© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb180703. doi:10.1242/jeb.180703

SHORT COMMUNICATION

In vivo aerobic metabolism of the rainbow trout gut and the effects of an acute temperature increase and stress event

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ABSTRACT

The fish gut is responsible for numerous potentially energetically costly processes, yet little is known about its metabolism. Here, we provide the first *in vivo* measurements of aerobic metabolism of the gut in a teleost fish by measuring gut blood flow, as well as arterial and portal venous oxygen content. At 10°C, gut oxygen uptake rate was 4.3±0.5 ml O_2 h^{-1} kg^{-1} (~11% of whole-animal oxygen uptake). Following acute warming to 15°C, gut blood flow increased ~3.4-fold and gut oxygen uptake rate increased ~3.7-fold (16.0±3.3 ml O_2 h^{-1} kg^{-1}), now representing ~25% of whole-animal oxygen uptake. Although gut blood flow decreased following an acute stress event at 15°C, gut oxygen uptake remained unchanged as a result of a ~2-fold increase in oxygen extraction. The high metabolic thermal sensitivity of the gut could have important implications for the overall aerobic capacity and performance of fish in a warming world and warrants further investigation.

KEY WORDS: Gastrointestinal, Teleost, Oxygen consumption, Metabolism, Energy expenditure

INTRODUCTION

The gut is a multifunctional organ system responsible for a range of crucial, intricately linked and potentially energetically costly physiological processes (Grosell et al., 2011). Essential gut functions in fish include digestion and absorption of nutrients (Bakke et al., 2011), ionic and osmotic regulation (Grosell, 2011), barrier and immune function (Cain and Swan, 2011), acid–base regulation (Taylor et al., 2011), endocrine/neuro-endocrine/paracrine functions (Takei and Loretz, 2011) and in some species even respiration (Nelson and Dehn, 2011). Although much is known about the functions of the gut and the pronounced implications for whole-animal integrative physiology, relatively little is known about the metabolic cost of the gut in fish (Seth et al., 2011).

In mammals, the gut is a highly perfused and metabolically active organ system with oxygen uptake rates ($\dot{M}_{O_2}$) of the gut constituting ~20–25% of whole-animal $\dot{M}_{O_2}$ in the fasted state (Greenway, 1981; Vaugelade et al., 1994; Wolff, 2007; Yen et al., 1989). Although a growing number of publications report that the gut of fish is also highly perfused and affected by various internal and external stimuli, no *in vivo* determinations of the metabolic costs of the gut in fish have yet been published (Seth et al., 2011). Currently, the limited amount of data stems from measurements of oxygen uptake by isolated intestinal segments in the gulf toadfish (*Opsanus beta*; Heuer and Grosell, 2016; Taylor and Grosell, 2009), and it has been estimated that the intestinal contribution to whole-animal $\dot{M}_{O_2}$ is ~5.6% (Taylor and Grosell, 2009).

In fasted fish, gut blood flow is regulated to a level sufficient for the gut to carry out basic homeostatic processes (e.g. regulation of acid–base and ion–water balance), as well as to provide for the housekeeping needs of the gut. Gut blood flow has been reported to range between 10% and 40% of cardiac output (see references within Seth et al., 2011). According to the Fick principle, the oxygen uptake of the gut equals the product of gut blood flow and oxygen extraction. Thus, considering the relatively high gut blood flow, and assuming that gut tissues have a similar oxygen extraction to other tissues, gut $\dot{M}_{O_2}$ should constitute a substantial proportion of whole-animal $\dot{M}_{O_2}$ in fish. Furthermore, gut blood flow increases by ~70–150% following a meal (Axelsson et al., 1989, 2000; Eliason et al., 2008; Gräns et al., 2009a; Seth et al., 2008; Thorarensen and Farrell, 2006), by ~100% when acclimating to seawater (Brijs et al., 2015, 2016) and by ~50–150% when acutely warmed (Gräns et al., 2009b, 2013). If these substantial increases in gut blood flow coincide with similar increases in gut $\dot{M}_{O_2}$, then the metabolic demands of the gut have the potential to limit the aerobic capacity for other activities such as locomotion (i.e. foraging and predator avoidance), reproduction and somatic growth. In contrast, gut blood flow has been demonstrated to decrease during hypoxia, exercise and acute stress (Altimiras et al., 2008; Axelsson et al., 2002; Axelsson and Fritsche, 1991; Dupont-Prinet et al., 2009; Gräns et al., 2009b; Sandblom et al., 2012). Presumably this is because blood flow is prioritized to other oxygen-demanding organs (e.g. heart and brain) and muscle tissues to support the fight or flight response (Farrell et al., 2001). Whether the oxygen demand of the gut decreases during such periods of transient stress or gut oxygen extraction increases to compensate for the decreased blood flow remains unknown. Therefore, it is necessary to obtain *in vivo* measurements of oxygen uptake in the gut of fish not only during the fasted state but also under metabolically demanding environmental and physiological states. This information will allow us to understand how the oxygen supply and demand of this essential organ system are regulated.

The overall aim of the present study was to obtain the first *in vivo* measurements of gut $\dot{M}_{O_2}$ in a teleost species and to determine the relative contribution of the gut to whole-animal aerobic metabolism. Furthermore, we investigated the effects of an acute temperature increase (i.e. an acute increase of 5°C) and an acute stress event (i.e. experimenter rapidly tapping or shaking the respirometer for 3 min) on gut metabolism. We hypothesized that gut $\dot{M}_{O_2}$ would increase in

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Received 13 March 2018; Accepted 21 May 2018

response to an acute temperature increase, whereas gut $\dot{M}_{O_2}$ would be maintained following the acute stress event as a result of increased tissue oxygen extraction compensating for an expected decrease in gut blood flow. The knowledge gained from this study is essential for understanding the energetic costs of the multifunctional gut of fish. Furthermore, it provides a methodological platform for future studies on the effects of environmental and/or anthropogenic stressors on gut function and overall energetics of fish.

MATERIALS AND METHODS

Experimental animals and holding conditions

Rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), of mixed sex, ranging between 564 and 839 g, were obtained from a local hatchery (EM-Lax AB, Fengersfors, Sweden). Fish were held at 10–11°C on a 12 h:12 h light:dark photoperiod in a 2000 l tank containing recirculating aerated freshwater for a minimum period of 3 weeks prior to experimentation. During this period, they were fed dry commercial trout pellets (9 mm Protec Trout pellets, Skretting, Stavanger, Norway) three times per week. Animal care and all experimental procedures were performed in accordance with national regulations and were covered by an ethical permit (165-2015) approved by the ethical committee on animal research in Gothenburg, Sweden.

Surgical procedures and instrumentation

Fish were fasted for approximately 1 week prior to surgery (n=8, body mass 719±32 g). Individual fish were anaesthetized in fresh water (10°C) containing 150 mg l^{-1} MS222 (ethyl-3-aminobenzoate methanesulphonic acid; Sigma-Aldrich Inc., St Louis, MO, USA) buffered with 300 mg l^{-1} $NaHCO_3$. Anaesthetized fish were transferred to an operating table covered with soft, water-soaked foam. To maintain anaesthesia, the gills were continuously flushed with aerated water containing 100 mg l^{-1} MS222 buffered with 200 mg l^{-1} $NaHCO_3$ at 10°C. To determine gut $\dot{M}_{O_2}$ *in vivo*, the fish was instrumented with catheters to allow the collection of arterial and portal venous blood samples, as well as a flow probe around the coeliacomesenteric artery to determine absolute gut blood flow as outlined in detail below.

The hepatic portal vein was cannulated using a custom-made catheter, which consisted of a 185 mm long piece of polyurethane tubing (2F-PU, Instech Solomon, Plymouth, UK) with two side holes (made using a punch constructed from a 23 G needle) located 2 mm from the tip. The polyurethane tubing was mechanically expanded to fit, with a ∼2 mm overlay, onto a 615 mm section of polyethylene tubing (PE-50, BD Intramedic™, Agnthos, Lidingö, Sweden). The catheter had a total volume of ∼200 µl and was pre-soaked with 100 IU ml^{-1} heparin (LEO Pharma AB, Mälmö, Sweden) in 0.9% saline. The hepatic portal vein was cannulated using the method described in Mclean and Ash (1989). Briefly, the fish was placed on its back and a ∼25 mm ventral incision was made between the pectoral and pelvic fins to access a ventral intestinal vein. The catheter was then inserted into the ventral intestinal vein and gently advanced along the vessel until the tip was located inside the hepatic portal vein. Correct positioning of the cannula was confirmed after each individual experiment. The catheter was secured in the vessel using 4.0 braided silk sutures (Vömel, Kronberg, Germany). The catheter exited the abdominal cavity via the incision and was secured to the skin with 3.0 braided silk sutures directly after the bubble on the polyethylene tubing. The incision was subsequently closed with interrupted 3.0 braided silk sutures (Vömel).

A 1.5 mm Transonic transit-time blood flow probe (B type; Transonic Systems Inc., Ithaca, NY, USA) was then placed around the coeliacomesenteric artery, which was accessed via a ∼25 mm ventrodorsal incision from the base of the right pectoral fin. The coeliacomesenteric artery branches from the dorsal aorta proximate to the efferent branchial vessels and divides progressively to supply the stomach, intestine, gallbladder, liver and gonads (Seth et al., 2011). The blood flow probe was placed around the coeliacomesenteric artery as described in detail by Brijs et al. (2016). Once the flow probe was in position, the lead was exited via the incision and secured to the skin with 3.0 braided silk sutures. The incision was subsequently closed with interrupted monofilament 3.0 Prolene sutures (Ethicon Inc., Somerville, NJ, USA).

The dorsal aorta was cannulated through the roof of the buccal cavity with a 950 mm long PE-50 catheter filled with 100 IU ml^{-1} heparin in 0.9% saline using a sharpened steel wire guide (Axelsson and Fritsche, 1994), with modifications by Kiessling et al. (1995). The catheter was exited through the snout and secured to the skin with 3.0 braided silk sutures.

Following surgery, fish were individually placed into one of two identical custom-made Perspex respirometers (volume: 10.00±0.01 l), which were submerged in a larger experimental tank with recirculating aerated freshwater at 10.0±0.1°C. The total time taken for the surgical procedures (i.e. time from when the fish was initially anaesthetized till its placement in the respirometer) was ∼1 h. Fish were left undisturbed to recover from surgery for at least 22 h.

Recovery period and intermittent flow respirometry

During the recovery period, whole-animal $\dot{M}_{O_2}$ was continuously measured using intermittent-flow respirometry (Clark et al., 2013). Briefly, the partial pressure of oxygen (P_{O_2}) in the water within the respirometer was measured continuously at 1 Hz using a FireSting O_2 system (PyroScience, Aachen, Germany). Automated flush pumps refreshed the water in the respirometers for 5 min every 15 min and the slope of the decline in the P_{O_2} in the water within the respirometers between flush cycles (i.e. when the respirometer was closed) was used to calculate whole-animal $\dot{M}_{O_2}$ using the following formula:

$$\text{Whole-animal } \dot{M}_{O_2} = [(V_r - V_f) \times \Delta C_{wO_2}]/(\Delta t \times M_f), \quad (1)$$

where V_r is the volume of the respirometer, V_f is the volume of the fish (assuming that the overall density of the fish is 1 g per ml of tissue, thus V_f=mass of the fish, M_f), ΔC_{wO_2} is the change in the oxygen concentration of the water within the respirometer (C_{wO_2} is the product of the partial pressure and capacitance of oxygen in the water, the latter being dependent on salinity and temperature) and Δt is the time during which ΔC_{wO_2} was measured (Clark et al., 2013).

Experimental protocol and data acquisition

Following the 22 h recovery period, the Transonic flow probes were connected to a Transonic flow-meter (model T206; Transonic Systems Inc.). The fish were then allowed a further 2 h to recover from the minimal disturbances associated with connecting the flow probes. All signals were relayed to a PowerLab 8/30 system (ADInstruments, Castle Hill, NSW, Australia) and data were collected continuously on a PC using ADInstruments acquisition software Chart™ 7 Pro v7.2.5, at a sampling rate of 10 Hz. Heart rate was derived from the pulsatile gut blood flow trace. The Transonic flow probes were calibrated at 10 and 15°C, according to the manufacturer's instructions, to account for the potential deviations in factory calibration and effects of temperature. However, instead of using a steady gravity-fed flow, we used an

air-driven syringe pump where we could set the rate and stroke volume to physiologically relevant levels. In addition, we used a 5 ml syringe in the setup to act as a Windkessel, whereby adjusting the amount of air in the syringe allowed us to generate a flow profile similar to what is observed *in vivo* in the fish.

By visually inspecting the traces used to calculate whole-animal $\dot{M}_{O_2}$, gut blood flow and heart rate of each individual fish 'on-line', it was possible to identify 'calm' periods (i.e. low activity; see Fig. S1) for the sampling of arterial and venous blood. The oxygen content of arterial and venous blood samples was determined using methods described in detail by Tucker (1967) using a modified TC500 Tucker Cell (Strathkelvin Instruments Ltd, North Lanarkshire, UK), which allowed the replacement of the original Strathkelvin oxygen electrode with a PyroScience oxygen optode (see http://bioenv.gu.se/personal/Axelsson_Michael/3d-design-interactive). The oxygen optode was calibrated by injecting 30 µl of distilled water, which had been oxygenated with pure oxygen for at least 1 h at 0°C (i.e. oxygen content of 4.8769 ml O_2 100 ml^{-1} at 1 atm), into the chamber containing a potassium ferricyanide solution (18 mmol l^{-1} $C_6FeK_3N_6$, 3 g l^{-1} saponin) and noting the change in P_{O_2}. Once the oxygen optode had been calibrated, the oxygen content of arterial and venous blood was determined for each individual. Directly after the recording of a 'calm' whole-animal $\dot{M}_{O_2}$ measurement, a 50 µl venous blood sample was withdrawn via the hepatic portal vein catheter using a 50 µl glass Hamilton syringe and the oxygen content was determined from duplicate 10 µl blood samples. A 50 µl arterial blood sample was then withdrawn from the dorsal aorta catheter with the glass Hamilton syringe and oxygen content was determined from duplicate 10 µl blood samples. An additional 300 µl sample of arterial blood was withdrawn from the dorsal aorta with a 1 ml syringe for haematological analyses. Haematocrit was determined as the fractional red cell volume upon centrifugation of a subsample of blood in 80 µl microcapillary tubes at 10,000 rpm for 10 min. Haemoglobin concentration was determined using a handheld Hb 201^+ meter (Hemocue® AB, Ängelholm, Sweden) and values were corrected for fish blood (Clark et al., 2008).

Following the determination of blood oxygen content and haematological parameters at 10.0±0.1°C, the water temperature was increased at a rate of 2.5°C for 2 h to reach a temperature of 15.0±0.1°C. Fish were then left undisturbed at this elevated temperature for 2 h. Following this 2 h period at 15.0°C, blood samples were withdrawn during 'calm' periods in the same manner as described above and analysed for oxygen content and haematological parameters. Once these measurements had been completed, the fish were acutely stressed while still maintained at 15.0±0.1°C. An acute stress response was invoked by the experimenter rapidly tapping or shaking the respirometer for 3 min. Following this acute stress event, whole-animal $\dot{M}_{O_2}$ and cardiovascular variables were recorded for 7 min before blood samples were again withdrawn and analysed for oxygen content. A significantly depressed gut blood flow at the moment of blood withdrawal suggests that the gut of the fish had not yet begun to recover from the stressor prior to the analysis of oxygen content. Furthermore, at the moment of blood withdrawal, the slope of the decline in the P_{O_2} in the water within the respirometers was still steep and was not decreasing, which further indicates that recovery had not yet begun. At the end of the experiment, the fish were subsequently killed with a sharp blow to the head.

To determine the relative mass of the different organs/tissues of the gut in rainbow trout in relation to total body mass, the stomach, gallbladder, liver, spleen, intestine, pyloric caeca and gonads were dissected out and weighed in a separate group of fish (n=8, 662±31 g). These fish had been used in a separate study but were sourced from the same hatchery as the abovementioned fish used for gut $\dot{M}_{O_2}$ measurements and housed under identical conditions. Using the method described in Seth et al. (2009), a cast of the gut vasculature was constructed with MICROFIL silicone casting material (MV-122, Flowtech, Carver, MA, USA) in one individual to check for any additional arterial or venous inputs to the gut. This fish was anaesthetized and injected with 5000 IU ml^{-1} heparin into the caudal vasculature to prevent blood clotting, and subsequently killed with a sharp blow to the head. The coeliacomesenteric artery was cannulated and a gill arch was cut before perfusing the entire vasculature with 0.9% saline containing 0.01 mol l^{-1} sodium nitroprusside in order to remove the blood and dilate the vasculature. The MICROFIL was then injected into the coeliacomesenteric artery and allowed to polymerize for at least 24 h. The gut was subsequently dissected out and placed in a bath to clear the tissue, which involved immersion in increasing concentrations of ethanol (25%, 50%, 75%, 95% and 100%, for 24 h at each step), followed by 24 h immersion in methyl salicylate. This approach confirmed that the coeliacomesenteric artery is indeed the main route for blood supply to the gut in rainbow trout (Seth et al., 2009). However, we observed some minor, previously undescribed, venous vessels originating from the ventral somatic musculature and pelvic fin region that emptied into the intestinal veins.

Data analyses

Measures of whole-animal $\dot{M}_{O_2}$, cardiovascular parameters (i.e. gut blood flow and heart rate) and haematological parameters (i.e. haematocrit and haemoglobin concentration) at 10 and 15°C were derived from a 10 min period when the respirometer was closed during the abovementioned 'calm' periods. The same measures, with exception of the haematological parameters, were also derived from a 7 min period when the respirometer was closed directly following the 3 min acute stress exposure. *In vivo* measures of gut $\dot{M}_{O_2}$ at 10 and 15°C and at 15°C following the acute stress event were determined using the Fick equation:

$$\text{Gut } \dot{M}_{O_2} = \text{gut blood flow} \times \text{arteriovenous oxygen content difference}, \quad (2)$$

where the arteriovenous oxygen content difference was determined by subtracting the oxygen content of the venous blood sample (i.e. collected from the hepatic portal vein) from the oxygen content of the arterial blood sample (i.e. collected from the dorsal aorta).

The proportion of oxygen extracted from the blood was determined using the following equation:

$$\text{Gut tissue oxygen extraction } (\%) = \frac{\text{arteriovenous oxygen content difference}}{\text{arterial blood oxygen content}} \times 100. \quad (3)$$

Rates of oxygen delivery to the gut (i.e. the product of gut blood flow and arterial oxygen content) were compared with gut $\dot{M}_{O_2}$ at 10 and 15°C and at 15°C following the acute stress event (Wolff, 2007). Q_{10} values were calculated for whole-animal $\dot{M}_{O_2}$ and gut $\dot{M}_{O_2}$ between 10 and 15°C using Van't Hoff's equation.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 21 (IBM Corp., Armonk, NY, USA). We used a one-way repeated measures ANOVA for the analysis of whole-animal $\dot{M}_{O_2}$, gut $\dot{M}_{O_2}$, gut

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blood flow and heart rate at 10 and 15°C, as well as following the acute stress event at 15°C. To meet the assumptions of this model, a logarithmic transformation was applied to whole-animal $\dot{M}_{O_2}$ and gut $\dot{M}_{O_2}$. The results for whole-animal $\dot{M}_{O_2}$ were also corrected using the Greenhouse–Geisser correction as the assumption of sphericity was violated (Greenhouse and Geisser, 1959). For comparisons between repeated dependent variables, a Bonferroni confidence-interval adjustment was used. We used a paired-samples *t*-test for analysis of haematological variables (i.e. haematocrit and haemoglobin concentration). *F*-, *t*- and *P*-values obtained from the models are reported in the figures or in Table S1, with significance defined at $P<0.05$. Unless otherwise specified, all data are presented as means±s.e.m.

RESULTS AND DISCUSSION

In vivo aerobic metabolism of the rainbow trout gut at 10°C

The rainbow trout gut is a highly metabolically active organ system, as gut $\dot{M}_{O_2}$ at 10°C was 4.3±0.5 ml O_2 h^{-1} kg^{-1} in the fasted state (Fig. 1; Table S1). Even though the relative mass of the gut represents less than 4% of the total body mass (Table S2), gut $\dot{M}_{O_2}$ of fasted rainbow trout constituted 11.2±1.2% of whole-animal $\dot{M}_{O_2}$ (Fig. 1; Table S1). The gut was perfused to a level whereby the rates of oxygen delivery to the gut exceeded the oxygen uptake of these tissues by ∼4- to 5-fold. This is similar to ratios found in mammals (e.g. ∼5- to 6-fold higher oxygen delivery than uptake in dogs, rats and baboons; Folkow and Neil, 1971; Wolff, 2007). It has been demonstrated in mammals that the minimum rate of oxygen delivery to the gut required for maintaining its oxygen uptake, independent of oxygen supply, is significantly greater than that for the body as a whole. This has been suggested to be due to the high specific metabolic rate of gut tissues combined with the lower capacity for extraction when compared with non-gut tissues (Cain and Chapler, 1978; Lutz et al., 1975; Nelson et al., 1987; Wolff, 2007). Because of the similar ratio between the delivery and uptake of oxygen in the gut of fish compared with that of mammals, it seems likely that this may also be the case in fish, although we cannot discount the possibility that the relatively high blood flow in the gut of fish may also be necessary for purposes unrelated to oxygen supply.

We most likely underestimated the metabolic costs of the gut in this study as we sampled the venous blood from the hepatic portal vein, which excludes the metabolic costs of the liver. The contribution of the gut (excluding the liver) to whole-animal $\dot{M}_{O_2}$ was ∼16% in a range of mammals (e.g. dogs, rats and baboons), whereas when the liver was included, the overall cost increased to ∼21% (Folkow and Neil, 1971; Wolff, 2007). Thus, assuming that the relative cost of the liver is similar in rainbow trout, the overall cost of the gut could potentially contribute up to ∼14–15% of whole-animal $\dot{M}_{O_2}$ at 10°C.

In vivo aerobic metabolism of the gut increases in response to acute warming

The acute increase in water temperature significantly increased the overall oxygen demand of rainbow trout by ∼1.7-fold (Q_{10} of 3.2±0.5). However, gut $\dot{M}_{O_2}$ increased by ∼3.7-fold (Q_{10} of 15.0±4.8), which meant that the gut (excluding the liver) required 24.8±5.7% of whole-animal $\dot{M}_{O_2}$ at 15°C (Fig. 1; Table S1). Although the underlying reasons for the high temperature sensitivity and associated oxygen demand of the gut following the acute temperature increase remain unknown and warrant further investigation, this could represent a potential constraint on the aerobic capacity of fish during acute warming. However, further investigations using more ecologically relevant temperature changes over longer time scales are required to substantiate this.

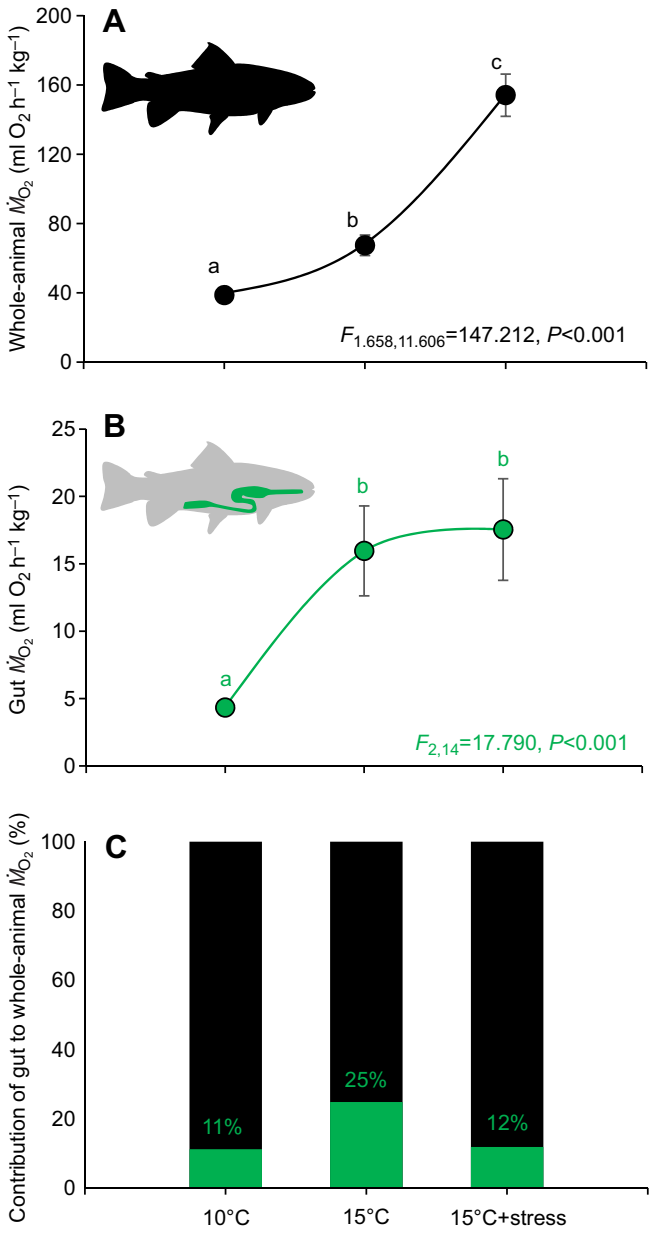

Fig. 1. Whole-animal and gut oxygen uptake rates ($\dot{M}_{O_2}$) of rainbow trout before and after an acute temperature increase and acute stress event. (A) Whole-animal $\dot{M}_{O_2}$ and (B) gut $\dot{M}_{O_2}$ of rainbow trout at 10°C, following an acute temperature increase to 15°C and following an acute stress event at 15°C (n=8). (C) The relative contribution of the gut (green bar) to whole-animal $\dot{M}_{O_2}$ (black bar). Statistical analyses for A and B were performed using a one-way repeated measures ANOVA and a Bonferroni confidence-interval adjustment was used for comparisons between repeated dependent variables. Model outputs are summarized at the bottom of each panel. Significant differences (P<0.05) between the repeated measures are indicated by different lowercase letters. Data are presented as means±s.e.m.

Prior to the acute temperature increase, gut blood flow was 2.9±0.3 ml min^{-1} kg^{-1} and the oxygen extraction of the gut tissues was 24.1±2.9%. The elevated oxygen demand of the gut during warming was primarily supplied by a more than 3-fold increase in gut blood flow, while oxygen extraction of the gut tissues did not significantly change (Fig. 2; Table S1). Significant increases in gut blood flow due to acute warming have also been reported in shorthorn sculpin (*Myoxocephalus scorpius*, ∼50% increase; Gräns

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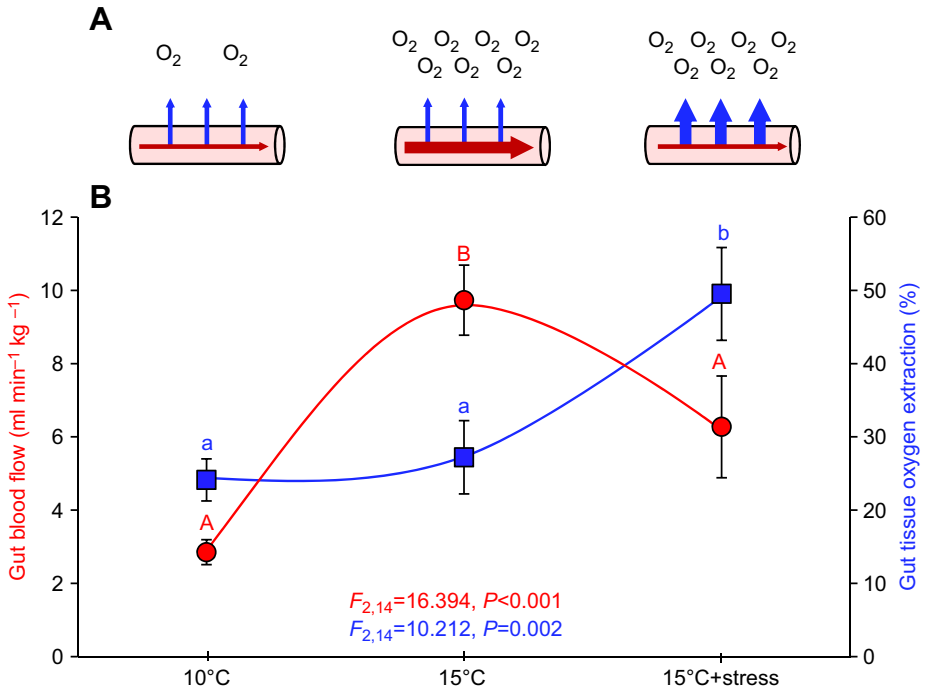

Fig. 2. Gut blood flow and gut tissue oxygen extraction of rainbow trout. (A) A simple schematic drawing illustrating how gut blood flow (red arrows), gut tissue oxygen extraction (blue arrows) and gut oxygen uptake rates ('O_2') change following an acute temperature increase to 15°C, as well as after an acute stress event at 15°C. The thickness of the arrow and 'O_2' number represent the relative changes that occur for each specific variable at each stage. (B) Mean gut blood flow (red line) and gut tissue oxygen extraction (blue line) of rainbow trout at 10°C, following an acute temperature increase to 15°C and following an acute stress event at 15°C (n=8). Statistical analyses were performed using a one-way repeated measures ANOVA and a Bonferroni confidence-interval adjustment was used for comparisons between repeated dependent variables. Model outputs are summarized in red (gut blood flow) and blue (gut tissue oxygen extraction) at the bottom of the panel. Significant differences (P<0.05) between the repeated measures are indicated by different uppercase (gut blood flow) and lowercase letters (gut tissue oxygen extraction). Data are presented as means±s.e.m.

et al., 2013) and green sturgeon (*Acipenser medirostris*, up to 150% increase; Gräns et al., 2009b). Depending on their capacity for long-term thermal acclimation, the substantially elevated gut blood flow in response to elevated temperature may constrain the ability of fish to further increase gut blood flow to process a meal or osmoregulate in seawater (Brijs et al., 2015, 2016; Eliason et al., 2008; Gräns et al., 2009a). As these questions remain open at this stage, investigations using thermally acclimated fish are warranted to gain insight into the long-term *in vivo* metabolic and circulatory thermal responses of the gut, as well as the pronounced implications for whole-animal integrative physiology.

In vivo aerobic metabolism of the gut is maintained during acute stress

The acute stress event invoked a more than 2-fold increase in whole-animal $\dot{M}_{O_2}$ when compared with the resting value obtained at 15°C (Fig. 1; Table S1). Gut $\dot{M}_{O_2}$ did not change following the acute stress event, but now only constituted 11.8±2.5% of whole-animal $\dot{M}_{O_2}$ because of the substantial increase in the latter (Fig. 1; Table S1). The rate of oxygen delivery to the gut decreased following the acute stress event and was only 2.3±0.5 times higher than gut $\dot{M}_{O_2}$. This was primarily due to a significant decrease in gut blood flow (Fig. 2; Table S1), as blood flow was most likely prioritized to muscle tissues (i.e. fight or flight response) and other high oxygen-demanding organs (i.e. heart and brain) (Farrell et al., 2001). However, although gut blood flow significantly decreased during the acute stress event, gut $\dot{M}_{O_2}$ was maintained as oxygen extraction of the gut tissues increased from ∼27% to ∼50%. Visual inspection of gut blood flow traces revealed that following recovery from the acute stress event, gut blood flow returned to pre-stress levels within minutes but no overshoot was observed. This suggests that, at least in the short term, normal metabolic processes of the gut in rainbow trout could be maintained by increased oxygen extraction, which is similar to what occurs in humans, as the gut only becomes hypoxic once oxygen extraction exceeds 70% (Nelson et al., 1988; Takala, 1996; Zhang et al., 1995).

Conclusions

The present study provides the first ever *in vivo* measurements of the aerobic costs of the gut in a teleost species, and demonstrates the metabolic responses to an acute temperature increase and acute stress event. Our findings reveal that the gut of fasted rainbow trout is highly metabolically active and contributes to more than 11% and 24% of whole-animal aerobic metabolism at 10 and 15°C, respectively. Furthermore, the oxygen demand of the gut and the associated changes in gut blood flow in response to an acute temperature increase and an acute stress event may have implications for the aerobic and circulatory capacity of fish to perform other essential activities (e.g. locomotion, digestion, osmoregulation, reproduction and growth). Further development and application of the methodology described in the present study will be beneficial for future studies attempting to investigate the metabolic and circulatory responses of the gut over longer time periods (e.g. thermal acclimation, postprandial responses, etc.), as well as the pronounced implications for whole-animal integrative physiology.

Competing interests

The authors declare no competing or financial interests.

Funding

This research was funded by the Swedish Research Council (Vetenskapsrådet) (M.A., E.S.); Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Svenska Forskningsrådet Formas) (A.G., E.S.); Stiftelsen Wilhelm och Martina Lundgrens Vetenskapsfond (J.B.); Herbert och Karin Jacobssons Stiftelse (J.B.); Kungliga Vetenskaps- och Vitterhets-Samhället (J.B.); and Helge Ax:son Johnsons Stiftelse (J.B.).

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.180703.supplemental

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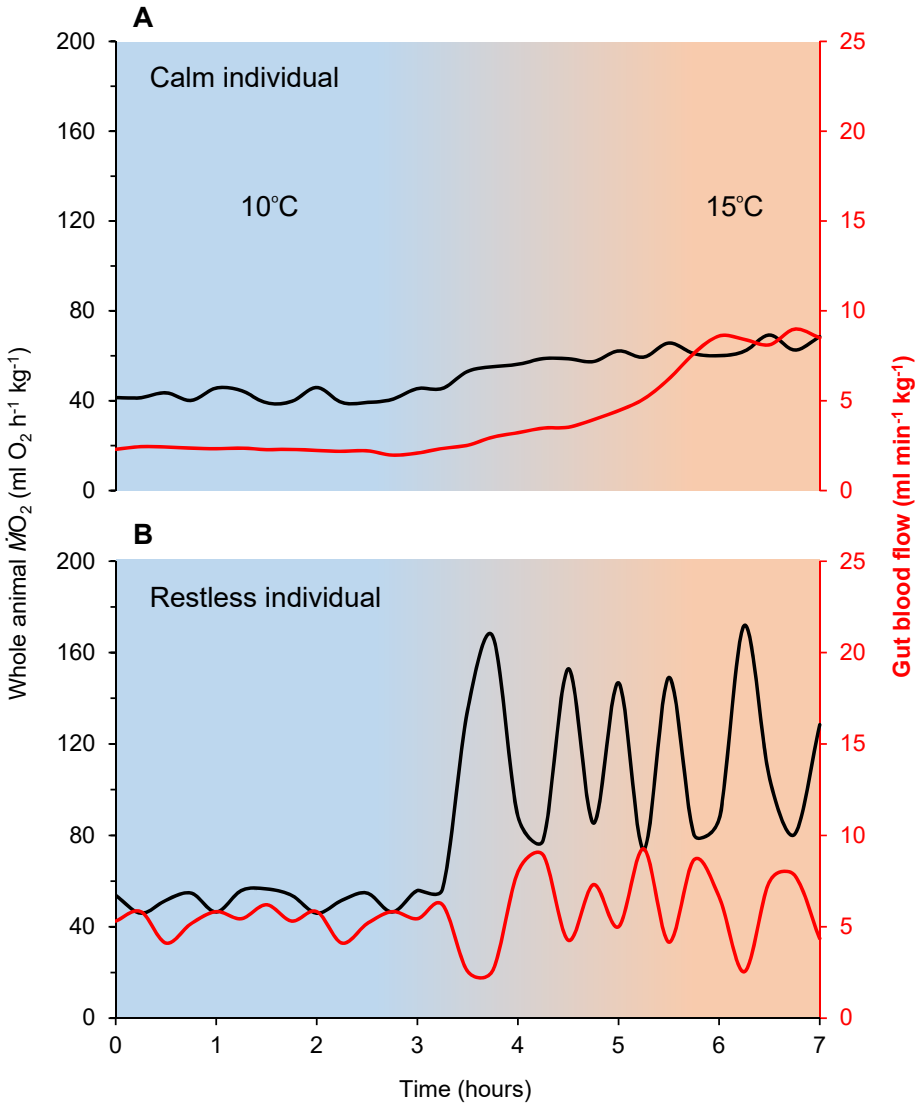

Fig. S1. Representative experimental traces from a 'calm' and 'restless' individual during the experiment. (A) Individual traces of whole animal $\dot{M}O^2$ (black line) and gut blood flow (red line) before, during, and after an acute increase in temperature (blue shaded area is at 10°C, gradient from blue to red represents temperature increase and red shaded area is at 15°C) in a 'calm' individual. In this particular individual, arterial and venous blood samples could be taken during the majority of the experiment due to stable values of whole animal $\dot{M}O^2$ and gut blood flow. (B) In contrast, in the 'restless' individual, a clear inverse relationship can be observed between whole animal $\dot{M}O^2$ and gut blood flow during alternating periods of activity (*i.e.* high whole animal $\dot{M}O^2$ and low gut blood flow) and rest (*i.e.* low whole animal $\dot{M}O^2$ and high gut blood flow). Thus, in this case, it is important to monitor whole animal $\dot{M}O^2$ and gut blood flow on-line to wait for a 'calm' period before sampling arterial and venous blood for the determination of oxygen content.

Table S1. Summary of metabolic, cardiovascular and haematological variables in rainbow trout.

	Model output	10°C	15°C	acute stress at 15°C
Aerobic metabolic parameters				
Whole animal $\dot{M}_{O_2}$ (ml O_2 h^{-1} kg^{-1})	$F_{1.658,11.606}$: 147.212, P<0.001	38.7±1.8^a	67.4±5.9^b	154.1±12.2^c
Gut $\dot{M}_{O_2}$ (ml O_2 h^{-1} kg^{-1})	$F_{2,14}$: 17.790, P<0.001	4.3±0.5^a	16.0±3.3^b	17.5±3.8^b
Arterial blood oxygen content (ml O_2 ml^{-1})	$F_{2,14}$: 0.816, P=0.462	0.11±0.01^a	0.11±0.01^a	0.11±0.01^a
Venous blood oxygen content (ml O_2 ml^{-1})	$F_{2,14}$: 9.908, P=0.002	0.09±0.01^a	0.08±0.01^a	0.05±0.01^b
Arteriovenous O_2 difference (ml O2 ml^{-1})	$F_{2,14}$: 10.765, P=0.001	0.03±0.00^a	0.04±0.01^a	0.07±0.01^b
Gut tissue O_2 extraction (%)	$F_{2,14}$: 10.212, P=0.002	24.1±2.9^a	27.2±5.0^a	49.5±6.3^b
Oxygen delivery:oxygen uptake of the gut	$F_{2,14}$: 3.899, P=0.045	4.6±0.6^a	5.9±1.1^a	2.3±0.5^b
Cardiovascular and haematological parameters				
Gut blood flow (ml min^{-1} kg^{-1})	$F_{2,14}$: 16.394, P<0.001	2.9±0.3^a	9.7±1.0^b	6.3±1.4^a
Heart rate (beats min^{-1})	$F_{2,14}$: 21.732, P<0.001	61.5±2.6^a	83.6±4.0^b	79.4±5.1^b
Haematocrit (%)	t_7: -1.881, P=0.102	19.4±1.5^a	20.3±1.5^a	-
Haemoglobin conc. (g dl^{-1})	t_7: -0.297, P=0.775	5.9±0.3^a	6.0±0.3^a	-

Statistical analyses were performed using one-way repeated measures ANOVA or paired samples t-tests (for haematocrit and haemoglobin concentration). For the comparisons between repeated dependent variables, a Bonferroni confidence-interval adjustment was used. Significance differences (P<0.05) between the repeated measures are indicated by different lower-cased letters. Data are presented as means±s.e.m (n=8).

Table S2. Absolute and relative organ masses supplied by the coeliacomesenteric artery.

	Mass (g)	% of whole animal
Liver	4.98±0.25	0.76±0.03
Gallbladder	0.28±0.07	0.04±0.01
Stomach	8.33±0.63	1.29±0.13
Pyloric caeca	8.20±1.05	1.25±0.16
Intestine	3.26±0.28	0.49±0.04
Gonads	0.51±0.13	0.07±0.02
Total	25.36±1.38	3.88±0.25

Data are presented as means±s.e.m (n=8).