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Effect of hypoxia on specific dynamic action and postprandial cardiovascular physiology in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Fish routinely encounter hypoxic environments, which may have detrimental effects on digestion and performance. The present study measured oxygen consumption (MO_2), gastrointestinal blood flow (GBF), cardiac output (V_b) and heart rate (f_H) in rainbow trout *Oncorhynchus mykiss* at 10 °C–11.5 °C while exposed to a 1.5-h stepwise hypoxia treatment (80%, 60% and 40% saturation = 16.7, 12.6 and 8.4 kPa, respectively), which began 4 h after being fed 1% of their body mass. GBF and f_H significantly decreased by 41 and 25%–29%, respectively, at the most severe hypoxia step (40% saturation), while MO_2 and V_b were maintained throughout the entire hypoxia exposure. Thus, GBF and f_H were more sensitive to hypoxia than MO_2 or V_b in digesting rainbow trout. Subsequent to the hypoxic exposure, the fish were returned to normoxia and monitored for a total of 50 h after feeding. While the magnitude of SDA was unaffected, peak postprandial MO_2 was reduced by 17%, and the duration of specific dynamic action (SDA) was prolonged by 6 h in hypoxia-treated fish when compared to control fish. In conclusion, digestive performance was compromised both during and after the hypoxic exposure, which could lead to negative effects on growth.

1. Introduction

Fish feed in order to obtain nutrients and energy that are essential for growth, health, reproduction and body maintenance. The digestion, absorption and distribution of nutrients require numerous physiological changes such as increases in oxygen consumption (MO_2), gut activity and blood flow to the gastrointestinal system (GBF) (Farrell et al., 2001; Halver and Hardy, 2002; McCue, 2006). The metabolic cost of processing a meal is termed specific dynamic action (SDA, also termed heat increment of feeding) and is primarily associated with protein handling (McCue, 2006). GBF typically increases by 42%–136% following feeding in order to transport nutrients and to supply O_2 to metabolising tissues (Farrell et al., 2001; Axelsson et al., 2002; Eliason et al., 2008).

Fish are routinely exposed to hypoxic environments (a reduced water oxygen level), both in nature and in aquaculture. Even at sublethal levels, hypoxia can have profound effects on fish digestion and performance. Hypoxia reduces aerobic scope [the difference between maximum and minimum metabolic rate, which represents the amount of oxygen available for all aerobic activities (Fry, 1947)], which can limit the peak in MO_2 that follows a meal and prolong the duration of SDA (Jordan and Steffensen, 2007). As a result, growth can be compromised under hypoxic conditions (Chabot and Dutil, 1999). Furthermore, since fish cannot simultaneously perfuse all their capillaries, blood flow must be prioritized among competing organs according to the capacity of the heart. Un-fed fish redistribute blood away from the gastrointestinal system under hypoxic conditions (Axelsson and Fritsche, 1991; Fritsche et al., 1993). However, studies with European sea bass *Dicentrarchus labrax* and Atlantic cod *Gadus morhua* have shown that hypoxia does not compromise GBF in fed fish (Axelsson et al., 2002; Dupont-Prinet et al., 2009; Behrens et al., 2012), demonstrating a prioritization of gastrointestinal perfusion during digestion.

The aim of the present study was to determine how the cardiorespiratory system of rainbow trout *Oncorhynchus mykiss* responds to three different levels of hypoxia (80%, 60% and 40% saturation ≅ 16.7, 12.6 and 8.4 kPa, respectively) while digesting a meal of 1% of body mass. We hypothesized that MO_2, GBF and heart rate (f_H) would be compromised during the hypoxia exposure. Furthermore, we hypothesized that the duration and magnitude of SDA would increase following hypoxia exposure, prolonging the digestive process.

2. Materials and methods

2.1. Fish husbandry

Rainbow trout (*O. mykiss*, Salmonidae; 904.5 ± 83.3 g, $N = 20$) obtained from a local fish hatchery (Richard Henley Farm, Langley,

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BC, Canada) were held at the Department of Fisheries and Oceans Centre for Aquaculture and Environmental Research (West Vancouver, BC, Canada) in outdoor 4,000-L tanks in freshwater between 9 °C and 11 °C under natural photoperiod. The fish were fed 9 mm salmonid pellets three times a week (Skretting, Vancouver, BC, Canada: crude protein 37%, crude fat 33%, crude fibre 1.5%, ash 12%, sodium 0.4%, calcium 1.3%, phosphorous 1%, vitamin A 5000 IU kg^{-1}, vitamin D3 3000 IU kg^{-1} and vitamin E 200 IU kg^{-1}). All experiments were approved by the UBC Animal Care Committee, in accordance with the Canadian Council of Animal Care.

2.2. Surgery

Fish were anaesthetized in buffered MS-222 (0.1 g L^{-1} MS-222 and 0.2 g L^{-1} $NaHCO_3$) and transferred to a surgery table where their gills were continuously irrigated with a maintenance dose of chilled, aerated and buffered anaesthetic solution (0.05 g L^{-1} MS-222 and 0.1 g L^{-1} $NaHCO_3$). Surgical procedures to fit the ventral aorta with a flowprobe follow those previously reported (Thorarensen et al., 1996; Steffensen and Farrell, 1998). Briefly, the ventral aorta was isolated under the right operculum by gently lifting and removing the thin layer of connective tissue covering the vessel. A small piece of moist 2–0 suture silk was slipped under the vessel and used to lift the vessel and guide the Transonic flowprobe (2 RB or 2 SL, Transonic systems, Ithaca, NY, USA) around the ventral aorta in order to measure cardiac output (V_b). The flowprobe was secured in place with two silk sutures in the opercular cavity. The gastrointestinal arteries were then fitted with a flowprobe following the procedures described previously (Thorarensen et al., 1993; Eliason et al., 2008). A small incision was made just posterior to the pectoral fin and the gastric and intestinal arteries were carefully isolated so as not to damage any nerves. A single Transonic flowprobe (1 or 1.5 RB, Transonic systems) was placed around both the gastric and intestinal arteries to measure gastrointestinal blood flow (GBF). The wound was closed with 2–0 silk sutures, and the leads from both flowprobes were secured to the body wall of the fish and led off the leading edge of the dorsal fin.

2.3. Experimental protocol

Oxygen consumption (MO_2) was measured in individual fish using a custom made 4-chamber intermittent-flow through respirometry system (Loligo Systems, Hoboro, Denmark). Each 9.9-L chamber received aerated well water at 5 L min^{-1} (10–11.5 °C). The chambers were equipped with a recirculation pump that only turned on when the inflow water was turned off during a measurement period in order to ensure sufficient water mixing and to minimize intermittent disturbances to the fish. The flush cycle was 10.5 min, the wait period was 30 s and the recirculation period for measurement was 4 min. An oxygen probe (MINI-DO; Loligo Systems, Hoboro, Denmark) was placed directly in the chamber and measured oxygen content of the water every second during the measurement period. Therefore, a value for MO_2 was measured every 15 min throughout the experiment for each of the chambers. Background MO_2 was assessed for at least 1 h before and after each experiment and was determined to be negligible.

Fish were fasted for at least 48 h and then underwent surgery before being placed in a respirometry chamber. The fish were maintained under continuous light while in the respirometer chamber to reduce spontaneous activity associated with lights turning on and off (Eliason et al., 2008). Routine MO_2 was continuously monitored while the fish recovered for 36–48 h. The fish were then removed from the chamber, lightly anaesthetized (0.08 g L^{-1} MS-222 and 0.16 g L^{-1} $NaHCO_3$) and force-fed by gavage 1% of their body weight using the same feed as during holding (see fish husbandry above). Any regurgitated pellets were counted and determined to be negligible relative to the size of the meal. The fish were immediately replaced into their chamber and monitored for a further 50 h. Control fish ($n = 9$) were maintained in normoxia throughout the entire 50-h postprandial period. Hypoxia-treated fish ($n = 11$) were exposed to a step-wise hypoxic treatment beginning at 4 h postprandial because previous studies using a sham handling treatment with rainbow trout determined that handling effects were negligible after 4 h (Eliason et al., 2007, 2008). The hypoxic steps were 30 min at 80% saturation (≅16.7 kPa, 8.9 mg O_2 L^{-1}), 30 min at 60% saturation (≅12.6 kPa, 6.7 mg O_2 L^{-1}) and finally 30 min at 40% saturation (≅8.4 kPa, 4.4 mg O_2 L^{-1}). Therefore, the hypoxia-treated fish were exposed to each hypoxic step for 2 full MO_2 measurements for a total hypoxic exposure of 1.5 h before normoxia was restored for the 44.5 h of the experiment that remained. At the end of the experiment, the fish were euthanized by cerebral concussion and any food or feces remaining in the stomach and intestines were weighed.

2.4. Data and statistical analysis

MO_2 was recorded using LoliResp4 software (Loligo Systems). Cardiac output (V_b) and gastrointestinal blood flow (GBF) were recorded using MP100 BioPac Acknowledge software (BIOPAC Systems Inc., Santa Barbara, CA, USA). Heart rate (f_H) was determined from the blood flow traces using the automated software, which was confirmed by visually counting pulsatile traces. Stroke volume (V_s) was calculated as $V_s = V_b / f_H$. The percentage of V_b directed to the gastrointestinal tract (%V_b to GI) was calculated as %V_b to GI = (GBF / V_b)100. Estimated oxygen extraction rates (E_{O2}) were calculated as $E_{O2} = MO_2 / V_b$ after converting MO_2 from mg O_2 kg^{-1} h^{-1} to mL O_2 kg^{-1} min^{-1}. V_b and GBF could not be simultaneously and continuously recorded from some of the fish because four flowmeters were not available. Measurements were made for at least 1 h on each fish at 6, 12, 24 and 48 h postprandial. In addition, postprandial V_b, V_s, %V_b to GI and E_{O2} are only reported for hypoxia-treated fish because too few normoxic fish had a flowprobe that continued to function throughout the entire >86 h experiment.

The assessment of standard metabolic rate (SMR) and specific dynamic action (SDA) was based on Eliason et al. (2007), who have detailed the challenges associated with determining these values in active fish such as trout. Briefly, SMR, baseline V_b, GBF and f_H, were determined for each fish that had continuous measurements as the average of the six lowest hourly values over the entire trial, excluding the first 4 h after the fish were placed in the respirometer. Peak postprandial MO_2, V_b, f_H, V_s, GBF, %V_b to GI and E_{O2} were determined for each fish that had continuous measurements as the highest hourly block value for each variable, excluding the first time point (6 h) after the fish was returned to normoxia to avoid the brief, stress-related elevation in MO_2 that sometimes accompanied the switch back to normoxic water. Time-to-peak MO_2, V_b, f_H, V_s, GBF, %V_b to GI and E_{O2} was the number of hours postprandial to reach the peak value. The duration of the postprandial SDA response for individual fish was the time when postprandial MO_2 returned to within 10% of SMR, or the end of the 50-h experiment if it never reached this level. The duration of the mean postprandial SDA response was determined as the time when postprandial MO_2 was no longer significantly different from SMR.

The SDA for each individual fish was estimated by subtracting SMR from postprandial MO_2 and integrating under the curve. Since the first 4 h of the postprandial period was excluded from all analysis (previous studies have demonstrated that the effect of sham-feeding lasted up to 4 h; Eliason et al., 2007, 2008), the initial postprandial period was estimated by assuming a linear relationship between the SMR and the 7-h postprandial value. The cost of SDA as a % of digestible energy intake (SDA coefficient) was estimated by assuming that 1 g of O_2 is associated with the release of 13.6 kJ of energy (Cho et al., 1982). The SDA and the SDA coefficient were estimated for 12, 24, 36 and 50 h after feeding.

Mean values are presented ± standard error of the mean (SEM). A p-value of less than 0.05 was considered statistically significant, and all statistical tests were conducted using SigmaPlot 11.0. Differences in

the amount of feces present, weight, length, peak MO_2, GBF, f_H, time-to-peak MO_2, GBF, f_H, SDA and SDA coefficient between hypoxia and normoxia-treated fish were compared using a Student's *t*-test. The effect of the hypoxic treatment on postprandial MO_2, GBF and f_H were compared using a 2-way repeated measured ANOVA. A post hoc Holm–Sidak multiple comparisons procedure was used to test for differences among groups.

3. Results

Body mass and fork length did not significantly differ between normoxia- and hypoxia-treated fish (body mass: 1029 ± 129 g and 911 ± 71 g, respectively; fork length: 41.9 ± 1.9 cm and 41.3 ± 1.1 cm, respectively). Notably, 25% of normoxia-treated fish and 56% of hypoxia-treated fish had food remaining in the stomach at the end of the experiment (range: 0–6.9 g and 0–19.5 g, respectively). The amount of feces present in the lower intestine did not significantly differ between groups (overall mean ± SEM: 5.2 ± 0.7 g). Representative traces of the overall experimental protocol for a hypoxia-treated fish are shown in Fig. 1.

A repeated-measures ANOVA did not reveal any significant change in MO_2 during the step-wise hypoxia challenge (Fig. 2). In contrast, GBF was significantly reduced by 41% for the hypoxia-treated fish at the 40% O_2 saturation step when compared to normoxic fish (Fig. 2). Heart rate was significantly 25%–29% lower in fish exposed to 40% O_2 saturation compared to 60 and 80% O_2 saturation (Fig. 2).

MO_2 increased immediately and briefly following the hypoxia challenge for many fish, although the magnitude of this response was variable (see representative trace shown in Figs. 1 and 3). Peak postprandial MO_2 was significantly lower in hypoxia-treated fish when compared to control fish, being increased by 35% and 62% above SMR, respectively (Table 1). Mean postprandial MO_2 did not significantly differ between hypoxia-treated and normoxia-treated fish at any time except at 6 h (Fig. 3). Mean postprandial MO_2 was no longer significantly different from SMR after 37 h for control fish but after 43 h for hypoxia-treated fish, indicating that the hypoxia treatment had prolonged SDA (Fig. 3). Notably, postprandial MO_2 did not return back to SMR for some individual fish (14% of control and 50% of hypoxia fish), indicating that digestive processes and SDA were not complete for some fish at the end of the 50-h postprandial period. SDA and SDA coefficient were not significantly different as a result of the hypoxic treatment at any of the time periods (12, 24, 36 or 50 h; Table 2).

Peak postprandial GBF increased from baseline levels by 111% and 102% for normoxia- and hypoxia-treated fish, respectively (Table 1). Postprandial V_b increased from baseline levels by 84% in hypoxia-treated fish (Table 1). Before feeding, GBF was 27.2% of V_b, and this increased significantly after feeding to 40.5% (Table 1). Postprandial GBF did not significantly differ between treatments and was significantly elevated above baseline at 12 h postprandial (Fig. 4). Similarly, peak postprandial GBF and time-to-peak postprandial GBF did not significantly differ between treatments (Table 1).

Postprandial f_H was significantly higher immediately following the hypoxia challenge (at 6 h) in hypoxia-treated fish compared to normoxia-treated fish (Fig. 4). Postprandial f_H remained significantly elevated above baseline at 6, 12 and 24 h postprandial in hypoxia-treated fish but only at 12 h postprandial in normoxia-treated fish (Fig. 4). Peak postprandial f_H and time-to-peak postprandial f_H did not significantly differ between groups (Table 1).

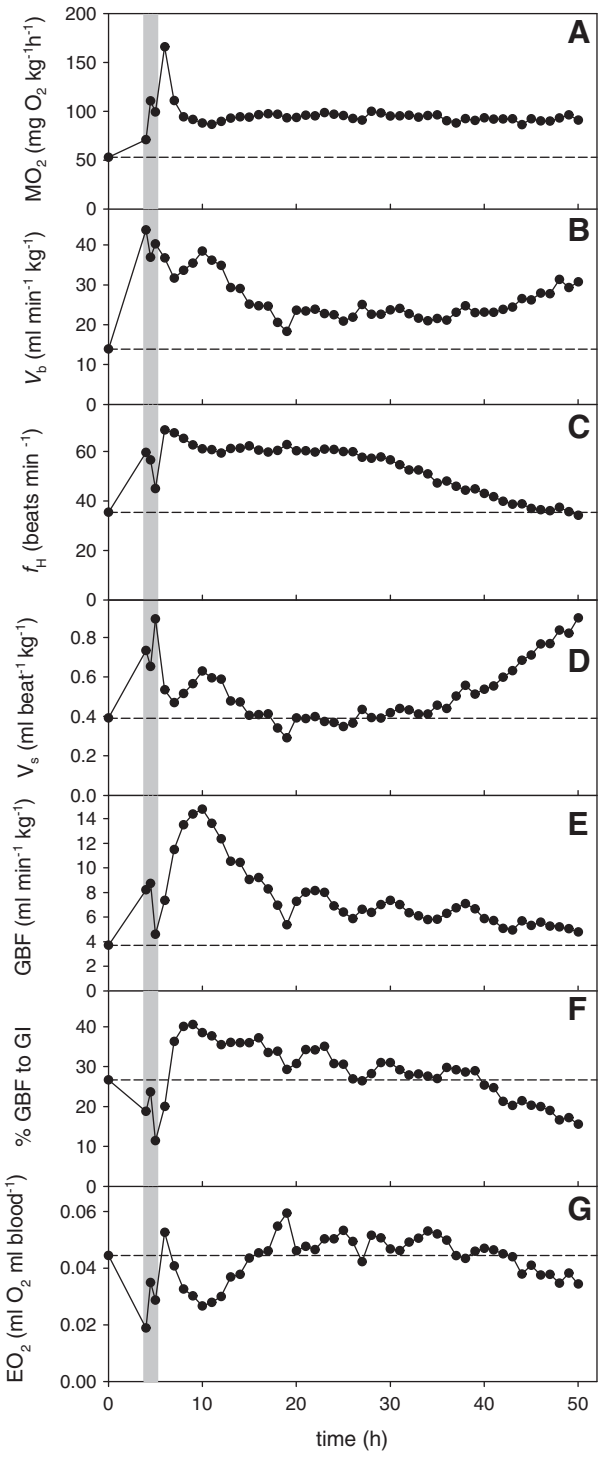

Fig. 1. Representative trace for a 709-g hypoxia-treated fish. The fish was fed at time 0, the horizontal grey bar indicates when the 1.5-h step-wise hypoxia treatment occurred and the dashed lines indicate baseline values. (A) Oxygen consumption rate (MO_2), (B) cardiac output (V_b), (C) heart rate (f_H) and (E) gut blood flow (GBF) were simultaneously measured. (D) Stroke volume (V_s) was calculated as $V_s = V_b / f_H$. (F) The percentage of V_b directed to the gut was calculated as (GBF / V_b)100, and (G) estimated oxygen extraction rate (E_{O2}) was calculated as $E_{O2} = MO_2 / V_b$ after converting MO_2 from mg O_2 kg^{-1} h^{-1} to mL O_2 kg^{-1} min^{-1}.

4. Discussion

The primary finding of the present study is that blood flow to the gastrointestinal tract and heart rate can become compromised when a digesting rainbow trout becomes hypoxic. The magnitude of the hypoxic exposure needed to generate this response was quite severe (40% saturation) since postprandial GBF and f_H were maintained throughout 30-min exposures to 80% and 60% O_2 saturation. In contrast, postprandial MO_2 was unchanged throughout the entire hypoxia exposure (presumably through a compensatory increase in oxygen extraction since arterial oxygen content would have decreased during hypoxia while V_b was maintained). Thus, the cardiovascular system was more sensitive to

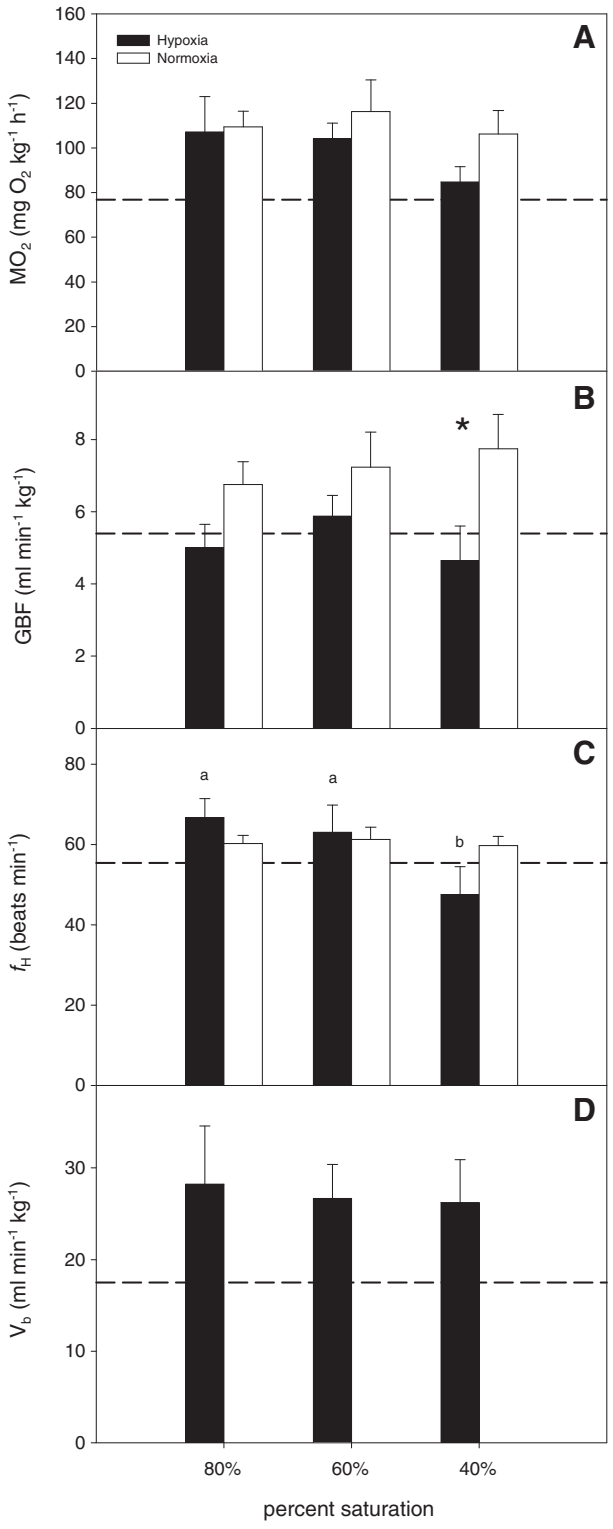

Fig. 2. Postprandial (A) oxygen consumption rate (MO_2), (B) gastrointestinal blood flow (GBF), (C) heart rate (f_H) and (D) cardiac output (V_b) for rainbow trout exposed to a 1.5-h step-wise decrease in water oxygen saturation (hypoxia = black bars) or constant water oxygen saturation (normoxia = white bars) are presented as the mean ± SEM. Differing letters indicate a statistically significant difference among hypoxia levels within a treatment. An asterisk indicates a significant difference between hypoxia and normoxia-treated fish ($p < 0.05$). Dashed line indicates baseline values. Unfortunately, there were insufficient normoxia-treated fish with functioning cardiac output flowprobes during the hypoxic treatment to be included in panel D.

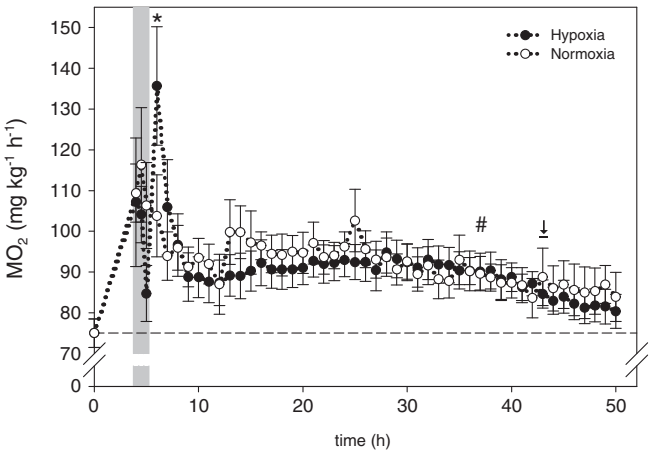

Fig. 3. Postprandial oxygen consumption (MO_2) for normoxia (white) and hypoxia-treated (black) fish are presented as mean ± SEM. Fish were fed at time 0, the horizontal grey bar indicates when the 1.5-h step-wise hypoxia treatment and the dashed line indicates standard metabolic rate (SMR). An asterisk indicates a significant difference between hypoxia and normoxia-treated fish. Postprandial MO_2 was no longer significantly different from SMR by 37 and 43 h after feeding for normoxia (#) and hypoxia-treated fish (↓), respectively.

hypoxia compared to whole animal MO_2 and postprandial oxygen delivery to the gastrointestinal system as well as nutrient assimilation and transport may become compromised at an oxygen tension when whole animal MO_2 is being maintained. Moreover, a number of recent studies suggest that the critical oxygen level for maximum growth of fish is higher still (Foss et al., 2002; Thorarensen et al., 2010; Remen et al., 2012), indicating perhaps that the assimilation process may be the most sensitive phase of digestion.

Table 1
Baseline and postprandial physiological values [standard metabolic rate (SMR), oxygen consumption rate (MO_2), cardiac output (V_b), heart rate (f_H), stroke volume (V_s), gut blood flow (GBF), percentage of V_b directed to the gastrointestinal tract (%V_b to GI) and estimated oxygen extraction rates (E_{O2})] are presented for rainbow trout at 10 °C–11.5 °C during digestion of 1% of body mass (mean ± SEM). Hypoxic fish underwent a 1.5 h step-wise hypoxia treatment 4 h after feeding (to 80%, 60% and 40% O_2 saturation), while normoxic fish remained at full O_2 saturation for the entire duration of the postprandial period. See text for details of the calculations that were performed; only fish with continuous measurements for the duration of the entire experiment were included.

Baseline			
SMR (mg O_2 kg^{-1} h^{-1})			75.0 ± 3.4
GBF (mL min^{-1} kg^{-1})			5.3 ± 0.5
f_H (beats min^{-1})			53.9 ± 2.8
V_b (mL min^{-1} kg^{-1})			17.5 ± 1.0
V_s (mL $beat^{-1}$ kg^{-1})			0.31 ± 0.03
%V_b to GI			27.2 ± 3.6
E_{O2} (mL O_2 mL $blood^{-1}$)			0.051 ± 0.002
Postprandial	**Hypoxia**	**Normoxia**	***p*-value**
Peak			
MO_2 (mg O_2 kg^{-1} h^{-1})	101.3 ± 4.5	121.8 ± 5.9	**0.015**
GBF (mL min^{-1} kg^{-1})	10.7 ± 1.1	11.2 ± 2.3	0.817
f_H (beats min^{-1})	73.4 ± 3.6	68.2 ± 2.1	0.306
V_b (mL min^{-1} kg^{-1})	32.2 ± 5.0	na	
V_s (mL $beat^{-1}$ kg^{-1})	0.6 ± 0.06	na	
%V_b to GI	40.5 ± 6.7	na	
E_{O2} (mL O_2 mL $blood^{-1}$)	0.063 ± 0.007	na	
Time-to-peak (h)			
MO_2	26.0 ± 2.5	24.8 ± 3.1	0.760
GBF	10.9 ± 0.6	16.8 ± 7.1	0.933
f_H	10.5 ± 2.1	14.0 ± 2.8	0.127
V_b	11.8 ± 3.1	na	
V_s	20.3 ± 9.8	na	
%V_b to GI	10.0 ± 1.0	na	
E_{O2}	18.3 ± 5.5	na	

Table 2
Specific dynamic action (SDA) was calculated for 12, 24, 36 and 50 h postprandial for hypoxia- and normoxia-treated fish. The cost of the meal as a percentage of dietary digestible energy intake (SDA coefficient) was calculated assuming 1 g of O_2 is associated with the release of 13.6 kJ of energy (Cho et al., 1982).

	Hypoxia	Normoxia	*p*-value
SDA (mg O_2 kg^{-1})			
12 h	290.8 ± 68.5	206.2 ± 23.7	0.694
24 h	523.1 ± 107.5	464.1 ± 60.9	0.654
36 h	766.1 ± 140.5	655.6 ± 66.4	0.510
50 h	955.6 ± 183.3	787.1 ± 80.7	0.463
SDA coefficient (%)			
12 h	2.2 ± 0.5	1.6 ± 0.2	0.694
24 h	4.0 ± 0.8	3.6 ± 0.5	0.654
36 h	5.9 ± 1.1	5.0 ± 0.5	0.510
50 h	7.3 ± 1.4	6.0 ± 0.6	0.463

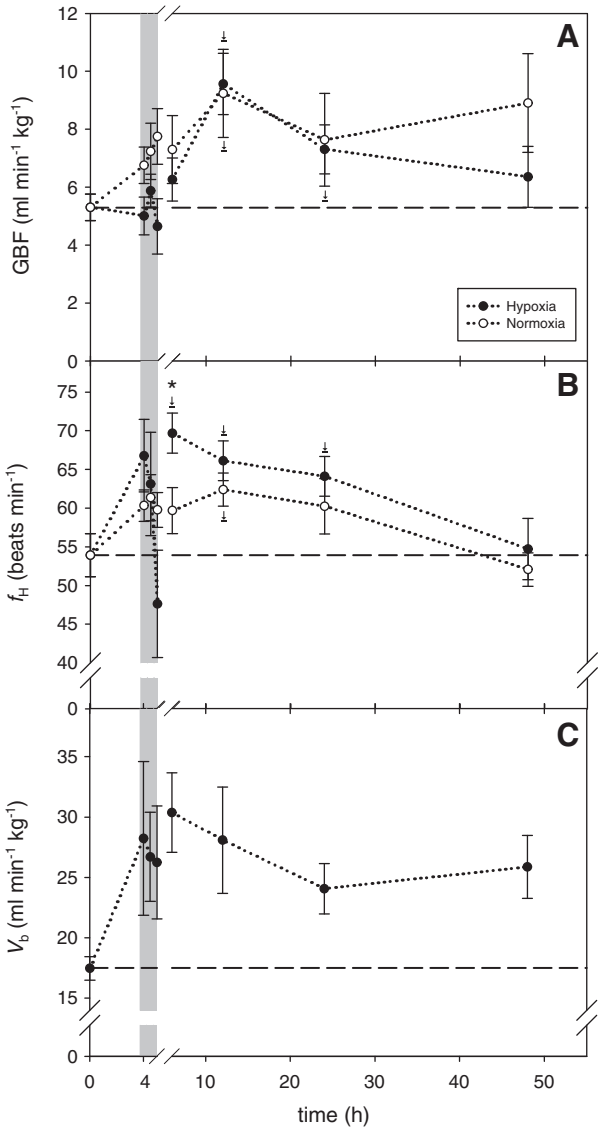

Fig. 4. Postprandial (A) gastrointestinal blood flow (GBF), (B) heart rate (f_H) and (C) cardiac output (V_b) for fish treated with hypoxia or normoxia are presented as mean ± SEM. Fish were fed at time 0, the horizontal grey bar indicates when the step-wise hypoxia treatment occurred and the dashed line indicates baseline. An asterisk indicates a statistically significant difference between the hypoxia- and normoxia-treated fish and an arrow indicates a statistically significant difference from baseline. Unfortunately, there were insufficient normoxia-treated fish with functioning cardiac output flowprobes during the hypoxic treatment to be included in panel C.

Digestive performance was also compromised after the 1.5-h hypoxia exposure. Fish exposed to hypoxia had a 17% lower peak postprandial MO_2 compared to fish held in normoxia, though this effect was not evident in the mean response of postprandial MO_2 (Fig. 3) because of the variation in timing of the peak among individuals (range: 16–37 h). In addition, mean SDA duration was prolonged by 6 h in hypoxia-exposed fish. Postprandial f_H was significantly higher and took longer to return to baseline levels in hypoxia-treated fish, indicating that the hypoxia-treatment likely induced a stress response since f_H has been demonstrated to be highly sensitive to stressful conditions. Consistent with these findings was the observation that twice as many hypoxia-treated fish had food remaining in their stomach at the end of the experiment compared with control fish. The magnitude of SDA did not differ between hypoxia and normoxia-treated fish, though we caution that SDA was likely slightly underestimated here since postprandial MO_2 had not returned to SMR for all individual fish. This was especially evident for hypoxia-treated fish (50% of hypoxia-treated fish compared to 14% of control fish did not return to SMR). As such, we may have been unable to detect subtle differences in the magnitude of SDA between groups.

Collectively, these findings raise an important concern for rainbow trout encountering hypoxia, both in nature and in the aquaculture industry. A more prolonged exposure to hypoxia than the short-term, moderate one used here could further reduce GBF and impair oxygen delivery to the gastrointestinal tract, presumably decreasing gut transit time, absorption and assimilation efficiency and thus compromising digestive performance and growth.

4.1. Response to hypoxia exposure

Hypoxia reduces aerobic scope, and so the prediction is that a fish becomes more sensitive to hypoxia during digestion when metabolism increases and aerobic scope is utilized (feeding can typically double SMR). The critical oxygen tension (P_{crit}; the oxygen tension when SMR starts to decline) has been predicted to increase in fed fish (Claireaux and Lagardère, 1999; Wang et al., 2009), which was confirmed in perch *Perca fluviatilis* (Thuy et al., 2010). Here, MO_2 was maintained throughout the entire hypoxia exposure. P_{crit} is ~2.8 kPa or ~13.5% saturation for fasted rainbow trout at 10 °C (Ott et al., 1980; Svendsen et al., 2012), which is well below the lowest hypoxia level tested here. Therefore, we predict that P_{crit} for a digesting rainbow trout is likely between 2.8 and 8.4 kPa or between 13.5% and 40% saturation, although this remains to be tested experimentally.

Since all capillary beds cannot be simultaneously perfused, fish must prioritize regional blood flow according to their needs. Numerous studies with unfed fish have shown that GBF decreases with hypoxia (Axelsson and Fritsche, 1991; Fritsche et al., 1993; Axelsson et al., 2002), exercise (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Thorarensen et al., 1993; Thorarensen and Farrell, 2006; Altimiras et al., 2008) and hypercapnia (Crocker et al., 2000). Thus, GBF cannot be a priority circulation when fish are not digesting. However, when digesting fish are exposed to challenging conditions such as hypoxia or exercise, greater and conflicting demands are placed on the cardiovascular system. GBF was unchanged in fed Atlantic cod exposed to 35% saturation (~7 kPa) compared to normoxia (Behrens et al., 2012). Similarly, GBF and $\%V_b$ to GI did not differ in fed European sea bass exposed to 50% saturation (~10 kPa) compared to normoxia (Dupont-Prinet et al., 2009). Also in sea bass, absolute postprandial GBF significantly decreased with a more severe hypoxia (3.9 kPa, ~20% saturation), but relative GBF ($\%V_b$ to GI) did not change (Axelsson et al., 2002). All three studies suggested that postprandial GBF was spared at some level when these species were exposed to hypoxia, demonstrating a prioritization of gastrointestinal perfusion during digestion. In contrast, the present study demonstrates a low priority in rainbow trout since postprandial GBF and $\%V_b$ to GI both decreased at 40% saturation (~8.4 kPa).

The differences in prioritization of gastrointestinal perfusion during digestion among species may be attributed to differences in life histories and hypoxia tolerance. For example, Atlantic cod and sea bass are found in marine environments where hypoxic water is common (Diaz and Rosenberg, 2008). As such, local control mechanisms may have evolved to spare postprandial GBF during moderate hypoxic conditions in order to ensure efficient digestion (Behrens et al., 2012). In contrast, rainbow trout typically inhabit well-oxygenated waters (a notable exception is overwintering underneath ice at northern latitudes, but then prey intake dramatically declines as the fish's energetic requirements are reduced at the very low water temperatures). Therefore, species differences likely exist in the central and local control of regional blood flow. The differences among species may also be attributed to differences in the hypoxic threshold for compromised GBF. Presumably, GBF must decrease in all species at some level of hypoxia because cardiorespiratory capacity is not limitless. These ideas need to be tested experimentally.

The decrease in f_H at the most extreme hypoxia exposure (40% saturation, ~8.4 kPa) was expected since reflex bradycardia is a well-documented and common cardiac response to hypoxia in fishes (for a review, see Farrell, 2007) and the threshold for bradycardia is ~45% saturation or 9.3 kPa in rainbow trout at 12 °C (Marvin and Heath, 1968; Gamperl and Driedzic, 2009). Notably, f_H paralleled the GBF response during the step-wise hypoxia exposure, and this continued throughout the 50-h postprandial period after the fish were returned to normoxia, supporting previous observations with rainbow trout (Eliason et al., 2008). Given the tight association between f_H and GBF, digestion in rainbow trout could be assessed remotely using biotelemetry for f_H under both normoxic and hypoxic conditions.

As expected, V_b was maintained throughout the hypoxia treatment via a compensatory increase in V_s. Fish commonly increase V_s as bradycardia develops during hypoxia, enabling V_b to be maintained (see Farrell, 2007; Gamperl and Driedzic, 2009), as has been previously demonstrated for rainbow trout exposed to moderate hypoxia levels [~6.7–7.3 kPa (Wood and Shelton, 1980; Sandblom and Axelsson, 2005)].

4.2. Baseline physiology and general postprandial response

All baseline values (SMR, V_b, GBF, f_H, V_s, E_{O2} and $\%V_b$ to GI) were within the expected ranges for rainbow trout at 10 °C–11.5 °C. SMR (75.0 ± 3.4 mg O_2 kg^{-1} h^{-1}) and baseline f_H (53.9 ± 2.8 beats min^{-1}) were at the high end of the reported range of 48–80 mg O_2 kg^{-1} h^{-1} and 28–68 beats min^{-1}, respectively, for rainbow trout (Webb, 1971; Kiceniuk and Jones, 1977; Pagnotta and Milligan, 1991; Gallaugher et al., 1995; Taylor et al., 1996; Alsop and Wood, 1997; Claireaux et al., 2005; Eliason et al., 2008; Grans et al., 2009; Seth and Axelsson, 2010). In this regard, we cannot exclude the possibility that a 24-h continuous light photoperiod (used to minimize diurnal changes) may have elevated overall metabolic rate. All the same, 27% of total V_b was directed to the gastrointestinal tract in resting, unfed rainbow trout, which is within previously reported range of 10%–30% for other fish species (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Fritsche et al., 1993; Thorarensen et al., 1993; Axelsson et al., 2000; Farrell et al., 2001; Dupont-Prinet et al., 2009; Sandblom et al., 2012).

Peak postprandial GBF increased from baseline levels by 102%–111% (from 5.3 to 10.7–11.2 mL min^{-1} kg^{-1}), which is within the expected range of 60%–136% (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Axelsson et al., 2000, 2002; Eliason et al., 2008; Sandblom et al., 2012). This postprandial response was due to an increase both in postprandial V_b (by 84%) and the proportion of V_b directed to the GI (from 27% to 41%), as has been previously demonstrated in rainbow trout (Seth et al., 2009). Peak postprandial f_H (68–73 beats min^{-1}) represented a 27%–36% increase above baseline levels. Maximum f_H for rainbow trout swimming at 9 °C–12 °C ranges between 51 and 70 beats min^{-1} (Kiceniuk and Jones, 1977; Butler et al., 1986). Thus, postprandial f_H may have been approaching maximum f_H in the present study.

The postprandial cardiovascular response peaked earlier (time-to-peak GBF, V_b and f_H ranged between 11 and 17 h) than whole animal MO_2 (time-to-peak MO_2 ranged between 25 and 26 h), consistent with previously findings (Eliason et al., 2008). This result supports the idea that postprandial MO_2 remains elevated long after nutrients have been absorbed and transported from the gut in order to fuel nutrient processing and assimilation, especially the processes associated with protein handling.

The magnitude of SDA was likely slightly underestimated in the present study since some individual fish had not returned to SMR and had food remaining in their stomach at the end of the 50-h postprandial period. This was unexpected since a similar study by our group that fed rainbow trout twice as much food (2% of body mass) at a slightly warmer temperature (11 °C–16 °C) found that postprandial MO_2 had returned to SMR by 41 h postprandial (Eliason et al., 2008). SDA was lower here for a meal of 1% of their body mass (787–956 mg kg^{-1}) compared to a meal of 2% of their body mass [989–1163 mg kg^{-1} (Eliason et al., 2007, 2008)]. The SDA coefficient was slightly higher in the present study (6%–7%) compared to previous work [4%–5% (Eliason et al., 2007, 2008)], though still below previous estimates of between 8% and 29% (Cho et al., 1982; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Beamish and Trippel, 1990).

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