AKADEMISK AVHANDLING

On the Regulation of Postprandial Gastrointestinal Blood Flow in Teleost Fish

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DISSERTATION ABSTRACT

Henrik Seth (2010) **On the Regulation of Postprandial Gastrointestinal Blood Flow in Teleost Fish** Department of Zoology/Zoophysiology, University of Gothenburg, Box 463, 405 30 Göteborg.

The regulation of the cardiovascular changes, in particular the increase in gastrointestinal blood flow that follows after feeding has received little attention in teleost fish. Therefore, the aim of the research that led to this thesis was to discern some of the mechanisms behind the postprandial cardiovascular response.

Several methods, described within this thesis, were used in order to study, *in vivo*, the influence of both mechanical as well as chemical stimuli in triggering the increase in gastrointestinal blood flow that occurs after feeding in fish. Furthermore, additional methods, combining *in vivo* and *in situ* pharmacology were used to study the regulatory mechanisms in more detail.

The results indicate that both mechanical as well as chemical stimuli are important during the postprandial response. Mechanical stimuli within the stomach evoke an increased adrenergic tone and chemical stimuli induce a subsequent hyperemia that is localized within the gastrointestinal tract. The response to chemical stimuli is also influenced by the composition of the diet. Furthermore, even though the extrinsic innervation (*sympathetic* and *parasympathetic*) of the gastrointestinal tract is important in controlling the routine tone of the gastrointestinal vasculature, it is of little importance during the postprandial hyperemia. In contrast, the intrinsic innervation (*enteric*) within the gastrointestinal tract is of fundamental importance to this hyperemia. In addition, the response is most likely modulated, in response to the diet composition, by endocrine and paracrine factors, such as the gastrointestinal hormone cholecystokinin.

In conclusion, the regulation of the gastrointestinal vasculature after feeding is very complex and several mechanisms contribute to the cardiovascular response that will depend on the composition of the diet as well as surrounding environmental factors such as temperature, oxygen levels and stress.

Key words: Rainbow trout (*Oncorhynchus mykiss*), Shorthorn sculpin (*Myoxocephalus scorpius*), Diet composition, Oxygen consumption, Mechanical stimuli, Chemical stimuli.

To my wife – My everything

PAPERS

This thesis is based on the following papers, which in the text are referred to by their Roman numerals:

I. Seth H, Sandblom E, Holmgren S, Axelsson M. (2008) Effects of gastric distension on the cardiovascular system in rainbow trout (*Oncorhynchus mykiss*). Am J Physiol Regul Integr Comp Physiol. **294**, R1648-1656.

II. Seth H, 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**, R171-177.

III. Seth H, Sandblom E, Axelsson M. (2009) Nutrient-induced gastrointestinal hyperemia and specific dynamic action (SDA) in rainbow trout (*Oncorhynchus mykiss*) - Importance of proteins and lipids. Am J Physiol Regul Integr Comp Physiol. **296**, R345-352.

IV. Seth H, Axelsson M. (2010) Sympathetic, parasympathetic and enteric regulation of the postprandial gastrointestinal hyperemia in rainbow trout (*Oncorhynchus mykiss*). (*Under revision*).

V. Seth H, Gräns A, Axelsson M. (2010) Cholecystokinin (CCK) as a potential regulator of cardiac function and postprandial gut blood flow in rainbow trout (*Oncorhynchus mykiss*). Am J Physiol Regul Integr Comp Physiol. **298**, R1240-1248.

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ABBREVIATIONS

5-HT	Serotonin
ACh	Acetylcholine
ATP	Adenosine triphosphate
BW	Body weight
CCK	Cholecystokinin
CGRP	Calcitonin gene-related peptide
CM	Circular muscle layer
CMA	Coeliacomesenteric artery
CNS	Central nervous system
C_v	Vascular compliance
CO	Cardiac output
GBF	Gastrointestinal blood flow
GET	Gastric emptying time
GI	Gastrointestinal
HI	Heat increment
HR	Heart rate
IPAN	Intrinsic primary afferent neuron
LM	Longitudinal muscle layer
M	Mucosa
MCFP	Mean circulatory filling pressure
MO_2	Metabolic oxygen consumption
MP	Myenteric plexus
NE	Norepinephrine
NO	Nitric oxide
NTS	Nucleus tractus solitarius
PO_2	Partial pressure of oxygen
Q_{cma}	Coeliacomesenteric blood flow
R_{cma}	Coeliacomesenteric vascular resistance
R_{coel}	Coeliac vascular resistance
R_{sys}	Systemic vascular resistance
R_{res}	Somatic vascular resistance
SDA	Specific dynamic action
SEM	Standard error of mean
SM	Submucosa
SMP	Submucosa plexus
SNP	Sodium nitroprusside
SV	Stroke volume
TTX	Tetrodotoxin
VIP	Vasoactive intestinal polypeptide
VMC	Vasomotor center

INTRODUCTION

Since the origin of multicellular animals, there has been a specialization of different cells into discrete tissues. The subsequent evolution of specialized organs such as diverse excretory organs, a cardiovascular system and the gastrointestinal tract made these organisms efficient and allowed an increase in size. Today, we see animals that have tailored their lifestyle to fit environments ranging, from the dry deserts to the hydrothermal vents of the deep oceans. The gastrointestinal tract must enable an efficient digestion and absorption of a wide variety of nutrients derived from meals, ranging in quality and composition, and therefore different animals show an enormous diversity when it comes to the morphology and physiology of the gastrointestinal tract. Also within the paraphyletic group of fish there are a wide variety of specializations or adaptations that enable an efficient digestion, absorption and redistribution of nutrients.

The gastrointestinal blood flow is very important to these physiological processes and it should come as no surprise that it is closely regulated with respect to food intake and nutrient composition. The gastrointestinal vasculature supplies the gastrointestinal tissues with oxygenated blood and facilitates the transport of hydrolyzed and absorbed nutrients from the gastrointestinal mucosa to other parts of the intestine as well as the liver and the systemic circulation. However, it is still unclear how the gastrointestinal blood flow is regulated both in unfed and fed teleost fish and how it is influenced by various external and internal factors. Furthermore, there is limited knowledge concerning the link between the postprandial (i.e. after eating) hyperemia and the postprandial increase in oxygen consumption.

Gastrointestinal vasculature

The gastrointestinal (GI) tract of most teleost species studied to date is, in contrast to most mammalian species, supplied mainly (with minor exceptions) via one major vessels, the coeliacomesenteric artery (CMA), which branches off the dorsal aorta (Figure 1). The CMA then divides into two major arteries, the larger intestinal artery (also often referred to as the mesenteric artery) and the smaller gastric artery (coeliac artery) (for more

details see **Paper II** and **III)**. These vessels then divide into progressively smaller vessels, supplying, for example, the gonads, the stomach and the intestine as well as the liver with blood. The liver also receives venous blood via the portal circulation (Thorarensen et al., 1991). In elasmobranchs (sharks, rays and skates) the GI tract receives blood via several distinct vessels and in the spiny dog fish (*Squalus acanthias*) these are referred to as the coeliac artery, the mesenteric artery and the lienogastric artery (Farrell et al., 2001; Holmgren et al., 1992). Whereas in mammals they are instead called the coeliac artery, the superior mesenteric artery and the inferior mesenteric artery (Matheson et al., 2000).

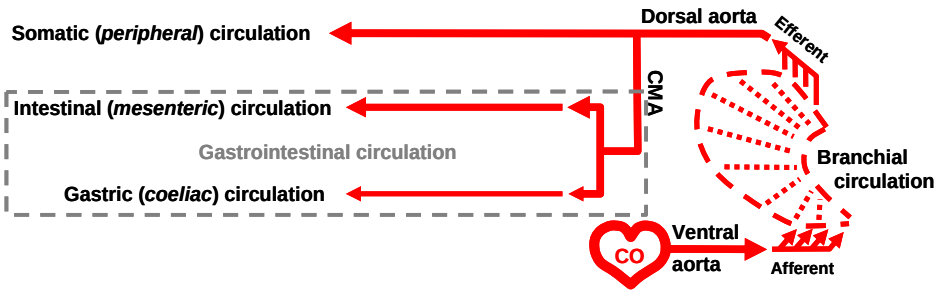

Figure 1. Schematic illustration of the vasculature from a teleost fish. The somatic circulation supplies most tissues, such as muscles and skin, with blood. The gastrointestinal tract is supplied via the coeliacomesenteric artery (CMA) that divides into two major circulations, the intestinal and the gastric. Afferent and efferent denotes the vasculatures leading to and from the gills (branchial circulation), respectively.

The anatomy of the vasculature of teleost fish as well as elasmobranchs has traditionally been investigated using the corrosion cast technique (Murakami, 1975). In this technique the vasculature of the euthanized animal is first perfused with saline with an added vasodilator such as sodium nitroprusside. Once the vasculature has been fully perfused a two-component epoxi plastic is carefully injected and allowed to cure. The organic tissues of the animal are then removed using potassium hydroxide to expose the cast of the vasculature.

This technique has revealed several differences among different teleost species (Farrell et al., 2001), possibly reflecting adaptations of the circulatiory system depending on the habitat and feeding regime. In the rather sedentary benthic shorthorn sculpin (*Myoxocephalus scorpius*), which has a very well vascularised GI tract, receiving a relatively large portion of cardiac output, there seems to be a peculiar anastomose where the branchial arteries unit to form the dorsal aorta, possibly enabling the animal to shunt oxygenated blood from the gills directly to the GI tract (**Paper II**).

In addition to the CMA there is also blood supply of the hind gut via two unpaired arteries connecting the dorsal aorta to the distal portion of the intestinal artery (Thorarensen et al., 1991). These vessels are smaller in diameter compared with the CMA, but their relative contribution is at present unknown. Consequently measurements of blood flow in only the CMA will lead to an underestimate of the total gastrointestinal blood flow (GBF).

Microvasculature of the gastrointestinal tract

Like in most tissues a substantial portion of the blood flow distribution in the GI tract is controlled at the level of the GI microvasculature, in contrast to the larger arteries. For example, in the sea raven (*Hemitripterus americanus*) adrenaline induce a substantial increase in the GI vascular resistance with no pressure difference between the dorsal aorta and the intestinal artery (Axelsson et al., 1989). Therefore, the adrenergic tone apparently operates at the arteriolar level. However, little is know about the anatomy of the GI microcirculation in fish and the main focus has been on the structures of the microvasculature within the gills (Dunel-Erb and Laurent, 1980; Laurent and Dunel, 1980; Olson, 2002; Sundin and Nilsson, 1992, 1997; Wilson and Laurent, 2002). Therefore it is at present unknown to what extent the control is mediated via changes in arteriolar diameter or pre-capillary sphincters (Soldatov, 2006).

The microvasculature of mammals has been extensively reviewed from both an anatomical and a physiological perspective (Gore and Bohlen, 1977; Rhodin, 1967).

Gastrointestinal blood flow

In the resting, undisturbed and unfed state, which in this text will be referred to as *routine*, the blood flow through the GI tract is regulated in order to maintain a flow that is sufficient for the housekeeping requirements. These include inter-digestive motility, basal secretion, osmotic regulation through for instance water uptake/excretion, as well as the regular metabolism of the cells of the GI tract. Several changes occur after the ingestion of a meal, both to the general circulation as well as more specifically to the GI circulation.

Regulation of routine blood flow

Nervous and humoral control

During routine conditions the GI tract in fish receives between 10% and 40% of cardiac output, with the lowest routine values reported in sea raven (Axelsson et al., 1989) and sea bass (*Dicentrarchus labrax*) (Dupont-Prinet et al., 2009) (Table 1). In contrast, the GI tract of the Atlantic cod (*Gadus morhua*) receives as much as 40% of cardiac output (Axelsson and Fritsche, 1991). This range is similar to the range reported for mammals (Matheson et al., 2000). The nervous regulation of blood flow could either be intrinsic (i.e. local within the gut) or extrinsic and thus mediated via the central nervous system. There has been a limited focus on the regulation of the GBF during routine conditions in fish. Based on mammalian studies an overview of the three major pathways that regulates the routine tone of the GI vasculature is presented in Figure 2.

Table 1. Cardiac output and gut blood flow in selected unfed fish as well as the percentage of cardiac output that passes through the coeliacomesenteric artery (ratio).

Species	Temp °C	Cardiac output (ml min-1 kg-1)	Gut blood flow (ml min-1 kg-1)	Ratio (%)	Source
Sea raven (*Hemitripterus americanus*)	10-12	18.8	2.9	15	Axelsson et al., 1989
Atlantic cod (*Gadus morhua*)	10-11	19	4.1/3.5	40	Axelsson and Fritsche., 1991
Red Irish lord (*Hemilepidotus hemilepidotus*)	7-9	24	4.1/4.9	34	Axelsson et al., 2000
Sea bass (*Dicentrarchus labrax*)	16	40	9.6	24	Axelsson et al., 2002
Sea bass (*Dicentrarchus labrax*)	22-23	51.4	13.8	27	Altimiras et al., 2008
Sea bass (*Dicentrarchus labrax*)	19	43.4	4.3	10	Dupont-Prinet et al., 2009
Rainbow trout (*Oncorhynchus mykiss*)	11-16	N/A	4-6	N/A	Eliasson et al., 2008
Chinook salmoon (*Oncorhynchus tshawytscha*)	8-11	33	12-14.2	36	Thorarensen and Farrell., 1993

Multiple values (X/Y) indicate flow through the celiac (X) and mesenteric artery (Y), respectively. N/A denotes a missing value.

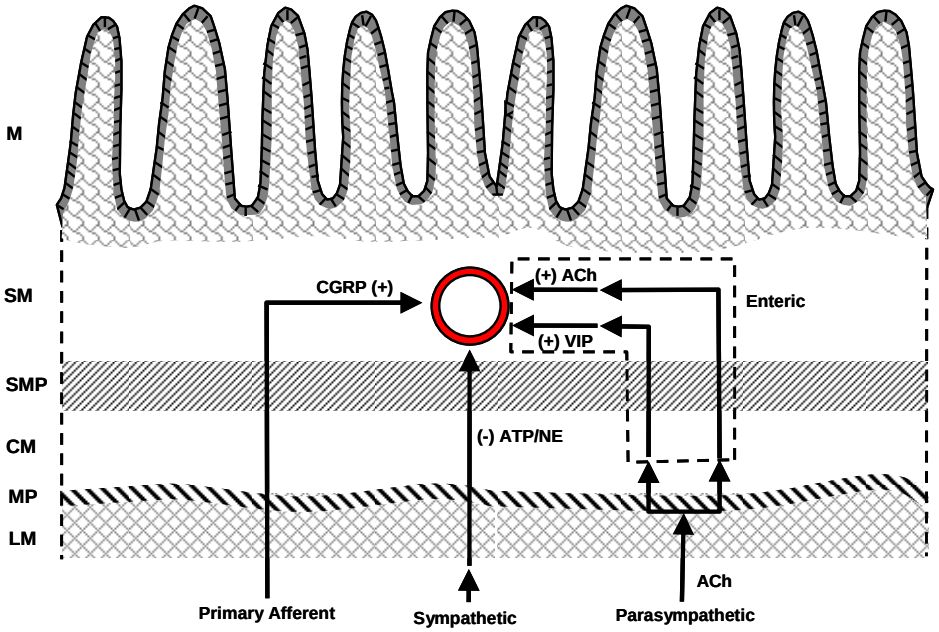

Figure 2. The three major pathways in which routine gastrointestinal blood flow is regulated, at the level of the submucosal arteriole, in mammals. Sympathetic input maintains a vasoconstrictive tone, whereas, primary afferents and parasympathetic innervation, either directly or indirectly (via enteric neurons) impose a vasodilator tone. ACh: Acetylcholine, ATP: Adenosine triphosphate, CGPR: Calcitonin gene-related peptide, CM: Circular muscle layer, LM: Longitudinal muscle layer, M: Mucosa, MP: Myenteric plexus, NE: Norepinephrine (noradrenaline), SM: Submucosa, SMP: Submucosa plexus, VIP: Vasoactive intestinal polypeptide.

In fish, most studies have focused on the regulation of the microvasculature within the gills (Nilsson and Sundin, 1998; Stenslokken et al., 2006; Sundin and Nilsson, 1997; Sundin and Nilsson, 1992; Sundin et al., 2003). A few other studies have focused on organs such as the head-kidneys (Brown, 1985; Elger et al., 1984) and the coronary circulation (Axelsson and Farrell, 1993; Mustafa and Agnisola, 1998).

There are, however, a few reports on the regulation within the GI tract in unfed fish. In sea raven there is an adrenergic tone that maintains the resistance of the GI vasculature (Axelsson et al., 1989). Such a mechanism is also present in unfed red Irish lord (*Hemilepidotus hemilepidotus*), where α-adrenergic blockade with phentolamine lowers the vascular resistance of both the coeliac and the mesenteric artery (Axelsson et al., 2000). This is in contrast

to the Atlantic cod, in which the adrenergic tone is limited to the mesenteric circulation (Axelsson and Fritsche, 1991). In the Altantic cod, the adrenergic tone was mediated via both humoral and nervous mechanisms, but it is often difficult to distinguish between the effects of circulating catecholamines and catecholamines released from nerves.

However, under routine conditions the circulating levels of catecholamines are often too low to significantly contribute to the resting adrenergic tone on the heart or the circulation in fish (Axelsson, 1988; Axelsson et al., 1987; Axelsson and Nilsson, 1986). Others have shown that in the trout at least the adrenergic tone on the heart can be influenced by circulating catecholamines, especially at low temperatures (Graham and Farrell, 1989). At present the general opinion is that the adrenergic tone on the GI vasculature in fish is chiefly derived from nerves. The presence of a nerve mediated sympathetic tone on the GI vasculature was also confirmed in **Paper IV**.

In mammals, nitric oxide (NO) is important in the regulation and control of resting GBF (Alemayehu et al., 1994). However, it seems as though fish (at least salmonids), in contrast to mammals, lack a endothelial-derived non-prostanoid relaxing factor (i.e. NO) (Olson and Villa, 1991). Therefore it is still under debate whether or not NO is synthesized and released from nerves only or if there is also an endothelial subform in fish (Olson and Donald, 2009). It is likely that prostaglandins might provide a function, in fish, comparable to that of NO in mammals (Jennings et al., 2004; Shahbazi et al., 2002) and prostaglandins could thus be important in maintaining the routine vascular tone (Kågström and Holmgren, 1997) as well as having a potential postprandial role.

Autoregulation

In mammals the GI vasculature, particularly the distal parts (i.e. the colon) is relatively poorly autoregulated (Granger et al., 1982; Kvietys et al., 1980b), i.e. there is a limited ability of the organ in itself to maintain a constant blood flow during fluctuations in arterial blood pressure. Whether or not this holds true also in fish remains to be determined, but it indicates that there is an increased dependence of the vasculature on a coordinated central input, *via* for example the autonomic nerves, in addition to myogenic as well as metabolic mechanisms (Kvietys et al., 1980b). Overall it seems as though arteriolar vasoconstriction is mainly metabolically induced or myogenic whereas autonomic innervation predominates in the control of the precapillary sphincters, at least in mammals (Shepherd, 1982).

Arteriolar vasoconstriction or precapillary sphincters

A few studies in fish have revealed structures similar to the pre-capillary sphincters of the mammalian vasculature. In the spleen of the rainbow trout (*Oncorhynchus mykiss*) there are sphincter-like structures that probably regulate the blood flow through this organ (Kita and Itazawa, 1990). In addition, peculiar pericyte like structures filled with actin filaments have been found in arterioles of the sheepshead minnow (*Cyprinodon variegatus*) (Couch, 1990). These structures could potentially have a similar function to mammalian precapillary sphincters but their contribution to the resting tone of the vasculature remains to be determined. In mammals, the contribution of either arteriolar diameter or precapillary sphincters depends on the stimuli. During a modest decrease in the tissue oxygen tension, the perfusion of the downstream tissue is regulated mainly by means of the precapillary sphincters. However, when the metabolically induced lowering of the tissue PO_2 becomes more severe there is shift in the control from the precapillary sphincters towards the upstream arterioles (Granger et al., 1975; Granger and Shepherd, 1973).

Therefore, if the regulation of GI blood flow in fish would depend on both precapillary sphincters and arteriolar tone, it is important to acknowledge that there are important physiological differences in how these regulate vascular resistance and the distribution of blood flow. Precapillary sphincters regulate the number of open GI capillaries and as such adjust the diffusive distance of oxygen and the capillary exchange rate, whereas changes in arteriolar diameter influence the vascular resistance and thus flow. A change in arteriolar diameter thus controls and maintains capillary PO_2, whereas precapillary sphincters regulate the diffusion of oxygen from the capillaries to the tissue. In theory the arteriolar diameter and the precapillary sphincters should be controlled independently although a change in either will affect the other.

Regulation of GI blood flow after feeding

Even before food enters the GI tract, several physiological cascades are initiated, at least in mammals ("Pavlonian reflex" and the cephalic gastrointestinal phase). These events include an increased secretion in various parts of the stomach (Lin and Alphin, 1957) and the intestine (Sarles et al., 1968), changes in the GI motility (Katschinski et al., 1992), and several hemodynamic changes (Fronek and Stahlgren, 1968; Vatner et al., 1970b; Vatner et al., 1974). However, little is known about these initial events in the regulation of GBF in teleosts.

Magnitude of the postprandial response

The increase in GBF after the ingestion of a normal sized meal (Table 2) ranges from around 70% in the sea bass (*Dicentrarchus labrax*) (Axelsson et al., 2002) to over 150% in the sea bass and the rainbow trout (Dupont-Prinet et al., 2009; Gräns et al., 2009).

Table 2. Relative changes in postprandial cardiac output and gut blood flow in selected fish species as well as the percentage of cardiac output that passes through the coeliacomesenteric artery (ratio).

Species	Temp °C	Meal size (% b.w.)	Cardiac output (%)	Gut blood flow (%)	Ratio (%)	Source
Sea raven (*Hemitripterus americanus*)	10-12	10-20	15.4	100	27-30	Axelsson et al., 1989
Atlantic cod (*Gadus morhua*)	10-11	2.2-3.5	23	72/42	52	Axelsson and Fritsche., 1991
Red Irish lord (*Hemilepidotus hemilepidotus*)	7-9	10-15	90	112/94	40	Axelsson et al., 2000
Sea bass (*Dicentrarchus labrax*)	16	2.9	13.5	71	34	Axelsson et al., 2002
Sea bass (only 6hr) (*Dicentrarchus labrax*)	22-23	2.7	22	82	40	Altimiras et al., 2008
Sea bass (*Dicentrarchus labrax*)	19	3	27	160	20	Dupont-Prinet et al., 2009
Rainbow trout (*Oncorhynchus mykiss*)	11-16	2	N/A	136	N/A	Eliasson et al., 2008
Rainbow trout (*Oncorhynchus mykiss*)	9-10	2	23-42	156	N/A	Gräns et al., 2009
Short-horn sculpin (*Myoxocephalus scorpius*)	10	8-10	50	93	N/A	Study II
Chinook salmoon (*Oncorhynchus tshawytscha*)	9-10	2	N/A	81	N/A	Thorarensen and Farrell., 2006

Multiple values (X/Y) indicate flow through the coeliac (X) and mesenteric (Y) artery, respectively. N/A denotes a missing value. Meal size is given as percentage of body weight (% bw).

These results are however difficult to compare given there are substantial differences in the experimental protocols as well as the methods used to measure blood flow. The blood flow in the sea raven was for example measured proximal to the bifurcation of the coeliacomesenteric artery whereas the blood flow in the red Irish lord was measured at both of its major branches, the mesenteric as well as the coeliac artery. The increase in the coeliac artery was 112% while the increase in the mesenteric artery was 94%. The cardiovascular response may also vary depending on factors such as meal size, temperature and the physical status of the animal. Nevertheless, in general there does not seem to be much difference depending on the lifestyle,

habitat or feeding regime, as the increase in GBF of sedentary ambush predators such as the shorthorn sculpin (**Paper II**) or the red Irish lord (Axelsson et al., 2000) is very similar compared with more active agile swimmers, such as salmonids like the Chinook salmon (*Oncorhynchus tshawytscha*) (Thorarensen and Farrell, 2006), or the rainbow trout (Eliason et al., 2008) as well as the sea bass (Altimiras et al., 2008; Axelsson et al., 2002; Dupont-Prinet et al., 2009).

Temporal pattern of the postprandial response

Most of the studied fish species show a relatively slow increase in the GBF after feeding (Figure 3). In the red Irish lord (Axelsson et al., 2000) there is a clear temporal pattern with an initial increase in the blood flow through the gastric artery (coeliac) and a subsequent increase in the blood flow through the intestinal artery (mesenteric), which corresponds to the movement of the chyme within the GI tract. A similar temporal pattern has also been seen in the Atlantic cod (Axelsson and Fritsche, 1991). In other species, such as, the sea raven (Axelsson et al., 1989) and the shorthorn sculpin (**Paper II**) the response lasts over several days, with a maximal increase at around 24 h post-feeding.

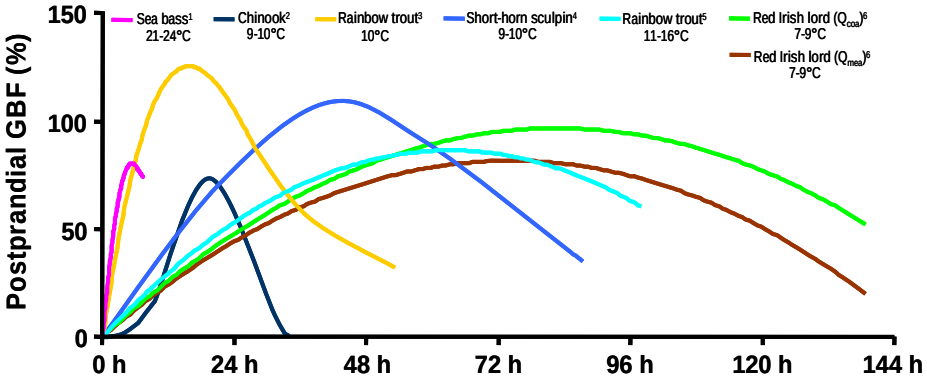

Figure 3. Illustration of the temporal pattern of the postprandial changes in gastrointestinal blood flow in selected fish. Feeding induces a substantial increase in the blood flow reaching the gastrointestinal tract, and the timing and duration of the response, varies between species and the ingested diet, but usually develops over 10-20 h and may persist for well over 72 h. [1]Altimiras et al., 2008; [2]Thorarensen and Farrell, 2006; [3]Gräns et al., 2009; [4]Seth and Axelsson, 2009; [5]Eliason et al., 2008; [6]Axelsson et al., 2000.

There are, however, exceptions and in the sea bass (*Dicentrarchus labrax*) there are reports of an almost instantaneous increase in the GBF (within 1 h) and after 6 hours it had increased by 82% (Altimiras et al., 2008). There is also a comparatively rapid increase in the rainbow trout, with the maximal increase occurring at roughly 12 h after feeding (Gräns et al., 2009). This is in contrast to another study in the rainbow trout where GBF increased slowly and persisted for well over 96 h.

The reason for these differences remains to be determined as there are no clear trends. The water temperature as well as the activity level of the animal most likely influences GBF, and it seems as though less active animals at lower temperatures show a prolonged response compared with more active swimmers such as the sea bass. However, this does not explain the difference between, for example, the two studies conducted in rainbow trout, which most likely reflect differences in the experimental protocols.

Table 3. Gastric emptying time including the initial lag phase in a number of fish species fed various diets.

Species	Temp °C	Meal (% of b.w.)	Lag phase (h)	Gastric emptying (h to 50%)	Gastric emptying (h to 100%)	Source
Rainbow trout (*Oncorhynchus mykiss*)	10-13	2% dry pellet	4	12-24	96	Bucking and Wood, 2006
Yellow perch (*Perca flavescens*)	22	0.4% dry pellet[1]	<2	6-7	10-14	Garber, 1983
		0.8% dry pellet[1]	<2	8-9	16-18	
Plaice (*Pleuronectes platessa*)	5	0.5 ml (0kJ/ml) kaolin[1]	N/A	~19	~37	Jobling, 1980
	5	0.5 ml (1.78kJ/ml) fish paste[1]	N/A	~23	~45	
	5	0.5 ml (2.97kJ/ml) fish paste[1]	N/A	~28	~56	
	5	0.5 ml (5.20kJ/ml) fish paste[1]	N/A	~34	~66	
Plaice (*Pleuronectes platessa*)	5	0.5 ml fish paste[1]	N/A	~36	<72	Jobling and Davies, 1979
	10	0.5 ml fish paste[1]	N/A	~20	~36	
	21	0.5 ml fish paste[1]	N/A	~12	<24	
Dab (*Limanda limanda*)	16	1% fish paste[1,2]	N/A	N/A	12.0	Jobling et al., 1977
		2% fish paste[1,2]	N/A	N/A	17.2	
		5% fish paste[1,2]	N/A	N/A	26.5	
Rainbow trout (*Oncorhynchus mykiss*)	12	0.5% dry pellet	0-5	16-24	>40	Olsson et al., 1999
Rainbow trout (*Oncorhynchus mykiss*)	10-12	3.2% fish	<10[3]	N/A	41-81	Ruohonen et al., 1997
		0.8% dry pellet	>20[3]	N/A	51-81	
Short-horn sculpin (*Myoxocephalus scorpius*)	10	8-10% fish	<1	35	>72	Study II
Rainbow trout (*Oncorhynchus mykiss*)	15	0.65% dry pellet	N/A	9	>24	Windell et al., 1969
	15	1.00% dry pellet	N/A	11	>24	
Rainbow trout (*Oncorhynchus mykiss*)	10	1.5% Oligochaetes	N/A	11.3	38.3	Windell et al., 1976
	20	2.0% Oligochaetes	N/A	4.5	16.4	
	10	0.97% Pellet	N/A	15.1	44.2	
	20	0.97% Pellet	N/A	5.6	16.4	

[1]Force-feeding, [2]Differently sized animals fed a 1 g diet, [3]4 out of 6 and 5 out of 6 animals, respectively, showed this response. Meal size is given as percentage of body weight (% bw).

Other factors, which should influence the GBF, is the postprandial gastric lag phase, i.e. the time it takes for the stomach to start emptying its content into the proximal intestine and the gastric emptying time (GET), i.e. the time it takes to empty the stomach (Table 3). Gastric emptying time and the length of the lag phase will depend on both the texture of the meal (solid to liquid) (Bucking and Wood, 2006; Ruohonen et al., 1997) as well as the temperature (Jobling and Davies, 1979; Windell et al., 1976) and the caloric content (Jobling, 1980). Sometimes there is also a lag phase of at least 2-3 h in animals fed a diet consisting of dry pellets (Bucking and Wood, 2006; Olsson et al., 1999; Ruohonen et al., 1997; Windell et al., 1969). In contrast there is virtually no lag phase in shorthorn sculpins fed a wet diet (**II**). The GET takes about 48-96 h, which about equals the time it takes for the GBF to return to pre-feeding values.

The start of gastric emptying and the GET is much more rapid in mammals, again depending on the texture of the diet. The shorter lag phase and the more rapid gastric emptying with a subsequent hydrolysis and release of composite macromolecules in the intestine in combination with the substantially higher core body temperature explains the more rapid increase in GBF of mammals compared with the values reported above for fish. Most studies in mammals report an increase in GBF within 5-10 minutes after feeding, with the maximal response occurring during the next 6 hours, whereby GBF returns to pre-feeding values (Fronek and Fronek, 1970; Fronek and Stahlgren, 1968; Hopkinson and Schenk, 1968; Takagi et al., 1988; Vatner et al., 1970a, 1974).

How is an increased gastrointestinal blood flow maintained?

An increase in the blood available to the GI tract during a hyperemia can be achieved either through an increase in the blood volume pumped by the heart per time unit (i.e. cardiac output; CO) and/or a redistribution of blood to the GI circulation from other systemic vascular beds without a concomitant increase in CO. The relative contribution of each factor is strongly influenced by the physical status of the animal (i.e. exercise or other stressors) as well as the environmental factors such as oxygen availability. Consequently, the postprandial increase in GBF will depend on both the decrease in resistance of the GI vasculature, i.e. the hyperemia, and how much blood that is available to the GI tract, either by an increase in CO or a redistribution of blood. Therefore, the terms hyperemia and GBF are not necessarily the same, although a GI hyperemia is usually associated with a concomitant increase in GBF, unless there is a decrease in the systemic blood pressure.

In most of the fish species studied the increase in GBF is sustained almost entirely through an increase in CO. In rainbow trout the postprandial increase in CO appears large enough to sustain, if not all, at least most of the increase in GBF (Eliason et al., 2008; Gräns et al., 2009). A large postprandial increase in CO has also been seen in Atlantic cod, sea raven and red Irish lord (Axelsson et al., 1989, 2000; Axelsson and Fritsche, 1991). Even though there is no redistribution of blood from the somatic circulation (i.e. systemic circulation excluding the visceral organs) there can still be a shift in the amount of CO reaching the GI tract. As discussed above, about 10-40% of CO reaches the GI tract in unfed fish. However, after feeding over 50% of CO reaches the GI circulation in the Atlantic cod (Axelsson and Fritsche, 1991). A shift in the amount of CO reaching the GI vasculature is mediated via a decrease in the resistance of the GI vasculature and a maintained or increased resistance of other systemic vascular beds.

These results are in strong contrast to what happens postprandially in mammals such as dogs. Several studies have shown that there is a limited increase in the CO after feeding in a stationary dog. The increase in GBF is therefore almost entirely due to a redistribution of blood. However, if the animal goes from beeing stationary to being more active there is in general also an increase in cardiac output in order to maintain the postprandial increase in GBF in dogs (Burns and Schenk, 1969; Gallavan et al., 1980; Hopkinson and Schenk, 1968; Vatner et al., 1970b). This has also been seen in primates (Vatner et al., 1974).

Triggers for postprandial increase in gastrointestinal blood flow

There are several possible ways in which the ingestion of food can trigger the postprandial increase in GBF. The cephalic phase which induces increased salivation and increased production of gastric acid is probably not important for a subsequent increase in GBF in dogs (Takagi et al., 1988). Whether or not this holds true in fish remains to be determined and there is little known about how the presence of "a meal" affects the GI physiology in fish, via for example olfaction or gustation. Therefore, the remaining sections will focus on the mechanical and chemical stimuli that occur when food enters the stomach and intestine.

Mechanical stimuli

One possible trigger for the cardiovascular changes associated with feeding in fish is the distension of the stomach when a meal is ingested. Previous studies in fish indicate that mechanical stimuli are of importance to,

for example, the relaxation of the stomach during a meal (Grove and Holmgren, 1992a, b), but there is a limited knowledge as to how mechanical stimuli might influence gut blood flow.

In mammals, several studies have focused on the cardiovascular effects of gastric distension and the general consensus is that the mechanical distension of the stomach accounts for most if not all of the increase in sympathetic activity that occurs soon after feeding (Longhurst et al., 1980, 1981; Longhurst and Ibarra, 1982, 1984; Nosaka et al., 1991; Pittam et al., 1988; Pozo et al., 1985). The increased sympathetic activity leads to an increased blood pressure with additional effects on the heart. However, there is no increase in the GBF with gastric distension alone. In contrast some studies indicate that a mechanical stimulus within the intestine induce a subseguent vasodilation, at least in cats (Biber et al., 1970, 1971).

The presence of food in the stomach is detected by mechanoreceptors. Vagal mechanoreceptors in the GI tract of mammals are of two main types based on their morphology. Stretch receptors function like muscle spindle afferents and tension receptors resemble the Golgi tendon organ. However, electrophysiological studies in mammals have so far only been able to identify one type of receptor that has the properties of an in-series tension receptor (Phillips and Powley, 2000), which is strange given that there is a need for two different types of receptors in order to be able to discriminate between active relaxation-contraction and the passive distension of the stomach during filling. Despite the conflicting results these receptors are usually defined as low-threshold receptors, found within the muscular layers of the GI tract that mediate the sensation of fullness or satiation (Ozaki et al., 1999). The perception of fullness is also most likely mediated via stretch receptors, although tension receptors might contribute depending on the activity of the stomach (Carmagnola et al., 2005). These vagal receptors are also involved in the gastrocolic reflex, which regulates the emptying of the bowels and are senzitised by various types of stimulus such as GI hormones as well as other substances such as glycerol.

In contrast to the low-threshold mechanoreceptors, high-threshold mechanoreceptors mediate the sensation pain and noxious stimuli via the splanchnic nerves (spinal nerves) in the rat (Ozaki and Gebhart, 2001), although it has been suggested that these spinal nerves are also activated by low-threshold stimuli (Furness et al., 1999). There are also additional rapidly adapting receptors in the mucosa of the GI tract that function more in an on-off manner and thus respond to changes in the movement of, for example, chyme through the intestine (Leek, 1977).

Vagal mechanoceptors show an increased response with an increase in the stretching of the stomach (Ozaki et al., 1999). The level of stretch will

depend on the amount of food ingested but also on the compliance (ability to stretch) of the stomach. Therefore, depending on the feeding habit of a fish the stomach will stretch more or less. A salmonid, like for example the rainbow trout, that feeds on small prey like insects and larvae will probably stretch the stomach to a lesser extent compared with an ambush predator such as the shorthorn sculpin that feeds on larger prey, sometimes up to 50-80% of their own bodyweight. These larger meals are also ingested more rapidly, leading to a very rapid and profound stretching of the stomach. However, the level of stretch will also depend on the unstretched size of the stomach and a larger meal does not necessarily stretch the stomach more.

Chemical stimuli

When food enters the stomach and subsequently the intestine, the meal is digested i.e. enzymatically broken down into smaller components by carbohydrases, lipases and proteases (Kitamikado and Tachino, 1960a, b, c). It is likely that it is these hydrolyzed products that induce the subsequent GI hyperemia and in mammals the largest increase in GBF occurs as food is hydrolyzed in the intestine (Chou and Coatney, 1994; Chou et al., 1978). However, even though there is a limited nutrient uptake in the stomach, there are gastric chemoceptors, that have the capability to detect the presence of, for example, noxious stimuli (Rozengurt, 2006) and/or the presence of certain food components (Nakamura et al., 2008; Tsurugizawa et al., 2009), and it is possible that these gastric receptors are also important in triggering an increase in the blood flow to the stomach as well as the rest of the GI tract.

In the red Irish lord (Axelsson et al., 2000), and perhaps also in the cod (Axelsson and Fritsche, 1991), there is a shift in the blood flow distribution when hydrolyzed food enters the intestine, from the coeliac artery supplying the stomach, towards the mesenteric circulation, supplying the major portion of the proximal and distal intestine as well as other vascular beds within the GI tract. This local increase in blood flow that coincides with the presence of food along the GI tract, seen also in most mammals (Gallavan and Chou, 1985; Matheson et al., 2000), can best be explained by a chemically induced hyperemia, i.e. a decrease in the vascular resistance.

At least in mammals, the composition of the ingested diet influences the cardiovascular response (Chou and Coatney, 1994; Gallavan and Chou, 1985). For example in dog, fatty acids induced the largest increase in GBF compared with carbohydrates and amino acids (Chou and Coatney, 1994), and bile enhace the response to most nutrients (Kvietys et al., 1980a, 1981a).

In fish it remains to be determined to what extent different nutrients influence and adjust this response. As shown in **Paper III**, a diet with a

composition similar to what the rainbow trout normally ingests gave the most profound hyperemia, indicating that there are indeed differences. This reasonable given that the physiology of the GI tract is adjusted to the composition of the most frequently ingested diet (Buddington et al., 1987, 1997; Buddington and Hilton, 1987). Still the GI tract can show remarkable plasticity with changes in the diet (Buddington and Hilton, 1987). For example, a carnivorous fish, like the rainbow trout that ingests very little carbohydrates and instead relies on proteins and fats for its metabolic energy needs, will most likely show a lesser response to carbohydrates.

It is also unclear whether or not the caloric content of the diet affects the hyperemic response. A recent study in rainbow trout indicated that the caloric content might be a key factor and there was no difference in the GBF with different iso-caloric diets (Eliason et al., 2008). However, as discussed below (see results and discussion) the connection between caloric content and GBF has not been established possibly due to a lack of knowledge concerning the postprandial GI metabolism.

Regulation of postprandial blood flow

Even though there are some basic ideas as to how the postprandial increase in GBF is elicited, the regulation that underlies this is complex and as a consequence there is, at present, no simple explanation as to how the postprandial GI hyperemia is regulated. However, a few possible mechanisms have been suggested, some of which are presented below. Even though one might predominate, several of these regulatory mechanisms are most likely involved in ultimately fine-tuning the response.

Central nervous control

In order to respond to changes in the demand at the tissue or organ level, there has to be a coordinated and continuous redistribution of blood within the cardiovascular system. The autonomic nervous system, divided into the sympathetic, parasympathetic and enteric is of fundamental importance in regulating the distribution of blood within an animal. However, tissues that depend on a continuous supply of oxygenated blood, and are crucial for the direct survival of the animal, such as the heart and brain, rely very little on extrinsic autonomic nerves (i.e. sympathetic and/or parasympathetic) for the control of blood flow, but more on local signals. In fish, the vascular system is inmost species under the control of the sympathetic innervation as well as circulating catecholamines (Axelsson et al., 1989, 2000; Axelsson and Fritsche, 1991). The exception being the vasculature of the gills that is also dependent

on a parasympathetic input (Nilsson and Sundin, 1998; Sundin and Nilsson, 1992, 1997).

Combining information from mammalian studies and the studies conducted in fish, it seems likely that the routine tone of the GI resistance vessels is under the control of the sympathetic nervous system via the splanchnic nerve as describe in Figure 2. However, the postprandial hyperemia does not seem to depend on an extrinsic GI innervation in sea bass as there is only a minor decrease in postprandial GBF during hypoxia. In contrast, unfed sea bass there is a substantial reflex vasoconstriction during hypoxia, therefore the lack of a GI vasoconstriction in postprandial sea bass, suggests that local control mechanisms predominate (Axelsson et al., 2002). In the red Irish lord a decrease in the α-adrenergic tone on the GI vasculature explain only a small portion of the postprandial increase in GBF and local mechanisms also predominates in this species (Axelsson et al., 2000). The notion that the there is a sympathetic tone on the GI vasculature, that is of a limitied importance during the postprandial hyperemia was also confirmed in **Paper IV**.

This has also been shown in mammals indicating that the sympathetic and parasympathetic nervous systems are of little importance to the postprandial hyperemia across species. Sectioning the extrinsic nerves innervating the GI tract, or blocking the effects of these nerves using antagonists like for example atropine (muscarinic antagonist), have a very limited effect on the postprandial GBF (Nyhof and Chou, 1981, 1983, 1985).

Endocrine control

Hormones like cholecystokinin (CCK) and gastrin could be importance in regulating and coordinating the GBF with other functions of the GI tract, especially given their wide range of functions in the gut. The effects of numerous GI hormones (i.g. pentagastrin, secretin and CCK) on the GI vasculature in mammals have been reviewed (Chou et al., 1984), therefore, the reminder of this section will focus on cholecystokinin.

In mammals, several studies have shown that CCK is involved and modulates the postprandial GI hyperemia (Chou et al., 1977; Granger et al., 1980). This mechanism probably involves a chemical stimulation of certain cells of the gastric mucosa, which leads to the release of CCK from so called type 1 endocrine cells (Verberne et al., 2003). Subsequently CCK act in a paracrine fashion to activate afferent vagal neurons through a CCK-A receptor as well as through a 5-HT (serotonin) dependent mechanism (Saita and Verberne, 2003). This leads to a selective inhibition of the sympathetic outflow to the GI tract and a decreased vasomotor tone (Sartor and Verberne, 2002; Verberne and Guyenet, 1992). This pathway, which is similar to the

classic baroreflex, where an increased arterial blood pressure leads to a decrease in the sympathetic tone on the heart and the vasculature, involves glutamate mediated activation of GABA-ergic neurons within the CNS (Verberne and Sartor, 2004) (Figure 4).

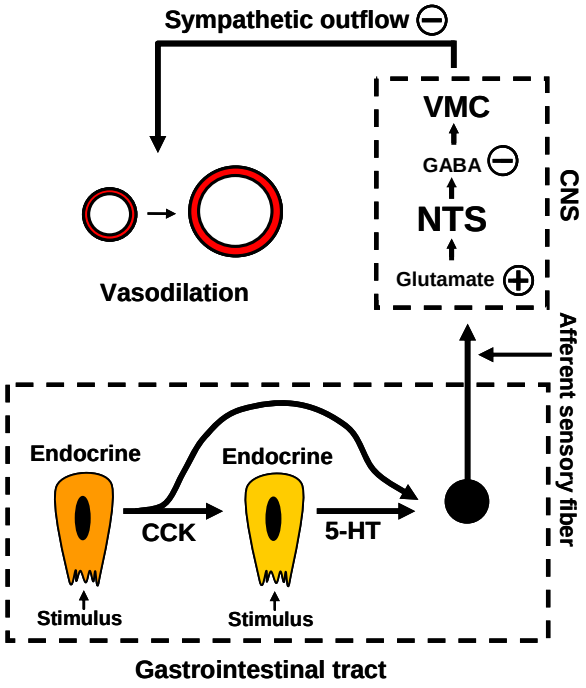

Figure 4. Reflex mediated decrease in the tone of the gastrointestinal vasculature (hyperemia) via the release of cholecystokinin (CCK) and/or 5-HT (serotonin) upon a chemical stimulation of mucosal endocrine cells. This illustration is based on the results from several mammalian studies (see text), but a similarly intricate series of events might very well occur in teleost fish, in order to modulate the postprandial gastrointestinal blood flow. CNS: central nervous system. NTS: nucleus tractus solitarius, VMC: vaso motor center. + and – denotes an activation or an inhibition, respectively.

In the rainbow trout, CCK is found in several different subforms (Jensen et al., 2001) and it is involved in the control of gastric emptying (Olsson et al., 1999) as well as gall bladder contractions, leading to the secretion of bile into the proximal intestine (Aldman et al., 1992). These functions are similar to what have been found in mammals. The circulating level of CCK is also influenced by the diet (Jönsson et al., 2006) and it is thus possible that CCK could be a potential regulator of GBF also in fish. As shown in **Paper V** and further discussed below, CCK do increase GBF when injected intra-arterially in rainbow trout.

Local (enteric) nervous control

The GI tract of fish is innervated by a large number of intrinsic neurons that are commonly referred to as the enteric nervous system. The enteric nervous system is an independent part of the autonomic nervous system and

the structure as well as physiology has been extensively reviewed for fish (Olsson and Holmgren, 2009). Even though it has the capacity to function on its own it receives information from extrinsic sympathetic and parasympathetic nerves in order to coordinate the activities within an animal. It also sends sensory information from the GI tract via the extrinsic nerves to the CNS. The enteric nervous system is involved in most aspects of the GI function including gut motility and secretion of digestive enzymes (Olsson and Holmgren, 2001; Olsson et al., 2009). Therefore, it should also have the capacity to modulate the vasculature and enteric nerves are commonly found around the GI vasculature. Despite this there is a limited knowledge concerning the circulatory control imposed by the enteric neurons.

In mammals, enteric cholinergic neurons are probably important and regulate the GI microcirculation during various conditions, such as after feeding (Vanner and Surprenant, 1996). More recent studies in mammals have shown that enteric nerurons respond to both mechanical and chemical stimuli and subsequently influence the vasculature (Vanner and Macnaughton, 2004). The importance of local nerve reflexes has been confirmed by several studies demonstrating that the response to mechanical or chemical stimuli persists even after the blockade of the extrinsic nerves (Biber, 1973; Biber et al., 1970, 1971, 1974).

Due to the difficulty in blocking the enteric nervous system in *in vivo* experiments most work in mammals have involved *in situ* or *in vitro* techniques in order to map the enteric circuitry involved in control of the vasculature (Vanner and Macnaughton, 2004). For example the use of tetrodotoxin (TTX) revealed a vasodilatory response that was both TTX-sensitive and TTX-insensitive in guinea pigs, thus probably involving neural as well as non-neural mechanisms (Vanner et al., 1993). However, TTX failed to block the chemically induced hyperemia in GI segments of dogs indicating that the hyperemia was more closely related to a direct change in metabolism (Nyhof and Chou, 1983).

Prostanoids and histamines released from enteric nerves or the surrounding tissue after feeding are probably also involved in the local control of the vasculature (Chou et al., 1989; Chou and Kvietys, 1981; Chou and Siregar, 1982).

Several studies in different fish species have identified substances, within the GI tract, that indeed influence the GBF (Table 4). Tachykinins, including substances such as substance P and neurokinin A, have been found in the GI tract of an elasmobranch, the spiny dogfish (Kågström et al., 1996a) as well as in teleosts, the Atlantic cod and the rainbow trout (Jensen et al., 1993). These substances are released from local enteric nerves or endocrine cells.

In rainbow trout, there is a triphasic response to substance P, with an

initial increase in GBF, followed by a decrease to baseline and a subsequent increase (Jensen et al., 1991). This effect is probably local and mediated via changes in the resistance of the GI vasculature and an increase in cardiac output. Vasoactive intestinal polypeptide (VIP) had a similar effect on GBF but decreased gut motility.

Table 4. *In vivo* cardiovascular responses to selected substances found within the gastrointestinal tract.

Species	Temp °C	Substance P	Neurokinin A	VIP	Scyliorhinin I	Scyliorhinin II	Source
Spiny dogfish (*Squalus acanthias*)	10	GBF ↑; CO ↑; HR ↑	N/A	GBF ↓; R_{coel} ↑; HR ↑	N/A	N/A	Holmgren et al., 1992
Atlantic cod (*Gadus morhua)*	10-11	GBF↑↓↑ ; CO ↑	N/A	GBF ↑; CO ↑	N/A	N/A	Jensen et al., 1991
Rainbow trout (*Oncorhynchus mykiss*)	10	N/A	N/A	N/A	R_{coel} ↓↑; CO ↓↑	R_{coel} ↓↑; CO ↓↑	Kågstrom et al., 1994
Rainbow trout (*Oncorhynchus mykiss*)	10	R_{coel} ↑; CO ↓	R_{coel}↑; CO ↓	N/A	N/A	N/A	Kågstrom et al., 1996b
Spiny dogfish (*Squalus acanthias*)	10	GBF ↑; R_{coel} ↓	GBF ↑↓; R_{coel} ↓↑	N/A	GBF ↑; R_{coel} ↓	GBF ↑; R_{coel} ↑↓	Kågstrom et al., 1996a

↑↓ and ↑↓↑ denotes a bi-phasic and a tri-phasic response, respectively. GBF: gut blood flow; CO: cardiac output; R_{coel}: vascular resistance of gastrointestinal (coeliac) artery; HR: heart rate. N/A means that data is missing.

However, in another study in rainbow trout substance P caused a vasoconstrictor response and a subsequent increase in dorsal aortic pressure (Kågström et al., 1996b), while VIP was shown to be a potent vasorelaxant acting through an endothelium NO independent mechanism (Kågström and Holmgren, 1997). In the spiny dogfish substance P caused an increase in the GBF, whereas VIP decreased GBF (Holmgren et al., 1992). Whether or not this variation represents true species differences or effects of the experimental procedure remains to be established. It is hard to make any generalized conclusions from these results and it is also unknown to what extent these substances influence the postprandial GBF.

As demonstrated in **Paper IV** and discussed below, a blockade of the enteric nervous system completely abolished the postprandial hyperemia. Consequently local nerves within the enteric nervous system are important and control the postprandial GBF in fish, but if the enteric nerves exert their effect via an increased or decreased release of, for example, tachykinins remains to be determined.

Local enteric nerves could also function as sensors. For instance enteric sensory nerves or intrinsic primary afferent neurons (IPANs) could relay signals from the mucosa where the major change in oxygen content/tension and osmolarity occurs (Bohlen, 1980a, 1982), via

interneurons to vasomotor neurons innervating the submucosal arterioles, where a substantial portion of the vascular control resides.

Metabolite induced control

A change in the oxygen tension or content and the osmolarity of the tissue due to metabolic processes such as secretion, absorption, and assimilation, remains the most probable cause for the chemically induced hyperemia in mammals (Bohlen, 1998a; Matheson et al., 2000). There could either be direct effects on the vasculature or a release of vasoactive metabolites such as lactate and hydrogen ions as well as other vasoactive substances such as ADP and NO (Bohlen, 1980a, b, 1998a, b). In mammals it has been shown that lowering the oxygen content of the vasculature leads to a release of ATP from red blood cells (Gonzalez-Alonso et al., 2002). ATP produces vasodilation thus further contributing to the hyperemia. This metabolic theory would explain the fact that the hyperemia is localized to the parts of the intestine that is filled with hydrolyzed food as is evident from both fish and mammalian studies.

In fish (and mammals), the postprandial increase in oxygen consumption is not necessarily coupled to an increase in GBF, which could be explained by that, most likely, as much as 70-80% of the postprandial increase in oxygen consumption is due to processes outside of the GI tract and depend on the assimilation of amino acids through catabolic and anabolic processes (Brown and Cameron, 1991a, b). Proteins are potent in inducing a postprandial increase in total oxygen consumption, but this does not necessarily mean that there is an equally large increase in the oxygen consumption of the GI tract. Therefore it is possible that lipids and carbohydrates increase the oxygen consumption and metabolism of the GI tissues more than proteins. If a metabolic control of the GI vasculature is predominant in fish, one would expect a diet composition that increases the oxygen consumption of the GI tract to also increase GBF. This question was addressed in **Paper III**.

Furthermore, it has not been established how a change in the oxygen tension or osmolarity of the mucosa, induce to a subsequent change in the status of the submucosal vasculature, where the majority of flow regulation occurs, but enteric nerves probably contribute as mentioned above.

Indirect effects of gut motility on gastrointestinal blood flow

Processes such as gut motility and peristalsis cause only limited changes in the PO_2 of the GI tract and seem to be of a limited importance to the overall postprandial increase in blood flow (Fara et al., 1972). In mammals an

increase in the motility of the gut can, however, lead to a redistribution of blood from the mucosa and the submucosa to the muscular tissue layers within the GI wall, which will have an increased oxygen demand (Chou, 1982). Furthermore, the flow profile of the GBF is influenced by the GI motility, yet again with no change in the total flow (Fioramonti and Bueno, 1984).

A simple way to separate the effects of gut motility on GBF from other effects, in fish, would be to use unabsorbable ballotini beads (or kaolin) that stimulate the motility of the gut, as previously described in rainbow trout (Bucking and Wood, 2006). These beads are not hydrolyzed or absorbed after being ingested or placed into the intestine of an experimental animal, and consequently there is no subsequent chemical stimulus.

Postprandial metabolism

Assuming that a metabolic component is of fundamental importance to the postprandial increase in GBF, any change in the metabolism of the GI tract should influence the GBF. The metabolic response after feeding has been studied in more than 250 vertebrate and invertebrate species and represents one of the most studied physiological phenomena in fish (Secor, 2009). In contrast, virtually nothing is known about the routine as well as postprandial metabolism that is intrinsic to the GI tract, partially owing to the difficulty in isolating the oxygen consumption of the GI tract from other metabolically active tissues.

There are a few exceptions in mammals and *in vivo* studies in pigs have revealed that as much as 20-25% of the arterial oxygen is consumed by the GI tract, which represent about 5% of the total body mass, and the oxygen consumption increases after feeding (Vaugelade et al., 1994; Yen et al., 1989). In these studies the oxygen content of the portal vein outflow was measured and the oxygen consumption was calculated by means of the Fick-equation. In one very recent study in toad fish (*Opsanus beta*), segments of the GI tract, which in total represented 2% of body mass, was isolated before and after feeding (Taylor and Grosell, 2009). The routine oxygen consumption of the GI tract was estimated to be 5.6% of the total oxygen consumption, and although it was calculated that the oxygen consumption of the GI tract increased by 100% after feeding, the proportion of oxygen consumed by the GI tract was only 4.8%. This confirms the notion that a substantial portion of the increase in postprandial metabolism is due to proceses outside of the GI tract.

However, the main focus of this section will be the much more studied postprandial change in total metabolic rate, which although not restricted to the GI tract, should influence the postprandial GBF.

The rapid postprandial increase in total metabolic rate, followed by a more gradual return to the fasted metabolic rate characterizes what is commonly known as the specific dynamic action or SDA. A comprehensive review of the GI metabolism in mammals was published by McCue (2006). In fish, most studies have been conducted on economically important commercial or recreational species such as salmonids, Atlantic cod, catfish and sea bass. This has been done in order to increase the amount of energy that can be allocated to sustain grown, by finding the optimal meal size and composition as well as the most optimal environmental conditions regarding temperature, that decrease the SDA response (Couto et al., 2008; Enes et al., 2009; Fu et al., 2005; LeGrow and Beamish, 1986; Medland and Beamish, 1985).

Specific dynamic action

Originally referred to as "spezifisch dynamische wirkung" (Rubner, 1902) and later translated to specific dynamic action (SDA), this term describes the postprandial increase in heat production that occurs due to an increase in metabolism. Some scientists refer to the postprandial increase in heat production as heat increment (HI) of feeding or apparent heat increment. This acknowledges that the absolute increase in heat production and hence oxygen consumption is the result of several accumulative metabolic processes, rather than a single "action" (Secor, 2009). Other terms such as thermogenic effects of feeding and postprandial thermogenesis have also been used. For traditional reasons and clarity, I use the original translated term: specific dynamic action or SDA.

SDA-coefficient

The SDA can also be represented in terms of the SDA-coefficient, i.e. the cost of digestion and assimilation. The SDA coefficient is the SDA expressed as a proportion of the ingested dietary energy. The SDA coefficient is independent of meal size (Jordan and Steffensen, 2007), and this makes the SDA-coefficient very useful when comparing different species and studies. The SDA could also be expressed as the proportion of the ingested energy that is digested and absorbed (Beamish, 1974), and since most of the SDA is believed to reside within post-absorptive processes in fish (Brown and Cameron, 1991a, b; Jobling and Davies, 1980), reptiles (Coulson et al., 1978)

and mammals (Ashworth, 1969; Wilhelmj and Bollman, 1928), this might prove to be an even more accurate variable.

Effects of feeding

It is generally assumed that the SDA is largely a post-absorptive phenomenon and evidence for the dependence of post-absorptive processes stems from the fact that there is little difference in the SDA-response whether the diet is ingested or infused intravenously in fish (Brown and Cameron, 1991a; Jobling and Davies, 1980). Therefore, the SDA probably involves intestinal catabolic processes (deamination and formation of excretion products) as well as anabolic processes within and/or outside of the GI tract. By injecting cyclohexamide, a potent inhibitor of protein synthesis, into channel catfish (*Ictalurus punctatus*), Brown and Cameron were able to almost completely abolish the SDA-response (Brown and Cameron, 1991b). The same has also been shown in reptiles, where cyclohexamide removed about 70% of the SDA (McCue et al., 2005).

The ingestion and swallowing of food might also contribute to the SDA, as could the production and secretion of gastric proteolytic enzymes. In mammals these contributions are probably small, whereas in reptiles, the gastric phase sometimes constitutes a major portion of the SDA (Secor, 2003). Ultimately, the time that food resides within the stomach influences the contribution of this phase. Furthermore, there is a limited contribution of the GI motility to the SDA in fish, as shown in plaice fed an indigestible diet (Jobling and Davies, 1980). The indigestible diet will increase gut motility, but since the diet cannot be digested and absorbed there are no subsequent post-absorptive processes. To what extent digestion and absorption (as well as secretion) contributes to the SDA remains to be determined in fish, but in alligators the digestion and absorption of amino acids are of little importance, whereas the subsequent protein synthesis increases the oxygen consumption (Coulson et al., 1978; Coulson and Hernandez, 1979).

Amplitude and duration of the SDA

In the investigated species the amplitude of the SDA ranges from a 10-fold increase in metabolic rate for the European eel (*Anguilla anguilla*) (Owen, 2001) to a 30% increase in the bluegill (*Lepomis macrochirus*) (Pierce and Wissing, 1974). On average the SDA is associated with an approximately 2-fold increase in metabolic rate in fish, which is similar to what has been reported for many mammalian species, but the duration is almost always shorter in mammals compared with most fish (McCue, 2006; Secor, 2009).

The SDA coefficient ranges from 1.6% in rainbow trout (Smith et al., 1978) to 58% in the cyprinid top-mouth gudgeon (*Pseudorasbora parva)* (Cui and Liu, 1990). Larger values indicate a higher cost of digestion and absorption, with less absorbed energy available for growth.

The duration of the SDA ranges from 3-6 h for rainbow trout and Atlantic cod (Peck et al., 2003; Smith et al., 1978) to 390 h for the Antarctic spiny plunderfish (*Harpagifer antarcticus*) (Boyce and Clarke, 1997). Longer durations are almost always associated with low temperatures.

Effects of the chemical composition of the diet

Most animals, including fish, show a larger SDA for a protein-rich meal, which is consistent with the aforementioned proteins synthesis and in largemouth bass (*Micropterus salmoides*) (Tandler and Beamish, 1980) and rainbow trout (LeGrow and Beamish, 1986) the amplitude of the SDA increased with an increased protein content (and energy content). The duration remained the same irrespective of the diet composition and energy content.

However, the SDA in sea bass is not entirely dependent on protein, and there was no additional increase in SDA when increasing the protein content of the diet (Peres and Oliva-Teles, 2001). A similar pattern was also seen in rainbow trout as well as Atlantic cod fed iso-caloric diets with different protein contents and the SDA reflected the energy content of the diet (Eliason et al., 2007; Juan et al., 2010). The response to lipid content varies among species (Medland and Beamish, 1985; Ross et al., 1992). Still in **Paper III** the SDA differs depending on the diet composition and different nutrient components superimpose their effects in a mixed diet. Therefore, it is now believed that the SDA will depend, not only on the protein content, but also the interactions with other components of the diet. As with most aspects of fish physiology there are substantial differences among different studies, even within the same species, and more studies are needed in order to establish how the chemical composition of the diet affects the SDA.

AIMS

The overall aim of the studies included in this thesis was to increase the knowledge regarding the postprandial regulation of gastrointestinal blood flow in teleost fish.

More specific aims:

I. To determine the importance of mechanical stimuli within the stomach in the regulation of gastrointestinal blood flow in the rainbow trout (*Oncorhynchus mykiss*).

II. To compare the results from the rainbow trout with another teleost species, the shorthorn sculpin (*Myoxocephalus scorpius*), with a different feeding regime.

III. To determine the contribution of different macronutrients to the postprandial hyperemia as well as the postprandial increase in oxygen consumption (SDA), in the rainbow trout (*Oncorhynchus mykiss*).

IV. To determine the importance of autonomic innervation, extrinsic as well as intrinsic, in the regulation of routine and postprandial blood flow, in the rainbow trout (*Oncorhynchus mykiss*).

V. To determine if cholecystokinin (CCK) is an important component in the regulation of postprandial gastrointestinal blood flow and cardiac function, in the rainbow trout (*Oncorhynchus mykiss*).

METHODOLOGICAL CONSIDERATIONS

All experiments that form the base for this thesis were performed on teleost fish. The term fish is used to describe a paraphyletic assemblage of some 28 500 (Baille et al., 2004; Nelson, 1994) to 31 600 species (*Fishbase; http://www.fishbase.org/*), and the teleost fish (infraclass: *Teleostei*) comprise about 20 000 of these. The teleosts are separated from other bony fish by having several movable jawparts (maxillae and premaxillae) and a flexible musculature that enables these parts to protrude outwards from the mouth. Other characteristic are a homocercal hind/caudal fin and a vertebral column that does not extend into the caudal fin.

The text below represents a summary of some of the experimental procedures used in **Paper I**, **II**, **III**, **IV** and **V**. In addition the text also includes a discussion of certain methodological aspects. For a more detailed description of, for example, the surgical procedures and the experimental protocols the reader is referred to the respective paper.

Basic terminology

Several terms are commonly used to describe the physiological status of an animal before, for example, the distribution of a drug or exercise. In this thesis and the included papers, "Routine" is used to describe a physiological variable that is measured under normal spontaneous activity not necessarily in a post-absorptive animal, unless stated as unfed. The term "unfed" or "fasted" will be used to describe an animal that has not been feed for at least a week prior to the experimental protocol, which based on previous studies is enough for cardiovascular variables to reach post-absorptive levels. No animals were starved, with signs of inanition (e.g. catabolysis), which takes several weeks at the relatively low temperatures used in the included studies.

Sometime the term "baseline" is used to describe the stable period of a certain cardiovascular variable that is subsequently used in a comparison with the values after a certain treatment. Additionally, "control" is used describe an experimental protocol, which is designed to reveal and compensate for the effects of, for example, the injection of a drug or the handling of an animal. A saline injection could identify subsequent cardiovascular effects that are not pharmacological but due to the injection and sham-feeding the animal removes confounding effects from the handling during force-feeding.

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) ranging in size between 380g and 750 g were acquired from a local hatchery (Antens laxodling AB) and used for the studies presented in **Paper I**, **III**, **IV** and **V**. Shorthorn sculpins (*Myoxocephalus scorpius*) ranging in size between 145g and 380 g were caught by a local fisherman outside the Swedish west coast (Gullmarsfjorden, Sweden) and used in the study presented in **Paper II**. Ethical permits *64/2004* and *13/2007* from the Animal Ethics Committee of Gothenburg covered all experiments reported in this thesis.

The rainbow trout, which is referred to as *Salmo gairdneri* in earlier publications, is an active freshwater salmonid that in contrast to the related anadromous salmonids species never enters the ocean. The GI tract is characterized by a relatively small (in comparison with the shorthorn sculpin) but muscular stomach that is associated with an abundance of pyloric caeca originating from the proximal intestine. These pyloric caeca together with a short intestine often characterize the GI tract of a carnivorous fish (Buddington et al., 1997).

Shorthorn sculpins are instead inactive ambush predators that inhabit the ocean floor and belong to the cottid family. Sculpins devour their prey, rapidly in one large bite, which is in strong contrast to the active foraging of smaller food item by the rainbow trout. This makes these two species interesting when investigating how mechanical stimuli during the ingestion of a meal contribute to and modulate the postprandial cardiovascular changes (**Paper I** and **II**). In captivity the rainbow trout were fed a diet consisting of commercial dry trout pellets, whereas the shorthorn sculpins were fed pieces of hoki (*Macruronus novaezelandiae*).

These two fish species were chosen as experimental animals for several different reasons. Both are robust and easily kept in a laboratory setting. They can also be acquired easily within a small radius from the department.

Vascular anatomy - corrosion casts

In order to visualize the vasculature, and in particular the GI portion, vascular corrosion casts were made of both the rainbow trout (**Paper I**) and the shorthorn sculpin (**Paper II**). This was vital for the proper placement of the flow probes during surgery.

The corrosion cast technique, which has been described by Murakami (1971; 1975) involves filling the entire vasculature or selected tissues, with an epoxy plastic and then removing the organic material with potassium hydroxide (KOH) and calcified tissues, such as bones, with hydrochloric acid (HCl). To enable an good filling of the vascular beds, the vasculature the was

flushed with a saline mixture containing the nitric oxide donor sodium nitroprusside (SNP). SNP release of NO, which dilates the perfused vessels fully and thus decreases the pressure needed to infuse the highly viscous epoxy plastic. Even so, it is difficult to get a sufficient perfusion of the entire vasculature without a subsequent destruction of more fragile vessel in, for example the gills.

Experimental and surgical procedures

Anesthesia

Animals were anaesthetized prior to surgery using MS-222 (ethyl 3-aminobenzoate methanesulfonate salt) is a sodium channel blocker, which readily passes the blood-brain barrier and thus affects the CNS to induce a generalized anesthesia. For the freshwater-living rainbow trout, the dosage was set to 150 mg l^{-1} and the water was buffered with sodium bicarbonate (300 mg l^{-1}) to avoid a lowering of the pH. For the marine shorthorn sculpin the dose was set to 100mg l^{-1} and due to the presence of bicarbonates and other buffering minerals there was no need to buffer the seawater. During the entire experimental protocol, the animals were kept under surgical anesthesia using a lower maintenance dose.

Blood pressure measurements

Blood pressure on the arterial side of the circulatory system can be measured in either the ventral aorta or the dorsal aorta. Depending on the resistance of the branchial circulation, the blood pressure in the ventral aorta is higher than that of the dorsal aorta. The ratio between the dorsal and ventral aortic pressure depends on the temperature as well as the environmental PO_2, but on average the ventral aortic pressure is around 30-50% higher than the dorsal aortic pressure (Holeton and Randall, 1967; Stevens and Randall, 1967).

A dorsal aortic cannula was used to measure the dorsal postbranchial aortic pressure in all of the studies included in this thesis. A rapid and minimally invasive method originally published by Smith and Bell (1964) and now routinely used was utilized in the rainbow trout. A polyethylene catheter was inserted into the dorsal aorta via the roof of the buccal cavity, a procedure that, even though the vessel is not visualized, can be done with accuracy and high success rate. In the shorthorn sculpin, the postbranchial blood pressure was measured via a thin catheter inserted into the third efferent branchial artery.

Venous pressure was measured, either in the *sinus venosus* of the rainbow trout or via a catheter in one of the portal veins leading into the *sinus venosus* of the shorthorn sculpin. Venous pressure is arduous to measure and great care was taken to place the recording catheter with millimeter precision in order to minimize noise and instability.

Blood flow measurements

Blood flow was measured by means of either a *pulsed doppler* (**Paper I**, **II**, **III** and **V**) or a *transit-time* (**Paper IV**) technique. The use of the pulsed doppler technique, which exploits the doppler shift to calculate the relative speed of the blood, can be advantageous under certain circumstances. These flow probes are small and customized to fit perfectly on a vessel. In addition, they have very thin and compliant connecting wires. In contrast, the transit time technique measures the difference in transit time, of ultrasonic pulses passed in either the upstream or the downstream direction, which is directly related to volume flow and therefore this technique measures the absolute flow through a vessel. Under some circumstances this is crucial, such as when comparing the flow among separate groups of fish. These probes are, on the other hand, much larger with a thick and stiff connecting wire that easily either dislodges the probe or bends the blood vessel obstructing the flow through it.

Mechanical distension of the stomach

To study the cardiovascular effects of mechanical distension of the stomach in **Paper I** and **II**, in order to mimic what happens during the ingestion of a meal, without any chemical stimuli, an inflatable nitrile balloon of fixed size was placed in the stomach of the experimental animal (Figure 5A).

A similar procedure has been used earlier to study, for example, gastric relaxation and the control of stomach volume in fish (Grove and Holmgren, 1992a, b). Similar procedures have also been used in mammals to study the cardiovascular effects of stomach distention in cats (Longhurst and Ibarra, 1982, 1984; Longhurst et al., 1981), rats (Molinari et al., 2006; Nosaka et al., 1991; Ozaki et al., 1999; Pittam et al., 1988; Pozo et al., 1985) as well as pigs (Molinari et al., 2003; Vacca et al., 1996; Vacca and Vono, 1993).

The nitrile balloon is connected to a thin and flexible polyethylene catheter, which allows filling the balloon with fluid. By connecting the balloon to a pressure gauge via a three-way valve, the intra-gastric pressure can be measured during and after filling the balloon with a specified volume of fluid. This enables the subsequent study of changes in the force and frequency of

contraction with different distension volumes. An alternative to a balloon is to use the stomach itself as the balloon. By tying off the stomach it is possible to create a closed volume that can be filled by means of a catheter that enters the stomach via a purse-string-suture. Depending on the method used the recorded response may vary, and for example rapidly adapting mechanoceptors are only triggered when using an inflatable balloon (Ozaki and Gebhart, 2001; Ozaki et al., 1999; Pittam et al., 1988). An inflatable balloon should, however, be more physiological in fish, since a meal seldom fill the entire stomach uniformly, especially not when a shorthorn sculpin ingests an entire fish.

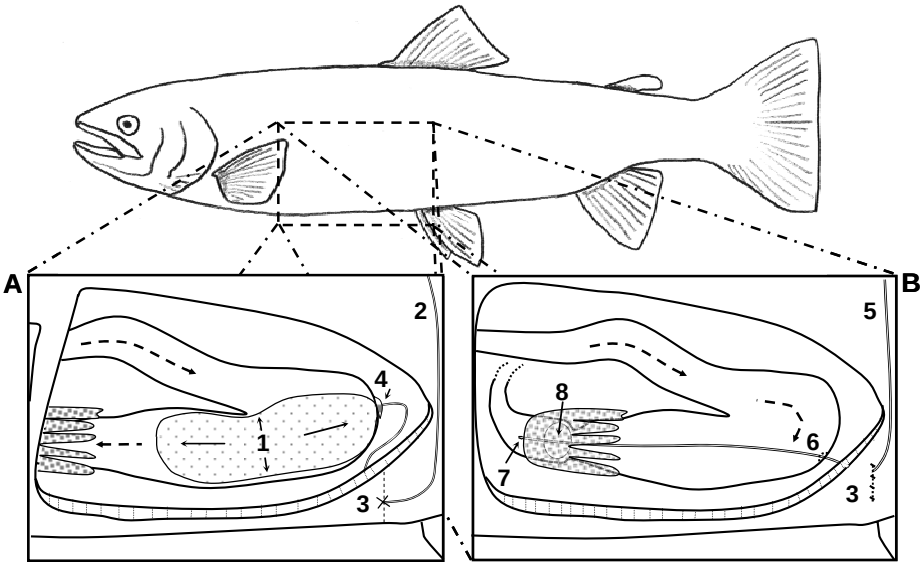

Figure 5. Schematic illustration showing: **A,** the placement of a fixed volume nitrile balloon (**1**) into the stomach. The balloon is filled with fluid via a thin cathether (**2**) that is passed through the body wall via a small incision (**3**) and into the stomach via a small puncture in the stomach wall that is sealed using a small custom made plastic cap (**4**). **B**, the placement of a double lumen Fogarty embolectomy catheter (**5**) into the proximal intestine of a rainbow trout (*Oncorhynchus mykiss*). The double lumen Fogarthy embolectomy catheter was inserted through the body wall via a small incision (**3**) and secured at its passage through the stomach wall with a purse string suture (**6**). The embolectomy catheter was placed so that the hollow point (**7**) was in the proximal intestine and subsequently secured by inflating the embolectomy bubble (**8**) at the pyloric junction. *Modified from* ***Paper I*** *and* ***III***.

Infusion of a nutrient solution into the proximal intestine

How the GI hyperemia is influenced by the nutrient composition has traditionally been studied in anaesthetized mammals by infusing a predigested nutrient solution of a known composition into isolated or resected segments of the intestine (Chou and Coatney, 1994; Chou et al., 1978, 1985; Kvietys et al., 1980a, 1981a; Siregar and Chou, 1982).

For the studies that are presented in **Paper III** and **IV** a new improved method was developed that allowed the injection of food directly into the GI tract in unanaesthetized animals. A customized double lumen Fogarty embolectomy catheter was introduced into the proximal intestine via the stomach and a small incision in the body wall (Figure 5B). The catheter was secured in place within the proximal intestine by inflating the small inflatable bubble at the end of the catheter. The bubble was then passively deflated over the next 12 h. This meant that the catheter tip remained in its designated position during the initial struggling behavior most often displayed post-surgically in fish. However, after the initial 12 h there was no distending stimulus, which could otherwise have affected the response of the subsequent experimental protocol.

This approach allowed the infusion of different predigested diets of known volume and composition into unanaesthetized fish. There was no need to sedate and force-feed the fish and due to the predigested nature of the diet, the hyperemic response was rapid both in onset and duration This is particularly beneficial when carrying out respirometric measurements (see below) of the postprandial metabolism, such as those in **Paper III,** as any stressor, such as force-feeding, will influence the metabolism for up to several hours. A direct infusion of a predigested diet in to the proximal intestine also allowed the study of how TTX-treatment in anaesthetized fish influences the hyperemia, something that would otherwise have been impossible (**Paper IV**).

Respirometry

There are several possible methods of measuring the metabolism of an animal, for example after feeding (**Paper III**). These range from direct calorimetric measurements of the energy produced by the animal to the measurement of changes in the respiratory gases (O_2, CO_2).

In fish, being a water breathing animal, it has most often involved measuring the consumption of oxygen by the animal in a closed chamber of known volume. As discussed by Steffensen (1989), the use of an intermittent flow respirometer has proven to be a very reliable method, with several benefits compared with for example, closed respirometery. Intermittent flow respirometry involves placing the fish in a gas tight cylindrical chamber (Figure

6). The chamber is then supplied with oxygenated water by means of a submersible time-controlled circulation pump. The oxygen tension/content in the chamber is monitored by either measuring the oxygen tension directly in the chamber or by continuously circulating water from the chamber through a flow through oxygen electrode setup. By switching off the time-controlled circulation pump the oxygen consumption can be calculated as the decrease in oxygen content in the chamber over a given time (see Data analysis).

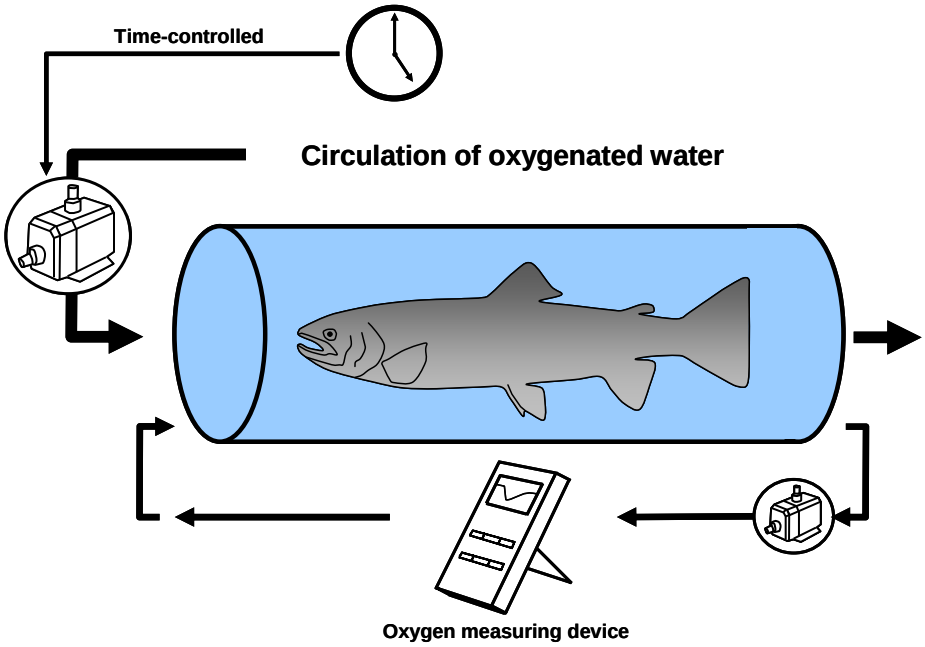

Figure 6. The experimental setup used to measure the oxygen consumption in rainbow trout (*Oncorhynchus mykiss*). The fish is placed in a gas tight respirometer of known volume and well aerated water is supplied by means of a large pump. The oxygen level in the water is monitored by circulating water through an oxygen measuring device by means of a small er pump. The larger pump is time controlled and at regular intervals this pump is stopped and the decrease in the oxygen level, due to the oxygen consumption of the experimental animal is monitored over time, whereby the larger pump is then started and the oxygen level is allowed to return to its original value.

Denervation of the gastrointestinal tract

The GI tract of a rainbow trout is innervated by means of the paired vagi as well as the splanchnic nerve (Figure 7) as revealed by ocular inspection (**Paper IV**). This is in conformity with previous results of Burnstock (1959) and the vagi innervates the major portion of the stomach while its innervation

of the intestine is probably limited in salmonids, which is in contrast to stomachless fish, such as zebra fish, where the entire GI tract is innervated by the vagus (Catharina et al., 2008).

The two bilateral branches of the vagus originate from the skull base, with the cell bodies of sensory nerves in the nodose ganglion just outside of the cranium, and then split up to innervate the gills, the heart and the stomach. Via a small incision in the thin membrane covering the oesophagus, the duct of Cuvier and the sinus venosus the vagal branches are easily visualized. The cardiac and gastric branches travel along the duct of Cuvier and the sinus venosus until the gastric branch makes a sharp turn and passes through a muscular wall into the abdominal cavity to innervate the stomach. In order to denervate the stomach the vagus was cut bilaterally just where it passes through the muscular layer (**Paper IV**).

In salmonids the splanchnic nerve travels along the coeliacomesenteric artery to innervate the major portion of the GI tract. It is therefore easy to identify and the nerve was cut just after placing a flow probe around the artery, thereby limiting the amount of surgery needed (**Paper IV**).

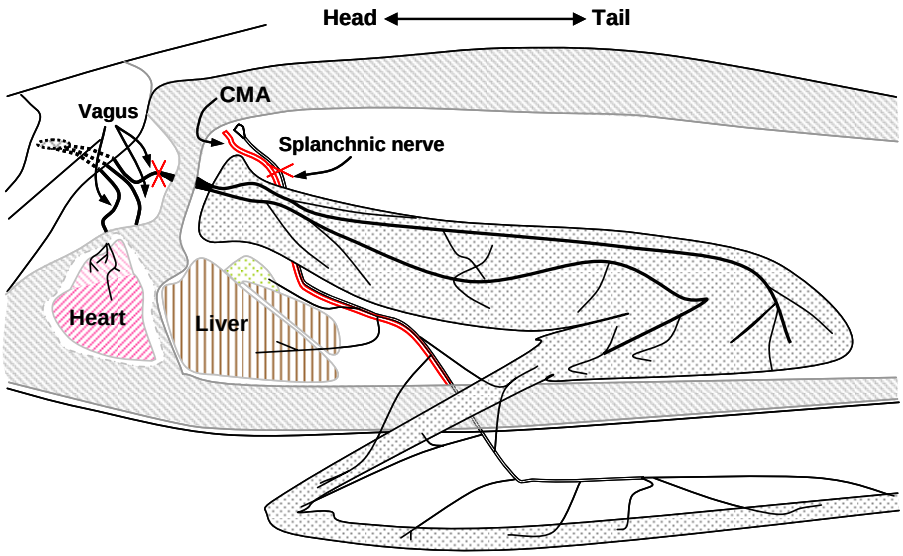

Figure 7. Schematic illustration of the innervation of the gastrointestinal tract via the paired vagus (only one side is shown) and the splanchnic nerve that runs along the coeliacomesenteric artery (CMA) and its branches. The vagus originates from the brain and innervates the major portion of the stomach (as well as the heart etc) with few or no fibers reaching the proximal intestine in the salmonids. The splanchnic nerve innervates most of the intestine as well as several other organs including the stomach. Red **X** denotes where the sections were done *Modified from* ***Paper IV***.

In situ characterization of cardiovascular variables

In order to study how the GI hormone cholecystokinin influences the GBF in more detail (**Paper V**), we used vascular preparations as well as *in situ* perfused hearts.

Vascular preparations

Second- and third-order branches of the coeliacomesenteric artery were carefully removed and mounted on force transducers. The vessels were kept under tension (~10-15 mN) and pre-contracted using adrenaline to measure the isometric wall tension. The endothelium was left intact, to avoid disrupting any mechanisms involved in the release of endothelial-derived relaxing factors such as endothelia dependant prostaglandins (Jennings et al., 2004; Kågström and Holmgren, 1997), or possibly NO if at all released from the endothelium in fish (Olson and Donald, 2009; Olson et al., 1991).

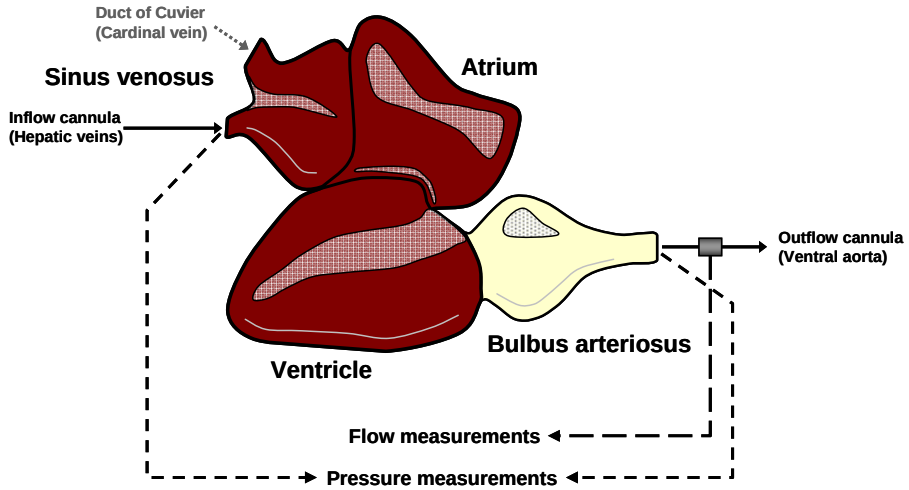

Figure 8. The basic principles of the *in situ* perfused heart. The preparation was kept within an intact pericardium (omitted for clarity). The inflow cannula was inserted via one of the hepatic veins and the remaining veins were tied off. The outflow cannula was attached to the ventral aorta before the division into the efferent branchial arteries. The inflow pressure (preload) and the outflow pressure (afterload) was adjusted to within physiological values and continuously monitored along with the cardiac output.

In situ perfused hearts

To study the pharmacological effects of CCK on cardiac tissue alone, hearts from rainbow trout were perfused *in situ* as has been previously

described by Franklin and Axelsson (1994) (Figure 8). The pericardium as well as the surrounding nerves was left intact for the entire experimental protocol.

The fact that the pericardium was left intact is important, considering that an open pericardium severely limits the hearts ability to do pressure work in the eel (*Anguilla sp.*) (Franklin and Davie, 1991). In the rainbow trout there is a significant decrease in the routine cardiac output if the pericardium is open (Farrell et al., 1988). In elasmobranchs with their more rigid pericardium, the effects of an open pericardium is considerable and there is a clear shift in the Starling curve of the heart, such that the hearts goes from *vis-a-fronte* filling to *vis-a-tergo* filling (Franklin and Davie, 1993; Sandblom et al., 2006).

Experimental protocols

Below follows a brief summary of the experimetal protocols used, for more details the reader is referred to the individual papers.

Mechanical stimuli

A distensible balloon of a fixed volume was used to distend the stomach in order to mimic the mechanical distention that occurs during the ingestion of a meal (**Paper I** and **II**). The balloon was filled with water that had the same temperature as the animal (10ºC). The filling took place over several minutes, although no stressful response occurred with a more rapid inflation. In contrast a rapid deflation or emptying of the balloon seemed to induce struggling behavior though only for a short period of time, indicating that this was a stressful stimulus. This is perhaps logical considering that the stomach might fill quite rapidly, especially in the shorthorn sculpin. In contrast gastric emptying is always slow and a rapid emptying would only occur during vomiting, a response that indicates that something is wrong either with the ingested food, the environment (i.e. hypoxia) or the animal.

Four different volumes were used to distend the stomach in the rainbow trout, and a volume corresponding to 2% of body weight was considered a normal sized meal (Grove and Holmgren, 1992a; Thorarensen and Farrell, 2006). The stomach was also distended for different durations.

Prazosin a potent α-adrenoceptor antagonist that is selective for the α_1-adrenoceptors found on the smooth muscle of the vasculature was used to determine if the pressor response that occurred following the mechanical distension of the stomach was mediated via an adrenergic reflex. Due to its selective nature it is useful when trying to discern whether or not a pressor response is mediated via vascular α_1-adrenoceptor, but no attempt was made

to distinguish between nervous or humoral components of the response. Administration of prazosin lowered the arterial blood pressure and consequently there was a barostatic reflex increase in heart rate and contractility. After the recovery period (~3 h) the hemodynamic variables were stabilized.

In **Paper I** the nitrile balloon was emptied quit rapidly, whereas in **Paper II** the volume of the balloon was decreased over a 72 h period, which resembled the dynamics of the gastric emptying in the shorthorn sculpin.

Chemical stimuli and diet composition

Three different diets were used to study how the nutrient composition influences the postprandial GI hyperemia as well as the SDA in rainbow trout (**Paper III**). All diets were predigested using physiological concentrations of bile and pancreatic enzymes. Bile was sampled from the gallbladder of the experimental animal during surgery using a small needle.

No pancreatic enzymes were obtained from the experimental animals since, unlike in mammals, there is no distinct organ, and aggregates of pancreatic and islet tissue is instead found around the pyloric ceca (Epple and Brinn, 1975). In addition smaller brockmann bodies, consisting of a core of endocrine cells surrounded by exocrine cells, are scattered around the gall bladder. The pancreatic enzymes were instead a commercially available mixture of enzymes, including amylases, trypsinases, lipases, ribonucleases as well as proteases all commonly found in the GI tract of rainbow trout (Hidalgo et al., 1999; Kitamikado and Tachino, 1960a, b, c).

The use of a commercial mixture of digestive enzyme should not have limited the proteolytic breakdown of the various diets given that there are no differences in the structure of the, for example the proteins, ingested by a fish, although the composition might vary. Furthermore, the pancreatic enzyme mixture was used under optimal conditions concerning pH and temperature.

The balanced diet resembling the normal pellet diet contained 50% protein and 25% fat, the high lipid diet contained 15% protein and 60% fat and the high protein diet contained 70% protein and 5% fat. These non-isocaloric diets were prepared using fish protein, fish oil and carbohydrates. In order to resemble the normal conditions of the rainbow trout proximal intestine (Bucking and Wood, 2006), the water content, pH and osmolarity were adjusted to 70-80%, 7.3-7.4 and 380-400 mOsm, respectively.

During the experimental procedure a small volume of either of the diets was injected. The injected volume corresponding to the amount that enters the proximal intestine during a 2-5 h period, after an average sized meal (2%), (Olsson et al., 1999; Windell et al., 1969). To control for any affects of the

injection, for example due to a mechanical stimulation of the mucosa, a subsequent control injection using saline was made.

Denervation of the gastrointestinal tract

The importance of the extrinsic innervation of the GI tract, in triggering and maintaining the postprandial hyperemia, was tested by cutting the vagal and the splanchnic innervation of the GI tract in one group of fish and then comparing the *in vivo* response to the injection of a pre-digested diet into the proximal intestine, with that in a group of untreated fish (**Paper IV**). Furthermore, to test whether the splanchnic nerve is important in maintaining the routine tone of the GI vasculature in fish, as previously shown in mammals (Furness and Costa, 1974; Vanner and Surprenant, 1996), the routine GI blood flow was measured before and after sectioning the splanchnic nerve. To avoid any confounding effects of the sectioning, routine blood flow was also compared between two separate groups, one in which the splanchnic nerve was left intact and one the in which the nerve had been sectioned (**Paper IV**).

TTX-treatment

Tetrodotoxin a very potent blocker of fast voltage-gated sodium channels was used to completely block the extrinsic, i.e. sympathetic and parasympathetic and the enteric innervation of the GI tract. This was done in order to investigate, in anaesthetized fish, the importance of enteric neurons in inducing and maintaining the postprandial hyperemia (**Paper IV**).

Anaesthetized fish were pre-treated with TTX at a given concentration prior to the injection of the pre-digested diet. The results from the TTX-treated group were compared with the response of an untreated group, and a control group, which received an injection of pure saline only, instead of the pre-digested diet.

Not all tissues are equally sensitive to TTX and several of the less rapid voltage gated sodium channels found in the mammalian heart, for example in the pacemaker cells, are resistant due to the low TTX affinity of the cardiac sodium channel called Na(v)1.5. In contrast, the voltage-gated sodium channels found in fish heart are a 1000-fold more sensitive (Haverinen et al., 2007). Therefore the correct dose was investigated in *in situ* perfused hearts. Based on the results from the perfused hearts and additional *in vivo* trials the dose of TTX to be used was set to 4 pM kg^{-1}. This dose had minimal effects on the heart *in vivo*, apart from an initial decrease in rate and contractility, while reducing most spontaneous bodily movements such as the ventilatory

movements of the opercula. The oxygenation of the animal was maintained by a constant flow of oxygenated water over the gills.

Injections of CCK *in vivo*

To test the potential of CCK as a regulator of the GBF especially during the postprandial phase, rainbow trout received an injection of CCK while several cardiovascular variables were monitored.

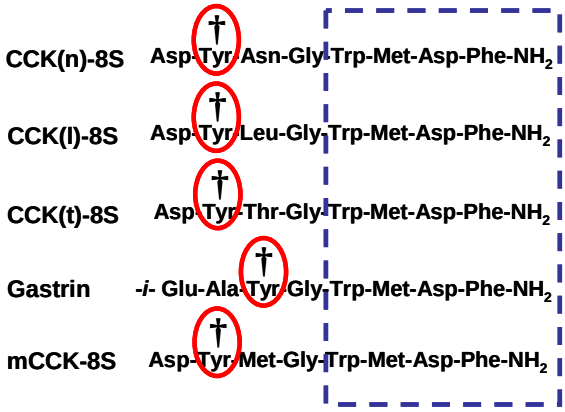

Figure 9. Amino acid sequence of three different trout CCK- 8S, a gastrin and the mammalian CCK-8S used in **Paper V**. The conserved region at the amidated c-terminal, which is also the active site, is included within the hatched box. The sulfated tyrosines are indicated by daggers. *-i-* indicates that the peptide exists in several forms of varying length.

In **Paper V** a mammalian CCK-8S, differing from the trout forms in having a methionine in the sixth position from the fully amidated C-terminal, compared with leucine (CCK-l), asparagine (CCK-n) and threonine (CCK-t) (Jensen et al., 2001), was used. However, since the biologically active site is situated near the C-terminal just by the sulfated tyrosine residue within a conserved region that is shared among all the rainbow trout and mammalian iso-forms (Johnsen, 1998), it should be equally effective as any of the trout iso-forms. The region that lies outside of the conserved region might, however, influence the folding of the molecule and ultimately its specificity, although there seems to be little discrimination in fish as long as the molecule is sulphated (Himick et al., 1996; Oliver and Vigna, 1996; Vigna et al., 1986).

The lower concentrations used in **Paper V** corresponded to the circulating levels seen after feeding in rainbow trout (Jönsson et al., 2006). However, the concentration of CCK at discreet locations is most likely much higher than the circulating levels.

Isolated vessel preparations

Vascular preparations were precontracted with adrenaline and allowed to reach a stable baseline, in order to study whether CCK has a direct pharmacological effect on the vasculature of the GI tract (**Paper V**). CCK was added to the Ringer solution in stepwise increments from 0.1 nM to 100nM. Thereafter the potential effect of a cholinergic vasodilatory mechanism as well and the ability of the preparations to relax was examined using atropine (0.1 mM). Atropine in addition to being a muscarinic receptor antagonist is also a very potent vasorelaxant, via a non-muscarinic mechanism, in adrenergically stimulated vessels (Kwan et al., 2003; Liu et al., 2004).

The use of isolated strip reparations enabled the separation between a local effect on the vasculature (Chou et al., 1977; Guth and Smith, 1976) and a neurogenic mechanism outside of the vasculature (Saita and Verberne, 2003; Sanchez-Fernandez et al., 2004; Sartor and Verberne, 2008; Verberne and Sartor, 2004).

In situ perfused hearts

The *in situ* perfused hearts were mounted as described in **Paper V** and inflow (preload) and outflow (afterload) pressures were adjusted to match physiological values (0 ± 0.20 kPa and 4.5 ± 0.50 kPa, respectively). The viability of the preparations was confirmed using a standard power test on each preparation by a stepwise increase in the outflow pressure. After a baseline recording, CCK was added to the Ringer solution in stepwise increments from 0.1 nM to 100 nM. The preparations were allowed to stabilize between each increment.

Data analysis and statistics

All data were sampled in accordance with the Nyquist–Shannon sampling theorem (Nyquist, 1928; Shannon, 1949) stating that the sampling frequency should be at least twice the recorded frequency response i.e. the *Nyquist rate*. Heart rate (HR) was obtained from the phasic pulsating pressure traces and cardiac stroke volume (SV) was calculated as SV = CO/HR.

Calculation of vascular resistance

In order to calculate the vascular resistance in both the systemic and the GI vasculature two assumptions was made (**Paper I**, **II** and **IV**). Firstly, the driving force for the blood flow through the coeliacomesenteric artery is the dorsal aortic blood pressure and secondly, the blood viscosity does not change

during the experimental protocol. In **Paper IV,** since there was no measurement of the central venous pressure, it was assumed that the central venous pressure did not change and thus remained close to 0 kPa.

The vascular resistance was calculated from raw data values using a modification of the Ohm's equation:

$$R_{sys} = (P_{da}-P_{ven})/CO \quad (1)$$
$$R_{cma} = (P_{da}-P_{ven})/Q_{cma} \quad (2)$$
$$R_{sys} = R_{cma}+R_{res}, \quad (3)$$

R_{sys}, R_{cma} and R_{rest} are the systemic vascular resistance, the coeliacomesenteric vascular resistance and the vascular resistance in the system except for the splanchnic resistance (i.e. somatic), respectively. CO is the cardiac output and Q_{cma} is the gastrointestinal blood flow. P_{da} and P_{ven} denote the dorsal aortic and central venous pressure. The logical consequence of (3) is that when there is change in the vascular resistance of the GI tract (splanchnic) there is also a change in the systemic vascular resistance and these resistances are consequently dependent upon each other.

Calculation of oxygen consumption

To calculate the oxygen consumption (MO_2) of the experimental animal within the respirometer the time-controlled pump was set so that the circulation of water through the respirometer was stopped for 10 min, at intervals of 50 min.

To calculate M_{O2} the following formula was used:

$$MO_2 = (\Delta[O_2] \times \alpha_{O2} \times v)/(m \times t) \quad (4)$$

where $\Delta[O_2]$ is the difference in oxygen concentration (mg l^{-1}) before and at the end of the 10 min period when the circulation through the respirometer was closed; α_{O2} is the oxygen content (mg l^{-1}) of water at the particular temperature and barometric pressure; v is the volume of the water enclosed in the respirometer minus the volume of the fish, m is the mass of the fish and t is the time.

Of critical importance are the duration when there is no circulation through the respirometer and the volume of the respirometer. The circulation should be closed no longer than what is needed in order to get a measurable decrease in the oxygen tension to accurately determine the slope using a linear regression and thus calculate the $\Delta[O_2]$. If the circulation is closed for too long, the oxygen tension will fall to a level where there could be effects on the experimental animal. The volume of the respirometer is also important since

an excessively large respirometer, compared with the size of the experimental animal, will require that the recirculation is closed for a longer period of time, resulting in a lower temporal resolution with fewer measurements over time.

Characterization of the phasic cardiac output flow profile

The phasic cardiac output flow profile of the perfused hearts before and after te administration of CCK (**Paper V)** was analysed using the systolic flow slope (+dQ/dT), the diastolic flow slope (-dQ/dT), the amplitude as well as the time to peak and the time to return was obtained from each flow pulse and subsequently averaged over a arbitrarily chosen period of time for each treatment.

Statistical analysis

A repeated measure ANOVA was utilized to analyse changes over time in a paired experimental protocol, followed by a Bonferroni or Dunnet post-hoc test to analyze individual points. Unpaired as well as paired comparisons between treatments were tested using a parametric two-sample or paired Student's t-test assuming equal variance and multiple comparisons were corrected for using a modified Holm-Bonferroni algorithm.

In all of the studies presented within this thesis, mean values are shown ± standard error of mean (SEM). All statistical analysis was performed on untransformed raw data values. A significant difference from the routine or control was assumed when $p<0.05$.

RESULTS AND DISCUSSION

Below follows the most important results and a discussion of their relevance in respect to the general aim of this thesis – that is the regulation of postprandial GBF in teleost fish.

Mechanical stimuli and gastric distension

In accordance with studies in mammals (Longhurst and Ibarra, 1981, 1982, 1984) mechanical stimuli alone is not sufficient to evoke a significant increase in the GBF, in neither the rainbow trout nor the shorthorn sculpin (**Paper I** and **II**). There are exceptions and in, for example, dogs stomach distenstion induce a hyperemia, although short lived (Kato et al., 1989).

In rainbow trout, there is a rapid increase in the dorsal aortic blood pressure following distension of the stomach and this increase persists for the entire duration of the distension, or at least for up to 60min (**Paper I**). The increase in dorsal aortic blood pressure (i.e. pressor response) was mediated via an α-adrenergic increase in the resistance of the systemic vasculature as it could be blocked with the α-adrenergic antagonist prazosin. Since it is unlikely that there is such a rapid increase in the circulating levels of plasma catecholamines, the response is most likely evoked by a generalized increase in the adrenergic nerve activity, which is similar to what has been reported earlier during the ingestion of a meal in mammals (Fronek and Stahlgren, 1968; Vatner et al., 1970a, b). The purpose of this pressor response is not entirely clear but part of it might facilitate a subsequent increase in the GBF through a redistribution of blood flow.

A postprandial increase in sympathetic activity leading to a pressor response is also evident in the shorthorn sculpin, both during the mechanical distension of the stomach and following the ingestion of a meal (**Paper II**). By feeding one group of sculpins their normal diet consisting of pieces of fish and sham-feeding another group followed by gastric distension in the same experimental setting it was possible to discern in more detail the contribution of mechanical stimuli to the postprandial cardiovascular response. When distending the stomach mechanically there is a prolonged pressor response that persists over several hours. With feeding there is still a significant increase in the dorsal aortic blood pressure, however, the pressure begins to decrease within an hour after feeding, coinciding with a decrease in the GI vascular resistance due to the chemical stimuli. This decrease in arterial pressure and

GI vascular resistance marks the start of the gastric emptying phase as determined by feeding experiments, whereby the contents of the gut was measured at regular intervals after spontaneous feeding (**Paper II**). These results confirm the original notion that it is the entry of food into the proximal intestine (i.e. duodenum in mammals) and its subsequent hydrolysis (i.e. chemical stimuli) that triggers the hyperemia.

The mechanism by which the gastric distension is sensed and relayed to the central nervous in fish has not been established. In rainbow trout (**Paper IV**) the stomach is innervated via the paired vagus and the splanchnic nerve (Figure 7), with the former innervating the major portion of the stomach (Burnstock, 1959). It is thus likely that a mechanical stimulus is sensed by mechano/stretch-receptors in the wall of the stomach as is the case in mammals (Carmagnola et al., 2005; Ozaki and Gebhart, 2001; Ozaki et al., 1999). These receptors, located within the circular muscular layer, and the myenteric plexus, relay the signal via vagal afferents to the central nervous system where it is processed (Berthoud and Neuhuber, 2000; Pittam et al., 1988). In mammals, a sympathetic efferent pathway regulates the tone of the vasculature, a concept that is corroborated in view of these results.

Additionally, several studies in cats have shown that mechanical stimuli within the intestine may induce a GI hyperemia by mechanisms involving vasoactive intestinal polypeptide and acetylcholine (Biber et al., 1970, 1971; Sjöqvist et al., 1980). No such response is seen in unanaesthetized rainbow trout when the proximal intestine is stimulated mechanically with saline (**Paper III**).

In addition to the large increase in gut blood flow, seen once food starts to enter the proximal intestine of the sculpin, there is also an initial smaller increase while most if not all of the food still remained within the stomach. Again this increase was not seen with gastric distension alone and must have dependent on some sort of chemical stimuli.

Finally, there does not seem to be any differences in the response to gastric distension between the rainbow trout and the shorthorn sculpin, despite the different feeding habits.

Chemical stimuli and diet composition

Gastrointestinal blood flow

In **Paper III**, a predigested diet was injected into the proximal intestine of unanaesthetized rainbow trout. Subsequently, there was a rapid increase in the GBF and the magnitude of the response depended on the nutrient composition of the diet. The most profound hyperemia was attained with a

diet resembling the "normal" diet (see Methodological considerations for details). The normal diet also produces the most profound increase in the postprandial GI oxygen consumption or metabolism (i.e. SDA). Aside from a difference in the amplitude of the response there is also a substantial difference in the temporal pattern. Increasing the protein content of the diet delays the increase in GBF compared with the normally ingested diet as well as a diet rich in lipids/fats. This difference probably reflects the different ways in which nutrients are digested, absorbed and metabolized and a rapid uptake could lead to a more prominent change in the osmolarity as well as the oxygen tension of the tissue.

The fact that a nutrient injection but not mechanical stimuli (the saline injection) evoked a GI hyperemia corroborated the results in **Paper I** and **II**. A chemical stimulus is the key in inducing the GI hyperemia, which is localised to where the digested food is positioned. A local hyperemia would also explain why the response in study **III** was quite small compared with, for example study **II**. The volume of the injected diet was relatively small and would fill only a very limited portion of the intestine as well as of course being rapidly absorbed. In addition, it could very well be that the mechanical stimuli during a meal, although not an initiating factor on its own, potentiate the response and assist in the shunting of blood from the systemic circulation to the GI tract via a constriction of the somatic vasculature.

As shown in study **III**, the initial increase in gut blood flow is maintained almost exclusively via a redistribution (shunting) of blood and there is a delayed increase in cardiac output. Therefore, since many studies in fish (Axelsson et al., 1989, 2000; Axelsson and Fritsche, 1991) attribute a large part of the increase in gut blood flow to an increase in cardiac output it is likely that the initial increase in cardiac output is dependent on mechanical stimuli and a subsequent increase in the sympathetic activity, while the meal is ingested. Nevertheless, the results indicate that a redistribution of blood does indeed occur in fish, as described earlier in several mammalian studies, where there is often a limited increase in cardiac output (Burns and Schenk, 1969; Fronek and Stahlgren, 1968; Vatner et al., 1970b, 1974).

Other studies suggest that it is not the diet composition but the energy or caloric content that is the central factor in altering the postprandial GBF (Eliason et al., 2008). However, it is unclear how the caloric content of the diet would be "sensed" by the GI tract and the exact mechanism linking caloric content and nutrient composition remains to be determined. Especially, since the postprandial oxygen consumption of the GI tract, the SDA is larger with a high protein diet compared with a high fat diet. Therefore, since the oxygen status of the GI tissues is one of the most important regulators of the GI

blood flow it is not easy to delineate how the caloric content of the diet would influence the response.

Results in **Paper II** suggest that gastric chemoreceptors might be present in the shorthorn sculpin and regulate the GBF after feeding. A similarly rapid increase in gut blood flow is also seen in sea bass but it was not determined whether or not this effect was mediated via a chemical or mechanical stimulus (Altimiras et al., 2008). Similar responses have also been reported in mammals and the placement of milk in the stomach produce a substantial, although short lived hyperemia in dogs (Kato et al., 1989).

The importance of these potential chemoreceptors warrants further investigations, but it seems unlikely that such a beneficial regulatory mechanism should be restricted to mammals alone and thus either have been lost by most fish or have evolved after the divergence from a common ancestor. In mammals there are mucosal chemoreceptors in the stomach that sense the presence or absence of certain nutrients such as glutamate or lipids in the diet (Blackshaw et al., 1987; Nakamura et al., 2008; Raybould, 1999) as well as the presence of noxious stimuli/taste (Rozengurt, 2006). Such a mechanism would prove vital to most if not all organisms and it is thus unlikely to have been lost over time.

The afferent limb responsible for relaying information regarding the presence and quantity of certain types of chemical stimuli, whether nutrient components or hormones resides within the vagus (Verberne et al., 2003) as well as possibly via an insulin dependent mechanism (Tsurugizawa et al., 2009).

Postprandial metabolism

Similar to the cardiovascular response, there was also a difference in the SDA that was dependant on the composition of the diet (**Paper III**). A diet that resembled the normal diet in rainbow trout produced a small but still significant response that followed rapidly after the nutrient injection. In contrast to this, there was a larger yet delayed response to a diet rich in protein. A diet consisting of mostly lipids gave no significant response at all. This is in concordance with the postulation that protein metabolism accounts for most of the SDA and that as much as 80% of the SDA response, to mainly proteins, reside outside of the GI tract (Brown and Cameron, 1991a, b). However, several more recent studies indicate that it is the energy content that is the the most important determinant (Eliason et al., 2007; Juan et al., 2010).

The fact that the response was small, irrespective of the diet composition, is reasonable given that most studies in fish have shown a clear

relationship between meal size and the SDA (Fu et al., 2005, 2006; Jobling and Davies, 1979; Jordan and Steffensen, 2007).

The central question that remains to be determined is to what extent the GBF is influenced by the postprandial increase in the GI metabolism and vice versa. A substantial portion of the postprandial increase in GBF, is due a decrease in the oxygen content or tension GI tissues in mammals (Bohlen, 1980a, b) and therefore any change in the GI metabolism should be important.

The result presented in **Paper III**, suggests that a substantial portion of the SDA is due to processes within the GI tract. The increase in oxygen consumption is rapid and occurs simultaneously with the increase in GBF. Therefore, the initial SDA is most likely due to processes within the GI tract, whereas at later stages processes outside of the GI tract dominate. This would explain why the SDA is maintained for a longer period of time compared with the increase in GBF. Additional evidence for this hypothesis comes from a study in sea bass (Dupont-Prinet et al., 2009). When challenged with hypoxia, sea bass maintain the postprandial GBF while decreasing the SDA and it was estimated that this was due to decreases in the portion of the SDA that occurs outside of the GI tract.

Consequently, the fact that there does not seem to be a 1:1 relation between the SDA and the GBF probably reflects that the SDA is the increase in total oxygenconsumption, not the GI metabolism *per se*. A substance or component that increases the metabolism of the GI tract, and thus the oxygen consumption, would lead to a decrease in PO_2 and therefore the oxygen content of that GI tissue. However, the SDA represents the total increases in metabolism. Therefore, parts of the SDA might even counteract the increase in GBF as there is also a decrease in PO_2 outside of the GI tract, which results in a competition for the available blood.

Also, the oxygen content does not necessarily have to be the only determinant and studies in mammls have shown that the post-absorptive tissue osmolarity is important in inducing and maintaining a postprandial hyperemia (Bohlen, 1982). As described below, hormonal components could also be important and modulate the response depednig on the diet.

Hormonal control mechanisms

Studies in mammals have shown that several hormones commonly found within the GI tract influence the GI vasculature (Chou et al., 1977). These hormones include gastrin (Guth and Smith, 1976; Kato et al., 1989) and cholecystokinin (CCK), which is a potent vasodilator in the GI tract (Chou et

al., 1984; Granger et al., 1980; Premen et al., 1985). Since it has been shown that the circulating levels of CCK increase after feeding in fish and vary with the diet (Jönsson et al., 2006) it was hypothesized the CCK might modulate the postprandial cardiovascular response in fish (**Paper V**).

Gastrointestinal blood flow

When infusing a small dose of CCK, into the arterial circulation of unanaesthetized rainbow, which corresponds to physiological postprandial plasma levels in fish (Jönsson et al., 2006) there was a significant increase in the GBF (**Paper V**). The fact that the response was small could be because the measured circulating levels of CCK represents a "spill over" into the systemic circulation. Locally along the gut, i.e. where the hydrolyzed food is, the levels of CCK probably reach much higher levels and CCK work mainly in a paracrine fashion.

When increasing the injected dose there was instead a decrease in the response compared with the lower dose. This can best be explained by a secondary effect of CCK on the branchial circulation leading to an increased vascular resistance of the gills and a subsequent decrease in both dorsal aortic pressure and cardiac output, as shown in Atlantic cod (Sundin and Nilsson, 1992). This further strengthens the notion that CCK acts as a local modulator, in the context that other factors such as oxygenation and osmolarity probably induce the majority of the postprandial hyperemia, with CCK acting to fine-tune the response to the composition of the diet.

In **Paper V** there was no response when treating isolated vascular preparations with CCK, which indicates that the mechanism is indirect and outside of the vessel itself. It could be that the CCK-mediated increase in GBF is limited to specific organs or regions within the GI tract. In dogs, CCK released from endocrine cells within the mucosa circulates to the pancrease and increase blood flow through the pancreatic circulation (Nakajima et al., 2001). This effect is probably due to an increased secretion of pancreatic enzymes with a subsequent increase in metabolism and oxygen consumption. In accord with this, the oxygen consumption of the pancrease and gut increases in the presence of GI substances such as secretin or CCK, even when the blood flow is held constant to avoid effects of a limitation in the flow (Fara et al., 1972). The vasculature of the principal islets of the teleost pancreas also responds to different nutritive stimuli and perhaps also hormones (Syed Ali, 1985).

However, given the lack of a direct effect on the vasculature the mechanism probably involves an afferent sensory limb in the vagus with two possible effector mechanisms. Heinemann et al and Sanchez-Fernandez et al

(1996; 2004) demonstrated that CCK released from endocrine cells of the GI mucosa in rats, is detected by afferents of the vagal limb leading to CNS-mediated adjustments in the outflow of the efferent vagal limb. The consequence of this is an increased release of acetylcholine and a secondary release of NO from the endothelium, leading to vasodilation. Another possible mechanism that has been studied in detail in rats includes changes in the sympathetic outflow to the GI tract. CCK released from the intestinal mucosa activate CCK-A receptors on afferent sensory nerves (Sartor and Verberne, 2002), either directly or indirectly via a 5-HT dependant mechanism (Saita and Verberne, 2003). This leads to an activation of the afferent limb of the vagus (see Figure 4). Subsequently there is a decrease in the sympathetic outflow to the vasomotor neurons of the GI tract that is dependent on mechanisms within the central nervous system (Sartor and Verberne, 2006; Verberne and Sartor, 2004). Since the sympathetic vasomotor neurons are essential to the tone of the GI vasculature this leads to a vasodilation (Vanner and Surprenant, 1996).

In fish, the presence of CCK-A and B receptors is still uncertain and a single ancestral form has been proposed in ectotherms such as frogs (Vigna et al., 1984, 1986), elasmobranchs (Oliver and Vigna, 1996) and in goldfish (*Carassius auratus*)(Himick et al., 1996). The so-called CCK-X receptor shows a higher affinity for the sulphated form of both CCKs and gastrin. The involvement of a CCK-receptor, either CCK-A or CCK-X, in the control of GBF in fish remains to be established.

The heart

CCK also had a significant influence on the function of the heart and it altered the phasic cardiac output flow profile (**Paper V**). There was no increase in cardiac output *per se* and therefore, the observed increase in cardiac output when injecting CCK in unanaesthetized rainbow trout was probably secondary to a decreased in blood pressure as a result of the dilated GI vasculature. Several studies, mainly in rats, have shown that CCK modulate the cardiac function in mammals, and there is a dose-dependant bradycardia that is mediated via CCK-A receptor, possibly on the membranes of pacemaker cells (Kurosawa et al., 2001; Marker and Roberts, 1988).

On the contrary, in the rainbow trout the change in the phasic flow profile is most likely due to a change in the compliance and/or tone of the bulbus arteriosus, but whether or not this involves a CCK-X receptor, remains to be determined. Since the bulbus is innervated by sympathetic fibers (Watson and Cobb, 1979), there is also the possibility that the effect is independent of a CCK receptor and instead mediated via an effect on these

fibers. A direct effect on the myocytes of the bulbus or a secondary effect via NO is also possible (Tota et al., 2005).

Most importantly, the physiological significance of such a mechanism is far from clear. It is, however, tempting to speculate that a change in the pulse profile represents a way to synchronize the flow through the gills with the ventilatory movements (Farrell et al., 1980). The bulbus, aside from its wind-kessel effect (Farrell, 1979), enhances the pumping capacity of heart in the winter flounder (*Pseudopleuronectes americanus*) (Mendonca et al., 2007) and enables the large stroke volume seen in many fishes (Jones et al., 2005). Further pharmacological studies of the bulbus arteriosus will hopefully explain these effects, and also the origin of the CCK.

Neural mechanisms

The GI tract of a salmonid is innervated by entrinsic nerves, i.e. the paired vagus and the splanchnic nerve (Figure 7) as well intrinsic nerves, i.e. the enteric nervous system. This autonomic innervation is potentially important in the regulation of the GBF both under routine conditions and during the changes that follows the ingestion of a meal.

Routine gastrointestinal blood flow

When removing the sympathetic innervation of the GI tract by sectioning the splanchnic nerve, there was a significant decrease in the resistance of the GI vasculature, which led to an increase in absolute blood flow through the coeliacomesenteric artery (**Paper IV**). **Paper IV**, therefore, provides evidence to confirm the concept that sympathetic nerves regulate the routine tone of the GI vasculature, in fish. Previous studies in fish have used pharmacological blockade of adrenergic receptors to investigate if there is an adrenergic vasoconstrictor tone on the GI vasculature, and it has not been possible established whether the tone was due to catecholamines released from nerves or circulating catecholamines (Axelsson et al., 1989; Axelsson et al., 2000).

In Atlantic cod, however, the tone is dependant on both a nervous and a humoral component (Axelsson and Fritsche, 1991). Therefore, a blockade of α-adrenergic receptors could potentially have decreased the sympathetic vasoconstrictor tone on the GI vasculature in rainbow trout even further (**Paper IV**). Nevertheless, a sympathetic nervous tone on the vasculature is to be expected, especially since there is histochemical evidence of adrenergic

perivascular nerves at the level of the arteriole (Holmgren, 1978; Nilsson, 1973).

The results in **Paper IV** is similar to results from studies in mammals, which show that the main vasoconstrictor tone on the GI vasculature is mediated via sympathetic nerves and there is little evidence for an enteric vasoconstrictor tone (Vanner and Surprenant, 1996). The sympathetic tone on GI vasculature presumably functions at the level of resistance arterioles and precapillary sphincters in mammals (Furness and Costa, 1974), which is similar to what has been shown in Atlantic cod (Axelsson and Fritsche, 1991). Also there are reports of an additional vasoconstrictor tone on the GI vasculature by the vagus in mammals, and after vagotomy there is a decrease in blood flow (Bell and Battersby, 1968).

Postprandial gastrointestinal blood flow

When both the paired vagus and the splanchnic nerve were sectioned in **Paper IV,** there was no significant difference in the cardiovascular response to a predigested diet, when compared with untreated fish. This suggests that the extrinsic innervation of the GI tract is of limited importance to the postprandial hyperemia as suggested previously by Fara (1972) and Nyhof (1983) using direct denervation and pharmacological interventions in mammals. Others have, however, ascribed at least a partial importance of the extrinsic innervation of the GI tract, for example after mucosal stimulation (Vanner et al., 1993). This mechanism was dependant on a cholinergic vasodilation which is in contrast to several studies whereby atropine had limited effects on the postprandial hyperemia (Kvietys et al., 1981b; Nyhof et al., 1985). Some of these contradictions could most likely be explained by differences in the experimental protocol and foremost variations in the diets used. Thus it is not unlikely that the response in **Paper IV** would have changed, had another diet been used.

Although, the results in **Paper IV** indicate that extrinsic nerves are of a minor importance to the postprandial hyperemia in fish, there are numerous indications that the extrinsic innervation of the GI tract should be regarded as central to the overall cardiovascular regulation after feeding. First of all, the cardiovascular response to a mechanical stimulus (Blackshaw et al., 1987) would depend on sensory information relayed within the vagus, and mechanical stimuli is probably important as a modulator during the early phase of the cardiovascular adjustments after feeding **(Paper I** and **II)**. Secondly sensory afferents of the vagus, most likely, convey information of chemical stimuli, including that of GI hormones such as CCK to the CNS in mammals (Berthoud and Neuhuber, 2000; Verberne et al., 2003). Chemical

stimuli and CCK have the capability to act as modulators of the postprandial hyperemia (**Paper III** and **V**).

Such overall effects on the cardiovascular system were also seen in **Paper IV**. Albeit the removal of extrinsic innervation did not change postprandial GBF, except for a slight delay, there was indeed a change in cardiac output (CO). The increase in CO was smaller and delayed after the denervation, which indicates that the increase in CO is not entirely due to a decrease in the resistance of the gastrointestinal vasculature (i.e. hyperemia) but also other neural mechanisms. This delayed increase in CO also explains the slightly delayed increase in gastrointestinal blood flow. The mechanism by which chemical stimuli within the GI tract augments the postprandial increase in CO, warrants further investigation.

Also, since the gastric receptive relaxation in fish seems independent of the vagus (Grove and Holmgren, 1992a, b), it remains to be determined in fish, if the cardiovascular response to mechanical simuli is mediated via the vagus. It also remains to be determined whether CCK acts via afferent neurons of the vagus or if the mechanism resides within the enteric nervous system. A possibility is that the sensory afferent of the proximal intestine travel within the splanchnic nerve and cutting this nerve would thus influence the modulatory capacity to certain types of stimuli. In addition to the sensory nerves that travel within the vagus there are also low-threshold nerv fibers within the spinal afferents (Furness et al., 1999).

In contrast to the sectioning of the extrinsic innervation of the GI tract, the pretreatment with TTX, a potent blocker of fast voltage-gated sodium channels, removed the postprandial hyperemia completely (**Paper IV**). Pretreatment with TTX would block most if not all of the autonomic innervation of the GI tract, including both the extrinsic and the intrinsic innervation. TTX has previously been shown to be a potent inhibitor of the GI hyperemia (Vanner et al., 1993) as well as acetylcholine mediated vasodilation in guinea pig (Neild et al., 1990). However, TTX failed to inhibit the GI hyperemia after luminal placement of glucose in the anaesthetized dogs (Nyhof and Chou, 1983).

There are several TTX-sensitive sodium-channels within in the GI tract of mammals (Sage et al., 2007). Fish also posses TTX-sensitive sodium channels within the GI tract (Haverinen et al., 2007) and studies have shown that TTX inhibits, for example, gut motlilty in zebrafish (Holmberg et al., 2007).

However, the exact nature and function of the TTX-sensitive mechanism, other than enteric, that is critical to the postprandial regulation of the GBF remains to be established in fish. It is tempting to speculate that

enteric nerves contribute by relaying the signal of a change in the mucosal oxygen content or osmolarity to the submucosal arterioles.

A change in the mucosal oxygenation is detected directly by nerve endings within the mucosa or indirectly via the release of ATP from red blood cells, functioning as oxygen sensors (Ellsworth et al., 1995, 2009). These intrinsic primary afferent neurons (IPANs) relay the signal to myenteric interneurons that activate vasomotor neurons innervating the submucosal vasculature (Gore and Bohlen, 1977) and perhaps also more superficial arterioles or arteries in mammals (Holtzer et al., 1994). The perivascular vasomotor neurons probably release acetylcholine or VIP, with a subsequent release of endothelia derived NO. In fish, nitrergic perivascular nerves release NO (Jennings et al., 2004), but there could also be a VIP mediated release of prostaglandins (Kågström and Holmgren, 1997) or neuropeptide Y (Shahbazi et al., 2002).

As similar mechanism, which would explain why enteric neurons are critical to the postprandial hyperemia in fish, has been proposed in mammals and in addition, the IPANs may also influence submucosal arterioles in a more direct way without the need for interneurons (Vanner and Macnaughton, 2004).

Atropine

One of the more peculiar results was the fact that atropine injected directly into the intestine gave a profound vasodilator response and a subsequent increase in GBF.

The initial idea was to use atropine to block muscarinic receptors in order to investigate if a postprandial release of acetylcholine could be an important component contributing to the postprandial hyperemia in fish. Such a mechanism, either directly on the vasculature or indirectly via the release of vasoactive substances or an increase in motility, has been suggested in some studies (Fara et al., 1972; Heinemann et al., 1996; Sanchez-Fernandez et al., 2004; Vatner et al., 1970b) while dismissed by others (Nyhof et al., 1985). In theory, the inhibition of GI muscarinic receptors would have removed the postprandial cardiovascular response, at least in part, while having limited effects on the routine GBF.

However, in rainbow trout, an injection of atropine into the proximal intestine produced a rapid and substantial increase in GBF (**Paper IV**). The response was much larger than any previously documented postprandial responses in fish and likely represented a maximal dilation of the GI vasculature. This effect was also seen in isolated vessels of the GI tract from rainbow trout (unpublished observation). Whether it represents a direct

muscarinic effect on the vasculature or a pharmacological artifact remains to be determined. There are reports in rats of a similar response and this response was mediated via a non-cholinergic mechanism involving changes in the function potassium channels with subsequent hyperpolarisation of the smooth muscle cells (Kwan et al., 2003; Liu et al., 2004). This calls for caution when using atropine and the results of atropine treatment should not necessarily be interpreted as purely muscarinc.

SUMMARY AND CONCLUSIONS

The results provided within this thesis indicate that several factors contribute to the final cardiovascular response during and after the ingestion of a meal and the subsequent absorption and redistribution of nutrients also in teleost fish.

When food enters the stomach there is an increase in the sympathetic output and a subsequent increase in the dorsal aortic blood pressure. Following the progressive hydrolysis and breakdown of the ingested food, in the stomach and after the entry into the proximal intestine, smaller components such as amino acids and triglycerides are released. These components decrease the GI vascular resistance (i.e. hyperemia) and a together with a maintained or increased systemic vascular resistance, this leads to either a redistribution of blood flow from the systemic circulation or a larger fraction of the cardiac output reaching the GI tract in the event that cardiac output increases.

Several mechanisms such as changes in the oxygen content and osmolarity of the GI tissues, due to, for example absorptive processes, as well as the release of GI factors such as CCK contribute to or modulate the postprandial GBF. Enteric components are essential at this stage, whereas the extrinsic innervation of the GI tract maintain the routine tone of the GI vasculature.

Major findings

Paper I

A mechanical distension of the stomach in the rainbow trout elicits an increased adrenergic tone on the systemic vasculature. The increased adrenergic tone produces a vasoconstriction in the systemic circulation including the GI portion, and a subsequent increase in dorsal aortic pressure. This pressor response increases the driving force for the perfusion of the GI vasculature when chemical stimuli induce a decrease in the resistance of the GI vasculature.

Paper II

When comparing the cardiovascular response to either the distension of the stomach or the ingestion of a meal in the shorthorn sculpin, it was confirmed that mechanical stimuli alone is insufficient in inducing an increased GBF. It is instead the presence of chemical stimuli i.e. the digested components of the ingested food in the proximal intestine that induces a decreased resistance of the GI vasculature.

Paper III

The injection of different pre-digested diets into the proximal intestine of rainbow trout, verified that the hyperemia is influenced by the diet composition. Also, the hyperemia is maintained both by a redistribution of blood and an increase in cardiac output. The results also show that the diet composition influences the postprandial increase in oxygen consumption (SDA) and the GI hyperemia corresponded with the SDA.

Paper IV

The extrinsic sympathetic innervation of the GI tract via the splanchnic nerve maintains the routine tone of the GI vasculature in rainbow trout. However, the extrinsic innervation is of little importance to the postprandial hyperemia and the removal of this innervation had limited effects on the postprandial hyperemia. In contrast, components of the enteric nervous system are necessary and a blockade of the intrinsic innervation using TTX completely abolished the hyperemia.

Paper V

Cholecystokinin (CCK) had a stimulatory effect on the GBF in rainbow trout. The effect is probably mediated via a neural reflex pathway and there was no effect on isolated vessels. Surprisingly, CCK had additional cardiovascular effects and changed the characteristics of the cardiac output flow profile, possibly via an effect on the bulbus arteriosus. The physiologic significance of such an effect is a present unknown.

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REFERENCES

Aldman, G., Grove, D. and Holmgren, S. (1992). Duodenal acidification and intra-arterial injection of CCK8 increase gallbladder motility in the rainbow trout, *Oncorhynchus mykiss*. *Gen Comp Endocrinol* **86**, 20-25.

Alemayehu, A., Lock, K. R., Coatney, R. W. and Chou, C. C. (1994). L-NAME, nitric oxide and jejunal motility, blood flow and oxygen uptake in dogs. *Br J Pharmacol* **111**, 205-212.

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.

Ashworth, A. (1969). Metabolic rates during recovery from protein-calorie malnutrition: the need for a new concept of specific dynamic action. *Nature* **223**, 407-409.

Axelsson, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J Exp Biol* **137**, 287-301.

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.

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

Axelsson, M., Ehrenstrom, F. and Nilsson, S. (1987). Cholinergic and adrenergic influence on the teleost heart in vivo. *Exp Biol* **46**, 179-186.

Axelsson, M. and Farrell, A. P. (1993). Coronary blood flow in vivo in the coho salmon (*Oncorhynchus kisutch*). *Am J Physiol* **264**, R963-971.

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. and Nilsson, S. (1986). Blood pressure control during exercise in the Atlantic cod, *Gadus morhua*. *J Exp Biol* **126**, 225-36.

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]* **170**, 145-152.

Baille, J. E. M., Hilton-Taylor, C. and Stuart, S. N. (2004). A Global Species Assessement. In *2004 IUCN Red List of Threatened Species*, eds. J. E. M. Baille C. Hilton-Taylor and S. N. Stuart). Cambridge: The IUCN Species Survival Commission.

Beamish, F. W. H. (1974). Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. *J Fish Res B Can* **31**, 1763-1769.

Bell, P. R. and Battersby, C. (1968). Effect of vagotomy on gastric mucosal blood flow. *Gastroenterology* **54**, 1032-1037.

Berthoud, H. R. and Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci* **85**, 1-17.

Biber, B. (1973). Vasodilator mechanisms in the small intestine. An experimental study in the cat. *Acta Physiol Scand Suppl.* **401**, 1-31.

Biber, B., Fara, J. and Lundgren, O. (1974). A pharmacological study of intestinal vasodilator mechanisms in the cat. *Acta Physiol Scand* **90**, 673-683.

Biber, B., Jodal, M., Lundgren, O. and Svanvik, J. (1970). Intestinal vasodilatation after mechanical stimulation of the jejunal mucosa. *Experientia* **26**, 263-264.

Biber, B., Lundgren, O. and Svanvik, J. (1971). Studies on the intestinal vasodilatation observed after mechanical stimulation of the mucosa of the gut. *Acta Physiol Scand* **82**, 177-190.

Blackshaw, L. A., Grundy, D. and Scratcherd, T. (1987). Involvement of gastrointestinal mechano- and intestinal chemoreceptors in vagal reflexes: an electrophysiological study. *J Auton Nerv Syst* **18**, 225-234.

Bohlen, H. G. (1980a). Intestinal mucosal oxygenation influences absorptive hyperemia. *Am J Physiol* **239**, H489-493.

Bohlen, H. G. (1980b). Intestinal tissue PO2 and microvascular responses during glucose exposure. *Am J Physiol* **238**, H164-171.

Bohlen, H. G. (1982). Na+-induced intestinal interstitial hyperosmolality and vascular responses during absorptive hyperemia. *Am J Physiol* **242**, H785-789.

Bohlen, H. G. (1998a). Integration of intestinal structure, function, and microvascular regulation. *Microcirculation* **5**, 27-37.

Bohlen, H. G. (1998b). Mechanism of increased vessel wall nitric oxide concentrations during intestinal absorption. *Am J Physiol.* **275**, 542-550.

Boyce, S. J. and 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.

Brown, C. R. and Cameron, J. N. (1991a). The induction of specific dynamic action in channel catfish by infusion of essential amino acids. *Physiol Zool* **64**, 276-297.

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

Brown, J. A. (1985). Renal microvasculature of the rainbow trout, Salmo gairdneri: scanning electron microscopy of corrosion casts of glomeruli. *Anat Rec* **213**, 505-513.

Bucking, C. and Wood, C. M. (2006). Water dynamics in the digestive tract of the freshwater rainbow trout during the processing of a single meal. *J Exp Biol* **209**, 1883-1893.

Buddington, R. K., Chen, J. W. and Diamond, J. (1987). Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. *J Physiol* **393**, 261-281.

Buddington, R. K. and Hilton, J. W. (1987). Intestinal adaptations of rainbow trout to changes in dietary carbohydrate. *Am J Physiol* **253**, G489-496.

Buddington, R. K., Krogdahl, A. and Bakke-Mckellep, A. M. (1997). The intestines of carnivorous fish: structure and functions and the relations with diet. *Acta Physiol Scand Suppl* **638**, 67-80.

Burns, G. P. and Schenk, W. G., Jr. (1969). Effect of digestion and exercise on intestinal blood flow and cardiac output. An experimental study in the conscious dog. *Arch Surg* **98**, 790-794.

Burnstock, G. (1959). The innervation of the gut of the brown trout (*Salmo trutta*). *Quart J Microsc Sci* **100**, 199-219.

Carmagnola, S., Cantu, P. and Penagini, R. (2005). Mechanoreceptors of the proximal stomach and perception of gastric distension. *Am J Gastroenterol* **100**, 1704-1710.

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

Chou, C. C., Alemayehu, A. and Mangino, M. J. (1989). Prostanoids in regulation of postprandial jejunal hyperemia and oxygen uptake. *Am J Physiol* **257**, G798-808.

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

Chou, C. C., Hsieh, C. P. and Dabney, J. M. (1977). Comparison of vascular effects of gastrointestinal hormones on various organs. *Am J Physiol* **232**, H103-109.

Chou, C. C., Kvietys, P., Post, J. and Sit, S. P. (1978). Constituents of chyme responsible for postprandial intestinal hyperemia. *Am. J. Physiol.* **235**, H677-682.

Chou, C. C. and Kvietys, P. R. (1981). Physiological and pharmacological alterations in gastrointestinal blood flow. In *Measurments of blood flow - Applications to the splanchnic circulation*, eds. D. N. Granger and G. B. Bulkley). Baltimore: Williams and Wilkins.

Chou, C. C., Mangino, M. J. and Sawmiller, D. R. (1984). Gastrointestinal Hormones and Intestinal Blood Flow. *Physiology of the Intestinal Circulation (ed. Shepard, A.P and Granger, D.N)*, 121-130.

Chou, C. C., Nyhof, R. A., Kvietys, P. R., Sit, S. P. and Gallavan, R. H., Jr. (1985). Regulation of jejunal blood flow and oxygenation during glucose and oleic acid absorption. *Am J Physiol* **249**, G691-701.

Chou, C. C. and Siregar, H. (1982). Role of histamine H1- and H2-receptors in postprandial intestinal hyperemia. *Am J Physiol* **243**, G248-252.

Couch, J. A. (1990). Pericyte of a teleost fish: Ultrastructure, position, and role in neoplasia as revealed by a fish model. *Anat rec* **228**, 7-14.

Coulson, R. A., Herbert, J. D. and Hernandez, T. (1978). Energy for amino acid absorption, transport and protein synthesis in vivo. *Comp biochem Physiol* **60**, 13-20.

Coulson, R. A. and Hernandez, T. (1979). Increase in Metabolic Rate of the Alligator Fed Proteins or Amino Acids. *J Nutr* **109,** 538-550.

Couto, A., Enes, P., Peres, H. and Oliva-Teles, A. (2008). Effect of water temperature and dietary starch on growth and metabolic utilization of diets in gilthead sea bream (Sparus aurata) juveniles. *Comp Biochem Physiol A Mol Integr Physiol* **151**, 45-50.

Cui, Y. and Liu, J. (1990). Comparison of energy budget among six teleosts-II. Metabolic rates. *Comp Biochem Physiol* **97**, 169-174.

Dunel-Erb, S. and Laurent, P. (1980). Ultrastructure of marine teleost gill epithelia: SEM and TEM study of the chloride cell apical membrane. *J Morphol* **165**, 175-86.

Dupont-Prinet, A., Claireaux, G. and McKenzie, D. J. (2009). Effects of feeding and hypoxia on cardiac performance and gastrointestinal blood flow during critical speed swimming in the sea bass *Dicentrarchus labrax*. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **154**, 233-240.

Elger, M., Wahlqvist, I. and Hentschel, H. (1984). Ultrastructure and adrenergic innervation of preglomerular arterioles in the euryhaline teleost, *Salmo gairdneri*. *Cell Tissue Res* **237**, 451-8.

Eliason, E. J., Higgs, D. A. and 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.

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**, 380-2888.

Ellsworth, M. L., Ellis, C. G., Goldman, D., Stephenson, A. H., Dietrich, H. H. and Sprague, R. S. (2009). Erythrocytes: oxygen sensors and modulators of vascular tone. *Physiology (Bethesda)* **24**, 107-116.

Ellsworth, M. L., Forrester, T., Ellis, C. G. and Dietrich, H. H. (1995). The erythrocyte as a regulator of vascular tone. *Am J Physiol* **269**, H2155-2161.

Enes, P., Peres, H., Couto, A. and Oliva-Teles, A. (2009). Growth performance and metabolic utilization of diets including starch, dextrin, maltose or glucose as carbohydrate source by gilthead sea bream (*Sparus aurata*) juveniles. *Fish Physiol Biochem. In press*

Epple, A. and Brinn, J. E. (1975). Islet histophysiology: Evolutionary correlations. *Gen Comp Endocrinol* **27**, 320-349.

Fara, J. W., Rubinstein, E. H. and Sonnenschein, R. R. (1972). Intestinal hormones in mesenteric vasodilation after intraduodenal agents. *Am J Physiol* **223**, 1058-1067.

Farrell, A. P. (1979). The Wind-kessel Effect of the Bulbus Arteriousus in Trout. *J. Exp. Zool.* **209**, 169-173.

Farrell, A. P., Johansen, J. A. and Graham, M. S. (1988). The Role of The Pericardium in Cardiac Performance of The Trout (*Salmo gairdneri*). *Physio Zool* **61**, 213-221.

Farrell, A. P., Sobin, S. S., Randall, D. J. and Crosby, S. (1980). Intralamellar blood flow patterns in fish gills. *Am J Physiol* **239**, R428-36.

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 severe hypercapnia. *Comp Biochem Physiol A Mol Integr Physiol* **128**, 551-563.

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.

Franklin, C. and Axelsson, M. (1994). The Intrinsic Properties of an in Situ Perfused Crocodile Heart. *J Exp Biol* **186**, 269-288.

Franklin, C. and Davie, P. S. (1993). The role of the pericardium in cardiac function in the dogfish, *Squalus acanthias*. *J Fish Biol* **43**, 213-219.

Franklin, C. E. and Davie, P. S. (1991). The pericardium facilitates pressure work in the eel heart. *J Fish Biol* **39**, 559-564.

Fronek, K. and Fronek, A. (1970). Combined effect of exercise and digestion on hemodynamics in conscious dogs. *Am J Physiol* **218**, 555-559.

Fronek, K. and Stahlgren, L. H. (1968). Systemic and regional hemodynamic changes during food intake and digestion in nonanesthetized dogs. *Circ Res* **23**, 687-692.

Fu, S. J., Cao, Z. D. and Peng, J. L. (2006). Effect of meal size on postprandial metabolic response in Chinese catfish (*Silurus asotus* Linnaeus). *J Comp Physiol* **176**, 489-495.

Fu, S. J., Xie, X. J. and Cao, Z. D. (2005). Effect of meal size on postprandial metabolic response in southern catfish (Silurus meridionalis). *Comp Biochem Physiol A Mol Integr Physiol* **140**, 445-451.

Furness, J. B. and Costa, M. (1974). The adrenergic innervation of the gastrointestinal tract. *Ergeb Physiol* **69**, 2-51.

Furness, J. B., Kunze, W. A. and Clerc, N. (1999). Nutrient tasting and signaling mechanisms in the gut. II. The intestine as a sensory organ: neural, endocrine, and immune responses. *Am J Physiol* **277**, G922-928.

Gallavan, R. H., Jr. and Chou, C. C. (1985). Possible mechanisms for the initiation and maintenance of postprandial intestinal hyperemia. *Am J Physiol* **249**, G301-308.

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.

Garber, K. J. (1983). Effect of fish size, meal size and dietary moisture on gastric evacuation of pelleted diets by yellow perch, Perca flavescens. *Aquaculture* **34**, 41-49.

Gonzalez-Alonso, J., Olsen, D. B. and Saltin, B. (2002). Erythrocyte and the regulation of human skeletal muscle blood flow and oxygen delivery: role of circulating ATP. *Circ Res* **91**, 1046-1055.

Gore, R. W. and Bohlen, H. G. (1977). Microvascular pressures in rat intestinal muscle and mucosal villi. *Am J Physiol* **233**, H685-693.

Graham, M. S. and Farrell, A. P. (1989). The effect of temperature acclimation and adrenergic stimulation on performance of a perfused trout heart. *Physiol Zool* **62**, 38-61.

Granger, D. N., Mortillaro, N. A., Perry, M. A. and Kvietys, P. R. (1982). Autoregulation of intestinal capillary filtration rate. *Am J Physiol* **243**, G475-483.

Granger, D. N., Richardson, P. D., Kvietys, P. R. and Mortillaro, N. A. (1980). Intestinal blood flow. *Gastroenterology* **78**, 837-863.

Granger, H. J., Goodman, A. H. and Cook, B. H. (1975). Metabolic models of microcirculatory regulation. *Fed Proc* **34**, 2025-2030.

Granger, H. J. and Shepherd, A. P., Jr. (1973). Intrinsic microvascular control of tissue oxygen delivery. *Microvasc Res* **5**, 49-72.

Grove, D. J. and Holmgren, S. (1992a). Intrinsic Mechanisms Controlling Cardiac Stomach Volume of the Rainbow Trout (*Oncorhynchus mykiss*) Following Gastric Distension. *J Exp Biol* **163**, 33-48.

Grove, D. J. and Holmgren, S. (1992b). Mechanisms Controlling Stomach Volume of the Atlantic Cod Following Gastric Distension. *J Exp Biol* **163**, 49-63.

Gräns, A., Albertsson, F., Axelsson, M. and Olsson, C. (2009). Postprandial changes in enteric electrical activity and gut blood flow in rainbow trout (*Oncorhynchus mykiss*) acclimated to different temperatures. *J Exp Biol* **212**, 2550-2557.

Guth, P. H. and Smith, E. (1976). The effect of gastrointestinal hormones on the gastric microcirculation. *Gastroenterology* **71**, 435-438.

Haverinen, J., Hassinen, M. and Vornanen, M. (2007). Fish cardiac sodium channels are tetrodotoxin sensitive. *Acta Physiol (Oxf)* **191**, 197-204.

Heinemann, A., Jocic, M., Peskar, B. M. and Holzer, P. (1996). CCK-evoked hyperemia in rat gastric mucosa involves neural mechanisms and nitric oxide. *Am J Physiol* **270**, G253-8.

Hidalgo, M. C., Urea, E. and Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture* **170**, 267-283.

Himick, B. A., Vigna, S. R. and Peter, R. E. (1996). Characterization of cholecystokinin binding sites in goldfish brain and pituitary. *Am J Physiol Regul Integr Comp Physiol* **271**, R137-143.

Holeton, G. F. and Randall, D. J. (1967). Changes in blood pressure in the rainbow trout during hypoxia. *J Exp Biol* **46**, 297-305.

Holmberg, A., Olsson, C. and Hennig, G. W. (2007). TTX-sensitive and TTX-insensitive control of spontaneous gut motility in the developing zebrafish (*Danio rerio*) larvae. *J Exp Biol* **210**, 1084-1091.

Holmgren, S. (1978). Sympathetic innervation of the coeliac artery from a teleost, *Gadus morhua*. *Comp biochem Physiol* **60**, 27-32.

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

Holtzer, P., Livingston, E. H. and Guth, P. H. (1994). Neural, metabolic, physical, and endothelial factors in the regulation of the gastric circulation In *Physiology of the gastrointestinl tract* (ed. L. R. Johnson), pp. 1311-1329. New York: Raven Press.

Hopkinson, B. R. and Schenk, W. G., Jr. (1968). The electromagnetic measurement of liver blood flow and cardiac output in conscious dogs during feeding and exercise. *Surgery* **63**, 970-975.

Jennings, B. L., Broughton, B. R. and Donald, J. A. (2004). Nitric oxide control of the dorsal aorta and the intestinal vein of the Australian short-finned eel *Anguilla australis*. *J Exp Biol* **207**, 1295-1303.

Jensen, H., Rourke, I. J., Moller, M., Jonson, L. and Johnsen, A. H. (2001). Identification and distribution of CCK-related peptides and mRNAs in the rainbow trout, *Oncorhynchus mykiss*. *Biochim Biophys Acta* **1517**, 190-201.

Jensen, J., M., A. and S., H. (1991). Effects of substance P and vasoactive intestinal polypeptide on the gastrointestinal blood flow in the Atlantic cod, *Gadus morhua*. *J Exp Biol* **156**, 361-373.

Jensen, J., Olson, K. R. and Conlon, J. M. (1993). Primary structures and effects on gastrointestinal motility of tachykinins from the rainbow trout. *Am J Physiol* **265**, R804-810.

Jobling, M. (1980). Gastric evacuation in plaice, (*Pleuronectes platessa*) L.: effects of dietary energy level and food composition. *J Fish Biol* **17**, 187-196.

Jobling, M. and Davies, P. S. (1979). Gastric evacuation in plaice, (*Pleuronectes platessa*): effects of temperature and meal size. *J Fish Biol* **14**, 539-546.

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

Jobling, M., Gwyther, D. and Grove, D. J. (1977). Some effects of temperature, meal size and body weight on gastric evacuation time in the dab *Limanda limanda* (L.). *J Fish Biol* **10**, 291-298.

Johnsen, A. H. (1998). Phylogeny of the cholecystokinin/gastrin family. *Front Neuroendocrinol* **19**, 73-99.

Johnston, I. A. and Battram, J. (1993). Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. *Marine Biology* **115**, 7-14.

Jones, D. R., Perbhoo, K. and Braun, M. H. (2005). Necrophysiological determination of blood pressure in fishes. *Naturwissenschaften* **92**, 582-585.

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

Juan, C. P.-C., Santosh, P. L. and Gamperl, A. K. (2010). Effects of dietary protein and lipid level, and water temperature, on the post-feeding oxygen consumption of Atlantic cod and haddock. *Aquacult res* **41**, 198-209.

Jönsson, E., Forsman, A., Einarsdottir, I. E., Egner, B., Ruohonen, K. and Bjornsson, B. T. (2006). Circulating levels of cholecystokinin and gastrin-releasing peptide in rainbow trout fed different diets. *Gen Comp Endocrinol* **148**, 187-194.

Kato, M., Naruse, S., Takagi, T. and Shionoya, S. (1989). Postprandial gastric blood flow in conscious dogs. *Am J Physiol* **257**, G111-117.

Katschinski, M., Dahmen, G., Reinshagen, M., Beglinger, C., Koop, H., Nustede, R. and Adler, G. (1992). Cephalic stimulation of gastrointestinal secretory and motor responses in humans. *Gastroenterology* **103**, 383-391.

Kita, J. and Itazawa, Y. (1990). Microcirculatory pathways in the spleen of the rainbow trout *Oncorhynchus mykissJap J Ichthyol* **37**, 265-272.

Kitamikado, M. and Tachino, S. (1960a). Studies on the digestive enzymes of rainbow troue - I, Carbohydrates. *Bull Jap Soc of Sci Fish* **26**, 679-684.

Kitamikado, M. and Tachino, S. (1960b). Studies on the digestive enzymes of rainbow troue - II, Proteases. *Bull Jap Soc of Sci Fish* **26**, 685-690.

Kitamikado, M. and Tachino, S. (1960c). Studies on the digestive enzymes of rainbow troue - III, Esterases. *Bull Jap Soc of Sci Fish* **26**, 691-694.

Kurosawa, M., Iijima, S., Funakoshi, A., Kawanami, T., Miyasaka, K., Bucinskaite, V. and Lundeberg, T. (2001). Cholecystokinin-8 (CCK-8) has no effect on heart rate in rats lacking CCK-A receptors. *Peptides* **22**, 1279-1284.

Kwan, C. Y., Zhang, W. B., Kwan, T. K. and Sakai, Y. (2003). In vitro relaxation of vascular smooth muscle by atropine: involvement of K+ channels and endothelium. *Naunyn Schmiedebergs Arch Pharmacol* **368**, 1-9.

Kvietys, P. R., Gallavan, R. H. and Chou, C. C. (1980a). Contribution of bile to postprandial intestinal hyperemia. *Am J Physiol* **238**, G284-288.

Kvietys, P. R., McLendon, J. M. and Granger, D. N. (1981a). Postprandial intestinal hyperemia: role of bile salts in the ileum. *Am J Physiol* **241**, G469-477.

Kvietys, P. R., Miller, T. and Granger, D. N. (1980b). Intrinsic control of colonic blood flow and oxygenation. *Am J Physiol* **238**, G478-484.

Kvietys, P. R., Wilborn, W. H. and Granger, D. N. (1981b). Effect of atropine on bile-oleic acid-induced alterations in dog jejunal hemodynamics, oxygenation, and net transmucosal water movement. *Gastroenterology* **80**, 31-38.

Kågström, J., Axelsson, M. and Holmgren, S. (1994). Cardiovascular Responses To Scyliorhinin I And II In The Rainbow Trout, *Oncorhynchus Mykiss*, In Vivo And In Vitro. *J Exp Biol* **191**, 155-166.

Kågström, J., Axelsson, M., Jensen, J., Farrell, A. P. and Holmgren, S. (1996a). Vasoactivity and immunoreactivity of fish tachykinins in the vascular system of the spiny dogfish. *Am J Physiol* **270**, R585-593.

Kågström, J. and Holmgren, S. (1997). Vip-induced relaxation of small arteries of the rainbow trout, *Oncorhynchus mykiss*, involves prostaglandin synthesis but not nitric oxide. *J Auton Nerv Syst* **63**, 68-76.

Kågström, J., Holmgren, S., Olson, K. R., Conlon, J. M. and Jensen, J. (1996b). Vasoconstrictive effects of native tachykinins in the rainbow trout, *Oncorhynchus mykiss*. *Peptides* **17**, 39-45.

Laurent, P. and Dunel, S. (1980). Morphology of gill epithelia in fish. *Am J Physiol* **238**, R147-159.

Leek, B. F. (1977). Abdominal and pelvic visceral receptors. *Br Med Bull* **33**, 163-168.

LeGrow, S. M. and 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.

Lin, T. M. and Alphin, R. S. (1957). Cephalic Phase of Gastric Secretion in the Rat. *Am J Physiol* **192**, 23-26.

Liu, S. Q., Zang, W. J., Li, Z. L., Yu, X. J. and Li, B. P. (2004). Effect of atropine on denervated rabbit ear blood vessels. *J Cardiovasc Pharmacol* **43**, 99-105.

Longhurst, J. C., Ashton, J. H. and Iwamoto, G. A. (1980). Cardiovascular reflexes resulting from capsaicin-stimulated gastric receptors in anesthetized dogs. *Circ Res* **46**, 780-788.

Longhurst, J. C. and Ibarra, J. (1982). Sympathoadrenal mechanisms in hemodynamic responses to gastric distension in cats. *Am J Physiol* **243**, H748-753.

Longhurst, J. C. and Ibarra, J. (1984). Reflex regional vascular responses during passive gastric distension in cats. *Am J Physiol* **247**, R257-265.

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

Lyndon, A. R., Houlihan, D. F. and 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.

Marker, J. D. and Roberts, M. L. (1988). Chronotropic actions of cholecystokinin octapeptide on the rat heart. *Regul Pept* **20**, 251-259.

Matheson, P. J., Wilson, M. A. and 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 Mol Integr Physiol* **144**, 381-94.

McCue, M. D., Bennett, A. F. and Hicks, J. W. (2005). The effect of meal composition on specific dynamic action in Burmese pythons (Python molurus). *Physiol Biochem Zool* **78**, 182-192.

Medland, T. E. and 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.

Mendonca, P. C., Genge, A. G., Deitch, E. J. and Gamperl, A. K. (2007). Mechanisms responsible for the enhanced pumping capacity of the in situ winter flounder heart (*Pseudopleuronectes americanus*). *Am J Physiol Regul Integr Comp Physiol* **293**, R2112-2119.

Molinari, C., Battaglia, A., Grossini, E., Florio, S., Mary, D. A., Vassanelli, C. and Vacca, G. (2003). Activation of the renin-angiotensin system contributes to the peripheral vasoconstriction reflexly caused by stomach distension in anaesthetized pigs. *Exp Physiol* **88**, 359-367.

Molinari, C., Sabbatini, M., Grossini, E., Mary, D. A., Cannas, M. and Vacca, G. (2006). Cardiovascular effects and c-Fos expression in the rat hindbrain in response to innocuous stomach distension. *Brain Res Bull* **69**, 140-146.

Murakami, T. (1971). Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. *Arch Histol Jpn* **32**, 445-454.

Murakami, T. (1975). Pliable metacrylate casts of blood vessels: use in a scanning electron microscope study of the microcirculation in rat hypophysis. *Arch Histol Jpn* **38**, 151-168.

Mustafa, T. and Agnisola, C. (1998). Vasoactivity of adenosine in the trout (*Oncorhynchus mykiss*) coronary system: involvement of nitric oxide and interaction with noradrenaline. *J Exp Biol* **201**, 3075-3083.

Nakajima, M., Naruse, S., Kitagawa, M., Ishiguro, H., Jin, C., Ito, O. and Hayakawa, T. (2001). Role of cholecystokinin in the intestinal phase of pancreatic circulation in dogs. *Am J Physiol Gastrointest Liver Physiol* **280**, G614-620.

Nakamura, E., Torii, K. and Uneyama, H. (2008). Physiological roles of dietary free glutamate in gastrointestinal functions. *Biol Pharm Bull* **31**, 1841-3.

Neild, T. O., Shen, K. Z. and Surprenant, A. (1990). Vasodilatation of arterioles by acetylcholine released from single neurones in the guinea-pig submucosal plexus. *J Physiol* **420**, 247-65.

Nelson, J. S. (1994). Fishes of the World. New York: John Wiley & Sons.

Nilsson, S. (1973). Fluorescent histochemistry of the catecholamines in the urinary bladder of a teleost, *Gadus morhua Comp Gen Pharmacol* **4**, 17-21.

Nilsson, S. and Sundin, L. (1998). Gill blood flow control. *Comp Biochem Physiol A Mol Integr Physiol* **119**, 137-147.

Nosaka, S., Murase, S. and Murata, K. (1991). Arterial baroreflex inhibition by gastric distension in rats: mediation by splanchnic afferents. *Am J Physiol* **260**, R985-994.

Nyhof, R. and Chou, C. C. (1981). Absence of cholinergic or serotonergic mediation in food induced intestinal hyperemia (Abstract). *Federation Proc* **40**, 491.

Nyhof, R. A. and Chou, C. C. (1983). Evidence against local neural mechanism for intestinal postprandial hyperemia. *Am J Physiol* **245**, H437-446.

Nyhof, R. A., Ingold-Wilcox, D. and Chou, C. C. (1985). Effect of atropine on digested food-induced intestinal hyperemia. *Am J Physiol* **249**, G685-690.

Nyquist, H. (1928). Certain Topics in Telegraph Transmission Theory. *Trand, AIEE* **47**, 617-644.

Oliver, A. S. and Vigna, S. R. (1996). CCK-X receptors in the endothermic mako shark (*Isurus oxyrinchus*). *Gen Comp Endocrinol* **102**, 61-73.

Olson, K. R. (2002). Vascular anatomy of the fish gill. *J Exp Zool* **293**, 214-231.

Olson, K. R. and Donald, J. A. (2009). Nervous control of circulation--the role of gasotransmitters, NO, CO, and H2S. *Acta Histochem* **111**, 244-256.

Olson, K. R., Duff, D. W., Farrell, A. P., Keen, J., Kellogg, M. D., Kullman, D. and Villa, J. (1991). Cardiovascular effects of endothelin in trout. *Am J Physiol* **260**, H1214-1223.

Olson, K. R. and Villa, J. (1991). Evidence against nonprostanoid endothelium-derived relaxing factor(s) in trout vessels. *Am J Physiol* **260**, R925-933.

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.

Olsson, C., Holmberg, A. and Holmgren, S. (2008). Development of enteric and vagal innervation of the zebrafish (*Danio rerio*) gut. *J Comp Neurol* **508**, 756-770.

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

Olsson, C. and Holmgren, S. (2009). The Neuronal and Endocrine Regulation of Gut Function. In *Fish Neuroendocrinology*, vol. 28 eds. N. J. Bernier G. Van Der Kraak A. P. Farrell and C. J. Brauner, pp. 467-538. London: Academic Press, imprint of Elsevier.

Olsson, C., Jonz, M. and Zaccone, G. (2009). Nervous regulation of internal organs in fishes. Preface. *Acta Histochem* **111**, 173-175.

Owen, S. F. (2001). Meeting energy budgets by modulation of behaviour and physiology in the eel (*Anguilla anguilla* L.). *Comp Biochem Physiol A Mol Integr Physiol* **128**, 631-644.

Ozaki, N. and Gebhart, G. F. (2001). Characterization of mechanosensitive splanchnic nerve afferent fibers innervating the rat stomach. *Am J Physiol Gastrointest Liver Physiol* **281**, G1449-1459.

Ozaki, N., Sengupta, J. N. and Gebhart, G. F. (1999). Mechanosensitive properties of gastric vagal afferent fibers in the rat. *J Neurophysiol* **82**, 2210-2220.

Peck, M. A., Buckley, L. J. and Bengtsson, D. A. (2003). Energy losses due to routine and feeding metabolism in young-of-the-year juvenile Atlantic cod (*Gadus morhua*). *Can J Fish Aquat Sci* **60**, 927-937.

Peres, H. and Oliva-Teles, A. (2001). Effect of dietary protein and lipid level on metabolic utilization of diets by european sea bass (*Dicentrarchus labrax*) juveniles. *Fish Physiol Biochem* **25**, 269-275.

Phillips, R. J. and Powley, T. L. (2000). Tension and stretch receptors in gastrointestinal smooth muscle: re-evaluating vagal mechanoreceptor electrophysiology. *Brain Res Brain Res Rev* **34**, 1-26.

Pierce, R. J. and Wissing, T. E. (1974). Energy Cost of Food Utilization in the Bluegill (*Lepomis macrochirus*). *Trans Am Fish* **103,** 38-45.

Pittam, B. S., Ewart, W. R., Appia, F. and Wingate, D. L. (1988). Physiological enteric stimulation elicits cardiovascular reflexes in the rat. *Am J Physiol* **255**, G319-328.

Pozo, F., Fueyo, A., Esteban, M. M., Rojo-Ortega, J. M. and Marin, B. (1985). Blood pressure changes after gastric mechanical and electrical stimulation in rats. *Am J Physiol* **249**, G739-744.

Premen, A. J., Kvietys, P. R. and Granger, D. N. (1985). Postprandial regulation of intestinal blood flow: role of gastrointestinal hormones. *Am J Physiol* **249**, G250-255.

Raybould, H. E. (1999). Nutrient tasting and signaling mechanisms in the gut. I. Sensing of lipid by the intestinal mucosa. *Am J Physiol* **277**, G751-755.

Rhodin, J. A. (1967). The ultrastructure of mammalian arterioles and precapillary sphincters. *J Ultrastruct Res* **18**, 181-223.

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

Rozengurt, E. (2006). Taste receptors in the gastrointestinal tract. I. Bitter taste receptors and alpha-gustducin in the mammalian gut. *Am J Physiol Gastrointest Liver Physiol* **291**, G171-177.

Rubner, M. (1902). Die Gesetze des Energiever Brauchs bei der Ernahrung. Leipzig und Wien.

Ruohonen, K., Grove, D. J. and McIlroy, J. T. (1997). The amount of food ingested in a single meal by rainbow trout offered chopped herring, dry and wet diets. *J Fish Biol* **51**, 93-105.

Sage, D., Salin, P., Alcaraz, G., Castets, F., Giraud, P., Crest, M., Mazet, B. and Clerc, N. (2007). Na(v)1.7 and Na(v)1.3 are the only tetrodotoxin-sensitive sodium channels expressed by the adult guinea pig enteric nervous system. *J Comp Neurol* **504**, 363-378.

Saita, M. and Verberne, A. J. (2003). Roles for CCK1 and 5-HT3 receptors in the effects of CCK on presympathetic vasomotor neuronal discharge in the rat. *Br J Pharmacol* **139**, 415-23.

Sanchez-Fernandez, C., Gonzalez, M. C., Beart, P. M., Mercer, L. D., Ruiz-Gayo, M. and Fernandez-Alfonso, M. S. (2004). A novel role for cholecystokinin: regulation of mesenteric vascular resistance. *Regul Pept* **121**, 145-153.

Sandblom, E., Axelsson, M. and Farrell, A. P. (2006). Central venous pressure and mean circulatory filling pressure in the dogfish Squalus acanthias: adrenergic control and role of the pericardium. *Am J Physiol* **291**, R1465-1473.

Sarles, H., Dani, R., Prezelin, G., Souville, C. and Figarella, C. (1968). Cephalic phase of pancreatic secretion in man. *Gut* **9**, 214-221.

Sartor, D. M. and Verberne, A. J. (2002). Cholecystokinin selectively affects presympathetic vasomotor neurons and sympathetic vasomotor outflow. *Am J Physiol Regul Integr Comp Physiol* **282**, R1174-1184.

Sartor, D. M. and Verberne, A. J. (2006). The sympathoinhibitory effects of systemic cholecystokinin are dependent on neurons in the caudal ventrolateral medulla in the rat. *Am J Physiol Regul Integr Comp Physiol* **291**, R1390-1398.

Sartor, D. M. and Verberne, A. J. (2008). Abdominal vagal signalling: a novel role for cholecystokinin in circulatory control? *Brain Res Rev* **59**, 140-154.

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.

Secor, S. M. (2009). Specific dynamic action: a review of the postprandial metabolic response. *J Comp Physiol B* **179**, 1-56.

Shahbazi, F., Holmgren, S., Larhammar, D. and Jensen, J. (2002). Neuropeptide Y effects on vasorelaxation and intestinal contraction in the Atlantic cod Gadus morhua. *Am J Physiol Regul Integr Comp Physiol* **282**, R1414-1421.

Shannon, C. E. (1949). Communication in the presence of noise. *Proc Inst Radio Engin* **37**, 10-21.

Shepherd, A. P. (1982). Role of capillary recruitment in the regulation of intestinal oxygenation. *Am J Physiol* **242**, G435-441.

Siregar, H. and Chou, C. C. (1982). Relative contribution of fat, protein, carbohydrate, and ethanol to intestinal hyperemia. *Am J Physiol* **242**, G27-31.

Sjöqvist, A., Delbro, D., Jodal, M. and Lundgren, O. (1980). The effect of Apamin on nonadrenergic, noncholinergic nervous vasodilatations in the cat small intestine. *Experientia* **36**, 1202.

Smith, L. S. and Bell, G. R. (1964). A technique for prolonged blood sampling in free swimming salmon. *J Fish Res Bd Can* **21**, 711-717.

Smith, R. R., Rumsey, G. L. and Scott, M. L. (1978). Heat Increment Associated with Dietary Protein, Fat, Carbohydrate and Complete Diets in Salmonids Comparative Energetic Efficiency. *J Nutr* **108**, 1025-1032.

Soldatov, A. (2006). Organ blood flow and vessels of microcirculatory bed in fish. *J Evol Biochem and Physiol* **42**, 243-252.

Steffensen, J. (1989). Some errors in respirometry of aquatic breathers: How to avoid and correct for them. *Fish Physiol Biochem* **6**, 49-59.

Stenslokken, K. O., Sundin, L. and Nilsson, G. E. (2006). Endothelin receptors in teleost fishes: cardiovascular effects and branchial distribution. *Am J Physiol Regul Integr Comp Physiol* **290**, R852-860.

Stevens, E. D. and Randall, D. J. (1967). Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. *J Exp Biol* **46**, 307-315.

Sundin, L. and Nilsson, G. E. (1997). Neurochemical mechanisms behind gill microcirculatory responses to hypoxia in trout: in vivo microscopy study. *Am J Physiol* **272**, R576-585.

Sundin, L. and Nilsson, S. (1992). Arterio-Venous Branchial Blood Flow in the Atlantic Cod Gadus Morhua. *J Exp Biol*, **165**, 73-84.

Sundin, L., Turesson, J. and Taylor, E. W. (2003). Evidence for glutamatergic mechanisms in the vagal sensory pathway initiating cardiorespiratory reflexes in the shorthorn sculpin *Myoxocephalus scorpius*. *J Exp Biol* **206**, 867-76.

Syed Ali, S. (1985). Microvasculature of the principal islets in the scorpion fish, *Myoxocephalus scorpius*. *Arch Histol Jpn* **48**, 363-371.

Takagi, T., Naruse, S. and Shionoya, S. (1988). Postprandial celiac and superior mesenteric blood flows in conscious dogs. *Am J Physiol* **255**, 522-528.

Tandler, A. and Beamish, F. W. H. (1980). Specific Dynamic Action and Diet in Largemouth Bass, *Micropterus salmoides* (Lacepede). *J Nutr* **110**, 750-764.

Taylor, J. R. and Grosell, M. (2009). The intestinal response to feeding in seawater gulf toadfish, Opsanus beta, includes elevated base secretion and increased epithelial oxygen consumption. *J Exp Biol* **212**, 3873-3881.

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

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**, 525-532.

Tota, B., Amelio, D., Pellegrino, D., Ip, Y. K. and Cerra, M. C. (2005). NO modulation of myocardial performance in fish hearts. *Comp Biochem Physiol A Mol Integr Physiol* **142**, 164-177.

Tsurugizawa, T., Uematsu, A., Nakamura, E., Hasumura, M., Hirota, M., Kondoh, T., Uneyama, H. and Torii, K. (2009). Mechanisms of neural response to gastrointestinal nutritive stimuli: the gut-brain axis. *Gastroenterology* **137**, 262-273.

Vacca, G., Mary, D. A., Battaglia, A., Grossini, E. and Molinari, C. (1996). The effect of distension of the stomach on peripheral blood flow in anaesthetized pigs. *Exp Physiol* **81**, 385-396.

Vacca, G. and Vono, P. (19932). The primary reflex effects of distension of the stomach on heart rate, arterial pressure and left ventricular contractility in the anaesthetized pig. *Pflugers Arch* **425**, 248-255.

Vanner, S., Jiang, M. M. and Surprenant, A. (1993). Mucosal stimulation evokes vasodilation in submucosal arterioles by neuronal and nonneuronal mechanisms. *Am J Physiol* **264**, G202-212.

Vanner, S. and Macnaughton, W. K. (2004). Submucosal secretomotor and vasodilator reflexes. *Neurogastroenterol Motil* **16 Suppl 1**, 39-43.

Vanner, S. and Surprenant, A. (1996). Neural reflexes controlling intestinal microcirculation. *Am J Physiol* **271**, G223-30.

Vatner, S. F., Franklin, D. and Van Citters, R. L. (1970a). Coronary and visceral vasoactivity associated with eating and digestion in the conscious dog. *Am J Physiol* **219**, 1380-1385.

Vatner, S. F., Franklin, D. and Van Citters, R. L. (1970b). Mesenteric vasoactivity associated with eating and digestion in the conscious dog. *Am J Physiol* **219**, 170-174.

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

Watson, A. D. and Cobb, J. L. (1979). A comparative study on the innervation and the vascularization of the bulbus arteriosus in teleost fish. *Cell Tissue Res* **196**, 337-346.

Vaugelade, P., Posho, L., Darcy-Vrillon, B., Bernard, F., Morel, M. T. and Duee, P. H. (1994). Intestinal oxygen uptake and glucose metabolism during nutrient absorption in the pig. *Proc Soc Exp Biol Med* **207**, 309-316.

Verberne, A. J. and Guyenet, P. G. (1992). Medullary pathway of the Bezold-Jarisch reflex in the rat. *Am J Physiol* **263**, R1195-1202.

Verberne, A. J., Saita, M. and Sartor, D. M. (2003). Chemical stimulation of vagal afferent neurons and sympathetic vasomotor tone. *Brain Res Brain Res Rev* **41**, 288-305.

Verberne, A. J. and Sartor, D. M. (2004). CCK-induced inhibition of presympathetic vasomotor neurons: dependence on subdiaphragmatic vagal afferents and central NMDA receptors in the rat. *Am J Physiol Regul Integr Comp Physiol* **287**, R809-816.

Vigna, S. R., Steigerwalt, R. W. and Williams, J. A. (1984). Characterization of cholecystokinin receptors in bullfrog (Rana catesbeiana) brain and pancreas. *Regul Pept* **9**, 199-212.

Vigna, S. R., Thorndyke, M. C. and Williams, J. A. (1986). Evidence for a common evolutionary origin of brain and pancreas cholecystokinin receptors. *Proc Natl Acad Sci U S A* **83**, 4355-4359.

Wilhelmj, C. M. and Bollman, J. L. (1928). The specific dynamic action and nitrogen elimination following intravenous administration of various amino acids. *J Biol Chem* **77,** 127-129.

Wilson, J. M. and Laurent, P. (2002). Fish gill morphology: inside out. *J Exp Zool* **293**, 192-213.

Windell, J. T., Kitchell, J. F., Norris, D. O., Norris, J. S. and Foltz, J. W. (1976). Temperature and Rate of Gastric Evacuation by Rainbow Trout, *Salmo gairdneri*. *Trans Am Fish Soc* **105**, 712-717.

Windell, J. T., Norris, D. O., Kitchell, J. F. and Norris, J. S. (1969). Digestive response of rainbow trout, *Salmo gairdneri*, to pellet diets. *J Fish Res Board Can* **26**, 1801-1812.

Yen, J. T., Nienaber, J. A., Hill, D. A. and Pond, W. G. (1989). Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. *Proc Soc Exp Biol Med* **190**, 393-398.