (Axelsson et al., 1992; Campbell et al., 2009), much like other fishes (Farrell, 2009; Farrell and Smith, 2017). Also, while some studies report a high routine vagal tone in Antarctic notothenioids (Axelsson et al., 1992; Egginton and Campbell, 2016), Hemmingsen et al. (1972) reported only a small increase in f_H after atropine injection (from 16 to 18 beats min⁻¹) that could indicate stress. Therefore, we tested the possibility that earlier *in vivo* cardiovascular studies were affected by stress, which would have an impact on other measured variables such as \dot{Q} and cardiac power output. We used a minimally invasive method [electrocardiogram (ECG) measurements during an acute thermal ramp with and without autonomic vagal blockade with atropine] to provide insight into routine f_H , the level of vagal tone and the contribution of f_H in setting upper thermal tolerance (Farrell, 2009, 2016). The results from these minimally instrumented animals were used as a baseline for the more heavily instrumented animals that were part of the first comprehensive assessment of *in vivo* cardiorespiratory response of *C. aceratus* during activity and acute (sub-critical) warming. By simultaneously measuring a number of key cardiorespiratory variables, we could directly assess the flexibility and interplay of autonomic regulation and oxygen transport and address whether the Hb⁻ condition reduces cardiac scope and thus the ability of *C. aceratus* to tolerate temperature changes.

MATERIALS AND METHODS

Experimental animals

Given our concerns about physiological measurements being made on overly stressed animals, the ECG measurements on blackfin icefish, *Chaenocephalus aceratus* (Lönnerberg 1906) ($N=12$, body mass $M_b=1.55\pm 0.21$ kg, mean \pm s.d.), were also performed on the closely related Hb⁺ black rockcod, *Notothenia coriiceps* Richardson ($N=13$, $M_b=1.24\pm 0.32$ kg), to allow comparison with previously published data on other Hb⁺ Antarctic fishes. An additional group of *C. aceratus* were also instrumented for ECG measurements and used to ascertain autonomic tone ($N=4$, $M_b=1.587.5\pm 387.0$ kg). Finally, a separate group of *C. aceratus* ($N=16$, $M_b=0.85\pm 0.21$ kg) were used for the more invasive cardiorespiratory study. Adult fish were captured at 100–200 m depth using an otter trawl and baited benthic pots (for *N. coriiceps*) deployed from the ARSV *Laurence M. Gould* in Dallmann Bay (64°10'S, 62°35'W) and off the south-

western shore of Low Island (63°24'S, 62°10'W). Fish were held on the ship in circulating, aerated seawater tanks for up to 2 days before being transferred to Palmer Station, Antarctica, where they were held at ambient seawater temperature (0 \pm 1°C) for at least 72 h and up to 3 weeks before experiments commenced. *Notothenia coriiceps* were fed blocks of fish muscle fillet every other day, but not 24–48 h prior to surgery, whereas *C. aceratus* did not feed in captivity. All experiments involving live animals were approved by the University of Alaska, Fairbanks Institutional Animal Use and Care Committee (570217-9).

Surgical instrumentation for *in vivo* upper thermal tolerance test while measuring the ECG

Instantaneous ECGs were recorded with minimal surgical intervention using laterally staggered bipolar electrodes (7-strand Teflon-coated wire, 0.2 mm diameter; A-M Systems, Sequim, WA, USA). Fish were first anaesthetized in ice-chilled seawater containing MS-222 (100 mg l⁻¹) until unresponsive to touch but retaining opercular movements, then weighed and maintained in an anaesthetized state (gills irrigated with aerated MS-222, 50 mg l⁻¹) while electrodes were inserted through the ventral muscle overlying the pericardial cavity, taking care not to pierce the pericardial membrane (Campbell et al., 2004). To assess the influence of autonomic tone on thermal sensitivity, a catheter (PP10) was inserted through the skin at the base of the pectoral fins, fed into the pericardial region and externally secured to ECG wires. All surgery was performed in a cold room at ~5°C, and completed within ~10 min, with forced ventilation in a holding tank used to minimize the delay before regular ventilation was re-established. Fish recovered for 48–72 h in cradles covered with plastic netting suspended in darkened flow-through tanks until f_H was stable and consistently low. The cradles allowed space for fish to move without them turning around, and gave ready access to electrodes/cannulae in an unobtrusive manner.

Experimental protocol for *in vivo* upper thermal tolerance test

CT_{max} was measured on individual fish (both *C. aceratus* and *N. coriiceps*) held in 70 l insulated experimental tanks and exposed to an acute thermal ramping treatment (3.3°C h⁻¹; Beers and Sidell, 2011) by recirculating water through an in-line heater (AquaLogic, San Diego, CA, USA). This warming rate was chosen to ensure that core body temperature had sufficient time to equilibrate with environmental temperature, rather than slower ramping to avoid the opportunity for acclimation (Beers and Sidell, 2011). CT_{max} is usually defined as the temperature when righting ability is lost, i.e. loss of neuromuscular coordination, but in this study we adopted a more cardiocentric endpoint of prolonged periods of ventricular asystole (defined as a period lasting at least 10–20 cardiac cycles based on the f_H immediately prior to asystole). Cardiac asystole was chosen as an index of CT_{max} because it is probably most comparable with behavioural indices (loss of righting reflex) when brain blood flow becomes compromised. Following the appearance of sustained asystole, animals were stunned and the spinal cord transected; blood-free atrial and ventricular mass were then measured. The responses of intact fish were compared with those of fish following vagal blockade (atropine, 1 mg kg⁻¹).

Separately, an index of sympathovagal balance was calculated (Egginton and Campbell, 2016) using f_H measurements after vagal blockade (atropine, 1 mg kg⁻¹) and then after complete autonomic blockade (1 mg kg⁻¹ atropine+1 mg kg⁻¹ propranolol) in four *C. aceratus* at ambient temperature (~0°C). The acute effects of

atropine (tachycardia, increased ECG amplitude) and propranolol (bradycardia, decreased ECG amplitude) were evident within 3–4 min of injection, and effects were sustained for 12–24 h (i.e. substantially outlasting the experiments). Autonomic tone (atropine+propranolol) has previously been measured using this method in *N. coriiceps* (Egginton and Campbell, 2016), so instead of repeating the experiment, we provide relevant comparisons with previously published data (Table 1).

ECG signals were collected using a PowerLab (AD Instruments, Oxford, UK) with an animal bio-amplifier (1 kHz sampling, 5 Hz high-pass filter), and analysed using LabChart (version 7, AD Instruments). Fish were sensitive to external noise and so routine f_H (based on 10–20 consecutive heartbeats) was recorded at 06:00 h (local time) when fish were quiescent and external disturbance was minimal.

Heart rate often changes in a curvilinear manner with temperature, hence the use of inter-beat interval (R–R), which provides a linear relationship (Vornanen et al., 2014). Heart rate variability (HRV) analysis reflects the influence of the autonomic nervous system on the sinus node, when the heart is in regular sinus rhythm. Tachograms and period histograms were used to ensure data blocks were sufficiently stable. In addition to f_H (expressed as R–R interval), HRV indices were calculated in both the time (standard deviation of inter-beat intervals, SDNN; root mean square of successive differences, RMSSD) and frequency domains [power spectral analysis (PSA) using Fourier analysis; low frequency:high frequency (LF:HF) ratio of power distribution around the midpoint frequency]. At rest, up to 512 continuous heartbeats were sampled, but during the ramp test, this number was reduced to 256 or 128 heartbeats, as appropriate to avoid shifting baselines; spectral leakage was minimized by use of a Hanning window. A Poincaré plot of R–R intervals as a function of the subsequent R–R interval was used as an index of long-term (diagonal axis from the origin) and beat-to-beat variations (normal to the axis maxima) and provides a graphic parallel to SDNN and RMSSD indices, respectively (Campbell and Egginton, 2007).

Table 1. Thermal tolerance of *Chaenocephalus aceratus* and *Notothenia coriiceps*

	<i>C. aceratus</i>	<i>N. coriiceps</i>
Routine f_H (beats min^{-1})		
Intact	9.0±1.1 (4)	13.5±0.6 (7)*
Atropinized	16.8±1.5 (5)	24.6±2.2 (5)
Cholinergic tonus (%)	55.3±9.9 (4)	52.4 [‡]
Adrenergic tonus (%)	11.7±1.6 (4)	12.7 [‡]
8°C f_H (beats min^{-1})		
Intact	28.0±3.0 (4)	38.2±1.7 (7)*
Atropinized	33.2±0.6 (5)	39.0±1.2 (6)*
Peak f_H (beats min^{-1})		
Intact	38.2±4.2 (4)	54.8±2.2 (7)**
Atropinized	47.7±1.5 (5)	58.1±2.2 (6)*
BPT (°C)		
Intact	13.0±0.3 (4)	14.0±0.5 (7)
Atropinized	13.5±0.6 (5)	14.0±0.6 (6)
CT _{max} (°C)		
Intact	14.1±0.4 (4)	16.7±0.3 (7)
Atropinized	15.1±0.5 (5)	15.8±0.3 (6)

Routine heart rate (f_H) and autonomic tonus were obtained at ~0.6°C. Breakpoint temperature (BPT) was analysed with linear regression intercepts using transformed f_H and temperature data to determine the f_H at 8°C and the temperature at which peak f_H occurred. Critical thermal maximum (CT_{max}) was defined as the temperature at which persistent ventricular asystole occurred. Data are means±s.e.m. (N); * P <0.05, ** P <0.001 versus *C. aceratus* (ANOVA with Tukey *post hoc* test). [‡]Values from Egginton and Campbell (2016).

Surgical instrumentation for cardiorespiratory measurements

Chaenocephalus aceratus were anaesthetized in ice-chilled seawater containing MS-222 (140 mg l^{-1}) until unresponsive to touch but retaining opercular movements, and maintained in an anaesthetized state (gills irrigated with aerated MS-222, 70 mg l^{-1}) on the surgery table (on a foam pad covered with goatskin chamois to prevent desiccation and protect the scaleless skin). A ventral mid-line incision allowed placement of a 2.5 or 4 mm transit-time flow probe (model T420, Transonic Systems Inc., Ithaca, NY, USA) around the pericardium and ventral aorta immediately distal to the bulbus arteriosus to measure ventral aortic blood flow (13 fish). The fish were then placed on their right side to access the branchial arteries in the left third gill arch. The efferent branchial artery was occlusively cannulated using PE-50 tubing with a 2F polyurethane tip to measure dorsal aortic pressure (P_{da}) in 16 fish. The afferent branchial artery in the same gill arch was cannulated in seven fish to measure ventral aortic pressure (P_{va}) using a PE-50 cannula, which in smaller animals was tipped with heat-pulled PE-30. The left duct of Cuvier was non-occlusively cannulated in 5 fish to measure central venous pressure (P_{cv}) using PE-50 tubing tipped with 3F silicon (to prevent damage to the delicate venous vasculature), equipped with side-holes to maintain patency, which was advanced into the sinus venosus. The cannulae and electrical leads from the flow probe were sutured to the skin and the ventral incision was closed with 3-0 surgical silk. Surgery was performed in a cold room at ~2–3°C, and completed within 45–120 min, after which the gills were irrigated with fresh seawater until spontaneous ventilation recommenced.

After surgery, the fish were placed in custom-made 12.4 l PET (polyethylene terephthalate) respirometers (<https://skfb.ly/RMsV>), triangular in cross-section to accommodate the protruding pelvic fins that characterize *C. aceratus*. The 700 l tank contained two identical respirometers that were covered in black plastic. Icefish were permitted a minimum 40 h post-surgical recovery before experiments started. Submersible pumps (Eheim, Deizisau, Germany) recirculated water in the respirometers to attain complete mixing. Oxygen concentration (measured as percentage air saturation) was measured with a P_{O_2} optode (FireSting, Aachen, Germany) placed in-line with the mixing pump. \dot{M}_{O_2} was recorded as O_2 concentration decrease over a 10 min period, before a second Eheim pump started to flush the respirometers with fresh seawater for 15 min, thereby creating a 25 min cardiorespiratory measurement period. This regime ensured O_2 concentration never fell below 90% air saturation.

The flow probe was connected to a Transonic flow meter (T402; Transonic Systems, Inc.). The cannulae were attached via pinports (Instech Laboratories, Plymouth Meeting, PA USA) to pressure transducers (Medizintechnik, Kirchseeon, Germany), which were regularly calibrated against a static water column. The output signals from the pressure transducers were pre-amplified by a Senselab 4CHAMP amplifier (Somedic sales, Hörby, Sweden). The flow meter and 4CHAMP amplifier were connected to a PowerLab data acquisition system (ADInstruments, Castiel Hill, NSW, Australia), which provided input to, as well as data storage on, a computer running LabChart Pro (version 7; ADInstruments, Bella Vista, NSW, Australia).

Experimental protocol for cardiorespiratory measurements

The cardiorespiratory response to acute warming was assessed using in-line heating (AquaLogic) from ambient temperature (0.8±0.1°C) to 4°C over approximately 3 h and then to 8°C over a further 3 h. At

all three temperatures (maintained for at least 30 min each), routine cardiorespiratory variables were measured before inducing activity by agitating the respirometers for 1 min. For routine cardiorespiratory measurements, we ensured that the fish were not disturbed by external stimuli. Sustained activity is difficult to elicit in icefish (e.g. Egginton, 1997), but this method typically produced sustained tail flicking for several minutes without apparently exhausting the fish. Therefore, it is unlikely that this agitation method induced a maximum cardiorespiratory response.

\dot{M}_{O_2} was calculated according to the formula:

$$\dot{M}_{O_2} = ((\alpha_{O_2} \times V) \times (\Delta O_2 / \Delta t)) / M_b, \quad (1)$$

where α_{O_2} is the oxygen content of seawater at a given temperature, V is the volume of the respirometer, $\Delta O_2 / \Delta t$ is the change in oxygen concentration (% air saturation) per unit time and M_b is the fish body mass in kg.

Flow (\dot{Q}), pressure (P_{da} , P_{va} and/or P_{cv}) and heart rate (f_H ; derived in real time from the pulsatile flow or pressure trace) were measured simultaneously with \dot{M}_{O_2} at ambient temperature, 4°C and 8°C in quiescent fish and after activity. \dot{Q} values were normalized to body mass and the flow probes were calibrated for each temperature according to the instructions provided by the manufacturer. Stroke volume (V_S) was calculated as:

$$V_S = \dot{Q} / f_H. \quad (2)$$

In fish in which P_{cv} was measured, systemic conductance (G_{sys}) was calculated as:

$$G_{sys} = \dot{Q} / P_{da} - P_{cv}. \quad (3)$$

Otherwise, P_{cv} was assumed to be zero because measured P_{cv} was negligible.

Branchial conductance (G_{branch}) was calculated as:

$$G_{branch} = \dot{Q} / P_{va} - P_{da}. \quad (4)$$

Cardiac power output (CPO) was calculated as:

$$CPO = (\dot{Q} \times 1/60) \times P_{va}. \quad (5)$$

Statistical analysis for the upper thermal tolerance test

Differences between *C. aceratus* and *N. coriiceps* were determined using a multi-factorial ANOVA, with Fisher's protected least-significant difference *post hoc* test to discriminate amongst group means (StatView 5.0, SAS Institute Inc., Cary, NC, USA). Broken-line regression analyses of f_H versus temperature were conducted for each fish using the segmented package in R (v.3.1.0; <http://www.R-project.org/>) to determine the cardiac breakpoint temperature (BPT), i.e. when the increase in f_H with temperature first attenuated. The regression line fitted to this phase of increasing f_H was tested for differences in slope and intercept values using an ANOVA.

Three *C. aceratus* were excluded from subsequent analysis because routine f_H at ambient temperature was appreciably higher than in other fish ($P < 0.001$), suggesting stress, despite a similar peak f_H in both groups ($P = 0.16$). BPT was significantly lower in the outlier group ($P < 0.05$). Even so, the outlier group showed no overt signs of stress (e.g. banding of integument pigmentation or prolonged recovery from surgery) and therefore probably would have been considered unstressed without cardiovascular (ECG) data, potentially leading to erroneous conclusions (Fig. S1). In addition, one *N. coriiceps* was excluded because of apparent restlessness and periodic bouts of bradycardia throughout acute

warming (note: BPT and HRV values also deviated from those of the remaining fish).

Statistical analysis for the cardiorespiratory measurements

An unpaired *t*-test was used to investigate significant differences in routine f_H between *C. aceratus* instrumented with ECG electrodes and those with more extensive surgery for the cardiorespiratory measurements. The effect of temperature and activity on cardiorespiratory measurements in *C. aceratus* was investigated using a two-way repeated-measures analysis of variance (ANOVA), revealing the combined and isolated effects of warming and activity on each cardiorespiratory variable. We assumed acute warming to 8°C would impose an intolerable stress, which proved incorrect as revealed by subsequent fish responses, and so the first two fish tested were not agitated and therefore not included in the ANOVA model (to avoid Type II errors by using matched data for all measurement points). Nevertheless, these two fish are represented in the mean values in figures where data were obtained. The relative importance of f_H and V_S in meeting the increased oxygen demands following activity was elucidated at each temperature with a linear regression to correlate individual fish changes in \dot{Q} , f_H and V_S with respect to changes in \dot{M}_{O_2} . Statistical analysis was performed with GraphPad Prism (v.7.0d). Statistical significance was assigned to $\alpha \leq 0.05$ and data are presented as means \pm s.e.m.

Chemicals

Atropine methyl nitrate, propranolol hydrochloride and tricaine methanesulfonate (MS-222) were purchased from Sigma-Aldrich (St Louis, MO, USA).

RESULTS

Interspecific comparison of f_H and thermal tolerance to acute warming

Routine f_H was lower in *C. aceratus* than in *N. coriiceps* ($P < 0.05$), an absolute difference that widened at peak f_H ($P < 0.001$; Table 1). Consequently, the rise in f_H was slower and of a lower magnitude during a thermal ramp for *C. aceratus* (0 versus 10°C $\dot{Q}_{10} = 3.30$, $\Delta f_H = 21.2$ beats min^{-1}) than for *N. coriiceps* ($\dot{Q}_{10} = 2.74$, $\Delta f_H = 27.3$ beats min^{-1}). Periodic missed and ectopic beats were noted in *C. aceratus* around 8–10°C, while ventricular asystolic events (with P-wave presence) became more frequent and prolonged closer to BPT, suggesting a atrioventricular conduction failure (Fig. S2).

No significant difference in BPT was found between *C. aceratus* and *N. coriiceps* (Fig. 1) despite the large significant difference in peak f_H (38.2 ± 4.2 and 54.8 ± 2.2 beats min^{-1} , respectively; Table 1). Some *C. aceratus* individuals had a brief tachycardia after BPT, corresponding to the first observed change in fish behaviour. In *C. aceratus*, catastrophic cardiac failure rapidly ensued (Fig. 1), with prolonged ventricular asystolic periods (up to 8–10 s in duration) with a regular P-wave, interspersed with bursts of ventricular tachyarrhythmias of >40 beats min^{-1} , a situation that points to conduction failure (Fig. S2). In contrast, *N. coriiceps* displayed missed or ectopic beats 1–2°C above ambient temperature, with a transient increase in HRV around the mid-point of the ramp (5–10°C). Even so, regular sinus rhythm was re-established and maintained at a higher temperature than in *C. aceratus*. Again, heat-induced bradycardia was evident following the BPT, with cardiac failure preceded by progressively extreme periodicity (Figs S2 and S3).

Atropine nearly doubled routine f_H at 0°C in both species, and increased peak f_H by 25% ($P < 0.05$) and 5% (not significant, n.s.) in *C. aceratus* and *N. coriiceps*, respectively, but decreased both Δf_H

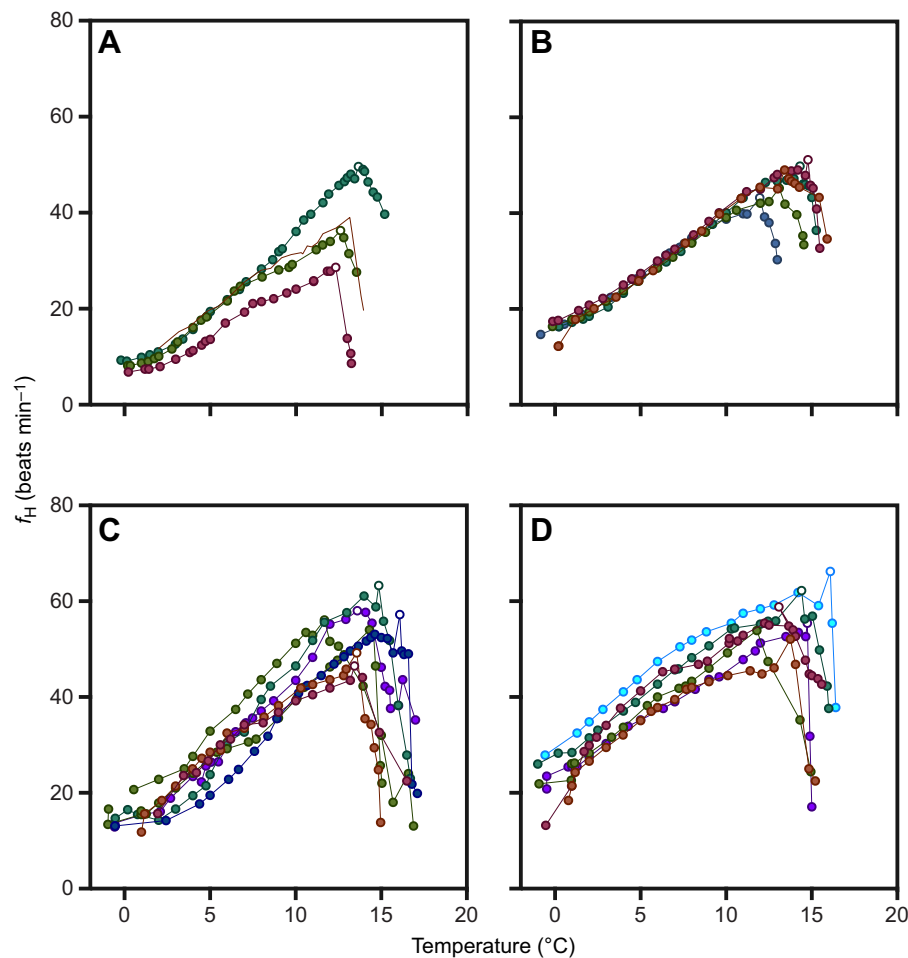


Fig. 1. Thermal sensitivity of notothenioid heart rate (f_H) with and without autonomic blockade. (A) Intact *Chaenocephalus aceratus*, (B) atropinized *C. aceratus*, (C) intact *Notothenia coriiceps*, (D) atropinized *N. coriiceps*. Individual animals are colour coded, and calculated breakpoint temperature (BPT) is shown as open circles. Note intraspecific variability, reduced following atropine, and the rapid onset of post-BPT bradycardia prior to cardiac failure.

ΔT (°C) (Table 1) and Q_{10} (2.42 and 2.03, respectively) (Table 2). Consequently, the difference in peak f_H between *C. aceratus* and *N. coriiceps* was reduced from $\sim 30\%$ to $\sim 20\%$ after atropine ($P < 0.05$), but peak f_H was reached at similar temperatures (n.s.). In addition, atropine substantially increased the temperature at which the first change in the ECG occurred in both species, making it closer to BPT; while ventricular asystolic periods progressively increased in duration, no tachyarrhythmia was observed. Vagal blockade with

atropine also reduced the difference in both BPT (Fig. 1) and especially, CT_{max} (Table 1) between the two species.

Interspecific comparison of *in vivo* HRV

The time- and frequency-domain indices of HRV showed post-surgery depression, were transiently elevated as anaesthesia wore off, and quickly stabilized within 12 h when HRV was proportional to R-R interval (Table S1). Although *C. aceratus* recovered from

Table 2. Time- and frequency-domain analysis of heart rate variability (HRV) during a thermal ramp to define CT_{max}

	<i>n</i>	NN (ms)		SDNN (ms)		Power ($\times 10^3$ ms ²)		LF:HF	
		Intact	Atropine	Intact	Atropine	Intact	Atropine	Intact	Atropine
<i>C. aceratus</i> (Hb ⁻ Mb ⁻)									
0°C	4	6838 \pm 1669	3599 \pm 336	427 \pm 121	9 \pm 9	120.7 \pm 44.7	0.07 \pm 0.10	0.96 \pm 0.52	0.58 \pm 0.46
5°C	4	3653 \pm 716	2284 \pm 60	187 \pm 182	7 \pm 10	22.1 \pm 30.2	0.02 \pm 0.04	1.65 \pm 1.01	1.11 \pm 0.88
10°C	4	2089 \pm 513	1482 \pm 128	115 \pm 78	2 \pm 1	8.5 \pm 9.2	0.004 \pm 0.002	1.01 \pm 0.82	2.08 \pm 2.11
15°C	2	2389 \pm 13.6	1415 \pm 68	2441 \pm 2032	15 \pm 9	2920.7 \pm 4062.8	0.3 \pm 0.2	0.60 \pm 0.09	2.52 \pm 1.65
CT_{max}	4	2641 \pm 413	2594 \pm 1947	1752 \pm 1575	932 \pm 128	2152.1 \pm 2701.1	312.4 \pm 327.8	2.39 \pm 2.61	3.43 \pm 1.43
Q_{10} (0,10)	3.30	2.42							
<i>N. coriiceps</i> (Hb ⁺ Mb ⁺)									
0°C	7	3915 \pm 651	2452 \pm 204	322 \pm 143	19 \pm 15	323.8 \pm 368.5	0.3 \pm 0.3	1.38 \pm 0.69	0.98 \pm 0.92
5°C	7	2319 \pm 419	1549 \pm 124	156 \pm 68	5 \pm 3	27.0 \pm 33.0	0.01 \pm 0.01	2.71 \pm 1.46	1.45 \pm 1.47
10°C	7	1402 \pm 101	1212 \pm 117	83 \pm 45	4 \pm 2	6.0 \pm 4.9	0.01 \pm 0.01	1.30 \pm 0.57	0.87 \pm 0.74
15°C	6	1399 \pm 269	1268 \pm 273	1778 \pm 2749	58 \pm 78	253.6 \pm 294.4	0.5 \pm 0.5	0.55 \pm 0.29	2.78 \pm 5.38
CT_{max}	7	3959 \pm 1784	1742 \pm 594	2816 \pm 934	2176 \pm 2157	1936.8 \pm 1817.6	1875.7 \pm 2230	1.01 \pm 0.77	1.16 \pm 0.29
Q_{10} (0,10)	2.74	2.03							

NN, interbeat intervals obtained by removing artifacts from the R-R series (i.e. 'normal' intervals); SDNN, standard deviation of NN intervals; Power, total spectral power in the analysis region; LF:HF, ratio between low frequency and high frequency power.

surgery more slowly than *N. coriiceps* (data not shown), interspecific differences in HRV were modest (Table 2; Table S1). Both short-range (RMSSD) and long-range (SDNN) indices of HRV generally declined with rising temperature to $\sim 10^{\circ}\text{C}$ in *C. aceratus* and to $\sim 12^{\circ}\text{C}$ in *N. coriiceps*. Hence, the Poincaré data cloud progressively narrowed with acute warming but widened as CT_{max} was approached (rapid collapse of *C. aceratus* after peak f_{H} precluded analysis at that point; Fig. S3). While total spectral power was heterogeneous among individuals, LF:HF ratio (a dynamic index of sympatho-vagal balance) progressively declined in both species to a temperature of 5– 10°C , but increased markedly at higher temperatures and especially at CT_{max} , indicating an autonomic conflict consistent with signs of ECG disruption (Table 2; Table S1). Only two *C. aceratus* reached 15°C with sinus rhythm intact, at which point all HRV variables (e.g. SDDNN, RMSSD, spectral power) were markedly different from those at 5 or 10°C (Table S1). Such changes became accentuated between BPT and CT_{max} .

Vagal blockade not only eliminated differences in BPT and CT_{max} between *C. aceratus* and *N. coriiceps* but also resulted in extremely low spectral power during the thermal ramp for both species, without changing the species difference in peak f_{H} (Table 1). All HRV indices were dramatically reduced by atropine, until just before BPT. The mid-range elevation of LF:HF by 10°C in atropinized *C. aceratus* is consistent with greater thermal sensitivity (probably sympathetic in origin), whereas LF:HF was minimal at this point in *N. coriiceps* (Table 2).

Routine cardiorespiratory status in *C. aceratus*

Routine \dot{Q} in *C. aceratus* at 0.8°C was $26.6 \pm 2.1 \text{ ml min}^{-1} \text{ kg}^{-1}$ at a f_{H} of $11.5 \pm 0.9 \text{ min}^{-1}$. Routine f_{H} measured in *C. aceratus* equipped with only ECG electrodes for the thermal tolerance test was not significantly different ($P=0.21$; $9.0 \pm 1.1 \text{ min}^{-1}$; Table 1), suggesting that fish had

recovered sufficiently from the more extensive surgical interventions to restore the high vagal tone characterizing unstressed fish.

Routine \dot{M}_{O_2} ($24.5 \pm 0.8 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$), as well as P_{va} ($2.06 \pm 0.05 \text{ kPa}$) and P_{da} ($1.81 \pm 0.06 \text{ kPa}$), were similar to previously reported values (Holeton, 1970; Hemmingsen and Douglas, 1972; Hemmingsen et al., 1972). P_{cv} has not previously been measured in *C. aceratus*, but the near-ambient central venous pressure ($0.06 \pm 0.03 \text{ kPa}$) was consistent with very low caudal venous pressures reported previously (Hemmingsen and Douglas, 1972).

Cardiorespiratory effects of acute warming in *C. aceratus*

Acute warming of *C. aceratus* from 0.8°C to 8°C increased routine \dot{M}_{O_2} by 2.4-fold to $59.5 \pm 2.4 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (Fig. 2A; $P < 0.001$) and \dot{Q} by 2.6-fold to $69.9 \pm 4.7 \text{ ml min}^{-1} \text{ kg}^{-1}$ (Fig. 2B; $P < 0.001$). The increase in \dot{Q} with temperature was entirely attributable to an increase in f_{H} to $29.1 \text{ beats min}^{-1}$ ($P < 0.001$), which compensated for a significant but small decrease in V_{S} ($P = 0.02$), which was most pronounced between 0 and 4°C (Fig. 2C). Importantly, f_{H} similarly increased from 9.0 ± 1.1 at 0.6°C to $28.0 \pm 3.0 \text{ beats min}^{-1}$ at 8°C in ECG-instrumented *C. aceratus* (cf. values for *N. coriiceps*: 13.5 ± 0.6 and $38.2 \pm 1.7 \text{ beats min}^{-1}$, respectively; Table 1), again suggesting that these fish had recovered sufficiently from extensive surgical intervention.

Despite the large increase in \dot{Q} in *C. aceratus* during warming, both P_{da} and P_{va} decreased with increasing temperature (Fig. 3; $P = 0.001$ and $P = 0.002$, respectively) because both G_{sys} ($P < 0.001$) and G_{branch} ($P = 0.001$) increased significantly with temperature and prominently at 8°C . P_{cv} tended to decrease with temperature ($P = 0.06$), while CPO increased significantly (Fig. 3; $P = 0.02$).

Effects of acute warming associated with enforced activity

At ambient temperature, enforced activity was associated with significant ($P < 0.001$) increases in \dot{M}_{O_2} , \dot{Q} , f_{H} and V_{S} in *C. aceratus*

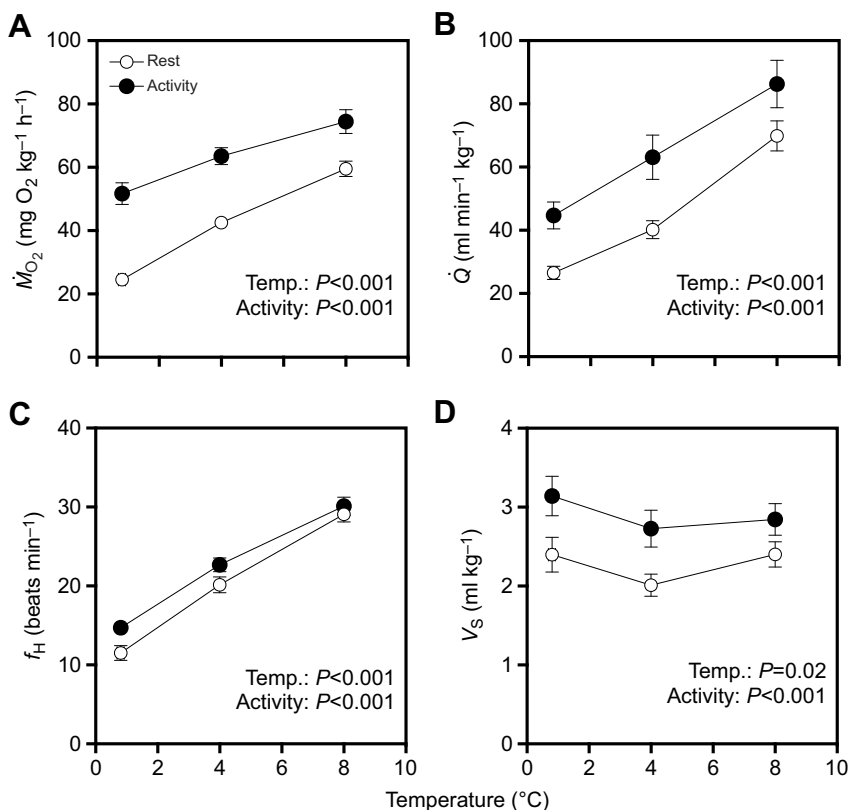


Fig. 2. Effect of temperature and activity on oxygen consumption and cardiac function in blackfin icefish (*C. aceratus*). (A) \dot{M}_{O_2} , oxygen consumption; (B) \dot{Q} , cardiac output; (C) f_{H} , heart rate; (D) V_{S} , stroke volume. $N=16$ for \dot{M}_{O_2} and f_{H} (except 8°C activity, $N=14$) and $N=13$ for \dot{Q} and V_{S} (except 8°C activity, $N=11$). P -values according to a two-way ANOVA. Values are means \pm s.e.m.

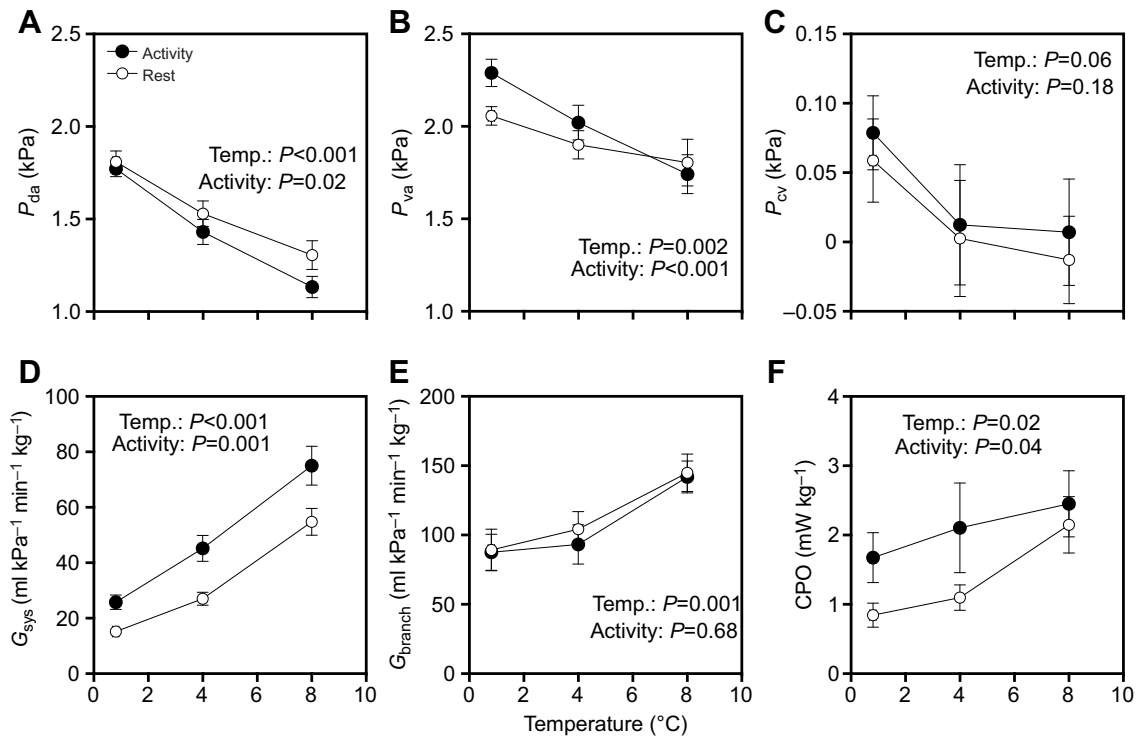


Fig. 3. Effect of temperature and activity on vascular pressure, conductance and cardiac power output. (A) P_{da} , dorsal aortic pressure; (B) P_{va} , ventral aortic pressure; (C) P_{cv} , central venous pressure; (D) G_{sys} , systemic conductance; (E) G_{branch} , branchial conductance; (F) CPO, cardiac power output. $N=16$ for P_{da} (except 8°C activity, $N=14$), $N=7$ for P_{va} , $N=5$ for P_{cv} (except 8°C activity, $n=4$), $N=13$ for G_{sys} (except 8°C activity, $N=11$), $N=6$ for G_{branch} and CPO. P -values according to a two-way ANOVA. Values are means \pm s.e.m.

(Fig. 2; Fig. S4). Although P_{da} decreased during activity ($P=0.02$), as with warming, P_{va} increased with activity ($P<0.001$) because G_{sys} ($P<0.001$) increased with activity but G_{branch} did not ($P=0.68$). There were no significant changes in P_{cv} from rest to activity, even though CPO increased (Fig. 3; $P=0.04$).

Individual changes in \dot{Q} and \dot{M}_{O_2} with temperature showed a strong positive correlation as *C. aceratus* transitioned from rest to activity at all test temperatures (Fig. 4). At ambient temperature, a strong relationship ($R^2=0.87$) existed between Δf_H and $\Delta \dot{M}_{O_2}$, without a significant correlation between ΔV_S and $\Delta \dot{M}_{O_2}$. At 4°C, however, the relationship between Δf_H and $\Delta \dot{M}_{O_2}$ weakened and a significant relationship between ΔV_S and \dot{M}_{O_2} developed. By 8°C, a strong correlation between ΔV_S and \dot{M}_{O_2} ($R^2=0.73$) emerged while the association between Δf_H and $\Delta \dot{M}_{O_2}$ had disappeared, reflecting the necessarily diminished potential change in f_H as peak heart rate was approached (Fig. 4).

DISCUSSION

In quiescent, undisturbed *C. aceratus* at 0.8°C, \dot{Q} was 26.6 ml min⁻¹ kg⁻¹. This value accords well with the partial measurements of flow inferred from Hemmingsen et al. (1972), but is much lower than the Fick estimate (100–150 ml min⁻¹ kg⁻¹; Holeton, 1970; Hemmingsen et al., 1972). This difference could be a consequence of well-vascularized icefish skin (Jakubowski, 1982), which provides an alternative pathway for oxygen uptake for which the Fick principle does not account (Farrell et al., 2014). Even though Hemmingsen et al. (1972) recognized this potential error, the considerably greater Fick-derived estimates were accepted without addressing the discrepancy with their direct flow measurements, a surprising conclusion considering that the same authors previously suggested that cutaneous oxygen uptake could

constitute up to 40% of total \dot{M}_{O_2} (Hemmingsen and Douglas, 1970). Similarly high (35%) cutaneous oxygen uptake has been independently measured in another scaleless Antarctic fish (*Rhizophila dearborni*; Wells, 1986). However, Holeton (1976) disputed such a significant role for a cutaneous oxygen supply in *C. aceratus* by arguing that branchial gas exchange must dominate because of a greater P_{O_2} diffusion gradient (i.e. between water and venous rather than arterial blood), a shorter blood-to-water distance, and gills receiving the entire \dot{Q} versus only a small portion to the skin. Thus, alternative factors, such as post-surgical stress, may explain the difference between current and earlier measurements of routine \dot{Q} .

Cardiac rhythmicity, which was linked with ventilatory activity, was evident in both *C. aceratus* and *N. coriiceps*, thus demonstrating good surgical recovery (see Campbell et al., 2004). The resting f_H (16–18 beats min⁻¹) reported by Hemmingsen et al. (1972) was close to the maximum f_H we recorded during activity at a similar temperature, again indicating that post-surgical stress could explain the extremely high Fick-derived estimates of routine \dot{Q} for *C. aceratus* at ~1°C (Hemmingsen et al., 1972). However, this concern would equally apply to the fish instrumented with electromagnetic flow probes in the same study and that yielded a lower routine \dot{Q} . Our assumption of equal blood flow in all branchial arteries may therefore not be correct, underestimating the inferred \dot{Q} , such that correspondence between our data and the estimated blood flows could be coincidental. Post-surgical stress was less likely to be an issue in Holeton's (1970) study, which reported the lowest \dot{M}_{O_2} measured in the period 2–10 days post-surgery and a relatively low routine f_H (13.8 beats min⁻¹), while \dot{Q} was calculated as 61 ml min⁻¹ kg⁻¹ using the Fick principle. This f_H was lower than that of Hemmingsen et al. (1972) but, rather than being close to our

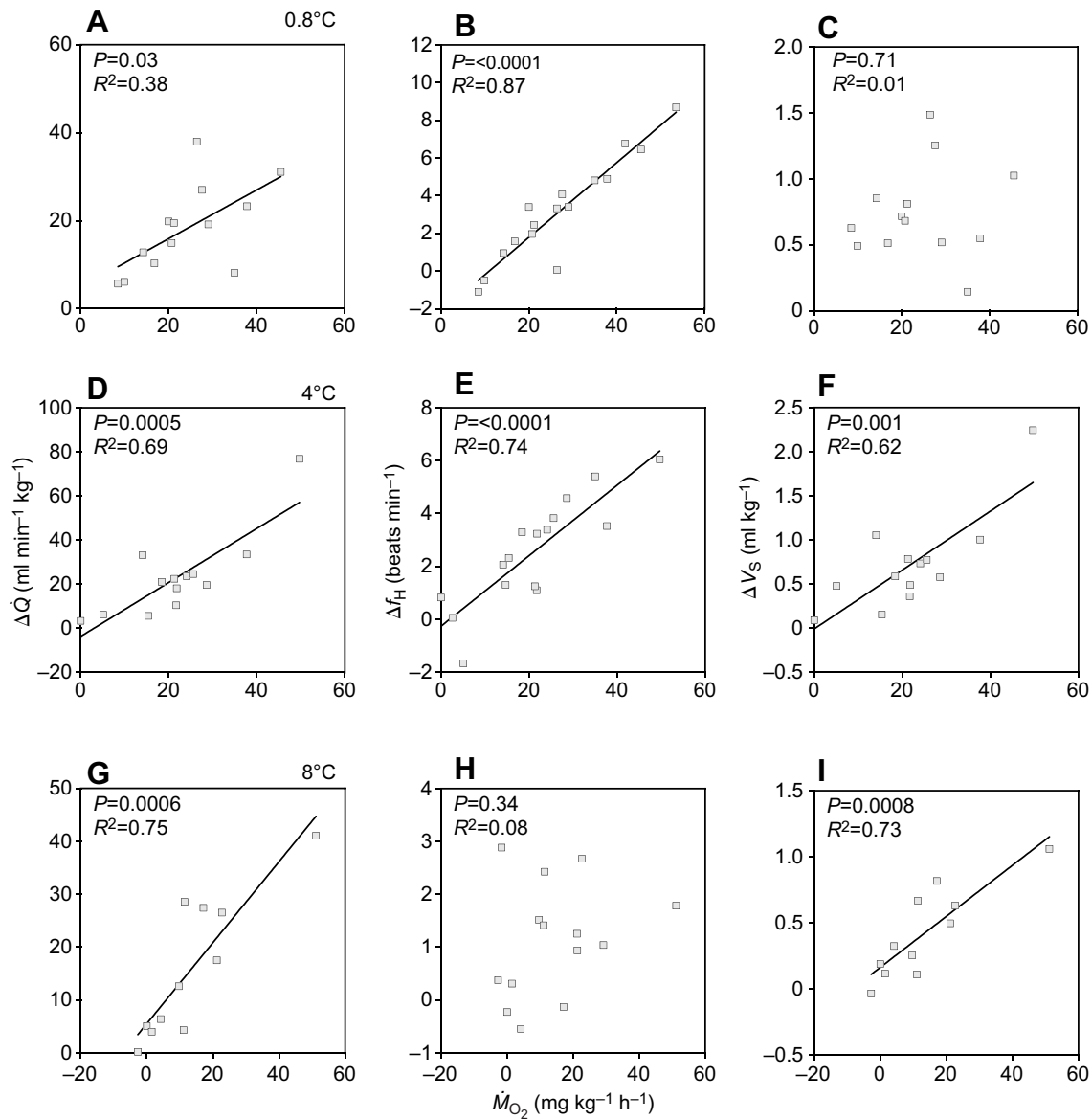


Fig. 4. Relationship between change in cardiac output, heart rate and stroke volume with change in oxygen consumption from rest to activity at different temperatures. (A–C) Ambient temperature (0.8°C); (D–F) 4°C; and (G–I) 8°C. ΔQ , change in cardiac output (A,D,G); Δf_H , change in heart rate (B,E,H); ΔV_S , change in stroke volume (C,F,I); $\Delta \dot{M}_{O_2}$, change in oxygen consumption. $N=16$ for A,D; $N=13$ for B,C,E,F; $N=14$ for G; $N=11$ for H,I. P -values and R^2 values calculated with linear regressions.

routine value (11.5 beats min⁻¹), it is closer to our value immediately following the 10 min of enforced activity (14.7 beats min⁻¹). We therefore conclude that a combination of cutaneous O₂ supply skewing the Fick calculation, post-surgical stress, and perhaps other methodological differences account for the previous overestimations of routine \dot{Q} in *C. aceratus*. This is an important conclusion, as we now have clear evidence that the low routine \dot{Q} provides a large scope for change and that the cardiorespiratory regulation may permit physiological flexibility during acute temperature challenges, something that appeared unlikely based on earlier measurements of routine \dot{Q} .

Interspecific comparison of *in vivo* cardiac electrical activity

In vivo ECGs have not been reported previously for *C. aceratus*, and the similarity of the ECGs between *C. aceratus* and *N. coriiceps* allowed our detailed assessment of disturbances to cardiac

conduction, which may be central to the failure of the heartbeat at high temperature in other fish species (Vornanen et al., 2014). Even though CT_{max} may vary with the extent of facilitated O₂ transport (Beers and Sidell, 2011), the current observations suggest that for acute warming this effect is modest, which is consistent with recent work showing that hyperoxia failed to increase the upper thermal tolerance of icefish (Devor et al., 2016).

Reduced HRV is often used as an index of diminished cardiovascular regulation in mammals, although changes in blood pressure may also induce changes in short-term HRV as a result of baroreflex control. Acute warming of both *C. aceratus* and *N. coriiceps* dramatically reduced HRV at around 10°C, before a marked elevation prior to BPT and CT_{max}, at which point ventricular asystole occurred, probably as a result of conduction failure. In combination, these data suggest a biphasic response to thermal stress. Using the LF:HF ratio as a simplified readout of

sympathovagal balance, the pharmacological blockade shows it to be a reasonable index of autonomic regulation in these species, as seen in other comparative studies (Campbell et al., 2004). Our data demonstrate that *C. aceratus* uses vagal tone to suppress thermally induced tachycardia more so than *N. coriiceps*, but similar to other Hb⁺ nototheniids (Axelsson et al., 1992; Franklin et al., 2001). While this vagal reflex during acute warming could minimize cardiac \dot{M}_{O_2} , acidosis or oxidative stress, it is likely to incur a cost of reducing the limits to thermal tolerance (see Sandersfeld et al., 2015). Thus, acute cardiac stress may have chronic downstream effects that have not been explored to date. Poincaré plots represent a more global view of HRV, and at this level there appears to be little difference between species, highlighting that additional influences on cardiac performance need to be considered (see below). For example, *C. aceratus* is relatively unresponsive to adrenaline, consistent with a low synthesis rate, and attenuated stress-induced release of catecholamines (Whiteley and Egginton, 1999), thereby obviating a humoral arm of cardiovascular control that could be available to other notothenioids, albeit to a lesser extent than in temperate species.

Regardless, it is clear that *C. aceratus* acutely stressed to temperatures well beyond current environmental conditions can mount a robust cardiovascular response, one that is similar to that of Hb⁺ nototheniids. An important difference is that while *N. coriiceps* retains a modest scope for f_H changes at the BPT (26%), *C. aceratus* retains a significantly higher scope for f_H change (81%), leading to a lower peak f_H . Thus, the cardio-protective strategy of imposing an autonomic brake on f_H in *C. aceratus* appears to be more robust, failing only *in extremis*. Indeed, indices of HRV (e.g. RMSSD, an index of the integrity of vagus-mediated autonomic control) are consistent with this suggestion. The HRV response to elevated temperature was similar among individual fish and between species, e.g. RMSSD tracked the change in SDNN in both *N. coriiceps* and *C. aceratus*, consistent with maintaining tight regulation of cardiac rhythmicity even in the Hb⁻ fish.

As noted above, icefish are clearly capable of developing a robust cardiorespiratory response to acute warming. The initially high Q_{10} probably reflects thermal history, as speciation in the extremely cold and stable aquatic environment is assumed to allow specializations that may blunt physiological plasticity (Hochachka and Somero, 2002). However, *N. coriiceps* displays greater vagal withdrawal in response to a temperature ramp, possibly because extreme sensitivity to metabolic acidosis accompanying the lack of proton buffering by Hb would otherwise drive a greater vagal suppression of thermally induced tachycardia in icefish. The reduction in Q_{10} for f_H with atropine suggests a similarity in fundamental thermal sensitivity that is overridden by vagal tone during a temperature increase, revealing a similarity of intrinsic f_H seen among notothenioids of varying ecotype (Campbell et al., 2009).

The cardiorespiratory effects of acute warming and activity in *C. aceratus*

In *C. aceratus*, both resting and active \dot{M}_{O_2} increased almost linearly between 0.8 and 8°C, whereas Hemmingsen and Douglas (1972) reported that \dot{M}_{O_2} only increased from 1 to 4°C and remained unchanged up to 10°C, consistent with stressed animals reaching maximum \dot{M}_{O_2} earlier in a temperature ramp. However, we used fish that were rapidly warmed (i.e. over several hours) compared with the earlier study where fish were incrementally warmed by 0.3–1°C per day and maintained at a given temperature for at least 48 h prior to \dot{M}_{O_2} measurements. Thus, fish may have become less stressed by the

slower warming speed, which permitted an overall reduction in oxygen demand, as seen in some temperate species (Gräns et al., 2014). However, it is unlikely that the fish were acclimating to a new temperature over a few days, as *N. coriiceps* does not exhibit reduced \dot{M}_{O_2} following acclimation (6–9.5 weeks) from 0 to 5°C (Egginton and Campbell, 2016; Joyce et al., 2018).

At 0.8°C, \dot{Q} reached 44.7 ml min⁻¹ kg⁻¹ during enforced activity, which is about 60% of the maximum value for \dot{Q} in the *in situ* perfused heart preparation (70 ml min⁻¹ kg⁻¹; S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished), an expected difference considering the relatively moderate activity imposed probably did not exploit maximal cardiac capacity. Also, we measured mean \dot{Q} concomitantly with \dot{M}_{O_2} over a period of several minutes, while peak \dot{Q} was transitory and higher than the mean value (Fig. S4; peak \dot{Q} immediately following agitation was 52.9±4.5 ml min⁻¹ kg⁻¹). Importantly, peak f_H (17.6±0.7 min⁻¹) following agitation was identical to that measured in *in situ* perfused hearts at the same ambient temperature (S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished), an indication of a complete loss of vagal tone with just moderate activity.

The implication of these data is that *C. aceratus* at 0.8°C has a considerable cardiac scope and that the heart is not pumping near its maximum capacity when the fish is in a quiescent, unstressed state. While an abnormally high cardiac preload may be used with perfused hearts to generate maximum \dot{Q} , the maximum \dot{Q} measured *in vivo* at high temperatures (86.3 ml min⁻¹ kg⁻¹) was close to the maximum \dot{Q} (98 ml min⁻¹ kg⁻¹) reported for perfused hearts at 4°C. Thus, we confirmed that the icefish heart is not only capable of pumping large volumes of blood at a near-freezing temperature but also retains a cardiac scope by maintaining vagal tone to increase f_H . Furthermore, the Hb⁻ *C. aceratus* can increase \dot{Q} with activity even at elevated temperature *in vivo* by elevating f_H . In fact, despite their similar routine \dot{Q} , maximum \dot{Q} in *C. aceratus* clearly exceeded that of the more active Hb⁺ nototheniid *Pagothenia borchgrevinki* (~60 ml min⁻¹ kg⁻¹; Franklin et al., 2007).

Whether fish primarily regulate V_S or f_H to change \dot{Q} during activity remains a subject of debate (Farrell, 1991; Altimiras and Larsen, 2000; Sandblom et al., 2005; Shiels and White, 2008; Farrell and Smith, 2017; Nelson et al., 2017). The present study informs this discussion because *C. aceratus* increased \dot{Q} during activity by elevating V_S and f_H to different degrees across temperatures. While $\Delta\dot{M}_{O_2}$ was closely correlated with Δf_H , as described previously in *N. coriiceps* (Campbell et al., 2008), this occurred only at 0.8°C. In fact, V_S increased by approximately 1 ml kg⁻¹ irrespective of the increase in \dot{M}_{O_2} across temperature, and became a primary determinant of the magnitude of $\Delta\dot{M}_{O_2}$ with activity at 8°C. This suggests that at ambient temperature, f_H is finely tuned to preserve adequate cardiovascular oxygen transport in the face of elevated metabolic demands, but as f_H moves closer to peak capacity at high temperatures, there is less scope to increase pacemaker activity and Δf_H can no longer be closely correlated with $\Delta\dot{M}_{O_2}$. Thus, the closer alignment of ΔV_S and $\Delta\dot{M}_{O_2}$ with temperature and the fact that the change in \dot{Q} with activity became predominantly volume regulated is more a reflection of a limit on peak f_H rather than a change in the ability to regulate V_S . The present data also support the general finding that changes in f_H are prioritized over V_S when a fish is acutely warmed, which is an unresolved issue because quiescent fish with an artificially depressed f_H can elevate V_S in a compensatory fashion (Gamperl et al., 2011), yet exercising fish that are warmed can certainly increase V_S (present study; Steinhausen et al., 2008; Eliason et al.,

Table 3. Routine cardiac output (\dot{Q}), ventral aortic pressure (P_{va}), central venous pressure (P_{cv}) and cardiac power output (CPO) in icefish (*C. aceratus*) and three red-blooded notothenioids

	\dot{Q} (ml min ⁻¹ kg ⁻¹)	P_{va} (kPa)	P_{cv} (kPa)	CPO (mW kg ⁻¹)
<i>Chaenocephalus aceratus</i>	26.6 ^a	2.06 ^a	0.06 ^a	0.89
<i>Trematomus bernacchii</i>	17.6 ^b	3.09 ^b	Not measured	0.91
<i>Pagothenia borchgrevinki</i>	29.6 ^b	3.60 ^b	0.11 ^c	1.72
<i>Notothenia coriiceps</i>	6.5 ^d	5.92 ^d	0.08 ^d	0.63

CPO was estimated based on mean \dot{Q} and pressure measurements for all species and calculated as: $CPO = (\dot{Q} \times 1/60) \times (P_{va} - P_{cv})$. For *Trematomus bernacchii*, P_{cv} has not been measured so we assumed it was zero. Body mass-specific CPO (cardiac work) in *C. aceratus* is within the range of that in red-blooded notothenioids. ^aPresent study; ^bAxelsson et al. (1992); ^cSandblom et al. (2009); ^dW.J., M.A., S.E., A.P.F., E.L.C. and K.M.O., unpublished data.

2011, 2013). Thus, while an increase in f_H reduces cardiac filling time and thus limits the capacity to increase V_S (Altimiras and Axelsson, 2004), at high temperature in *C. aceratus*, and in exercising fishes more generally, this confounding problem must be overcome for V_S to increase and contribute to increasing \dot{Q} .

During both warming and activity, we revealed surprisingly large (up to 5-fold) increases in G_{sys} , which indicates vasodilatation of the peripheral vasculature. A similar change has previously been reported in swimming *P. borchgrevinki* (Axelsson et al., 1992, 1994). This change in conductance can be attributed only in part to passive distension of the vasculature when accommodating greater \dot{Q} (e.g. Wood and Shelton, 1975) because P_{da} clearly decreased. Both activity and temperature increase the metabolic demand of tissues, which may release local vasoactive factors and induce a functional hyperaemia. Indeed, in humans it is believed that peripheral vasodilatation is the primary driver of the increased \dot{Q} during exercise (Bada et al., 2012).

Evolutionary consequences for the loss of Hb

In comparison with Hb⁺ Antarctic notothenioids, our measurements of routine \dot{Q} in *C. aceratus* (at 0.8°C, 26.6 ml min⁻¹ kg⁻¹) is not as divergent as previously reported. Indeed, routine \dot{Q} is lower (29 ml min⁻¹ kg⁻¹ at -0.5°C: Axelsson et al., 1992), or only marginally higher (24 ml min⁻¹ kg⁻¹ at 0°C: Sandblom et al., 2012; 22 ml min⁻¹ kg⁻¹ at -1.0°C: Franklin et al., 2007), than that previously reported in smaller specimens of the more active, cryopelagic *P. borchgrevinki*. In a more typically benthic species, *Trematomus bernacchii*, routine \dot{Q} was lower (17.6 ml min⁻¹ kg⁻¹; Axelsson et al., 1992). The greatest difference reported exists with another benthic species, *N. coriiceps* (6–7 ml min⁻¹ kg⁻¹; Egginton, 1997; Joyce et al., 2018) of similar body mass to *C. aceratus*. Thus, there is clearly considerable variation among notothenioid fishes. As a consequence of the lower routine \dot{Q} , the estimated routine CPO (which now also includes P_{cv}) places *C. aceratus* squarely within the range of their Hb⁺ relatives (Table 3). This suggests that cardiac pumping in *C. aceratus* is not necessarily twice as metabolically expensive as in Hb⁺ nototheniids (cf. Sidell and O'Brien, 2006), and the loss of haemoglobin may not be costly in terms of cardiac work. Furthermore, if recent estimates of maximum \dot{Q} with *in situ* perfused hearts are accurate (S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished), factorial scope for \dot{Q} is about 4 in *C. aceratus* at ~0°C, and is in line with that of other fish species such as rainbow trout (*Oncorhynchus mykiss*) and seabass (*Dicentrarchus laborax*) (Farrell and Smith, 2017).

Comparisons among related species provide valuable insight into the functional consequences of vicarious loss of individual gene products or wider disruption of gene expression, such as variation in cardiac performance within the Channichthyid icefish family, where six species lack not only haemoglobin but also myoglobin.

While *C. aceratus* occupies a largely benthic habitat, other icefish species are more pelagic (Rutschmann et al., 2011). In *C. aceratus*, relative ventricular mass is ~0.3% of body mass (Holeton, 1970; Robertson et al., 1998), whereas in other icefish species, *C. rastrispinosus*, *C. hamatus* and *Champscephalus gunnari*, it is over 25% greater (0.38–0.4%) (Tota et al., 1991; Robertson et al., 1998; S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished). Although resting values have not been reported, maximum \dot{Q} in *C. rastrispinosus* (128 ml min⁻¹ kg⁻¹) and *C. hamatus* (300 ml min⁻¹ kg⁻¹) perfused hearts are also greater than those in *C. aceratus* (70 ml min⁻¹ kg⁻¹) (Tota et al., 1991; S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished). *Chaenocephalus aceratus* is among the six out of 16 icefish species that does not express cardiac myoglobin (Moylan and Sidell, 2000; Sidell et al., 1997), the absence of which has been suggested to limit cardiac power, particularly in the face of increased afterload (Aciermo et al., 1997). *Chionodraco rastrispinosus* (Hb⁻Mb⁺) had a larger ventricle, a higher f_H and greater maximum V_S and \dot{Q} than *C. aceratus*, suggesting that cardiac Mb has functional relevance in supporting greater CPO *in vivo* (S. E. Egginton, M. Axelsson, E. L. Crockett, K. M. O'Brien and A. P. Farrell, unpublished).

Conclusions

Our measures show that routine \dot{Q} and CPO in *C. aceratus* under ambient temperatures are not as high as the values reported previously, but are still higher than those of benthic red-blooded (Hb⁺) notothenioids. In fact, routine \dot{Q} in *C. aceratus* is similar to \dot{Q} in *P. borchgrevinki*, a more active cryopelagic species. Furthermore, *C. aceratus* was also capable of increasing routine \dot{Q} severalfold to meet the increased \dot{M}_{O_2} when metabolic demands were elevated during an acute temperature challenge up to +8°C and stress-induced activity. Also, there was no difference in cardiac BPT in Hb⁺ *N. coriiceps* and Hb⁻ *C. aceratus* during acute temperature challenges.

Hemmingsen and Douglas (1972) were able to maintain *C. aceratus* 'in satisfactory condition' for at least 2 weeks at 8°C and only encountered aberrant behaviour at 10°C. Further, the Patagonian *Champscephalus esox* (which is also Hb⁻) occupies habitats in which water temperatures reach as high as 10°C, although this may be the edge of their thermal tolerance/ecological range because modelling studies conclude that at this temperature there is little scope for activity (Egginton et al., 2002).

Together, these data suggest that increased temperature – at least during acute warming – is surprisingly well tolerated by *C. aceratus*. However, it remains unresolved whether acute thermal tolerance leaves sufficient scope for sustained activity, digestion and reproduction, which are crucial for long-term species survival in a rapidly changing environment.

Acknowledgements

We are sincerely grateful to Amanda Biederman, Anna Rix and Elizabeth Evans for assistance with fishing and animal care. We are also indebted to the Masters and crew of the ARSV *Laurence M. Gould*, and the winter support staff at Palmer Station.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: W.J., S.E., A.P.F., E.L.C., K.M.O., M.A.; Methodology: W.J., S.E., A.P.F., E.L.C., K.M.O., M.A.; Formal analysis: W.J., S.E., M.A.; Investigation: W.J., S.E.; Writing - original draft: W.J.; Writing - review & editing: S.E., A.P.F., E.L.C., K.M.O., M.A.; Visualization: W.J., S.E.; Supervision: M.A.; Project administration: E.L.C., K.M.O.; Funding acquisition: S.E., A.P.F., E.L.C., K.M.O., M.A.

Funding

Funding was provided by grants from the National Science Foundation (ANT 1341602 to E.L.C. and ANT 1341663 to K.M.O.) and the Swedish Research council (Vetenskapsrådet; grant 2015-05286)

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.183160.supplemental>

References

- Acierno, R., Agnisola, C., Tota, B. and Sidell, B. D.** (1997). Myoglobin enhances cardiac performance in antarctic icefish species that express the protein. *Am. J. Physiol.* **273**, R100-R106.
- Altimiras, J. and Axelsson, M.** (2004). Intrinsic autoregulation of cardiac output in rainbow trout (*Oncorhynchus mykiss*) at different heart rates. *J. Exp. Biol.* **207**, 195-201.
- 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.
- Axelsson, M.** (2005). The circulatory system and its control. In *Physiology of Polar Fishes* (ed. J. Steffensen and A. P. Farrell), pp. 239-280. New York: Elsevier.
- Axelsson, M., Davison, W., Forster, M. E. and Farrell, A. P.** (1992). Cardiovascular responses of the red-blooded antarctic fishes *Pagothenia borchgrevinkii* and *P. borchgrevinkii*. *J. Exp. Biol.* **167**, 179-201.
- Axelsson, M., Davison, B., Forster, M. and Nilsson, S.** (1994). Blood pressure control in the Antarctic fish *Pagothenia borchgrevinkii*. *J. Exp. Biol.* **190**, 265-279.
- Bada, A. A., Svendsen, J. H., Secher, N. H., Saltin, B. and Mortensen, S. P.** (2012). Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. *J. Physiol.* **590**, 2051-2060.
- Beers, J. M. and Jayasundara, N.** (2015). Antarctic notothenioid fish: what are the future consequences of 'losses' and 'gains' acquired during long-term evolution at cold and stable temperatures? *J. Exp. Biol.* **218**, 1834-1845.
- Beers, J. M. and Sidell, B. D.** (2011). Thermal tolerance of Antarctic notothenioid fishes correlates with level of circulating hemoglobin. *Physiol. Biochem. Zool.* **84**, 353-362.
- Buckley, B. A., Hedrick, M. S. and Hillman, S. S.** (2014). Cardiovascular oxygen transport limitations to thermal niche expansion and the role of environmental Po₂ in Antarctic notothenioid fishes. *Physiol. Biochem. Zool.* **87**, 499-506.
- Campbell, H. A. and Egginton, S.** (2007). The vagus mediates cardio-respiratory coupling that changes with metabolic demand in a temperate notothenioid fish. *J. Exp. Biol.* **210**, 2472-2480.
- Campbell, H. A., Taylor, E. W. and Egginton, S.** (2004). The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin (*Myoxocephalus scorpius*). *J. Exp. Biol.* **207**, 1969-1976.
- Campbell, H. A., Fraser, K. P. P., Bishop, C. M., Peck, L. S. and Egginton, S.** (2008). Hibernation in an antarctic fish: on ice for winter. *PLoS ONE* **3**, e1743.
- Campbell, H., Davison, W., Fraser, K. P. P., Peck, L. S. and Egginton, S.** (2009). Heart rate and ventilation in Antarctic fishes are largely determined by ecotype. *J. Fish Biol.* **74**, 535-552.
- Clarke, A., Murphy, E. J., Meredith, M. P., King, J. C., Peck, L. S., Barnes, D. K. A. and Smith, R. C.** (2007). Climate change and the marine ecosystem of the western Antarctic Peninsula. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 149-166.
- Devor, D. P., Kuhn, D. E., O'Brien, K. M. and Crockett, E. L.** (2016). Hyperoxia does not extend critical thermal maxima (CT max) in white- or red-blooded Antarctic notothenioid fishes. *Physiol. Biochem. Zool.* **89**, 1-9.
- Egginton, S.** (1997). A comparison of the response to induced exercise in red- and white-blooded Antarctic fishes. *J. Comp. Physiol. B* **167**, 129-134.
- Egginton, S. and Campbell, H. A.** (2016). Cardiorespiratory responses in an Antarctic fish suggest limited capacity for thermal acclimation. *J. Exp. Biol.* **219**, 1283-1286.
- Egginton, S., Skillebeck, C., Hoofd, L., Calvo, J. and Johnston, I. A.** (2002). Peripheral oxygen transport in skeletal muscle of Antarctic and sub-Antarctic notothenioid fish. *J. Exp. Biol.* **205**, 769-779.
- Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., Gale, M. K., Patterson, D. A., Hinch, S. G. and Farrell, A. P.** (2011). Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109-112.
- Eliason, E. J., Clark, T. D., Hinch, S. G. and Farrell, A. P.** (2013). Cardiorespiratory performance and blood chemistry during swimming and recovery in three populations of elite swimmers: adult sockeye salmon. *Comp. Biochem. Physiol.* **166B**, 385-397.
- Farrell, A. P.** (1991). From hagfish to tuna: a perspective on cardiac function in fish. *Physiol. Zool.* **64**, 1137-1164.
- Farrell, A. P.** (2009). Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *J. Exp. Biol.* **212**, 3771-3780.
- Farrell, A. P.** (2016). Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J. Fish. Biol.* **88**, 322-343.
- Farrell, A. P. and Smith, F.** (2017). Cardiac form, function and physiology. In *The Cardiovascular System Morphology, Control and Function* (ed. A. K. Gamperl, T. E. Gillis, A. P. Farrell and C. J. Brauner). New York: Academic Press.
- Farrell, A. P., Eliason, E. J., Clark, T. D. and Steinhausen, M. F.** (2014). Oxygen removal from water versus arterial oxygen delivery: calibrating the Fick equation in Pacific salmon. *J. Comp. Physiol. B* **184**, 855-864.
- Feller, G. and Gerday, C.** (1997). Adaptations of the hemoglobinless Antarctic icefish (Channichthyidae) to hypoxia tolerance. *Comp. Biochem. Physiol. A Comp. Physiol.* **118**, 981-987.
- Franklin, C. E., Axelsson, M. and Davison, W.** (2001). Constancy and control of heart rate during an increase in temperature in the Antarctic fish *Pagothenia borchgrevinkii*. *Exp. Biol. Online* **6**, 1-8.
- Franklin, C., Davison, W. and Seebacher, F.** (2007). Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinkii*. *J. Exp. Biol.* **210**, 3068-3074.
- Gamperl, K. A., 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.
- Garofalo, F., Pellegrino, D., Amelio, D. and Tota, B.** (2009). The Antarctic hemoglobinless icefish, fifty five years later: A unique cardiocirculatory interplay of disaptation and phenotypic plasticity. *Comp. Biochem. Physiol. A* **154**, 10-28.
- Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I. et al.** (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO₂ in Atlantic halibut. *J. Exp. Biol.* **217**, 711-717.
- Harrison, P., Zummo, G., Farina, F., Tota, B. and Johnston, I. A.** (1991). Gross anatomy, myoarchitecture, and ultrastructure of the heart ventricle in the haemoglobinless icefish *Chaenocephalus aceratus*. *Can. J. Zool.* **69**, 1339-1347.
- Hemmingsen, E. A.** (1991). Respiratory and cardiovascular adaptation in hemoglobin-free fish: resolved and unresolved problems. In *Biology of Antarctic Fish* (ed. G. di Prisco, B. Maresca and B. Tota), pp. 191-203. New York: Springer-Verlag.
- Hemmingsen, E. A. and Douglas, E. L.** (1970). Respiratory characteristics of the hemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol. A Comp. Physiol.* **33**, 733-744.
- Hemmingsen, E. A. and Douglas, E. L.** (1972). Respiratory and circulatory responses in a hemoglobin-free fish, *Chaenocephalus aceratus*, to changes in temperature and oxygen tension. *Comp. Biochem. Physiol. A Comp. Physiol.* **43**, 1031-1043.
- Hemmingsen, E. A. and Douglas, E. L.** (1977). Respiratory and circulatory adaptations to the absence of hemoglobin in chaenichthyid fishes. In *Adaptations within Antarctic Ecosystems* (ed. G. A. Llano), pp. 479-487. Washington: Smithsonian Institution.
- Hemmingsen, E. A., Douglas, E. L., Johansen, K. and Millard, R. W.** (1972). Aortic blood flow and cardiac output in the hemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol. A Comp. Physiol.* **43**, 1045-1051.
- Hochachka, P. W. and Somero, G. N.** (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford: Oxford University Press.
- Holeton, G. F.** (1970). Oxygen uptake and circulation by a hemoglobinless Antarctic fish (*Chaenocephalus aceratus* Lönnberg) compared with three red-blooded Antarctic fish. *Comp. Biochem. Physiol. A Comp. Physiol.* **34**, 457-471.
- Holeton, G. F.** (1976). Respiratory morphometrics of white and red blooded antarctic fish. *Comp. Biochem. Physiol. A Comp. Physiol.* **54**, 215-219.
- IPCC** (2014). Climate change 2014: synthesis report. In *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. Core Writing Team, R. K. Pachauri and L. A. Meyer). Geneva, Switzerland: IPCC.
- Jakubowski, M.** (1982). Dimensions of respiratory of the gills and skin in the Antarctic white-blooded fish, *Chaenocephalus aceratus* Lönnberg (Chaenichthyidae). *Z. mikrosk-anatom. Forsch. Leipzig* **96**, 145-156.

- Meredith, M. P. and King, J. C.** (2005). Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century. *Geophys. Res. Lett.* **32**, 1-5.
- Moylan, T. J. and Sidell, B. D.** (2000). Concentrations of myoglobin and myoglobin mRNA in heart ventricles from Antarctic fishes. *J. Exp. Biol.* **203**, 1277-1286.
- Nelson, D., Stieglitz, J. D., Cox, G. K., Heuer, R. M., Benetti, D. D., Grosell, M. and Crossley, D. A.** (2017). Cardio-respiratory function during exercise in the cobia, *Rachycentron canadum*: the impact of crude oil exposure. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **201**, 58-65.
- O'Brien, K. M. and Crockett, E. L.** (2013). The promise and perils of Antarctic fishes. *EMBO Rep.* **14**, 17-24.
- Robertson, R. F., Whiteley, N. M. and Egginton, S.** (1998). Cardiac and locomotory muscle mass in antarctic fishes. In *Fishes of Antarctica* (ed. G. Di Prisco, E. Pisano and A. Clarke), pp. 197-204. Milano: Springer.
- Rutschmann, S., Matschiner, M., Damerou, M., Muschick, M., Lehmann, M. F., Hanel, R. and Salzburger, W.** (2011). Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. *Mol. Ecol.* **20**, 4707-4721.
- Ruud, J. T.** (1954). Vertebrates without erythrocytes and blood pigment. *Nature* **173**, 848-850.
- Sandblom, E., Farrell, A. P., Altimiras, J., Axelsson, M. and Claireaux, G.** (2005). Cardiac preload and venous return in swimming sea bass (*Dicentrarchus labrax* L.). *J. Exp. Biol.* **208**, 1927-1935.
- Sandblom, E., Axelsson, M. and Davison, W.** (2009). Circulatory function at sub-zero temperature: venous responses to catecholamines and angiotensin II in the Antarctic fish *Pagothenia borchgrevinki*. *J. Comp. Physiol. B.* **179**, 165-173.
- Sandblom, E., Davison, W. and Axelsson, M.** (2012). Cold Physiology: Postprandial Blood Flow Dynamics and Metabolism in the Antarctic Fish *Pagothenia borchgrevinki*. *PLoS ONE* **7**, e33487.
- Sandersfeld, T., Davison, W., Lamare, M. D., Knust, R. and Richter, C.** (2015). Elevated temperature causes metabolic trade-offs at the whole-organism level in the Antarctic fish *Trematomus bernacchii*. *J. Exp. Biol.* **218**, 2373-2381.
- Shiels, H. A. and White, E.** (2008). The Frank-Starling mechanism in vertebrate cardiac myocytes. *J. Exp. Biol.* **211**, 2005-2013.
- Sidell, B. D. and O'Brien, K. M.** (2006). When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. *J. Exp. Biol.* **209**, 1791-1802.
- Sidell, B. D., Vayda, M. E., Small, D. J., Moylan, T. J., Londraville, R. L., Yuan, M. L., Rodnick, K. J., Eppley, Z. A. and Costello, L.** (1997). Variable expression of myoglobin among the hemoglobinless Antarctic icefishes. *Proc. Natl Acad. Sci. USA* **94**, 3420-3524.
- Steinhausen, M. F., Sandblom, E., Eliason, E. J., Verhille, 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.
- Tota, B., Acierno, R. and Agnisola, C.** (1991). Mechanical performance of the isolated and perfused heart of the haemoglobinless Antarctic icefish *Chionodraco hamatus* (Lönnerberg): effects of loading conditions and temperature. *Philos. Trans. R. Soc. B Biol. Sci.* **332**, 191-198.
- Vornanen, M., Haverinen, J. and Egginton, S.** (2014). Acute heat tolerance of cardiac excitation in the brown trout (*Salmo trutta fario*). *J. Exp. Biol.* **217**, 299-309.
- Wells, R. M. G.** (1986). Cutaneous oxygen uptake in the antarctic icequab, *Rhizophila dearborni* (Pisces: Zoarcidae). *Polar Biol.* **5**, 175-179.
- Whiteley, N. M. and Egginton, S.** (1999). Antarctic fishes have a limited capacity for catecholamine synthesis. *J. Exp. Biol.* **202**, 3623-3629.
- Wood, C. M. and Shelton, G.** (1975). Physical and adrenergic factors affecting systemic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. Exp. Biol.* **63**, 505-523.
- Zummo, G., Acierno, R., Agnisola, C. and Tota, B.** (1995). The heart of the icefish: bioconstruction and adaptation. *Braz. J. Med. Biol. Res.* **28**, 1265-1276.