The Journal of Experimental Biology 205, 2891–2896 (2002)
Printed in Great Britain © The Company of Biologists Limited
JEB3987

Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*

Michael Axelsson[1,*], Jordi Altimiras[1] and Guy Claireaux[2]

[1]*Department of Zoology, University of Göteborg, Box 463, S-405 30 Göteborg, Sweden* and [2]*CREMA-L'Houmeau, (CNRS-IFREMER), France*

*Author for correspondence (e-mail: M.Axelsson@zool.gu.se)

Accepted 6 June 2002

Summary

The hypothesis that the increase in post-prandial splanchnic blood flow will be reduced during hypoxia to prioritise blood flow to other organs was tested by measuring cardiac output and gut blood flow during a stepwise hypoxic challenge (five steps, from 20.6 to 3.9 kPa, 5 min of exposure to each level) before and after feeding (equivalent to 2.9% of body mass). Splanchnic blood flow, both absolute and relative to cardiac output, increased after feeding. Mean post-prandial gut blood flow increased by 71% (from 9.6±1.6 to 14.9±1.6 ml min^{-1} kg^{-1}, means ± S.E.M.). Before feeding, gut blood flow was 24.0% of cardiac output, and this increased significantly 24 h after feeding to 34.0%. The absolute post-prandial increase in gut blood flow (5.3±0.9 ml min^{-1} kg^{-1}) was paralleled by an increase in cardiac output (5.4±2.1 ml min^{-1} kg^{-1}). Hypoxia decreased gut blood flow significantly from 9.6±1.6 to 3.7±1.1 ml min^{-1} kg^{-1}, corresponding to a decrease in relative gut blood flow from 24 % to 13%. Contrary to our initial hypothesis, and although post-prandial absolute blood flow decreases during hypoxia, the relative proportion of cardiac output reaching the gut did not decrease (34.6% pre-hypoxia *versus* 26.7% during hypoxia), unlike the situation in non-feeding fish. We propose that, following feeding, relative gut blood flow is maintained because splanchnic hyperaemia occurs as a result of the release of local factors; consequently the reflex vasoconstriction of the gastrointestinal vasculature during hypoxia is not as effective in decreasing gut blood flow as it was before feeding because local hyperaemia out-competes the reflex regulation.

Key words: gastrointestinal blood flow, cardiac output, hypoxia, post-prandial, sea bass, *Dicentrarchus labrax*.

Introduction

In the fish species studied so far, blood flow to the gastrointestinal tract in resting conditions ($\dot{q}_{GI}$) accounts for 20–30% of total cardiac output (Axelsson et al., 1989, 2000; Axelsson and Fritsche, 1991; Fritsche et al., 1993; Thorarensen et al., 1993; Thorarensen, 1994; Farrell et al., 2001). Like other vertebrates, fish cannot perfuse all the circulatory beds at the same time and have to prioritise the various vascular beds according to the capacity of the heart. When facing challenging conditions such as hypoxia or exercise, $\dot{q}_{GI}$ decreases and there is a general redistribution of blood flow to other organs (Axelsson and Fritsche, 1991; Fritsche et al., 1993; Thorarensen, 1994). It is also well documented that $\dot{q}_{GI}$ increases post-feeding in order to facilitate absorption of food and shorten digestion time.

In their normal habitat, fish have to adjust continuously to multiple stimuli such as changes in water temperature, salinity and oxygen levels; they also have to escape predators or forage and catch prey. If the cardiovascular system cannot fully adjust to these stimuli, compromises have to be made. This will become even more pronounced after feeding when a higher demand is put on the cardiovascular system.

It has been shown that marine coastal environments are exposed to large-scale and long-lasting hypoxic episodes (Diaz and Rosenberg, 1995). Even when not lethal, these conditions are liable to force fish to prioritize their energy demands to make them fit within a reduced metabolic scope (Priede, 1985). For example, in the Atlantic cod *Gadus morhua*, it was observed that fed fish voided their stomach contents when exposed to hypoxia, indicating that systems other than the gastrointestinal tract were prioritised during hypoxia (Claireaux et al., 2000).

The aim of the present study was to test whether the sea bass *Dicentrarchus labrax* shows a post-prandial increase in gastrointestinal blood flow and, if so, whether this increase is compromised during exposure to hypoxia. Sea bass was chosen as a model to test our hypothesis as it is exposed to hypoxia in its natural littoral habitat and its feeding habits are well documented because of its commercial importance. The experimental protocol involved the measurement of cardiac output and gut blood flow during a stepwise hypoxic challenge before and after feeding.

Materials and methods

Animal handling and maintenance

Sea bass *Dicentrarchus labrax* L., of either sex, were obtained from the Ferme Marine des Baleines (Ile de Ré, France) and maintained at the field facility of CREMA. Upon arrival at the laboratory, animals were transferred into a 0.4 m^3 indoor rearing tank supplied with natural sea water (29‰, 16°C). They were acclimated to laboratory conditions for 1 month under a natural photoperiod. During that time, they were fed twice a week on commercial dry pellets (2–4% of body mass). Feeding was discontinued 1 week prior to surgery.

Surgical procedures

Seven sea bass (body mass 356±24 g, mean ± S.E.M.) were individually anaesthetised in tricaine methane sulphonate (MS-222; 100 mg l^{-1}, Sigma) until breathing movements ceased, and then placed on their left side on an operating table. The gills were continuously irrigated with aerated sea water containing the same anaesthetic (50 mg l^{-1}). To measure cardiac output ($\dot{Q}$), the ventral aorta was exposed through an incision on the left side of the isthmus. Depending on the size of the animal, the freed portion of the ventral aorta was placed inside a 4S- or 2S-type Transonic ultrasound flow probe (resolution 0.8 and 0.1 ml min^{-1}, respectively; absolute accuracy ±15% for both probes) (Transonic System Inc., Ithaca, NY, USA). The opening of the probe head was closed either by the metallic lock (for 4S probes) or by a small piece of plastic glued to the probe head itself (for 2S probes). The lead from the probe was secured to the skin with two silk sutures. To record $\dot{q}_{GI}$, a 1 cm long incision was made just posterior to the left pectoral fin. After careful blunt dissection, the coeliac and mesenteric arteries were placed in a 2S- or 4S-type Transonic ultrasound flow probe. Care was taken not to damage the nerves running along the vessels. The leads from the probe were externalised through the incision and secured to the skin with two sutures. After surgery, the animals were transferred to the experimental chamber and allowed to recover for 24–36 h before experiments commenced.

Anatomical study of the gastrointestinal vasculature

Two animals were used to obtain corrosion casts of the main arteries. The animals were killed by MS-222 overdose (1 g l^{-1}). The heart was exposed through a midline incision, and a P120 catheter was secured in the ventricle. Physiological saline (0.9% NaCl) containing heparin (200 i.u. ml^{-1}) was used to rinse the vascular beds until the fluid that returned *via* the atrium was clear of red blood cells. Subsequently, Mercox resin (Ladd Research Industries Inc.) was slowly injected and allowed to polymerise overnight before the tissue was digested using 30% KOH solution. The cast was rinsed with distilled water and photographs were taken and digitised.

Gastrointestinal emptying time

A group of 18 sea bass (body mass 258±10 g, mean ± S.E.M.) were used to study gastric emptying time (GET) in non-instrumented animals. Fish were anaesthetized with MS-222 (100 mg l^{-1}) until righting reflexes disappeared and were then force-fed 2.9% of their body mass with blue mussels *Mytilus edulis*. The food ratio was chosen from the largest daily feeding rates observed in sea bass using *ad libitum* demand-feeding strategies (Azzaydi et al., 1998). The fish were placed randomly in three separate tanks maintained in identical conditions.

Three groups of six fish each were sampled after 24, 36 and 48 h. Each animal was killed by MS-222 overdose (1 g l^{-1}), and the stomach contents were carefully separated, blotted dry and weighed.

Experimental protocol: hypoxia exposure

After 24–36 h of post-operative recovery, the flow probes were connected to a two-channel Transonic flow meter (Transonic Systems Inc., model T206). The water oxygen concentration was continuously recorded using an Orbisphere 27141 oxygen meter and a nitrogen/air mixture was used to set each hypoxia level using calibrated glass flow meters. The experimental protocol started by recording $\dot{Q}$ and $\dot{q}_{GI}$ for 10 min at water P_{O_2}=20.6 kPa (normoxia) to obtain a stable control period. The animal was then exposed to a stepwise decrease in water P_{O_2} (to 14.2, 9.0, 6.4, 5.1 and 3.9 kPa, manually adjusted), each step being held for 5 min. After the last step, water P_{O_2} was returned to full saturation (20.6 kPa) and the fish was allowed to recover for 1 h. No struggling was observed during the hypoxia exposure.

The fish was then lightly anaesthetised in the experimental chamber until righting reflexes were lost, then force-fed 3% of its body mass with blue mussels *M. edulis*. The experimental chamber was flushed to eliminate the anaesthetic, and the animals were left to recover for 24 h before the stepwise hypoxia protocol was repeated. After the second hypoxic exposure, the animals were killed with MS-222 overdose (1 g l^{-1}) in the experimental chamber, and the stomach contents were carefully separated, blotted dry and weighed.

Data acquisition, calculations and statistical analysis.

The flow signals from the Transonic flow meter were fed directly into a Dell Latitude Xpi Laptop computer running Labview (National Instruments version 5.1). The flow signals were sampled at 20 Hz. Heart rate (f_H) was obtained from the pulsatile flow signals. Stroke volume (V_S) was calculated as $\dot{Q}/f_H$. $\dot{Q}$ and $\dot{q}_{GI}$ were normalized per animal body mass. Data are presented in graphs as means ± S.E.M.

Wilcoxon's signed-ranks test for paired (two-tailed) and Mann–Whitney *U* test for non-paired (two-tailed) samples were used to evaluate the statistical significance. The level of statistical significance was set to $P<0.05$. In cases of repeated tests, a modified Bonferroni procedure was used (Holm, 1979).

Results

The corrosion cast revealed that the common coeliaco-mesenteric artery (CMA) branches off at the root of the dorsal aorta, at the point where the bilateral efferent branchial arteries

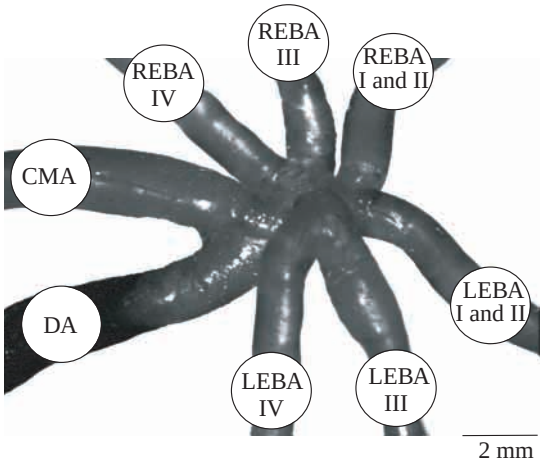

Fig. 1. Ventral view of a vascular cast showing the origin of the celiaco-mesenteric artery in a 360 g sea bass. REBA, right efferent branchial arteries (I and II, III, IV); LEBA, left efferent branchial arteries (I and II, III, IV); DA, dorsal aorta; CMA, coeliaco-mesenteric artery.

converge, as shown in Fig. 1. The mesenteric artery branches off the common coeliaco-mesenteric artery a few millimetres downstream. The stomach contents in non-instrumented fish (N=6) were voided within 48 h. 24 h after force-feeding with 2.9% of their body mass, the stomach contents had decreased to 28±5% of the amount fed, and this further decreased significantly to 7±3% after 36 h and to 0.5±3% after 48 h. In the instrumented fish (N=7), the stomach contents 24 h after feeding were significantly reduced to 62±4% of the amount fed, which was significantly higher in comparison than the value in non-instrumented fish 24 h post-feeding.

$\dot{Q}$ after feeding increased significantly by 15% (from 40.0±3.4 to 45.5±4.2 ml min^{-1} kg^{-1}, Fig. 2) and $\dot{q}_{GI}$ by 71% (from 9.6±1.6 to 14.9±1.6 ml min^{-1} kg^{-1}, Fig. 3) in normoxic conditions. Before feeding, $\dot{q}_{GI}$ constituted 24.0±3.2% of total cardiac output, and this increased significantly 24 h after feeding to 34.0±4.4% (Fig. 3).

During hypoxia alone, $\dot{Q}$ decreased significantly from 40.0±3.4 ml min^{-1} kg^{-1} in normoxia (20.6 kPa) to 29.8±2.5 ml min^{-1} kg^{-1} at P_{O_2}=3.9 kPa. f_H decreased significantly from 51.3±3.8 to 26.0±2.4 min^{-1}, while V_S increased significantly from 0.80±0.08 to 1.18±0.11 ml kg^{-1} (Fig. 2). Similar qualitative changes occurred 24 h after feeding, but $\dot{Q}$ post-feeding decreased to 24.7±1.7 ml min^{-1} kg^{-1}, which was significantly lower than pre-feeding values at this P_{O_2} (Fig. 2).

Hypoxia alone decreased $\dot{Q}_{GI}$ significantly from 9.6±1.6 ml min^{-1} kg^{-1} in normoxia (20.6 kPa) to 3.7±1.1 ml min^{-1} kg^{-1} at P_{O_2}=3.9 kPa (Fig. 3); this corresponded to a decrease in $\dot{q}_{GI}/\dot{Q}$ from 24±3 to 13±4%. After feeding, gastrointestinal blood flow decreased significantly from 14.9±1.6 ml min^{-1} kg^{-1} in normoxia (20.6 kPa) to 6.5±1.3 ml min^{-1} kg^{-1} at P_{O_2}=3.9 kPa (Fig. 3) but there was no change in $\dot{q}_{GI}/\dot{Q}$ because of the concomitant greater drop in $\dot{Q}$.

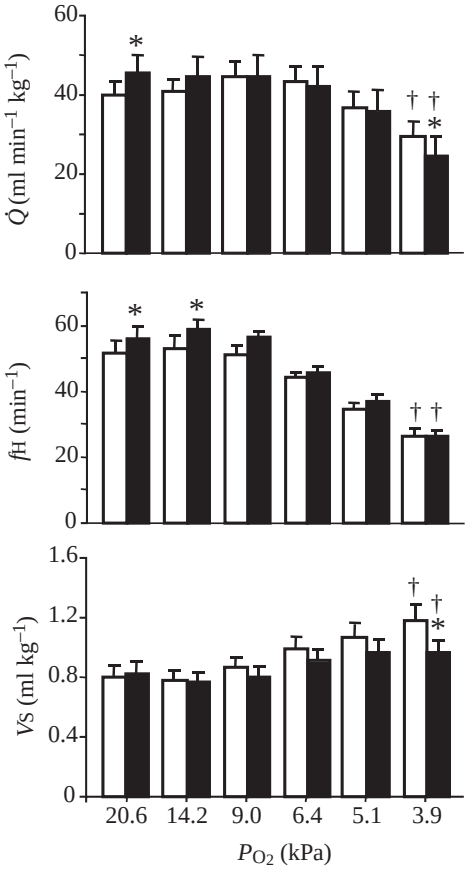

Fig. 2. Effects of a stepwise decrease in water oxygen partial pressure (P_{O_2}) on cardiac output ($\dot{Q}$), heart rate (f_H) and stroke volume (V_S) before (open columns) and after feeding (filled columns). Values are means ± S.E.M. (N=7). Asterisks indicate a significant difference between control (unfed) and fed group, daggers indicate significant differences from previous hypoxia level.

Discussion

To our knowledge, these are the first published data on the effects of hypoxia on gastrointestinal blood flow before and after feeding, a combination that these fish may encounter in their natural habitat. It is also the first cardiovascular study on sea bass involving variables other than heart rate. Our control heart rates were slightly higher than those recorded previously using telemetry (Lefrançois et al., 1998) (51 min^{-1} *versus* 40–45 min^{-1}). This is probably because our animals were directly attached to the recording equipment, it has been shown that heart rate can be affected by the experimental protocol (Altimiras and Larsen, 2000). Control cardiac output was higher than the values reported for any other teleost from temperate latitudes, but was lower than resting $\dot{Q}$ in tuna and Antarctic haemoglobinless species (Farrell and Jones, 1992). The coeliaco-mesenteric artery (CMA) in the sea bass arises as a single artery from the conjunction of all the branchial arteries and the dorsal aorta (Fig. 1). This is very similar to the anatomical arrangement in lingcod *Ophiodon elongatus* and sea raven *Hemitripterus americanus* (Farrell et al., 2001). In

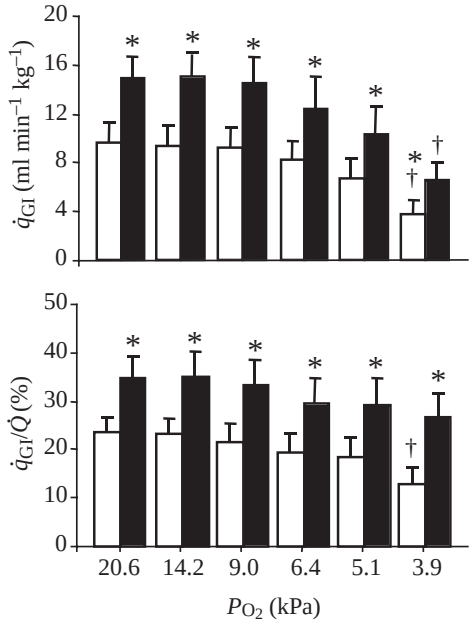

Fig. 3. Effects of a stepwise decrease in water oxygen partial pressure (P_{O_2}) on absolute ($\dot{q}_{GI}$) and relative ($\dot{q}_{GI}/\dot{Q}$) gastrointestinal blood flow before (open columns) and after feeding (filled columns). Values are means ± S.E.M. (N=7). Asterisks indicate a significant difference between control (unfed) and fed group, daggers indicate significant differences from previous hypoxia level.

other teleost species, such as the coho salmon *Oncorhynchus kisutch* and the Atlantic cod (*Gadus morhua*), the CMA branches from the dorsal aorta more posterior to the junction of the branchial efferent arteries. In the sea bass, the common CMA is short and branches to form the coeliac artery and the mesenteric artery. As in other teleosts, except salmonids, the coeliac artery has a larger diameter and supplies the stomach and the anterior portion of the intestine, while the smaller mesenteric artery supplies the remainder of the intestine. Coeliac blood flow is presumably greater than mesenteric blood flow, but in the present study blood flow was measured in both vessels simultaneously. In salmonids, the CMA branches into two vessels termed the gastric and intestinal arteries, the latter analogous to the mesenteric artery, and the CMA carries around 85% of the total gut blood flow (Thorarensen et al., 1991). The significance of these differences in the anatomy of the vasculature is not known.

The kinetics of digestion in fish has been studied in a variety of species, Sea bass of 26 g body mass fed 2% of their body mass emptied the stomach in 39 h at 15°C (Santulli et al., 1993). Assuming that the effect of body mass on gastric emptying time (GET) is similar to that in other species, a GET of 23 h is predicted for the sea bass used in the present study. Under similar conditions of body mass, amount of food and temperature, GET was 16 h for European plaice *Pleuronectes platessa* (Basimi and Grove, 1985) and 23 h for turbot *Scophthalmus maximus* and dab *Limanda limanda* (Grove et al., 1985; Gwyther and Grove, 1981).

GET in our non-instrumented fish was approximately 48 h, which is much greater than the predicted 23 h; it was even longer in the instrumented fish, in which the stomach contents after 24 h were still above 60%, indicating that force-feeding, anaesthesia and surgery delay gastric emptying, as previously reported in Atlantic cod (Dos Santos and Jobling, 1988) and rainbow trout *Oncorhyncus mykiss* (Olsson et al., 1999). The aim of the present study was not to determine the absolute gastric emptying time but to compare pre-prandial *versus* post-prandial states and the effects of hypoxia on gastrointestinal blood supply in these two different states. In the instrumented animals, the high gastric content 24 h after feeding was accompanied by an increased blood supply to the gut.

Relative splanchnic blood flow before feeding (24% of cardiac output) was within the range reported in other species: 40% in Atlantic cod (Axelsson and Fritsche, 1991), 36% in chinook salmon *Oncorhynchus tshawytscha* (Thorarensen et al., 1993), 35% in red Irish lord *Hemilepidotus hemilepidotus* (Axelsson et al., 2000), 25% in white sturgeon *Acipenser transmontanus* (Crocker et al., 2000) and 16% in sea raven (Axelsson et al., 1989). After feeding, splanchnic blood flow increased by 71±19% from 9.6±1.6 to 14.9±1.6 ml min^{-1} kg^{-1}). This increase is similar to that reported in Atlantic cod fed a similar food ratio (60%; Axelsson and Fritsche, 1991). Greater increases have been observed in sea raven (coeliac blood flow more than doubled; Axelsson et al., 1989) and red Irish lord (112% increase in coeliac flow 4 days after feeding; Axelsson et al., 2000) force-fed greater ratios (7–9% and 10–15% of body mass, respectively).

Red Irish lord and sea raven are both benthic ambush feeders with a relatively low level of activity compared with the pelagic highly active sea bass. In pelagic species, the active muscles need to be supplied with oxygen, and any increase in demand from other vascular circuits such as the gastrointestinal tract must be met either by a redistribution of blood or by an increased cardiac output. The post-prandial increase in gut blood flow of 5.3±0.9ml min^{-1} kg^{-1} is paralleled by an increase in cardiac output of 5.4±2.1 ml min^{-1} kg^{-1}. This increase is largely due to increase in heart rate, as in red Irish lord (Axelsson et al., 2000) and Atlantic cod (Axelsson and Fritsche, 1991). This match between the increase in gastrointestinal blood flow and cardiac output is in contrast to the post-prandial increase in splanchnic blood flow in mammals, which involves an additional redistribution of blood from other tissues (Matheson et al., 2000; Vatner et al., 1974).

During mild hypoxia, $\dot{Q}$ is maintained despite the reduction in f_H. This is due to a compensatory increase in V_H, similar to what has been reported in rainbow trout (Wood and Shelton, 1980) and Atlantic cod (Fritsche and Nilsson, 1989). At a P_{O_2} of 3.9 kPa $\dot{Q}$ decreases because of a significant bradycardia without inotropic compensation and, at the same time, $\dot{q}_{GI}$ decreases by a relatively greater percentage (61% at 3.9 kPa compared with normoxia). In Atlantic cod, coeliac blood flow decreased by more than 40% and mesenteric flow by more than 60% during hypoxia (Axelsson and Fritsche, 1991). Thus, these results provide ample evidence that hypoxia affects the

splanchnic blood flow in sea bass, similar to in other fish species, and that feeding itself also induces an increase in splanchnic blood flow.

Interestingly, and contrary to our initial hypothesis, relative splanchnic blood flow ($\dot{q}_{GI}/\dot{Q}$) during hypoxia did not decrease after feeding. Indeed $\dot{q}_{GI}/\dot{Q}$ decreased significantly before feeding but not after feeding (Fig. 3), indicating that, even when facing a hypoxic challenge, gut blood flow is maintained at the expense of other systems. Salmonids are able to digest food and swim at the same time, but this occurs at a cost, namely a 10–15% reduction in the maximum prolonged swimming performance (Thorarensen, 1994).

Similarly, regional blood flow must be readjusted when oxygen availability is reduced. Atlantic cod are known to regulate food intake during hypoxia. They exhibit a direct relationship between growth rate and oxygen availability, in the absence of changes in food conversion efficiency. Of the reduction in growth, 97% can be accounted for by reduced food consumption (Chabot and Dutil, 1999).

Although behavioural regulation of food intake may occur in sea bass as well, our results suggest that once food has been eaten it is digested and absorbed, even at the expense of a reduced oxygen supply to other organs.

A simple explanation of our results could be that sea bass lack reflex mechanisms to regulate $\dot{q}_{GI}$. However, we consider this highly unlikely, given that reflex control of the splanchnic circulation does occur in conditions such as fright or exercise (M. A., J. A. and G. C., unpublished data). During fright episodes, the reduction in $\dot{Q}$ is independent of the reduction in $\dot{q}_{GI}$, the latter recovering more slowly. This probably indicates an independent reflex vasoconstriction of the splanchnic circulation, as has been shown in other species (Farrell et al., 2001).

An alternative explanation for the post-prandial maintenance of $\dot{q}_{GI}/\dot{Q}$ during hypoxia could be as follows. Preprandial regulation of $\dot{q}_{GI}$ (as occurs during hypoxia or exercise) is largely dependent on reflex vasoconstriction of the gut vasculature. Following feeding, splanchnic blood flow increases as response to the release of local factors such as nitric oxide or adenosine (Matheson et al., 2000). Once this response has started, reflex vasoconstriction may not be as effective in decreasing $\dot{q}_{GI}$ because local hyperaemia outcompetes reflex regulation.

Although this hypothesis is indirectly supported by the present results, further studies are required to investigate the control mechanisms involved. In mammals, it has been shown that the initiation and maintenance of post-prandial hyperaemia is complex and involves gastrointestinal activity, tissue oxygenation levels, the enteric nervous system, gastrointestinal peptides and paracrine substances as well as sympathetic neuronal activity (Chou and Coatney, 1994; Vanner and Surprenant, 1996).

We are indebted to Michel Prineau and Marcel Guillaut for their technical assistance in different stages of the experiments. Travelling expenses were covered by IFREMER. The Conseil Régional Poitou-Charentes provided funds for accomodation and experimental costs. J.A. was in receipt of a EU postdoctoral fellowship (TMR Contract no. ERBFMBICT982940) and M.A. is supported by the Swedish Research Council.

References

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

Axelsson, M. and 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., Thorarensen, M., 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.* **170**, 145-152.

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! *Fish Physiol. Biochem.* **22**, 89-94.

Azzaydi, M., Madrid, J. A., Zamora, S., Sánchez-Vázquez, F. J. and Martínez, F. J. (1998). Effect of three feeding strategies (automatic, *ad libitum* demand-feeding and time-restricted demand-feeding) on feeding rhythms and growth in European sea bass (*Dicentrarchus labrax* L.). *Aquaculture* **163**, 285-296.

Basimi, R. A. and Grove, D. J. (1985). Gastric emptying rate in *Pleuronectes platessa*. *J. Fish Biol.* **26**, 545-552.

Chabot, D. and Dutil, J.-D. (1999). Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish Biol.* **55**, 472-491.

Chou, C. C. and Coatney, R. W. (1994). Nutrient-induced changes in intestinal blood flow in the dog. *Br. Vet. J.* **150**(5), 423-437.

Claireaux, G., Webber, D. M., Lagardère, J.-P. and Kerr, S. R. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *J. Sea Res.* **44**, 257-265.

Crocker, C. E., Farrell, A. P., Gamperl, A. K. and Cech, J. J., Jr (2000). Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *Am. J. Physiol.* **279**, R617-R628.

Diaz, R. J. and Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of the benthic fauna. *Oceanogr. Mar. Biol. Annu. Rev.* **33**, 245-303.

Dos Santos, J. and Jobling, M. (1988). Gastric emptying in cod, *Gadus morhua* L.: effects of food particle size and dietary energy content. *J. Fish Biol.* **33**, 511-516.

Farrell, A. P. and Jones, D. R. (1992). The heart. In *Fish Physiology*, vol. XIIB (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1-87. New York: Academic Press.

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

Fritsche, R. and Nilsson, S. (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Exp. Biol.* **48**, 153-160.

Fritsche, R., Axelsson, M., Franklin, C. E., Grigg, G. G., Holmgren, S. and Nilsson, S. (1993). Respiratory and cardiovascular responses to hypoxia in the Australian lungfish. *Resp. Physiol.* **94**, 173-187.

Grove, D. J., Moctezuma, M. A., Flett, H. J. R., Foott, J. S., Watson, T. and Flowerdew, M. W. (1985). Gastric emptying and the return of appetite in juvenile turbot, *Scophthalmus maximus* L. fed on artificial diets. *J. Fish Biol.* **26**, 339-354.

Gwyther, D. and Grove, D. J. (1981). Gastric emptying in *Limanda limanda* and the return of appetite. *J. Fish Biol.* **18**, 245-260.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65-70.

Lefrançois, C., Claireaux, G. and Lagardère, J.-P. (1998). Heart rate telemetry to study environmental influences on fish metabolic expenditure. *Hydrobiologia* **371/372**, 215-224.

Matheson, P. J., Wilson, M. A. and Garrison, R. N. (2000). Regulation of intestinal blood flow. *J. Surg. Res.* **93**, 182-196.

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

Priede, I. G. (1985). Metabolic scope in fish. In *Fish Energetics, New Perspectives* (ed. P. Tyler and P. Calow), pp. 33-64. London: Croom Helm.

Santulli, A., Modica, A., Cusenza, L., Curatolo, A. and D'Amelio, V. S. O. (1993). Effects of temperature on gastric evacuation rate and absorption and transport of dietary lipids in sea bass (*Dicentrarchus labrax*, L.). *Comp. Biochem. Physiol.* **105A,** 63-367.

Thorarensen, H. (1994). Gastrointestinal blood flow in chinook salmon (*Oncorhynchus tshawytscha*). PhD thesis. Vancouver: Simon Fraser University.

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 haematocrit on blood flow distribution. *J. Exp. Biol.* **179**, 115-129.

Thorarensen, H., McLean, E., Donaldson, E. M. and Farrell, A. P. (1991). The blood vasculature of the gastrointestinal tract in chinook, *Oncorhynchus tshawytscha* (Walbaum), and coho, *O. kisutch* (Walbaum), salmon. *J. Fish Biol.* **38**, 525-531.

Vanner, S. and Surprenant, A. (1996). Neural reflexes controlling intestinal microcirculation. *Am. J. Physiol.* **271**, G223-G230.

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

Wood, C. M. and Shelton, G. (1980). The reflex control of heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage, and systemic vasomotor tone. *J. Exp. Biol.* **87**, 271-284.