The Journal of Experimental Biology 212, 2550-2557
Published by The Company of Biologists 2009
doi:10.1242/jeb.030593

Postprandial changes in enteric electrical activity and gut blood flow in rainbow trout (*Oncorhynchus mykiss*) acclimated to different temperatures

Albin Gräns*, Fredrik Albertsson, Michael Axelsson and Catharina Olsson
Department of Zoology, University of Gothenburg, Göteborg, Sweden
*Author for correspondence (e-mail: albin.grans@zool.gu.se)

Accepted 6 May 2009

SUMMARY

Enteric electrical activity, cardiac output and gut blood flow were measured in rainbow trout (*Oncorhynchus mykiss*) acclimated to either 10°C or 16°C. Enteric electrical activity showed, in both the fasted and postprandial state, a distinct pattern with clusters of burst-like events interspersed by silent periods. The frequency of electrical events increased postprandially for both acclimation groups. Event frequency increased from 3.0±0.5 to 9.6±1.4 events min^{-1} and from 5.9±0.9 to 11.8±2.0 events min^{-1} in the 10°C and 16°C groups, respectively. Similarly, the number of events per cluster increased postprandially for both acclimation groups. Gut blood flow, cardiac output and heart rate increased after feeding. The gut blood flow significantly increased in both groups and peaked at 257±19% and 236±22% in the 10°C and 16°C groups, respectively. There was a strong correlation between the number of events and gut blood flow at both temperatures. Comparison between the two groups showed that fish acclimated to 16°C may have an increased cost of sustaining the basal activity of the gut compared with the group acclimated to 10°C. In conclusion, we have for the first time measured enteric electrical activity *in vivo* in a fish species and we have also demonstrated a strong correlation between gut blood flow and enteric electrical activity in fasted and postprandial fish.

Key words: teleost, electrical activity, postprandial, gut blood flow, temperature, feeding.

INTRODUCTION

Fish like other ectothermic animals have the same body temperature as their surrounding environment. This means that their physiological mechanisms need to work efficiently at the ambient temperature, which for many species fluctuates substantially over days/seasons/years. One of the essential mechanisms is the uptake of nutrients through the gut. The long-term aim of our project is to be able to predict what effects an increase in water temperature would have on the functionality of the fish gut. By using a comparative approach we will gain increased knowledge about the similarities and differences between ectothermic fish and endothermic mammalian models.

For an effective utilization of nutrients in a meal, processing and transport of food have to be optimized. This is achieved through neatly controlled patterns of movements (motility) and regulation of the blood supply (haemodynamic). In most animals studied, both gut blood flow and motility show distinct responses to feeding (see Chou, 1982).

Gut motility is created by contracting smooth muscles in the gut. The underlying mechanism for the contractions is depolarization of the muscle cells, which may be induced by intrinsic mechanisms in muscle cells, by interstitial cells of Cajal and by nerves and hormones (see Olsson and Holmgren, 2001). The electrical and contractile activity can be used as a measurement of gut motility, using electromyography, force transducers or video recordings. Depending on frequency, duration and amplitude of the contractions, different motility patterns are distinguished. These may be more or less confined to a particular region (such as mixing and propulsive movements) or involve large sections of the gut (Szurszewski, 1969). Mixing and propulsive motility patterns are generated and controlled locally in the gut by the enteric nervous system (see Olsson and Holmgren, 2001). The activities of the enteric nervous system are, however, constantly modified by signals originating from the central nervous system, *via* sympathetic and parasympathetic pathways, and from other regions of the gut. Intrinsic (local) and extrinsic reflexes are initiated by e.g. the presence of food in the gut *via* activation by mechanoreceptors or chemoreceptors in the gut wall.

The mechanical movements of the gut are supported by a finely tuned supply of blood to the different regions of the gut. As with gut motility, both intrinsic and extrinsic mechanisms are involved in the control of blood flow. While the former include local metabolic and myogenic control, local reflexes and locally produced vasoactive substances, the latter include sympathetic innervation, circulating vasoactive substances and changes in systemic haemodynamics. A profound understanding of how gut motility and gut blood flow are regulated *in vivo* is essential for advances in both comparative and clinical physiology studies.

The mammalian system is, by far, the most investigated, in terms of functional studies on gut motility and gut blood flow (Aviv et al., 2008; Chou and Grassmick, 1978; Takala, 1996). However, there are a growing number of studies on fish looking at the regulation of either motility (Grove and Holmgren, 1992b; Karila and Holmgren, 1995; Olsson and Holmgren, 1997; Olsson and Holmgren, 1998; Olsson and Holmgren, 2000; Olsson et al., 1999) or gut blood flow (Altimiras et al., 2008; Axelsson et al., 2002; Axelsson et al., 2000; Farrell et al., 2001; Gräns et al., 2009; Holmgren et al., 1992; Jensen et al., 1991; Seth and Axelsson, 2009; Seth et al., 2008). The fish literature provides central information on the evolution of vertebrate gut control. These studies indicate large similarities of control mechanisms in the gut between fish and mammals. A limitation in most studies on fish gut motility is that they have been conducted *in vitro* or *in situ*, which, in mammalian studies, has been shown to severely restrict the diversity of gut

motility patterns displayed (Bush et al., 2000; Longhurst et al., 1981). There are a number of *in vivo* studies on the control of blood flow in fish but we still have limited information about the coupling between the mechanical activity and the blood flow patterns seen during different kinds of external and internal stimuli.

It has been shown that gut motility changes from an interdigestive to a postprandial pattern after food intake. The interdigestive pattern in many vertebrate species is characterized by migrating motor complexes (MMCs) that pass along the entire gut as intense rhythmic contractions of the circular muscles (Husebye, 1999; Szurszewski, 1969). Generally, MMCs can be divided into three main phases, with phase three being the most active. It has been suggested that these complexes serve as housekeepers which prevent bacteria from overgrowing and remove waste products form the gut (Grzesiuk et al., 2001; Kruszewska et al., 2005; Szurszewski, 1969). Motility patterns characterizing the postprandial state include contractions that mix the food and peristaltic movements that propulse the food. Continuous feeders such as sheep and guinea pigs also maintain the MMCs after feeding while in intermittent feeders such as humans and dogs it is replaced by more ongoing phasic contractions.

The cardiovascular and in particular the gut blood flow response to feeding is characterized by a clear hyperaemia. This hyperaemia of the gut after feeding is well known in mammals (see Takala, 1996). It has also been shown in ectotherms including crocodiles (Axelsson et al., 1991) and various species of fish (Altimiras et al., 2008; Axelsson et al., 2002; Axelsson et al., 2000; Eliason et al., 2008; Gräns et al., 2009; Seth and Axelsson, 2009). The magnitude of the increase is determined by a range of factors (see Takala, 1996) and differs substantially between species. The magnitude of the postprandial gut blood flow increase ranges between 42% in sea bass (*Dicentrarchus labrax*) (Axelsson et al., 2002) and 112% in the red Irish lord (*Hemilepidotus hemilepidotus*) (Axelsson et al., 2000). All values reported on the postprandial effects on gut blood flow in fish are from studies using gavage feeding methods because instrumented and confined fish do not eat voluntarily, and this method also allows full control over the time of feeding and amount of food eaten. In mammals the hyperaemia is in most cases a true redistribution of blood without any change in total cardiac output, while in fish most of the studies show that the hyperaemia is fully compensated by an increase in cardiac output.

The aim of the present study was to develop an *in vivo* model for recording enteric electrical activity in order to be able to describe the interdigestive and postprandial electrical patterns of the gut in fish. Secondly we aimed to test whether there is a correlation between the enteric electrical activity and gut blood flow. We used two groups of animals acclimated to two different temperatures to study the effect of acclimation temperature on enteric electrical activity and blood flow.

MATERIALS AND METHODS

Animals used

Rainbow trout (*Oncorhynchus mykiss* Linnaeus), ranging in size between 440 and 1100 g, were used (N=25). The fish were obtained from a local hatchery (Antens Laxodling AB, Alingsås, Sweden). They were kept in fibreglass tanks which contained 2000 l of re-circulating aerated fresh water. They were acclimated to either 10°C or 16°C, for at least 4 weeks on a 12 h:12 h light:dark photoperiod and were fed commercial trout dry pellets, three times a week. Animal care and physiological experimental procedures were approved by the ethical committee of Göteborg, ethical permit 13-2007.

Surgical procedures

The animals were fasted for approximately 72 h before surgery. They were anaesthetized in water containing 150 mg l^{-1} MS-222 (ethyl 3-aminobenzoate methanesulphonic acid, $C_{10}H_{15}NO_5S$) buffered with 300 mg l^{-1} $NaHCO_3$. The fish were then placed on soft rubber foam, soaked in water. The gills were constantly irrigated with aerated 10°C fresh water, mixed with 75 mg l^{-1} MS-222 and buffered with 150 mg l^{-1} $NaHCO_3$, in order to keep the fish anesthetized during the surgical procedure.

The operculum and the gill arches on the left side were carefully lifted and the ventral aorta was exposed through a small skin incision and dissected free. A 20 MHz Doppler flow crystal (Iowa Doppler products, Iowa City, IA, USA) mounted in a 2.0 mm cuff was placed around the ventral aorta (see Fig. 1A), and the Doppler lead was secured to the back of the fish with a 3-0 silk suture. To access the celiacomesenteric artery a 30 mm incision was made dorsoventrally about 15 mm caudal to the operculum, and 10 mm ventral to the lateral line. The celiacomesenteric artery was dissected free, and care was taken to ensure the surrounding tissue and nerves were

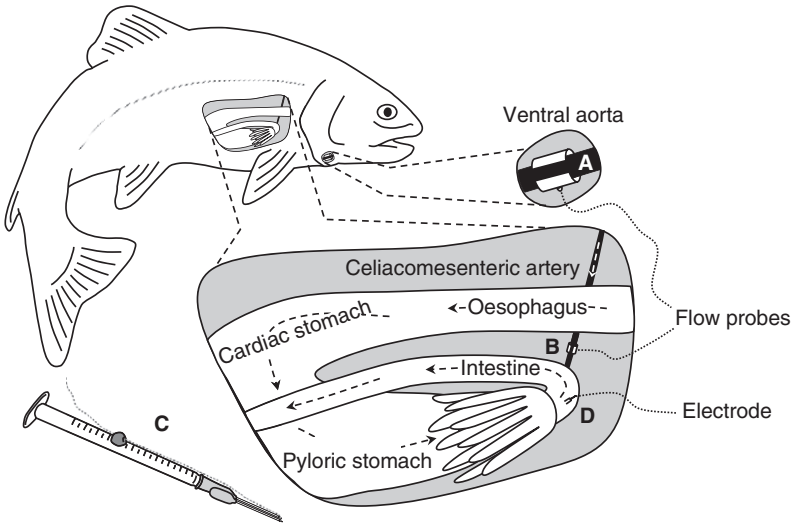

Fig. 1. A schematic picture of the Doppler flow probe positioned on the ventral aorta (A), the celiacomesenteric artery (B), needle and syringe-based instrument used to insert the electrodes into the intestine (C) and the electrode position in the intestine (D).

not damaged. A 20 MHz Doppler flow crystal mounted in a 1.3 mm cuff was placed around the celiacomesenteric artery (see Fig. 1B).

For recordings of enteric electrical activity, a pair of electrodes was inserted into the intestinal wall approximately 10 mm caudal to the pyloric caeca. Approximately 2 mm of the insulation on a twisted pair lead (model AS631-2, Cooner Wire, Chatsworth, CA, USA) was stripped off. To simplify insertion into the intestine, the stripped ends of the wires were inserted in two 23G 1 in (2.54 cm) hypodermic needles, glued 2 mm apart. The insulated lead was bent backwards and temporarily secured to a 1 ml syringe that acted as a handle for the two hypodermic needles (see Fig. 1C). The insertion method was based on that described for use in axial swimming musculature in fish (Bunt, 1999). Gluing two hypodermic needles together ensured that the electrodes were positioned at the same distance in all animals. The electrodes were exteriorized trough the same incision used to access the celiacomesenteric artery and secured with a 6-0 silk suture in the intestine (see Fig. 1D). Together with the celiacomesenteric artery Doppler lead, the enteric electrical activity (EEA, see below) lead was secured on the back of the fish with 3-0 silk sutures and the incision was closed with continuous sutures.

After surgery the fish was placed in an experimental tank 54 cm×13 cm×18 cm (L×W×D), with re-circulating aerated fresh water at the same temperature it had previously been acclimated to (10°C or 16°C, respectively). A plastic cover was placed over the container and the wires were connected to the equipment.

Experimental protocol

The fish were allowed to recover for at least 48 h before feeding or sham-feeding. For the two experimental groups (fish acclimated to 10°C, *N*=9 or 16°C, *N*=9), a 2 h control period was recorded before feeding (see below) and the recording started again approximately 15 min after feeding and continued 48 h after feeding. The fish were fed, by gavage, a meal that consisted of ground commercial trout dry pellets equivalent to 2% of the body mass, mixed with 0.5 ml water per gram of pellets.

One group of fish acclimated to 10°C (*N*=7) was used as sham-fed controls. For this group, recordings started immediately after surgery in order to study the time of recovery. The feeding procedure for the sham-fed group followed the same procedure as for the fed group but without injecting any food into the stomach. All animals were anaesthetized until the righting reflex was lost during the gavage- and sham-feeding procedure.

Post-mortem examinations of the gastrointestinal tract were conducted to confirm that the stomach and the intestine were empty when the experiments were finished.

Data acquisition and analysis

The leads from the Doppler flow probe were connected to a directional-pulsed Doppler flowmeter (model 545C-4, Iowa Doppler products), and the EEA lead was connected to a BIO Amp (model ML136, ADInstruments, Castle Hill, Australia). An electric ground wire was placed in the surrounding water. Both the Doppler flowmeter and the BIO Amp were connected to a PowerLab 8/30 system (ADInstruments). Data were collected on a PC using ADInstruments acquisition software Chart^{TM} 5 Pro v5.5.5, at a sampling rate of 1 kHz.

EEA recordings were conducted with the BIO Amp adjusted to: range (1 mV), low-pass filter (1 kHz), high-pass filter (0.3 Hz) and a 50 Hz notch filter activated. The absolute value of the EEA raw signal was filtered with a low-pass digital filter in Chart, set to 1 Hz, to filter out signal noise.

Calculations

The following EEA variables were calculated: event frequency (events min^{-1}), cluster frequency (clusters min^{-1}), number of events per cluster (events cluster^{-1}) and event amplitude (μV). An event was defined as a peak that exceeded the threshold that was set to 150% of the individual fish's noise value. The timing of each event was registered to locate clusters. For each fish a frequency histogram for the time between all consecutive events was created. From visual analysis of these histograms a minimum of 1 min was set as the criterion for cluster separation.

For clarification of the analysis method used, Fig. 2A,B shows the results of the filtering procedure. The shaded area in Fig. 2B shows a typical area that was used to calculate the noise level and set the threshold for the peak detection (150% of mean noise level).

Heart rate was calculated from the phasic cardiac output signal. Stroke volume was calculated by dividing cardiac output by heart rate.

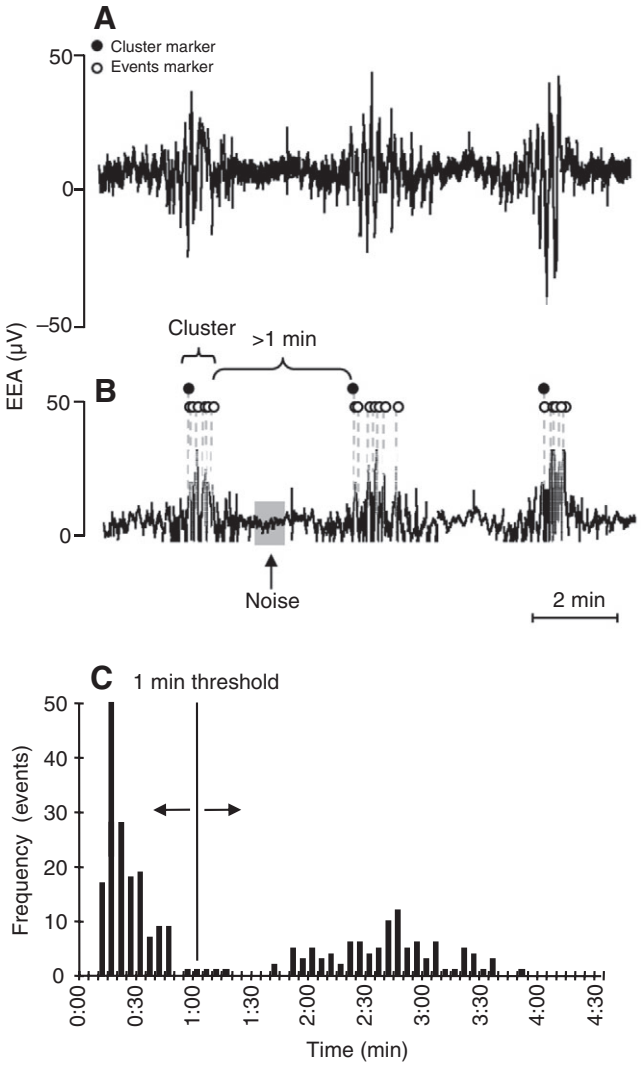

Fig. 2. (A) Representative enteric electrical activity (EEA) raw signal trace. (B) Absolute value of raw signal filtered with a 0.1 Hz low-pass digital filter. The shaded area is an example of a typical selected noise window. Events are marked with an open circle and clusters with a filled circle. (C) A frequency histogram, from which separation of clusters was made by visual analysis. The line indicates the 1 min threshold that was selected as a separator between clusters.

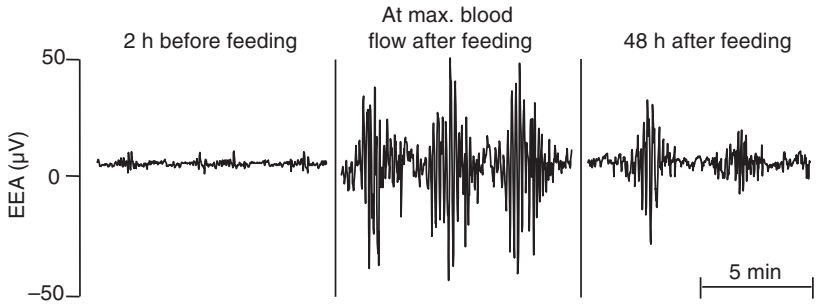

Fig. 3. The figure illustrates an example of cluster formations at three different time points during the experimental protocol.

Because of an inconsistency of definitions in earlier studies looking at electrical activity of the gut, we have used the term enteric electrical activity (EEA) throughout the paper as a summarizing term for all electrical activity derived from any part of the gastrointestinal tract.

Statistical analysis

All variables were calculated over 2 h periods, starting with the control period. The subsequent time points included 2 h around maximum gut blood flow (4–12 h), and 10–12 h, 22–24 h, 34–36 h and 46–48 h after feeding. For the sham-fed control group, additional periods were included (10–12, 22–24, 34–36 and 46–48 h after surgery). All data are presented as means ± s.e.m.

Statistical analyses were performed with GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). For comparison between acclimation temperatures, a two-tailed *t*-test assuming unequal variances was used. Within treatments, Dunnett's *post-hoc* test was used to compare the time point before feeding against all other time points. For the control group, the values from each time point after sham-feeding were compared with the 46–48 h value after surgery (corresponding to the control period in the experimental groups). Differences where $P<0.05$ were regarded as statistically significant.

Because the Doppler flow probe provides a relative measurement, the cardiovascular variables were calculated as a percentage, defining the 2 h control period before feeding as 100%. Consequently, no comparisons between the two experimental groups (10°C and 16°C) could be made. A correlation analysis was made between gut blood flow and the total number of events in the two experimental groups.

RESULTS

Enteric electrical activity (EEA)

The method used for measuring EEA proved to be robust and reliable. Based on the frequency histogram analysis the criterion for a new cluster was set to a minimum of 1 min between two events (Fig. 2C). A strong signal with well defined recurring clusters of events occurring approximately every 1 to 10 min could be identified. It was also apparent that feeding induced changes in these cluster patterns.

Visual and statistical analyses of the data revealed clear changes in the patterns between fed and unfed states. This is illustrated in Fig. 3, which shows effects on both the number of events per cluster and the amplitude of the events. Cluster formations were observed in all individuals studied. The event frequency but not the cluster frequency was significantly higher in the 16°C group than in the 10°C group during the control period (Fig. 4). Also, the number of events per cluster and the event amplitude were increased in the 16°C group compared with the 10°C group before feeding (Fig. 4).

Event frequency and number of events per cluster increased postprandially for both experimental groups. In the 10°C group, event frequency increased from 3.0±0.5 events min^{-1} to 9.6±1.4 events min^{-1} at maximum gut blood flow. In the 16°C group, event frequency increased from 5.9±0.9 events min^{-1} to 11.8±2.0 events min^{-1} at maximum gut blood flow (Fig. 4). Similarly, the number of events per cluster increased from 11.7±2.1 to 67.9±14.4 events $cluster^{-1}$ at maximum gut blood flow in the 10°C

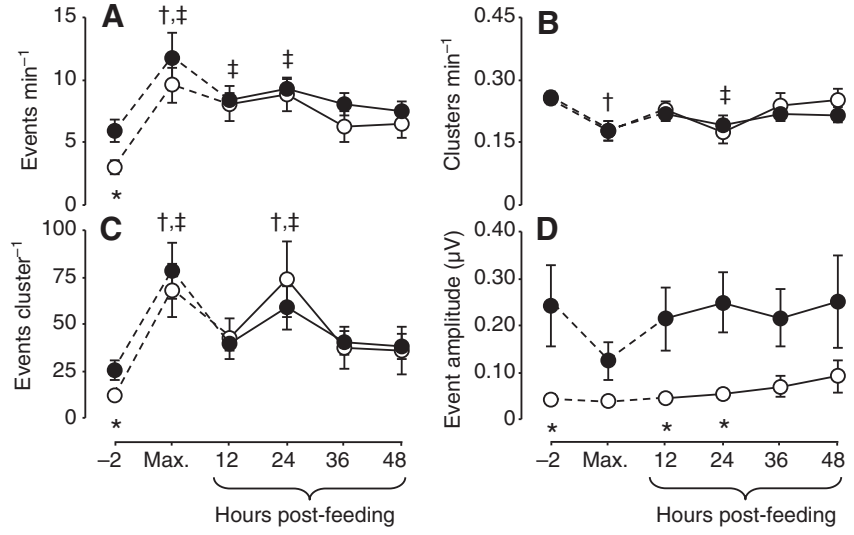

Fig. 4. EEA variables: (A) event frequency, (B) cluster frequency, (C) events per cluster and (D) event amplitude. All variables are shown for fish from two acclimation temperatures, 10°C (open circles) and 16°C (filled circles), before (–2) and after (Max. to –48) feeding. Data are presented as means (±s.e.m., $N=9$). ‡ and † indicate a significant difference ($P<0.05$) from values before feeding in 10°C and 16°C acclimated fish, respectively. * indicates a significant difference ($P<0.05$) between the two acclimation groups.

group, and from 25.1±5.3 to 78.1±14.8 events cluster^{-1} at maximum gut blood flow for fish acclimated to 16°C (Fig. 4). The event amplitude was not affected by feeding (see Fig. 4).

Cluster frequency decreased in the 10°C group from 0.26±0.01 to 0.18±0.02 clusters min^{-1} 24 h postprandially and from 0.25±0.01 to 0.18±0.02 clusters min^{-1} at maximum gut blood flow for fish acclimated to 16°C (Fig. 4).

In the sham-fed group, no differences were found for any of the four EEA variables compared with the control values (see Table 1).

Cardiovascular variables

The maximum postprandial gut blood flow increased in both groups and peaked at 256±19%, and 236±22% in the 10°C and the 16°C group, respectively (Fig. 5A). In both the 10°C and the 16°C acclimated groups, the increase in gut blood flow remained elevated for at least 36 h after feeding (Fig. 5A).

Cardiac output was increased, compared with the control, at the time for maximum gut blood flow in both the 10°C group (142±10%) and the 16°C group (123±5%) (Fig. 5B). In both the 10°C and the 16°C acclimated groups, the increase was still seen at 48 h after feeding (see Fig. 5B).

Stroke volume fluctuated in the 16°C group; it decreased initially (68±5% at maximum gut blood flow and 70±9% at 12 h after feeding), followed by an increase at 36 h (119±8%) and 48 h (120±5%) postprandially (Fig. 5C).

There was no difference in control heart rate between the two groups (35.8±3.9 b.p.m. in the 10°C group and 45.2±4.2 b.p.m. in the 16°C group). Fish acclimated to 16°C had a significantly higher heart rate than the group acclimated to 10°C at maximum blood flow, and 12, 24 and 48 h after feeding (Fig. 5D).

In both groups, heart rate and cardiac output increased after feeding while stroke volume was unchanged or even decreased, indicating that heart rate is the dominant factor for the cardiac output increase. Heart rate but not cardiac output showed the highest value at maximum gut blood flow with 57.5±2.3 and 80.9±2.7 b.p.m. for fish acclimated to 10°C and 16°C, respectively (Fig. 5D). In both acclimation groups the increase in heart rate was sustained for 24 h after feeding (Fig. 5D).

At both temperatures there was a strong correlation between the number of electrical events and gut blood flow; R^2=0.95 (P=0.007) and R^2=0.91 (P=0.001) for 10°C and 16°C, respectively (compare Fig. 4A and Fig. 5A).

A correlation between gut blood flow and EEA event frequency was also shown during periods of spontaneous swimming/thrashing as rapid reductions in gut blood flow as well as in event frequency (Fig. 6). The EEA parameters seemed to recover (to pre-stress levels) quicker than did gut blood flow.

No changes in gut blood flow or cardiac output were observed in the sham-fed group (see Table 1). Stroke volume was decreased for the first 12 h after surgery compared with the control period (48 h after surgery). After the initial decrease, there were no differences between any of the time points and the control values (Table 1). Heart rate was significantly elevated during the first 24 h after surgery (Table 1).

DISCUSSION

We have for the first time recorded gut smooth muscle electrical activity in adult unanaesthetized fish, using serosal recordings with surgically implanted electrodes *in vivo*. We show that the EEA is well correlated with blood flow to the gastrointestinal tract and that there is strong correlation between EEA activity, temperature and feeding.

Few studies have looked at gut motility *in vivo* in fish. Rhythmic contraction waves have been described in transparent larvae of Atlantic halibut (*Hippoglossus hippoglussus*) (Rönnestad et al., 2000) and zebrafish (*Danio rerio*) (Holmberg et al., 2003), using video analysis. A common way to measure gut motility *in vivo* in mammals is through inflating a balloon in the gut and measuring contraction through changes in pressure (Chou and Grassmick, 1978; Gwynne and Bornstein, 2007). However, the balloon distension method has several disadvantages compared with EEA measurements. When using an inflated balloon in the gut it is inevitable that the lumen will be blocked and this makes it impossible to measure basal and postprandial motility patterns.

The correlation between EEA and muscular contractions in the gut has been verified in several mammalian studies through simultaneous measurement of mechanical and electrical activity in isolated intestine, e.g. in cat (Perkins, 1971), as well as *in vivo*, e.g. in rat (Ferré and Ruckebusch, 1985) and dog (Aviv et al., 2008; Sanmiguel et al., 2007). However, to our knowledge this is the first study presenting data on EEA and gut blood flow in fish. There are a number of studies that have looked at gastrointestinal blood flow but the *in vivo* motility patterns of fish are largely unknown.

Basal activity

In many of the animals studied, the amplitude of the signal before feeding was low, but it was still possible to identify events and clusters of events. The occurrence of clusters appeared to be rhythmic with only a small variation in the time between them. The phases of quiescence or irregular contractions described in the fasted state of some mammals were not observed (see Husebye, 1999). The control group showed that by 12 h after surgery, all EEA variables were already stable and also that the mild anaesthesia during feeding did not affect the motility patterns 12 h after sham-feeding.

Table 1. Enteric electrical activity and cardiovascular variables from the control group during the recovery period and after the sham-feeding, indicated by the dashed line

	Recovery after surgery				At max. gut blood flow	Period after sham-feeding			
	12 h	24 h	36 h	48 h		12 h	24 h	36 h	48 h
Cardiac output (%)	88.2±7.2	90.3±5.7	97.9±6.0	100±0	114.6±15.8	103.4±9.8	100.1±9.8	115.4±17.7	108.0±14.9
Gut blood flow (%)	120.4±24.6	116.1±8.7	100.9±15.0	100±0	126.3±13.3	124.0±20.4	110.0±20.3	131.65±22.3	149.8±44.1
Stroke volume (%)	69.8±5.3†	75.2±4.0	96.0±5.7	100±0	107.1±15.4	107.1±12.6	114.5±16.4	139.7±21.3	129.0±20.3
Heart rate (b.p.m.)	51.9±2.5†	49.4±2.6†	42.3±2.9	41.2±2.1	44.3±2.6	40.0±3.1	37.5±2.4	34.0±1.8	35.2±1.4
Events (min^{-1})	5.5±0.8	4.1±0.5	3.8±0.7	4.6±0.4	4.7±0.8	5.6±1.1	5.3±0.8	5.0±0.8	5.2±1.0
Clusters (min^{-1})	0.19±0.02	0.28±0.03	0.21±0.02	0.24±0.02	0.28±0.05	0.20±0.02	0.22±0.04	0.24±0.03	0.24±0.03
Events cluster^{-1}	34.9±8.8	17.0±4.2	21.8±4.8	21.1±3.4	19.6±4.8	33.1±8.5	35.1±10.6	30.2±10.8	31.6±13.4
Event amplitude (mV)	20.4±3.7	17.2±2.9	19.7±4.0	31.0±8.2	29.6±4.8	26.7±5.6	26.7±5.7	29.9±4.7	31.9±3.6

Data are presented as means (±s.e.m., N=7). † indicates significant difference (P<0.05) from 46–48 h post-surgery and 2 h before sham-feeding.

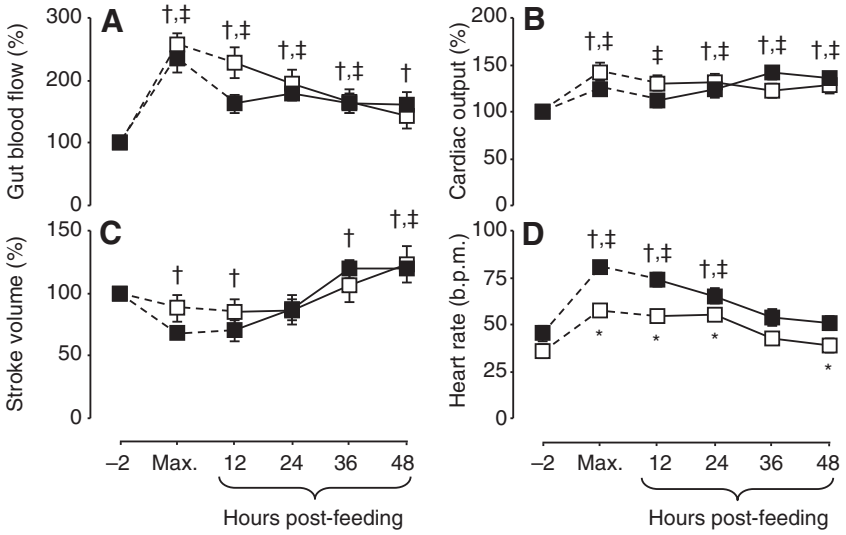

Fig. 5. Cardiovascular variables: (A) gut blood flow, (B) cardiac output, (C) stroke volume and (D) heart rate. All variables are shown for fish from two acclimation temperatures, 10°C (open boxes) and 16°C (filled boxes), before (–2) and after (Max. to –48) feeding. Data are presented as means (±s.e.m., N=9). ‡ and † indicate significant difference (P<0.05) from values before feeding in 10°C and 16°C acclimated fish, respectively. * indicates a significant difference (P<0.05) between the two acclimation groups.

The basal cluster frequency (0.26±0.01 clusters min^{-1} in the 10°C group) was lower than but in the same range as the contraction frequency (0.52±0.05 contractions min^{-1}, at 10°C) observed in isolated Atlantic cod intestine (Karila and Holmgren, 1995), but much lower than in guinea pig duodenum (~14 clusters min^{-1}, at 37°C) (Galligan et al., 1985). Somewhat surprisingly and in spite of the temperature difference, the cluster frequency observed in rainbow trout was also very similar to the rhythmic clusters of contractions observed in the human small intestine (Husebye and Engedal, 1992). MMCs travel along the entire gut at a speed of approximately 3–6 cm min^{-1} in mammals (Costa and Furness, 1976; Perkins, 1971; Szurszewski, 1969). The contractions observed in the cod migrated at a similar speed, 3.5±1.0 cm min^{-1} (Karila and Holmgren, 1995). In the present study, electrical activity was only registered at one location and therefore we could not study the propagation of the signal. Hence it could not be stated whether the clusters observed in the starved fish are equivalent to MMCs.

The resting heart rate in this study was low, 35 and 45 b.p.m. for 10°C and 16°C fish, respectively, compared with earlier studies with similar surgical instrumentation on fish from the same supplier. Sandblom and Axelsson (Sandblom and Axelsson, 2007) reported a resting heart rate of 46.8 b.p.m., and Seth and colleagues (Seth et al., 2008) one of 48.7 b.p.m. for rainbow trout acclimated to 10°C. Also, the heart rate from the group acclimatized to 16°C is in the lower limit when compared with resting heart rates of rainbow trout acclimated to 15°C (reviewed by Altimiras and Larsen, 2002), which had resting heart rates between 32 and 65 b.p.m. A low resting heart rate is normally an indication of a low stress level and in the laboratory environment it also reflects the postoperative recovery and postoperative stress due to the experimental setup (Altimiras and Larsen, 2000). From the control group we could also see that after a period of elevated values during the first 24 h after surgery heart rate was stabilized after 36 h and remained stable until data collection ended. This is a good indication that the feeding procedure *per se* had no evident stressful effect.

Postprandial activity

From the EEA results obtained in this study it is clear that the number of events and number of events per cluster increase postprandially. The increase in event frequency was expected and similar results have previously been reported from various species, including humans (Yin et al., 2004), dogs (Fioramonti and Bueno, 1984), rats and chickens (Rodriguez-Membrilla et al., 1995).

After feeding, motility is expected to change from patterns of maintenance to more active processing of the chyme. In rainbow trout this was seen as an increase in the number of events per cluster as the perfusion of blood to the gut was elevated. The fed state of mammals includes an elongation of irregular spiking activity, which makes separation between clusters impossible, and thereby leads to an increase of events in each cluster (Rodriguez-Membrilla et al.,

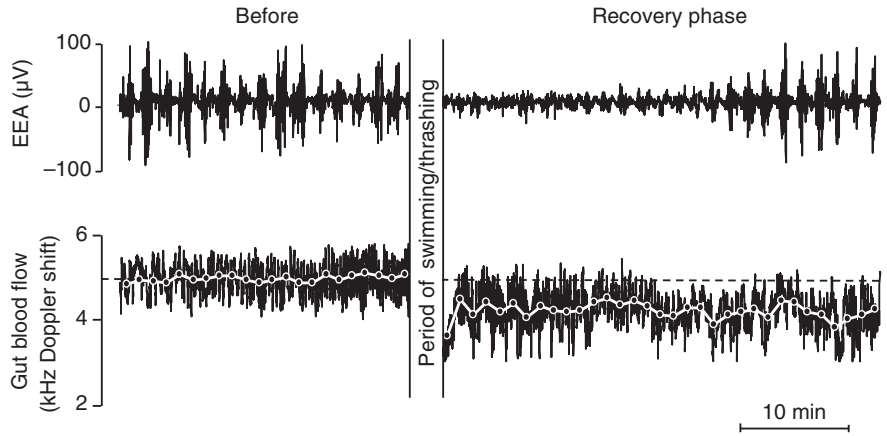

Fig. 6. Original raw data trace during a brief period of spontaneous swimming/thrashing. Note the simultaneous decrease in gut blood flow and EEA. The upper panel shows the EEA and the lower panel phasic blood flow to the gut. The white circles and lines in the lower trace represent the average gut blood flow.

1995). Also, in the fish the increased number of events per cluster made a distinction between individual clusters more difficult. However, in most cases clusters were identifiable. This pattern is similar to the maintenance of an unfed pattern observed in sheep and guinea pig, and like them rainbow trout have been shown to feed continuously when food is available (Jönsson et al., 2007). Whether the distinction between postprandial motility patterns and different feeding strategies also holds true for fish remains to be shown, possibly by a similar study conducted on a pronounced intermittent feeder, such as sculpin.

Both acclimation groups showed a postprandial increase in cardiac output and heart rate. This was expected following a meal as a response to the specific dynamic action and has been observed in all species studied so far (McCue, 2006). The increase in gut blood flow following a meal in the present study was higher than expected. This is probably due to the fact that our highest values were calculated for each fish individually and not the average from different fish at the same time after feeding. We chose to take the value at maximum blood flow for each individual instead of the blood flow at a fixed time after feeding because of the great individual variability in blood flow pattern in the first 12 h after feeding. This great variability between individuals has been described before in rainbow trout when looking at gastric emptying (Olsson et al., 1999). With this in mind, we found it more reasonable to take the values during the peak in each individual, instead of at a fixed time schedule. The timing of the peak in postprandial gut blood flow shown in this study resembles the initial peak recently shown by Eliasson and colleagues (Eliasson et al., 2008).

It has been shown that chyme has started to enter the intestine of rainbow trout by 10 h after feeding (Olsson et al., 1999). When comparing our peak gut blood flow data (occurring within the first 12 h) with the gastric emptying times, in rainbow trout, presented by Olsson and colleagues (Olsson et al., 1999), it looks like the main increase in gut blood flow occurs right before or during the first release of chyme from the stomach to the intestine. This comparison must, however, be made with caution as both the meal size and the handling/surgery procedure differ between the studies.

Intestinal motility and gut blood flow

Our results show not only that it is possible to measure EEA in the intestine of rainbow trout but also that EEA in rainbow trout is strongly correlated with gut blood flow. A correlation between mesenteric blood flow and motility has been shown before *in vivo* in conscious dogs (Fioramonti and Bueno, 1984). In addition, an increase in gut mucosal blood flow has been shown in conscious dogs (Gallavan et al., 1980). The increase in blood flow was confined to tissues involved in digestion, with a later increase of ileal blood flow compared with duodenal and jejunal blood flow (Gallavan et al., 1980). *In vitro* and *in situ* studies of rainbow trout have shown that balloon distension of the cardiac stomach increases muscular contractions (Grove and Holmgren, 1992b). Muscular contractions induced by distension have also been shown *in vivo* in Atlantic cod (*Gadus morhua*) and four species of flatfish (*Pleuronectes platessa*, *Limanda limanda*, *Scophthalmus rhombus* and *Scophthalmus maximus*) (Grove and Holmgren, 1992a). The only study in fish looking at stomach distension and gut blood flow was conducted in rainbow trout and no significant increase in gut blood flow following distension was found (Seth et al., 2008). Because gut blood flow evidently increases postprandially in fish (Axelsson et al., 2002; Axelsson et al., 2000; Gräns et al., 2009; Seth and Axelsson, 2009) but not *via* distension of a balloon in the stomach (Seth et al., 2008) it seems as though the increase in flow is only moderately induced by smooth muscle contractions. What effect stomach distension has on motility and the mechanisms coupling nutrient composition to motility is, however, still largely unknown in both mammals and fish. The rapid decrease in gut blood flow during a period of spontaneous swimming/thrashing appears to be analogous to the response seen during exercise, which has been described in several species of fish including Chinook salmon (*Oncorhynchus tshawytscha*) (Thorarensen et al., 1993), green sturgeon (*Acipenser medirostris*) (Gräns et al., 2009) and rainbow trout (Randall and Daxboeck, 1982). The sudden and simultaneous reductions in EEA and gut blood flow during a period of spontaneous swimming/thrashing strengthen the evidence of a strong correlation, and the slower onset of blood flow to the gut compared with the increase of electrical activity indicates that the motility can start before complete perfusion of the gut is restored.

Effect of temperature

The fact that there was no significant difference in resting heart rate between the two acclimation groups implies that the 16°C fish was successfully acclimated to the higher temperature. The much lower heart rate in our group acclimated to 16°C (45 b.p.m.) compared with the heart rates reported by Sandblom and Axelsson (Sandblom and Axelsson, 2007) (75.6 b.p.m.) in rainbow trout after exposure to an acute temperature increase from 10°C to 16°C is also a good indication of a successful acclimation period.

There was an increase in three out of the four EEA variables measured in the 16°C group compared with the 10°C acclimation group (no increase in cluster frequency was observed). An increase in contraction frequency when the temperature was increased has been shown before in isolated intestine from both mammals and ectothermic vertebrates (Studier et al., 1977). This increase in basal electrical activity of the gut suggests that there is a larger cost of sustaining an empty stomach when living in higher temperatures. The possibility that these differences would disappear with a longer acclimation time cannot, however, be ruled out, as there are no data on the effects of acclimation time on the EEA response in ectotherms.

The larger increase in gut blood flow and lower increase in heart rate shown in fish acclimated to 10°C compared with 16°C indicates that the group acclimated to 10°C have may have a larger cardiovascular capacity compared with the group acclimated to 16°C. That the reported ‘final preferendum’ (the temperature at which the animal should have optimal physiological functions) for rainbow trout is 11.3°C strengthens this hypothesis (McCaule et al., 1977). From earlier studies we can also conclude that a higher acclimation temperature leads to a higher basal metabolic rate for the group acclimated to 16°C (Beitinger and Fitzpatrick, 1979).

These results suggest that an increase in acclimation temperature from 10°C to 16°C decreases the cardiovascular capacity and increases the basal energy cost for the fish. This might leave the animals more vulnerable to commonly occurring environmental changes such as diurnal temperature fluctuations, oxygen availability, predator exposure and food availability.

Conclusion

We have developed and verified a method suitable for long-term *in vivo* recording of EEA in fish, using serosal recordings with surgically implanted electrodes. We have used this method to demonstrate fasted and postprandial EEA in fish. This method will be useful for future studies looking at motility patterns in fish. Our results show not only that it is possible to measure EEA in the intestine of rainbow trout but also that EEA is strongly correlated

with gut blood flow. The fish acclimated to the higher temperature (16°C) may have a smaller cardiovascular capacity due to an increase in the basal demand of the gut compared with the group acclimated to 10°C. The physiological and ecological implications of these temperature effects require further study.

We gratefully acknowledge Susanne Holmgren, Henrik Seth and Erik Sandblom for their comments and technical support throughout the process of this study. This research was supported by Göteborg University Research Platform on Integrative Physiology (GRIP), the Swedish Science Research Council grant numbers: 621-2005-2588 (M.A.) and 621-2004-3936 (C.O.), the Helge Ax:son Johnsson Foundation, and Wilhelm and Martina Lundgrens foundation (A.G.).

REFERENCES

Altimiras, J. and Larsen, E. (2000). Non-invasive recording of heart rate and ventilation rate in rainbow trout during rest and swimming: fish go wireless! *J. Fish Biol.* **57**, 197-209.

Altimiras, J., Claireaux, G., Sandblom, E., Farrell, A. P., McKenzie, D. J. and Axelsson, M. (2008). Gastrointestinal blood flow and postprandial metabolism in swimming sea bass *Dicentrarchus labrax*. *Physiol. Biochem. Zool.* **81**, 663-672.

Aviv, R., Policker, S., Brody, F., Bitton, O., Haddad, W., Kliger, A., Sanmiguel, C. P. and Soffer, E. E. (2008). Circadian patterns of gastric electrical and mechanical activity in dogs. *Neurogastroenterol. Motil.* **20**, 63-68.

Axelsson, M., Fritsche, R., Holmgren, S., Grove, D. J. and Nilsson, S. (1991). Gut blood-flow in the estuarine crocodile, Crocodylus-Porosus. *Acta Physiol. Scand.* **142**, 509-516.

Axelsson, M., Thorarensen, H., Nilsson, S. and 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 Biochem. Syst. Environ. Physiol.* **170**, 145-152.

Axelsson, M., Altimiras, J. and 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.

Beitinger, T. L. and Fitzpatrick, L. C. (1979). Physiological and ecological correlates of preferred temperature in fish. *Am. Zool.* **19**, 319-329.

Bunt, C. M. (1999). A tool to facilitate implantation of electrodes for electromyographic telemetry experiments. *J. Fish Biol.* **55**, 1123-1128.

Bush, T. G., Spencer, N. J., Watters, N., Sanders, K. M. and Smith, T. K. (2000). Spontaneous migrating motor complexes occur in both the terminal ileum and colon of the C57BL/6 mouse *in vitro*. *Auton. Neurosci.* **84**, 162-168.

Chou, C. C. (1982). Relationship between intestinal blood flow and motility. *Annu. Rev. Physiol.* **44**, 29-42.

Chou, C. C. and Grassmick, B. (1978). Motility and blood flow distribution within the wall of the gastrointestinal tract. *Am. J. Physiol.* **235**, H34-H39.

Costa, M. and Furness, J. B. (1976). The peristaltic reflex: an analysis of the nerve pathways and their pharmacology. *Naunyn Schmiedebergs Arch. Pharmacol.* **294**, 47-60.

Eliason, E. J., Higgs, D. A. and Farrell, A. P. (2008). Postprandial gastrointestinal blood flow, oxygen consumption and heart rate in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **149**, 380-388.

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

Ferré, J. P. and Ruckebusch, Y. (1985). Myoelectrical activity and propulsion in the large intestine of fed and fasted rats. *J Physiol.* **362**, 93-106.

Fioramonti, J. and Bueno, L. (1984). Relation between intestinal motility and mesenteric blood flow in the conscious dog. *Am. J. Physiol. Gastrointest. Liver Physiol.* **246**, G108-113.

Gallavan, R. H., Jr, Chou, C. C., kVietys, P. R. and Sit, S. P. (1980). Regional blood flow during digestion in the conscious dog. *Am. J. Physiol.* **238**, H220-225.

Galligan, J. J., Costa, M. and Furness, J. B. (1985). Gastrointestinal myoelectric activity in conscious guinea pigs. *Am. J. Physiol.* **249**, G92-G99.

Gräns, A., Axelsson, M., Pitsillides, K., Olsson, C., Hojesjo, J., Kaufman, R. and Cech, J. J. (2009). A fully implantable multi-channel biotelemetry system for measurement of blood flow and temperature: a first evaluation in the green sturgeon. *Hydrobiologia* **619**, 11-25.

Grove, D. J. and Holmgren, S. (1992a). Mechanisms controlling stomach volume of the Atlantic cod following gastric distension. *J. Exp. Biol.* **163**, 49-63.

Grove, D. J. and Holmgren, S. (1992b). Intrinsic mechanisms controlling cardiac stomach volume of the rainbow trout (*Oncorhynchus mykiss*) following gastric distension. *J. Exp. Biol.* **163**, 33-48.

Grzesiuk, E., Laubitz, D., Wojcik-Sikora, A., Zabielski, R. and Pierzynowski, S. G. (2001). Influence of intestinal myoelectrical activity on the growth of Escherichia coli. *Bioelectromagnetics* **22**, 449-455.

Gwynne, R. M. and Bornstein, J. C. (2007). Mechanisms underlying nutrient-induced segmentation in isolated guinea pig small intestine. *Am. J. Physiol.* **292**, G1162-G1172.

Holmberg, A., Schwerte, T., Fritsche, R., Pelster, B. and Holmgren, S. (2003). Ontogeny of intestinal motility in correlation to neuronal development in zebrafish embryos and larvae. *J. Fish Biol.* **63**, 318-331.

Holmgren, S., Axelsson, M. and Farrell, A. P. (1992). The effect of catecholamines, substance-P and vasoactive intestinal polypeptide on blood-flow to the gut in the dogfish *Squalus acanthias*. *J. Exp. Biol.* **168**, 161-175.

Husebye, E. (1999). The patterns of small bowel motility: physiology and implications in organic disease and functional disorders. *Neurogastroenterol. Motil.* **11**, 141-161.

Husebye, E. and Engedal, K. (1992). The patterns of motility are maintained in the human small-intestine throughout the process of aging. *Scand. J. Gastroenterol.* **27**, 397-404.

Jensen, J., Axelsson, M. and Holmgren, S. (1991). Effect of substance P and vasoactive intestinal polypeptide on gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. *J. Exp. Biol.* **156**, 361-374.

Jönsson, E., Forsman, A., Einarsdottir, I. E., Kaiya, H., Ruohonen, K. and Bjornsson, B. T. (2007). Plasma ghrelin levels in rainbow trout in response to fasting, feeding and food composition, and effects of ghrelin on voluntary food intake. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **147**, 1116-1124.

Karila, P. and Holmgren, S. (1995). Enteric reflexes and nitric-oxide in the fish intestine. *J. Exp. Biol.* **198**, 2405-2411.

Kruszewska, D., Podgurniak, P., Ljungh, A., Sebastian, A., Larsson, L., Zajdel-Dabrowska, J. and Pierzynowski, S. G. (2005). Extremely low electrical current generated by porcine small intestine smooth muscle alters bacterial autolysin production. *Exp. Physiol.* **90**, 855-863.

Longhurst, J. C., Spilker, H. L. and Ordway, G. A. (1981). Cardiovascular reflexes elicited by passive gastric distension in anesthetized cats. *Am. J. Physiol. Heart Circ. Physiol.* **240**, H539-545.

McCaule, R. W., Elliott, J. R. and Read, L. A. A. (1977). Influence of acclimation temperature on preferred temperature in the rainbow trout *Salmo gairdneri*. *Trans. Am. Fish. Soc.* **106**, 362-365.

McCue, M. D. (2006). Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **144**, 381-394.

Olsson, C. and Holmgren, S. (1997). Nitric oxide in the fish gut. *Comp. Biochem. Physiol. A Physiol.* **118**, 959-964.

Olsson, C. and Holmgren, S. (1998). PACAP inhibits spontaneous contractions in the intestine of the Atlantic cod, *Gadus morhua*. *Ann. NY Acad. Sci.* **865**, 512-514.

Olsson, C. and Holmgren, S. (2000). PACAP and nitric oxide inhibit contractions in the proximal intestine of the atlantic cod, *Gadus morhua*. *J. Exp. Biol.* **203**, 575-583.

Olsson, C. and Holmgren, S. (2001). The control of gut motility. *Comp. Biochem. Physiol. A Comp. Physiol.* **128**, 481-503.

Olsson, C., Aldman, G., Larsson, A. and Holmgren, S. (1999). Cholecystokinin affects gastric emptying and stomach motility in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **202**, 161-170.

Perkins, W. E. (1971). Method for studying electrical and mechanical activity of isolated intestine. *J. Appl. Physiol.* **30**, 768-771.

Randall, D. J. and Daxboeck, C. (1982). Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Can. J. Zool.* **60**, 1135-1140.

Rodriguez-Membrilla, A., Martinez, V., Jimenez, M., Gonalons, E. and Vergara, P. (1995). Is nitric-oxide the final mediator regulating the migrating myoelectric complex cycle. *Am. J. Physiol.* **31**, G207-G214.

Rönnestad, I., Rojas-Garcia, C. R. and Skadal, J. (2000). Retrograde peristalsis; a possible mechanism for filling the pyloric caeca? *J. Fish Biol.* **56**, 216-218.

Sandblom, E. and Axelsson, M. (2007). Venous hemodynamic responses to acute temperature increase in the rainbow trout (Oncorhynchus mykiss). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R2292-R2298.

Sanmiguel, C. P., Aviv, R., Policker, S., Haddad, W., Brody, F. and Soffer, E. E. (2007). Association between gastric electromechanical activity and satiation in dogs. *Obesity* **15**, 2958-2963.

Seth, H. and Axelsson, M. (2009). Effects of gastric distension and feeding on cardiovascular variables in the shorthorn sculpin (*Myoxocephalus scorpius*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R171-R177.

Seth, H., Sandblom, E., Holmgren, S. and Axelsson, M. (2008). Effects of gastric distension on the cardiovascular system in rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R1648-R1656.

Studier, E. H., Studier, A. L., Essy, A. J. and Dapson, R. W. (1977). Thermal sensitivity and activation energy of intrinsic intestinal motility in small vertebrates. *J. Therm. Biol.* **2**, 101-105.

Szurszewski, J. H. (1969). A migrating electric complex of canine small intestine. *Am. J. Physiol.* **217**, 1757-1763.

Takala, J. (1996). Determinants of splanchnic blood flow. *Br. J. Anaesth.* **77**, 50-58.

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

Yin, J., Levanon, D. and Chen, J. D. Z. (2004). Inhibitory effects of stress on postprandial gastric myoelectrical activity and vagal tone in healthy subjects. *Neurogastroenterol. Motil.* **16**, 737-744.