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Postprandial gastrointestinal blood flow, oxygen consumption and heart rate in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The present study is the first to simultaneously and continuously measure oxygen consumption (MO_2) and gastrointestinal blood flow (q_{gi}) in fish. In addition, while it is the first to compare the effects of three isoenergetic diets on q_{gi} in fish, no significant differences among diets were found for postprandial MO_2, q_{gi} or heart rate (f_H) in rainbow trout, *Oncorhynchus mykiss*. Postprandial q_{gi}, f_H and MO_2 were significantly elevated above baseline levels by 4 h. Postprandial q_{gi} peaked at 136% above baseline after 11 h, f_H peaked at 110% above baseline after 14 h and MO_2 peaked at 96% above baseline after 27 h. Moreover, postprandial MO_2 remained significantly elevated above baseline longer than q_{gi} (for 41 h and 30 h, respectively), perhaps because most of the increase in MO_2 associated with feeding is due to protein handling, a process that continues following the absorption of nutrients which is thought to be the primary reason for the elevation of q_{gi}. In addition to the positive relationships found between postprandial MO_2 and q_{gi} and between postprandial MO_2 and f_H, we discovered a novel relationship between postprandial q_{gi} and f_H.

Keywords: Digestion; Gut blood flow; Heat increment; MO_2; Rainbow trout; Specific dynamic action

1. Introduction

Digestion and absorption of food in vertebrates require increased gut activity, blood flow and oxygen consumption (MO_2). The increase in MO_2 following the ingestion of a meal is termed specific dynamic action (SDA) or heat increment of feeding (HiE). In practise, what is commonly referred to as SDA may or may not include the MO_2 associated with obtaining, grasping and chewing a meal (e.g.; Secor, 2003; Jordan and Steffensen, 2007). For physiological studies such as the one presented here, fish are typically fed by gavage and MO_2 is measured using respirometry. Therefore, to avoid confusion, we will use HiE to describe the metabolic costs of processing a meal after gavage. Numerous studies on fish have estimated HiE as between 4 and 29% of the digestible energy consumed in a given meal (Cho et al., 1982; Beamish and Trippel, 1990; Eliason et al., 2007), with peak postprandial MO_2 increasing from standard metabolic rate (SMR) by between 1.5- and 2.5-times (Jobling, 1981; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Ross et al., 1992; Boyce and Clarke, 1997; Hunt von Herbing and White, 2002; Peck et al., 2005). The duration of HiE depends largely upon temperature and meal size, with meal sizes of between 0.5 and 3.5% of body mass elevating MO_2 for between 12 and 76 h across a variety of fish species (Jobling and Davies, 1980; Tandler and Beamish, 1980; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Ross et al., 1992; Kaczanowski and Beamish, 1996; Eliason et al., 2007).

The various potential pre-absorptive, absorptive and post-absorptive costs associated with digestion have been recently summarized by McCue (2006) and the processes associated with protein handling have emerged as the primary contributor to HiE. This conclusion is supported by the observations that intra-arterial injections of amino acids in catfish result in similar elevations in MO_2 levels as the HiE and that this response is prevented by cycloheximide (a blocker of protein synthesis)

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(Brown and Cameron, 1991). Also, around 80% of the total MO_2 in trout hepatocytes was spent on protein synthesis (Pannevis and Houlihan 1992) and most tissues rapidly turnover protein following feeding (Lyndon et al., 1992; McMillan and Houlihan, 1992). We were particularly interested in the role of the absorptive processes in contributing to HiE. Although these are difficult to dissect out, associations can be made between postprandial changes in MO_2 and blood flow to the digestive tract, which are essential for the absorptive processes.

Gut blood flow increases postprandial to transport absorbed nutrients to the liver and beyond for modification, storage and use, and to supply O_2 to the metabolising tissues. The few studies that have studied the postprandial circulatory changes in fish show a 42–100% increase in gastrointestinal blood flow (q_{gi}) that reflects either a fixed percentage of the elevated cardiac output (Q) or an additional redistribution of blood flow towards the gut (for review, see Farrell et al., 2001; Axelsson et al., 2002). The postprandial increases in hepatic portal vein concentrations of amino acids, urea and ammonia in rainbow trout (Karlsson et al., 2006), appear to parallel these changes in q_{gi}, further suggesting a linkage between the increase in q_{gi} and nutrient transport. However, no one has reported simultaneous measurements of postprandial changes in MO_2 and q_{gi} in fish to test this idea. The present study simultaneously measured HiE (as MO_2) and q_{gi} in rainbow trout that had been reared for several months on three isoenergetic diets with varying protein and lipid ratios, but equal digestible carbohydrate content. These diets produced the same growth rates, standard MO_2 and HiE, but altered dietary protein utilization and whole body protein and lipid deposition (Eliason et al., 2007). Therefore, we were able to examine for the first time the general relationship between HiE and postprandial q_{gi} and the influence of dietary protein on q_{gi}. Given the important circulatory needs of digestion as a whole, we predicted a tight relationship between q_{gi} and HiE throughout the postprandial period, with perhaps a modulating effect of dietary protein levels. Specifically, we hypothesized that postprandial MO_2 would remain elevated above basal levels longer than q_{gi} due to post-absorptive processes such as nutrient handling and protein synthesis.

2. Materials and methods

2.1. Gastrointestinal blood flow surgery

Fish were anaesthetized in buffered 0.1 g l^{-1} MS-222 and were transferred onto a surgical table, ventral side up and right side exposed, where their gills were continually irrigated with a chilled, aerated, buffered anaesthetic solution (0.05 g l^{-1} MS-222). Surgical procedures to isolate the intestinal blood vessels followed those described previously (Thorarensen et al., 1993; Axelsson et al., 2002). An incision was made just posterior to the pectoral fin, extending from a few cm above the lateral line to a few cm below the midline. The gastric and intestinal arteries were carefully isolated so as not to disturb any nerves. A single Transonic flowprobe (either 1.0RB or 1.5RB; Transonic Systems, Ithaca, NY, USA) was fitted around both arteries. The incision was closed using interrupted 2-0 silk sutures. The probe lead was secured with 2-0 silk sutures at the incision site, to the skin and at the dorsal fin. The incision was lightly dusted with powdered penicillin before promptly returning the fish to the experimental chamber where its recovery was monitored visually.

2.2. Fish rearing and food composition

The twenty four rainbow trout (807.6±47.1 g, mean±SE) were maintained on their prescribed diets for 18 months prior to the present experiments. For the final 8 months, fish were held in 1000 l indoor fiberglass tanks that were supplied with running (10 l min^{-1}), aerated (dissolved O_2 >8.0 mg l^{-1}), dechlorinated fresh water at 11–16 °C, and a 12L:12D photoperiod at the University of British Columbia (UBC, Vancouver, BC, Canada). All experimental protocols were approved by the UBC Animal Care Committee in accordance with the Canadian Council of Animal Care. Fish were fed 4–5 days a week to near satiation (~2% of body mass) with one of three formulated dry diets: low (35%; LP), medium (45%; MP) and high (55%; HP) protein concentrations and, respectively, high (20%; HL), medium (15%; ML) and low (10%; LL) lipid concentrations. All diets had the same level of digestible carbohydrate (12%) and an identical balance of essential amino acids. The diets were isocaloric (16.7 MJ kg^{-1}). Details of diet preparation, ingredient and proximate compositions, and determined concentrations of digestible protein and energy are described fully by Eliason et al. (2007). The digestible protein to digestible energy ratios (DP:DE in g MJ^{-1}) were determined as 29.8, 24.8 and 19.8 in the HP:LL, MP:ML and LP:HL diet treatments, respectively. These bracket the recommended optimum range for good growth and protein utilization in trout, i.e. 22–25 g MJ^{-1} (Cho and Kaushik, 1990; Cho, 1992; Higgs et al., 1995). Eliason et al. (2007) previously demonstrated that these diets significantly alter dietary protein utilization and terminal whole body protein deposition without significantly affecting growth, feed intake and feed efficiency of rainbow trout.

2.3. Experimental protocol

MO_2 of individual fish was measured using a 4-chamber, intermittent-flow respirometry system (Loligo Systems, Hobro, Denmark). Each 9.9 l chamber received aerated water at 5 l min^{-1} and at the seasonal water temperature (range of 10.0–15.5 °C over the experimental period). We determined that it was preferable to maintain the fish at ambient water temperature over the 6-month experimental period rather than attempt to disrupt the normal seasonal variation in water temperature. The flush cycle was 10 min, the wait period was 30 s and the recirculation cycle for measurement was 5 min. The oxygen content of the water was measured every second during the 5 min recirculation cycle using a MINI-DO probe (Loligo Systems). These chambers had a recirculation pump in order to maintain water mixing when the inflow water was off and to minimize the effect of intermittent flow on the fish. The chambers were also submerged in a water bath to ensure a constant temperature ±0.5 °C during the 1- or 2-week experimental period. The oxygen probes were calibrated with oxygen-free

distilled water and fully aerated water prior to each replicate. MO_2 was recorded using LoliResp4 software (Loligo Systems).

An experiment on a single fish lasted either 7 (single feeding) or 14 (double feeding) days, during which MO_2 and q_{gi} were continuously measured. Fish were first starved for 48 h before being placed in a respirometry chamber and MO_2 was continuously monitored during a 24-h adjustment period. Fish were then removed for surgery and replaced afterwards for a 24-h recovery period. Following this recovery, fish were first sham-fed and then fed (2% of their body mass in pellet form) by gavage because of the repeated failure to induce voluntarily feeding in the respirometer in preliminary experiments. For sham-feeding, fish were lightly anaesthetized (loss of their righting ability when immersed in buffered 0.08 g l^{-1} tricainemethanesulfonate (MS-222), Sigma-Aldrich, Oakville, ON, Canada), and plastic forceps and a polyethylene tubing were repeatedly inserted into the esophagus for 5 min. Fish were immediately revived and returned to the respirometer, and MO_2 was followed for a further 24 h. For gavage, fish were re-anaesthetized, fed, revived and returned to the respirometer in the same manner. Any regurgitated pellets were counted (always <10% of the pellets). As a result of this protocol, MO_2 and q_{gi} were monitored for a minimum of 152 h and 128 h, respectively, and postprandial MO_2 and q_{gi} were monitored for 80 h. A total of 13 fish (n=3–6 fish per diet treatment) were tested in this manner for one feeding cycle.

Following a single feeding by gavage, postprandial MO_2 and q_{gi} remained significantly elevated above pre-feeding levels for the entire 80 h postprandial period (Figs. 1 and 2). This result suggested a delayed postprandial response, likely associated with stress and recovery from surgery. Consequently, the entire 7-day feeding cycle described above was repeated with an additional seven fish to determine if the longer recovery periods shortened the duration of HiE. In addition, q_{gi} was measured for a third group of fish (n=4 fish) that were monitored through two sham-feedings (24 h apart in one feeding cycle), but were not fed (the respirometry software failed during the postprandial time period in this group and this precluded the normal simultaneous measurements of MO_2).

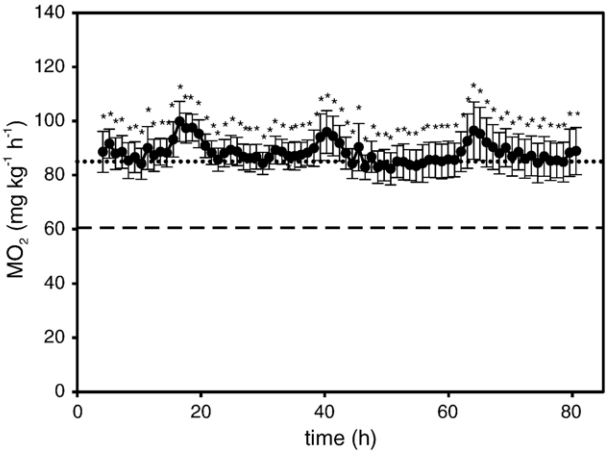

Fig. 1. Postprandial MO_2 for fish fed once (n=11). Fish were fed at time zero. All diet treatments are combined as the mean±SE. Routine metabolic rate (dotted line) and standard metabolic rate (dashed line) are indicated. A significant difference in MO_2 from SMR is indicated by an asterisk (p<0.05).

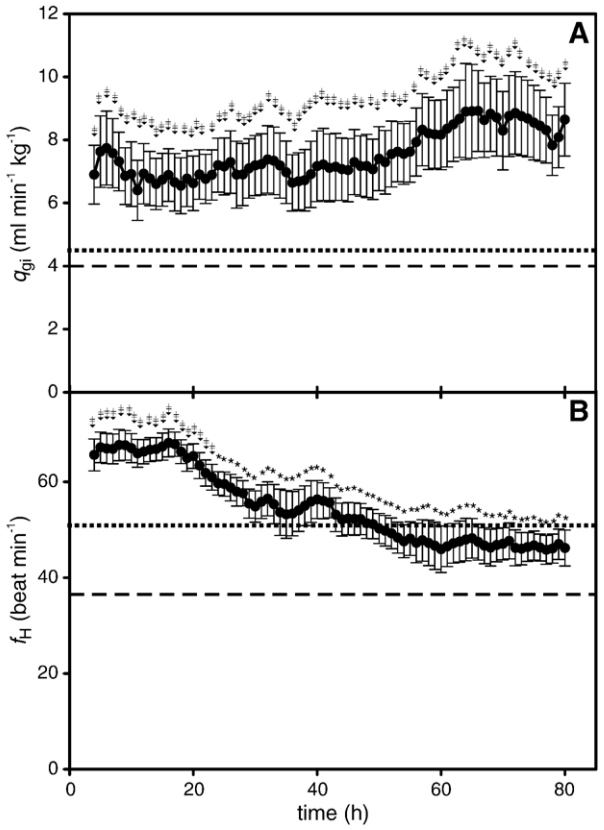

Fig. 2. Pooled (n=12) postprandial gut blood flow (q_{gi}) and heart rate (f_H) values for fish fed once (mean±SE). Fish were fed at time zero. Routine (dotted line) and baseline (dashed line) values are indicated. The symbol "‡" indicates a statistically significant difference from both routine and baseline and an asterisk indicates a statistically significant difference from baseline (p<0.05).

The fish were kept in darkness throughout the experiment to reduce entrained circadian rhythms and minimize the known stimulatory effects on MO_2 of lights being turned on and off (see Janz et al., 1991; Cheng and Farrell 2007). Though a circadian rhythm is perceptible in Fig. 1 following postprandial MO_2 in fish fed once, it was no longer apparent in fish fed a second time (Fig. 3). Nevertheless, experimental procedures and data analysis (see below) were based around a 24-h cycle to avoid bias. The temperature and O_2 levels (>9.0 mg l^{-1}) of the water supply were continually monitored throughout the experiment. Background MO_2 in each chamber was monitored for at least 1 h before and after each trial, and was determined to be negligible. At the end of the experiment, blood was sampled via caudal puncture to estimate hematocrit. All fish were euthanized by cervical dislocation and the tissues surrounding the probe were then inspected to ensure there were no signs of inflammation. Also, the stomach and intestines were checked for food and feces, and the liver and gonads were removed and weighed.

2.4. Data analysis

Block averages of q_{gi} and heart rate (f_H) were determined over 1 h using either WINDAQ (Dataq Instruments, Akron,

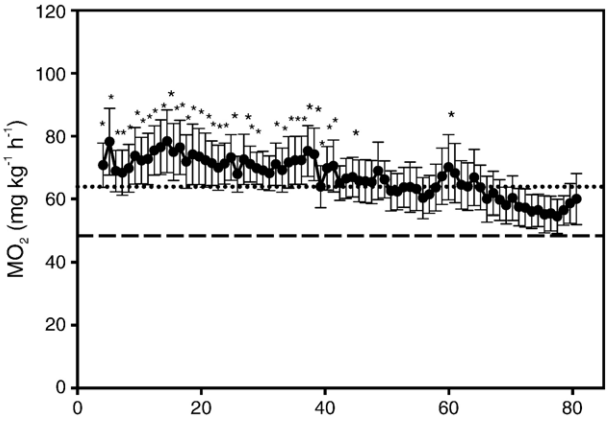

Fig. 3. Postprandial changes in MO_2 for fish fed twice (mean±SE; $n=6$). Fish were fed at time zero. Routine metabolic rate (dotted line) and standard metabolic rate (dashed line) are indicated. A significant difference in MO_2 from SMR is indicated by an asterisk ($p<0.05$).

Ohio, USA) or MP100 BioPac Acknowledge (BIOPAC Systems Inc., Santa Barbara, CA, USA) software sampling at a rate of 20 Hz. Standard metabolic rate (SMR), baseline q_{gi} and baseline f_H were estimated for each fish as the average of the six lowest hourly values over the entire trial, excluding the first 4 h recovery periods each time the fish was replaced in the chamber. Routine metabolic rate (RMR), routine q_{gi} and routine f_H were estimated for each fish using equal durations of "dark" and "light" periods prior to feeding. "Light" and "dark" periods corresponded to the times when the fish would have experienced light and dark conditions in their holding tanks prior to the experiment. The peak postprandial values for MO_2, q_{gi} and f_H were defined for each fish as the highest block value for each variable. The time-to-peak was the number of hours postprandial to reach the respective peak value. The variable effect of sham-feeding on MO_2, q_{gi} and f_H had subsided after 4 h. In addition, the double sham-feeding did not result in significantly different baseline or routine q_{gi} or f_H values. Therefore, the first 4 h of postprandial data are not included in the present analysis. The heat increment of feeding (HiE) is defined as the postprandial increase in MO_2 above SMR (Jobling, 1981; Beamish and Trippel, 1990). HiE was estimated as outlined in Eliason et al. (2007) using SMR and the lowest MO_2 value obtained for every hour postprandial. We assumed a linear relationship between SMR and the 4-h postprandial value to account for the first 4 h of postprandial data that were not included. The cost of HiE as a % of digestible energy intake ($C_{HiE}=(E_{HiE}/E_{meal})*100$) was estimated by assuming that 1 g of oxygen is associated with the release of 13.6 kJ of energy (Cho et al., 1982).

The total increase in postprandial blood flow to the gut was determined by integrating under the postprandial q_{gi} curve and subtracting baseline q_{gi}. Again, we assumed a linear relationship between baseline q_{gi} and the 4-h postprandial value.

The relationship between MO_2 and q_{gi} was assessed by comparing the difference between postprandial MO_2 from SMR (ΔMO_2) with the difference in postprandial q_{gi} from baseline (Δq_{gi}). The change in minimum postprandial MO_2 from SMR was plotted against the change in postprandial q_{gi} from baseline for each block interval. Δq_{gi} and Δf_H as well as ΔMO_2 and Δf_H were compared in a similar manner.

2.5. Statistical analysis

Mean values are presented ± standard error of the mean (SE). The effect of diet on postprandial MO_2, q_{gi} and f_H over time was assessed using 2-way repeated measures Analysis of Variance (ANOVA; SigmaStat 3.0). The pooled data from all diet treatments were assessed using a 1-way repeated measures ANOVA comparing MO_2, q_{gi} and f_H data to baseline and routine values over time. P values of less than 0.05 were considered to be statistically significant and the Holm–Sidak or Bonferonni multiple comparisons method was used to infer differences. Differences in haematocrit, SMR, RMR, baseline q_{gi} and f_H, routine q_{gi} and f_H, peak, time-to-peak, HiE, C_{HiE} and total q_{gi} were compared using ANOVA followed by the Holm–Sidak multiple comparisons test (SigmaStat 3.0). Data lacking a normal distribution and an equal variance were assessed using a nonparametric rank test followed by the Dunn multiple comparison test (SigmaStat 3.0).

3. Results

3.1. Fish assessment

At the end of the respirometry, all fish appeared to be in good health. The tissues surrounding the flow probe and the lead had no signs of inflammation, and the incision had already begun to heal in most fish. When compared with published values (Wells and Weber, 1991; Gallaugher et al., 1995), the hematocrit values (mean values were 37–45% for the three diets) suggested that blood loss from surgical incision was not a problem. Only one fish had remnants of the meal remaining in the stomach and eight fish had around 0.5–1.0 ml of feces remaining in the posterior portion of the intestine.

3.2. Effect of diet

Earlier, postprandial MO_2 on non-instrumented fish had been shown to be unaffected by the three diets used here (Eliason et al., 2007). Here we confirm that result and also show no statistical difference among diets for any of the cardiovascular parameters (data not shown). Therefore, cardiorespiratory data from all dietary treatments were pooled for further analysis to increase statistical power.

3.3. Fish fed once vs. twice

A single feeding, 2 days after surgery, resulted in prolonged digestion compared with our earlier study on non-instrumented fish (Eliason et al., 2007), with postprandial MO_2 and q_{gi} not completely returning to pre-feeding levels after 80 h (Figs. 1 and 2). Also, RMR and routine f_H were significantly higher during the first feeding cycle compared with the second feeding cycle (by 21 mg O_2 kg^{-1} h^{-1} and 16 beat min^{-1}, respectively). Likewise, peak postprandial MO_2 and f_H were significantly

Table 1
Standard metabolic rate (SMR), routine metabolic rate (RMR), peak postprandial MO_2, and time-to-peak postprandial MO_2 are presented for trout fed twice as the mean±SE

SMR (mg O_2 kg^{-1} h^{-1})	48.4±4.1
RMR (mg O_2 kg^{-1} h^{-1})	63.9±4.3
Peak MO_2 (mg O_2 kg^{-1} h^{-1})	94.6±10.4
Time-to-peak MO_2 (h)	26.9±8.1

Time (h)	HiE (mg O_2 kg^{-1})	Cost of HiE (%)
12	259.0±62.2	1.1±0.3
18	359.1±96.4	1.7±0.4
24	537.3±141.5	2.4±0.6
36	804.7±207.9	3.6±0.9
48	1021.2±260.6	**4.5±1.2**

Heat increment of feeding (HiE) values were calculated for 12, 18, 24, 36 and 48 h postprandial. The cost of the meal as a percent of dietary digestible energy intake (16.7 MJ kg^{-1} dry mass) was calculated assuming 1 g of O_2 is associated with the release of 13.6 kJ of energy (Cho et al., 1982). Bold font indicates the cost of HiE estimated after postprandial MO_2 had returned to SMR.

higher in fish fed once compared to fish fed twice, and by similar amounts as RMR and routine f_H (by 29 mg O_2 kg^{-1} h^{-1} and 14 beat min^{-1}, respectively). As a result, estimates of the cost of HiE (>9.7%) and total postprandial q_{gi} (>17.1 l kg^{-1}) were unrealistic. Consequently, we feel that the data for fish fed twice, as reported below, are a better representation of postprandial effects following a surgical intervention in the body cavity to measure gut blood flow.

3.4. Cardiorespiratory changes for fish fed twice

The metabolic rate data for fish fed twice (SMR, RMR, peak MO_2, time-to-peak MO_2, time course of HiE and cost of HiE) are shown in Table 1. The postprandial MO_2 data are illustrated in Fig. 3. By the 4th h postprandial, postprandial MO_2 was significantly elevated over SMR. MO_2 returned to SMR after 41 h postprandial, with the exception of two brief, significant subsequent increases (Fig. 3). RMR is indicated for reference in this figure.

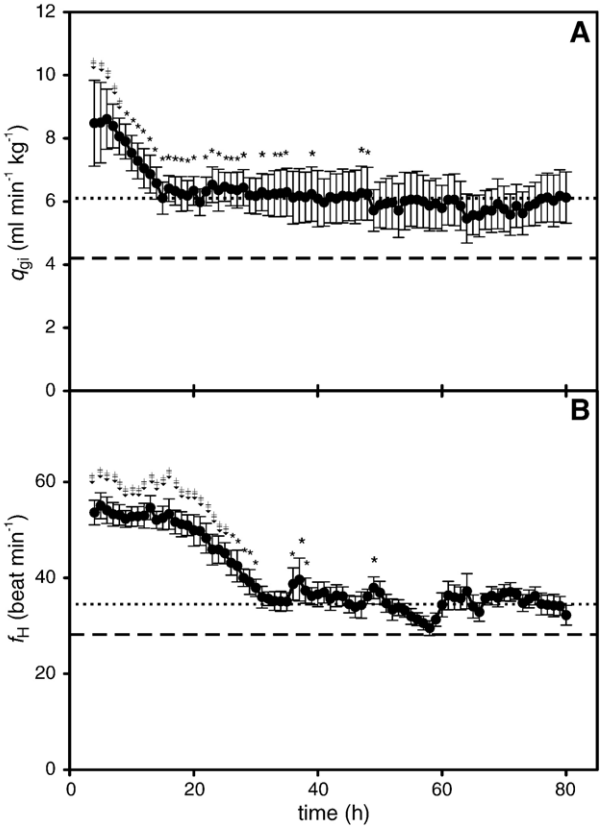

Fig. 4. Pooled (n=7) postprandial gut blood flow (q_{gi}) and heart rate (f_H) values for fish fed twice (mean±SE). Fish were fed at time zero. Routine (dotted line) and baseline (dashed line) values are indicated. The symbol "‡" indicates a statistically significant difference from both routine and baseline and an asterisk indicates a statistically significant difference from baseline (p<0.05).

The cardiovascular data for fish fed twice (baseline q_{gi} and f_H, routine q_{gi} and f_H, peak q_{gi} and f_H, time-to-peak q_{gi} and f_H, and the cumulative increase in q_{gi} from baseline) are shown in Table 2. The time courses of postprandial q_{gi} and f_H are illustrated in Fig. 4. Postprandial f_H and q_{gi} returned to baseline after 30 and 35 h, respectively, in fish fed twice (Fig. 4), with the

Table 2
Baseline, routine, peak and time-to-peak postprandial gut blood flow (q_{gi}) and heart rate (f_H) values for fish fed twice (mean±SE)

	q_{gi} (ml min^{-1} kg^{-1})	f_H (beat min^{-1})
Baseline	4.2±0.5	28.2±1.2
Routine	6.1±0.5	34.5±1.0
Peak	9.9±1.1	59.9±1.8
Time-to-peak (h)	10.9±4.7	13.5±4.9
Cumulative increase in q_{gi} from baseline (l kg^{-1}) [a]		
12 h	2.5±0.5	
18 h	3.7±0.6	
24 h	4.5±0.8	
36 h	**5.9±1.1**	

The totals for postprandial blood flow to the gut above baseline q_{gi} levels are indicated. Bold font indicates the total increase in q_{gi} estimated after postprandial q_{gi} had returned to baseline.

[a] Total increase in q_{gi} is calculated as the integral under the postprandial q_{gi} curve minus the baseline integral.

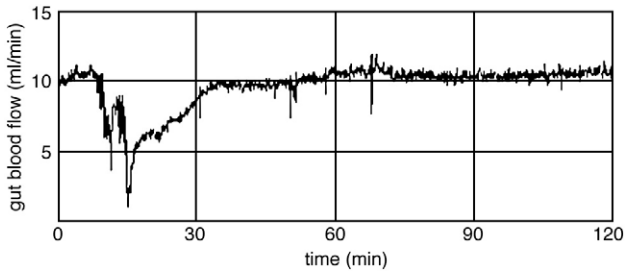

Fig. 5. Representative gut blood flow (q_{gi}) trace of an 811 g male rainbow trout, 2 days postprandial, that responded to a disturbance at the 10 min mark. The disturbance resulted in a 10% decrease in q_{gi} over the 2 h period (measured by subtracting the integral of the total 2 h period shown from a comparable 2 h period without a disturbance).

exception of some minor peaks. Routine q_{gi} and f_H are shown for reference in this figure.

Throughout these experiments and both pre- and postprandial, q_{gi} was sensitive to environmental disturbances including noises. Fig. 5 illustrates a 10% decrease in total q_{gi} during the 2 h post-disturbance period with no compensatory increase in q_{gi} afterwards. Postprandial events like this represent a delay in digestion through a lost opportunity for nutrient assimilation from the gut.

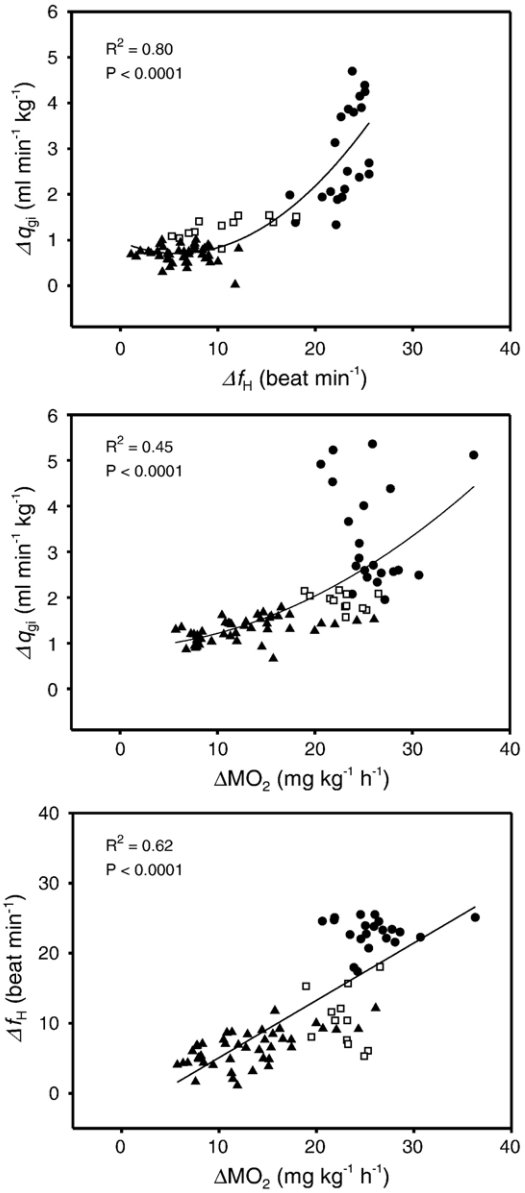

Fig. 6. (A) Changes in postprandial q_{gi} from baseline levels in relation to changes in the postprandial heart rate (f_H) from baseline levels ($\Delta q_{gi}=0.007\Delta f_H{}^2-0.087\Delta f_H+0.961$); (B) Changes in postprandial gut blood flow (q_{gi}) from baseline levels in relation to changes in postprandial oxygen consumption (MO_2) from standard metabolic rate (SMR) ($\Delta q_{gi}=0.003\Delta MO_2^2+0.007\Delta MO_2+0.893$); (C) Changes in postprandial f_H from baseline levels in relation to changes in postprandial MO_2 from SMR ($\Delta f_H=0.819\Delta MO_2-3.11$). The filled circles, open squares and filled triangles represent values from 4–24 h, 24–36 h and 36–80 h postprandial, respectively.

3.5. Associations between postprandial changes in q_{gi}, MO_2 and f_H

For fish fed twice, a quadratic relationship explained the association between the increases in q_{gi} and f_H during the first 24 h postprandial and their subsequent declines (Fig. 6A). A similar relationship was discovered between the postprandial changes in q_{gi} and MO_2 (Fig. 6B). Fig. 6C illustrates a clear linear relationship between the postprandial changes in MO_2 and f_H. Notably, postprandial MO_2 remained elevated above baseline longer than q_{gi}. No obvious patterns were observed in the relationships between the postprandial changes in q_{gi}, MO_2 or f_H for fish fed once (data not shown).

4. Discussion

As far as we are aware, the present study is the first to continuously and simultaneously monitor q_{gi} and MO_2 in fish following one or more meals. Several studies have followed cardiovascular changes for 24 h following a single meal [sea raven (*Hemitripterus americanus*), Atlantic cod (*Gadus morhua*) and sea bass (*Dicentrarchus labrax*) (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Axelsson et al., 2002)] and for up to 5 days following a single meal [red Irish lord (*Hemilepidotus hemilepidotus*) (Axelsson et al., 2000) and chinook salmon (*Oncorhynchus tshawytscha*) (Thorarenson and Farrell, 2006)]. Only Thorarensen and Farrell (2006) presented continuous recordings of q_{gi} and none of the previous studies measured MO_2 simultaneously. By simultaneously measuring q_{gi} and MO_2 in the present study and using fish fed twice, we discovered excellent relationships between the postprandial patterns for q_{gi} and MO_2, and that f_H was a predictor of MO_2.

4.1. Relationships between postprandial q_{gi}, MO_2 and f_H

Clear positive relationships were discovered between the postprandial increases in q_{gi}, f_H and MO_2 for trout fed twice (Fig. 6). Not surprisingly, there was a tighter relationship between q_{gi} and f_H compared with q_{gi} and MO_2. The relationship between postprandial q_{gi} and MO_2 was not a simple one. The increased scatter during the initial postprandial phase (4–24 h) of the q_{gi} and MO_2 relationship likely reflects the differing time course of the postprandial changes for the two variables. In this regard, postprandial q_{gi} peaked earlier (11 h), higher (136% above baseline) and returned to basal levels sooner (35 h) than MO_2 (which peaked after 27 h at 96% above baseline and returned to basal levels at 41 h). A similar phenomenon has been reported for the relationship between gastric evacuation rates and HiE (Jobling and Davies, 1980). This result is consistent with blood flow to the splanchnic circulation via the celiac and mesenteric arteries being down-regulated before postprandial MO_2 declines completely.

These relationships raise the important possibility that future studies of digestion in rainbow trout could benefit from biotelemetry of just f_H since biotelemetry techniques for f_H but not for MO_2 are well established for fish. Biotelemetry of f_H may then allow studies of digestion in freely feeding fish, which

would avoid confounding effects of confining fish in respirometry tubes and feeding by gavage.

4.2. Recovery and routine statuses of fish fed once or twice

The effect of sham-feeding either once or twice on MO_2, q_{gi} and f_H of trout appeared to have subsided within 4 h in this study. However, our results suggest that both RMR and routine f_H, but not routine q_{gi}, declined significantly between the first and second feedings. This finding is consistent with earlier studies showing that routine MO_2 is labile and habituation to the experimental apparatus may allow a decrease in routine MO_2 (Janz et al., 1991; Steffensen, 2005; Cheng and Farrell, 2007). Unsettled fish clearly confounded the present results for a first feeding. While digestion and assimilation were nearly completed after 80 h in fish fed once (given that little or no gut contents remained), this postprandial duration is about twice that for fish fed twice and for non-instrumented fish fed similarly sized meals in other studies.

Extensive research on gastric emptying time and duration of HiE in salmonids (for review see Brett and Groves, 1979; Fange and Grove, 1979; Jobling, 1981) suggests that digestion in satiated fish should last 24–48 h. In the present experiments, a second meal of 2% body mass resulted in a HiE lasting 41 h, while f_H and q_{gi} were elevated for 30 h and 35 h, respectively. Previous gavage feeding studies with non-instrumented rainbow trout using the same experimental apparatus have found similar digestion durations. HiE lasted 30–44 h for a meal of 2% body mass (Eliason et al., 2007). Similarly, following a meal of 1% body mass, peak concentrations in plasma amino acids that occurred between 4 h and 12 h postprandial had returned to baseline by 24 h (Murai et al., 1987; Ok et al., 2001). Likewise, Karlsson et al. (2006) observed that rainbow trout fed 1% of their body mass just 24 h after surgery to implant cannulae in the hepatic portal vein and dorsal aorta, exhibited peak concentrations in plasma amino acids between 6 h and 24 h postprandial, which then returned to baseline levels after 48 h.

Surgical recovery may be the most important factor for extending the postprandial responses following the first feeding. In fact, sea bass fitted with an intestinal flow probe delayed gastric evacuation time compared with non-instrumented sea bass (a 62% *vs*. a 28% stomach evacuation by 24 h postprandial) when fed a meal of 2.9% of their body mass (Axelsson et al., 2002). Even so, Thorarensen and Farrell (2006) reported peak postprandial q_{gi} at 23 h and recovery at 36 h in chinook salmon starved for 24 h before surgery and then allowed 24 h to recover before they were force fed 2% of their body mass. In red Irish lord that had been starved for 1 week before surgery and given 24 h to recover from surgery before force-feeding (10–15% of body mass), postprandial q_{gi} remained elevated above control levels for 6 days and small pieces of food and bones still remained in the stomach (Axelsson et al., 2000). The different meal composition (pieces of raw fish muscle *vs.* pelleted food) and its larger size likely contributed to a longer digestion time.

It should be noted that by feeding fish twice in two consecutive feedings, the possibility of an additive effect between gavages #1 and #2 cannot be discounted. The use of gavage is an unfortunate necessity when using instrumented, confined fish that won't freely feed, and all studies on q_{gi} to date have used a similar method. However, this is the first known study to attempt to distinguish between multiple feedings and to allow fish to recover considerably following surgery. The postprandial duration and physiological profiles found in our study using fish fed twice are comparable to those in the literature (described above and below), which suggests that digestion was proceeding normally and any additive effects were probably negligible.

4.3. Baseline physiology

SMR, baseline q_{gi} and baseline f_H values were all within expected ranges. SMR (48.4 ± 4.1 mg O_2 kg^{-1} h^{-1}) was at the low end of the reported range for SMR of 48–80 mg O_2 kg^{-1} h^{-1} in rainbow trout (Webb, 1971; Kiceniuk and Jones, 1977; Pagnotta and Milligan, 1991; Alsop and Wood, 1997; Claireaux et al., 2005; Simonot, 2005). Baseline q_{gi} was 4.2 ± 0.5 ml min^{-1} kg^{-1}, which represents 24% of the expected resting cardiac output (Q_{rest} = 17.6 ml min^{-1} kg^{-1}, Kiceniuk and Jones, 1977). Again, these values are within the reported range: sea raven, 2.9 ml min^{-1} kg^{-1} (celiac artery) or 15% of Q_{rest} (Axelsson et al., 1989); Atlantic cod, 4.1 ml min^{-1} kg^{-1} (celiac artery) and 3.5 ml min^{-1} kg^{-1} (mesenteric artery) or 40% of Q_{rest} (Axelsson and Fritsche, 1991); chinook salmon, 12.0–14.2 ml min^{-1} kg^{-1} (intestinal artery) or 36% of Q_{rest} (Thorarensen et al., 1993); red Irish lord, 4.1 ml min^{-1} kg^{-1} (celiac artery) and 4.9 ml min^{-1} kg^{-1} (mesenteric artery) or 34% of Q_{rest} (Axelsson et al., 2000); white sturgeon (*Acipenser transmontanus*), 8.9 ml min^{-1} kg^{-1} (celiacomesenteric artery) or 20% of Q_{rest} (Crocker et al., 2000); and sea bass, 9.6 ml min^{-1} kg^{-1} (coeliac and mesenteric arteries) or 24.0% of Q_{rest} (Axelsson et al., 2002). Baseline f_H (28.2 ± 1.2 beat min^{-1}) was slightly lower than most reported resting f_H values in rainbow trout, which range from 37.8 to 68.2 beat min^{-1} (Kiceniuk and Jones, 1977; Gallaugher et al., 1995; Taylor et al., 1996; Simonot, 2005). Therefore, we conclude that the baseline cardiorespiratory state of our fish was comparable with literature values.

4.4. Peak postprandial response

The peak postprandial response for fish fed twice can be summarized as follows. q_{gi}, f_H and MO_2 were all significantly elevated above baseline levels by 4 h postprandial. A peak value for q_{gi} of 136% was reached after 11 h, a peak value for f_H of 110% was reached after 11 h and a peak value for MO_2 of 96% was reached after 27 h.

The 96% postprandial increase in MO_2 above SMR in fish fed twice (94.6 ± 10.4 mg kg^{-1} h^{-1}) is consistent with the peak postprandial MO_2 values previously obtained for other fish, which increased by between 1.5 and 2.5 times SMR (Jobling, 1981; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Ross et al., 1992; Boyce and Clarke, 1997; Hunt von Herbing and White, 2002; Peck et al., 2005).

The peak postprandial increase in q_{gi} to 9.9 ± 1.1 ml min^{-1} kg^{-1} represented a 136% increase from baseline q_{gi}, which is a

slightly greater increase than previously observed. For example, postprandial blood flow increased by 100% in the celiac artery of sea raven fed 10–20% of body mass (Axelsson et al., 1989), 94% and 112% for the mesenteric and coeliac arteries, respectively, in red Irish lord fed 10–15% of body mass (Axelsson et al., 2000), 42% and 72% for mesenteric and coeliac arteries, respectively, in Atlantic cod fed 2.5–3.5% of body mass (Axelsson and Fritsche, 1991), and 71% in the combined coeliac and mesenteric arteries in sea bass fed 2.9% of body mass (Axelsson et al., 2002). While the relative size of the meal is expected to increase the magnitude of the q_{gi} response, as it does with HiE (Higgs et al., 1995), it appears that a relatively large meal in fish increases q_{gi} by 70–140%. In the present study, this corresponds to an additional 6 l of blood per kg directed to the gut during the digestion period.

The peak postprandial f_H (59.9±1.8 beat min^{-1}) represented a 110% increase above baseline f_H for fish fed twice. Simonot (2005) measured maximum f_H during swimming (80 beats min^{-1}) for the same stock of rainbow trout and temperature as we used. Consequently, postprandial f_H in our rainbow trout reached an estimated 75% of maximum f_H. In contrast to our findings for rainbow trout, Axelsson et al. (2002) and J. Altimiras (pers. comm.) found only a 10–20% rise in postprandial f_H in sea bass, and Axelsson and Fritsche (1991) observed no postprandial increase in f_H in Atlantic cod. These differences between studies are likely unrelated to the method of feeding (all used gavage), but may reflect species differences. Mammals typically increase in q_{gi} via a redistribution of Q (Vatner et al., 1974; Matheson et al., 2000). However, fish also increase Q postprandial to increase q_{gi}, via stroke volume and f_H (Axelsson and Fritsche, 1991; Axelsson et al., 2000; Axelsson et al., 2002). The fact that the postprandial changes in f_H in rainbow trout closely paralleled those in q_{gi} suggests to us that Q also increased during the first 24 h postprandial, although direct measurements of Q were not made.

4.5. Effect of dietary protein

As in an earlier study (Eliason et al., 2007), we confirmed that a wide range of DP:DE does not significantly influence postprandial MO_2 and additionally showed no significant change on q_{gi}. Others (Cho et al., 1976; Jobling, 1981; Cho et al., 1982; LeGrow and Beamish, 1986; Cho and Woodward, 1989) have reported effects (for a detailed discussion of these differences see Eliason et al., 2007). Of course it is possible that an almost two-fold difference in the dietary protein to lipid ratio may have been too small to resolve differences in either MO_2 or q_{gi}. This would mean that future studies would need to broaden the range in dietary protein and lipid levels well above and below that considered optimal for normal growth and protein utilization of rainbow trout, which would then preclude diets with equivalent digestible energy content, as were used here.

In summary, we demonstrated that positive relationships existed for postprandial increases in f_H, q_{gi} and MO_2 for rainbow trout provided they are given ample time to recover from surgery before the postprandial studies are performed.

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References

Alsop, D., Wood, C., 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 200, 2337–2346.

Axelsson, M., Fritsche, R., 1991. Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. J. Exp. Biol. 158, 181–198.

Axelsson, M., Driedzic, W.R., Farrell, A.P., Nilsson, S., 1989. Regulation of cardiac output and gut blood flow in the sea raven, *Hemitripterus americanus*. Fish Physiol. Biochem. 6, 315–326.

Axelsson, M., Thorarensen, H., Nilsson, S., Farrell, A.P., 2000. Gastrointestinal blood flow in the red Irish lord, *Hemilepidotus hemilepidotus*: long-term effects of feeding and adrenergic control. J. Comp. Physiol. B 170, 145–152.

Axelsson, M., Altimiras, J., Claireaux, G., 2002. Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. J. Exp. Biol. 205, 2891–2896.

Beamish, F.W.H., Trippel, E.A., 1990. Heat increment: a static or dynamic dimension in bioenergetic models? Trans. Am. Fish. Soc. 119, 649–661.

Boyce, S.J., Clarke, A., 1997. Effect of body size and ration on specific dynamic action in the Antarctic plunderfish, *Harpagifer antarcticus* Nybelin 1947. Physiol. Zool. 70, 679–690.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. Fish Physiol. 8, 279–352.

Brown, C.R., Cameron, J.N., 1991. The relationship between specific dynamic action (SDA) and protein synthesis rates in the channel catfish. Physiol. Zool. 64, 298–309.

Cheng, W.W., Farrell, A.P., 2007. Acute and sublethal toxicities of rotenone in juvenile rainbow trout (*Oncorhynchus mykiss*): swimming performance and oxygen consumption. Arch. Environ. Contam. Toxicol. 52, 388–396.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture 100, 107–123.

Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). In: Bourne, G.H. (Ed.), Aspects of Food Production, Consumption and Energy Values. World Rev. Nutr. Diet, vol. 61. Karger, Basel, pp. 132–172.

Cho, C.Y., Woodward, W.D., 1989. Studies on the protein to energy ratio in diets for rainbow trout (*Salmo gairdneri*). Energy Metabolism of Farm Animals. Proc. 11th Symposium, vol. 43. EAPP Publ, pp. 37–48.

Cho, C.Y., Bayley, H.S., Slinger, S.J., 1976. Energy metabolism in growing rainbow trout: partition of dietary energy in high protein and high fat diets. In: Vermorel, M. (Ed.), Proceedings of the 7th Symposium on Energy Metabolism. Bussac, Clermont-Ferrand, pp. 299–302.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. B 73B, 25–41.

Claireaux, G., McKenzie, D.J., Genge, A.G., Chatelier, A., Aubin, J., Farrell, A.P., 2005. Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. J. Exp. Biol. 208, 1775–1784.

Crocker, C.E., Farrell, A.P., Gamperl, A.K., Cech Jr., J.J., 2000. The effects of environmental hypercapnia on cardiac output and gut blood flow in the white sturgeon, *Acipenser transmontanus*. Am. J. Physiol. 279, R617–R628.

Eliason, E.J., Higgs, D.A., Farrell, A.P., 2007. Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout. Aquaculture 272, 723–736.

Fange, R., Grove, D., 1979. Digestion. Fish Physiol. 8, 161–260.

Farrell, A.P., Thorarensen, H., Axelsson, M., Crocker, C.E., Gamperl, A.K., Cech, J.J., 2001. Gut blood flow in fish during exercise and severe hypercapnia. Comp. Biochem. Physiol. A 128, 551–563.

Gallaugher, P., Thorarensen, H., Farrell, A.P., 1995. Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). Respir. Physiol. 279–292.

Higgs, D.A., Macdonald, J.S., Levings, C.D., Dosanjh, B.S., 1995. Nutrition and feeding habits in relation to life history stage. In: Groot, C., Margolis, L., Clarke, W.C. (Eds.), Physiological Ecology of Pacific Salmon. UBC Press, Vancouver, pp. 161–315.

Hunt von Herbing, I., White, L., 2002. The effects of body mass and feeding on metabolic rate in small juvenile Atlantic cod. J. Fish Biol. 61, 945–958.

Janz, D.M., Farrell, A.P., Morgan, J.D., Vigers, G.A., 1991. Acute physiological stress responses of juvenile coho salmon (*Oncorhynchus kisutch*) to sublethal concentrations of Garlon 4, Garlon 3A, and Vision herbicides. Environ. Toxicol. Chem. 10, 81–90.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. J. Fish Biol. 18, 385–400.

Jobling, M., Davies, P.S., 1980. Effects of feeding on metabolic rate, and the specific dynamic action in plaice, *Pleuronectes platessa* L. J. Fish Biol. 16, 629–638.

Jordan, A.D., Steffensen, J.F., 2007. Effects of ration size and hypoxia on specific dynamic action in the cod. Physiol. Zool. 80, 178–185.

Kaczanowski, T.C., Beamish, F.W.H., 1996. Dietary essential amino acids and heat increment in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 15, 105–120.

Karlsson, A., Eliason, E.J., Mydland, L.T., Farrell, A.P., Kiessling, A., 2006. Postprandial changes in plasma free amino acid levels obtained simultaneously from the hepatic portal vein and the dorsal aorta in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 209, 4885–4894.

Kiceniuk, J.W., Jones, D.R., 1977. The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. J. Exp. Biol. 69, 247–260.

LeGrow, S.M., Beamish, F.W.H., 1986. Influence of dietary protein and lipid on apparent heat increment of rainbow trout, *Salmo gairdneri*. Can. J. Fish Aquat. Sci. 43, 19–25.

Lyndon, A.R., Houlihan, D.F., Hall, S.J., 1992. The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. J. Comp. Physiol. B 162, 209–215.

Matheson, P.J., Wilson, M.A., Garrison, R.N., 2000. Regulation of intestinal blood flow. J. Surg. Res. 93, 182–196.

McCue, M.D., 2006. Specific dynamic action: a century of investigation. Comp. Biochem. Physiol. A 144, 381–394.

McMillan, D.N., Houlihan, D.F., 1992. Protein synthesis in trout liver is stimulated by both feeding and fasting. Fish Physiol. Biochem. 10, 23–34.

Medland, T.E., Beamish, F.W.H., 1985. The influence of diet and fish density on apparent heat increment in rainbow trout, *Salmo gairdneri*. Aquaculture 47, 1–10.

Murai, T., Ogata, H., Hirasawa, Y., Akiyama, T., Nose, T., 1987. Portal absorption and hepatic uptake of amino acids in rainbow trout force-fed complete diets containing casein or crystalline amino acids. Nippon Suisan Gakkaishi 53, 1847–1859.

Ok, I.H., Bai, S.C., Park, G.J., Choi, S.M., Kim, K.W., 2001. The patterns of plasma free amino acids after force-feeding in rainbow trout *Oncorhynchus mykiss* (Walbaum) with and without dorsal aorta cannulation. Aquac. Res. 32, 70–75.

Pagnotta, A., Milligan, C.L., 1991. The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and winter flounder (*Pseudopleuronectes americanus*). J. Exp. Biol. 489–508.

Pannevis, M.C., Houlihan, D.F., 1992. The energetic cost of protein synthesis in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. B 162, 393–400.

Peck, M.A., Buckley, L.J., Bengtson, D.A., 2005. Effects of temperature, body size and feeding on rates of metabolism in young of the year haddock. J. Fish Biol. 66, 911–923.

Ross, L.G., McKinney, R.W., Cardwell, S.K., Fullarton, J.G., Roberts, S.E.J., Ross, B., 1992. The effects of dietary protein content, lipid content and ration level on oxygen consumption and specific dynamic action in *Oreochromis niloticus* L. Comp. Biochem. Physiol. A 103, 573–578.

Secor, S.M., 2003. Gastric function and its contribution to the postprandial metabolic response of the Burmese python *Python molurus*. J. Exp. Biol. 206, 1621–1630.

Simonot, D.L. 2005. M.Sc. thesis. Cardiac remodeling in diploid and triploid rainbow trout (*Oncorhynchus mykiss* Walbaum). University of British Columbia, Vancouver, BC, Canada, pp. 1–130.

Steffensen, J.F., 2005. Respiratory systems and metabolic rates. Fish Physiol. 22, 203–238.

Tandler, A., Beamish, F.W.H., 1980. Specific dynamic action and diet in largemouth bass, *Micropterus salmoides* (Lacepede). J.Nutr. 110, 750–764.

Taylor, S., Egginton, S., Taylor, E., 1996. Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. J. Exp. Biol. 199, 835–845.

Thorarensen, H., Farrell, A.P., 2006. Postprandial intestinal blood flow, metabolic rates and exercise in chinook salmon (*Oncorhynchus tshawytscha*). Physiol. Biochem. Zool. 79, 688–694.

Thorarensen, H., Gallaugher, P.E., Kiessling, A.K., Farrell, A.P., 1993. Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. J. Exp. Biol. 179, 115–129.

Vatner, S.F., Patrick, T.A., Higgins, C.B., Franklin, D., 1974. Regional circulatory adjustments to eating and digestion in conscious unrestrained primates. J. Appl. Physiol. 36, 524–529.

Webb, P.W., 1971. The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. J. Exp. Biol. 521–540.

Wells, R.M.G., Weber, R.E., 1991. Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum). An interpretation of recent data based on blood viscosity measurements. J. Fish Biol. 38, 53–65.