



# **Gastrointestinal motility and blood flow in teleosts during digestion and osmoregulation**

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GASTROINTESTINAL MOTILITY AND BLOOD FLOW IN TELEOSTS  
DURING DIGESTION AND OSMOREGULATION

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# **DISSERTATION ABSTRACT**

Teleost fishes occupy virtually every aquatic habitat on earth and as a group face a wide range of environmental challenges in their natural habitats, as well as during their life histories. In order to survive and thrive in the face of these challenges, it is essential for them to maintain homeostasis, as well as to acquire and assimilate energy. For this to occur the gastrointestinal tract must function effectively and efficiently, which is achieved through a wide range of processes including tightly regulated gastrointestinal motility and blood flow responses. The overall aim of this thesis was to provide further insight into the importance of gastrointestinal motility and blood flow in teleosts by focusing on their role during digestion and osmoregulation.

Using a combination of methods, a range of propagating and non-propagating *in vivo* intestinal motility patterns were documented in shorthorn sculpin (*Myoxocephalus scorpius*) and rainbow trout (*Oncorhynchus mykiss*). Pre-prandially, both species of teleosts displayed a rhythmic, anally propagating motility pattern resembling and most likely sharing a similar 'housekeeper' function as mammalian migrating motor complexes. Following the ingestion of food, this motility pattern was reduced and replaced by irregular contractile activity in the shorthorn sculpin, whereas it persisted in the rainbow trout, which most likely reflects the differences in feeding strategy between the two species (*i.e.* intermittent vs. continuous feeders, respectively).

Gastrointestinal motility also plays an important role in osmoregulation. Euryhaline rainbow trout rapidly initiated a drinking response in order to maintain water balance when transitioning from freshwater to seawater. To promote water absorption in the intestine, imbibed seawater was substantially desalinated in the oesophagus. This was followed by a gradual increase in the contractile activity of the intestine, which plateaued after ~2 days to remain at a significantly elevated level in fully seawater-acclimated individuals. It seems that the teleost analogue of the mammalian migrating motor complexes may also play an osmoregulatory role, as their frequency was significantly higher in seawater. This motility pattern may be necessary for transporting and mixing imbibed seawater in an optimal manner for ion and water absorption, as well as preventing the mucosal accumulation of carbonate precipitates taking place in the intestine of teleosts living in the sea.

Furthermore, a raft of circulatory modifications occurs in rainbow trout heading to sea. Gastrointestinal blood flow, cardiac output and stroke volume began to increase after ~2 days in seawater and reached a level two-fold higher than in freshwater after 4 days, which was maintained in fully seawater-acclimated trout. The up-regulation of these cardiovascular processes is most likely essential for the maintenance of osmotic homeostasis and acid-base balance for teleosts living in the sea. My findings also suggest that the increased blood flow is mainly required for the transportation of products such as ions, water and metabolic wastes, as standard metabolic rate was not significantly affected. Furthermore, seawater-acclimated trout were still able to further increase gastrointestinal perfusion following a meal, although there were strong indications that these individuals were approaching their maximum threshold.

In conclusion, the findings of this thesis enable a greater insight into the importance of gastrointestinal motility and blood flow during the processing of food and maintenance of osmotic homeostasis, which ultimately underlies the relative fitness of marine and euryhaline teleosts living in the sea.

**Keywords:** teleosts, intestine, motility, blood flow, freshwater, seawater, feeding

# **LIST OF PUBLICATIONS**

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

- I. Brijs, J., Hennig, G., Axelsson, M. and Olsson, C. (2014). Effects of feeding on *in vivo* motility patterns in the proximal intestine of shorthorn sculpin (*Myoxocephalus scorpius*). *Journal of Experimental Biology* **217**: 3015-3027; doi:10.1242/jeb.101741
- II. Brijs, J., Hennig, G., Kellermann, A., Axelsson, M. and Olsson, C. (2017). The presence and role of interstitial cells of Cajal in the proximal intestine of shorthorn sculpin (*Myoxocephalus scorpius*). *Journal of Experimental Biology* **0**: 1-11; doi:10.1242/jeb.141523
- III. Brijs, J., Hennig, G., Gräns, A., Dekens, E., Axelsson, M. and Olsson, C. (2017). Exposure to seawater increases intestinal motility in euryhaline rainbow trout (*Oncorhynchus mykiss*). Submitted to *Journal of Experimental Biology*.
- IV. Brijs, J., Axelsson, M., Gräns, A., Pichaud, N., Olsson, C. and Sandblom, E. (2015). Increased gastrointestinal blood flow: An essential circulatory modification for euryhaline rainbow trout (*Oncorhynchus mykiss*) migrating to sea. *Scientific Reports* **5**: 10430; doi:10.1038/srep10430
- V. Brijs, J., Gräns, A., Ekström, A., Olsson, C., Axelsson, M. and Sandblom, E. (2016). Cardiorespiratory up-regulation during seawater acclimation in rainbow trout: Effects on gastrointestinal perfusion and post-prandial responses. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **310**: R858-R865; doi:10.1152/ajpregu.00536.2015.

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# **INTRODUCTION**

## **1.1. The world and its fishes**

Approximately 71% of the Earth's surface is covered with water. Oceans, seas and bays contribute to 96% of this coverage, lakes and rivers to slightly more than 1%, and the remaining water is locked up in ice and glaciers (Shiklomanov, 1993). Aquatic environments vary considerably in a range of physical features such as temperature, salinity, oxygen content, pressure and pH, and provide habitat for at least 33,000 fish species (Bone and Moore, 2008; Fishbase, [www.fishbase.org](http://www.fishbase.org)).

Fish are loosely defined as gill-bearing aquatic craniates that lack limbs with digits, and are classed as a paraphyletic group since they do not share a common ancestor (Gill and Mooi, 2002). This results in a highly diverse group consisting of Myxini (hagfish), Cephalaspidomorpha (lampreys), Elasmobranchii (sharks, rays and skates), Holocephali (sawfish and chimaeras), Sarcopterygii (lobe-finned fishes) and the Actinopterygii (ray-finned fishes) (Fishbase, [www.fishbase.org](http://www.fishbase.org)). Teleostei or the teleosts are the largest infraclass in the class Actinopterygii, constituting over 95% of all extant fish species and occupying virtually every aquatic habitat on earth (Nelson, 2006).

To occupy the vastly different and extreme habitats such as hot springs with temperatures exceeding 40°C or polar waters under the ice sheets approaching the freezing point of seawater, teleosts exhibit a high diversity of morphological, physiological and behavioural adaptations (Brix, 2002). The majority of teleosts also experience fluctuating environmental conditions within their natural habitat when they are stationary and/or during their life history when they are migratory (Bone and Moore, 2008). To survive and thrive under these conditions, they must be able to sustain the metabolic processes that are required for the maintenance of homeostasis, growth and reproduction. To accomplish this, it is crucial for them to acquire and assimilate sufficient amounts of energy from the surrounding environment, which is only possible through feeding and a functional gastrointestinal system.

## **1.2. The gastrointestinal tract**

The great diversity of teleosts and their respective life histories has led to a huge variation in the morphology, anatomy, histology and physiology of the gastrointestinal tract (Olsson, 2011a). Nevertheless, there are some key similarities. Thus, the gastrointestinal tract can be subdivided into the headgut (mouth and pharynx), foregut (oesophagus and stomach, although

some species are stomachless; Manjakasy et al., 2009), midgut (intestine) and hindgut (rectum) (Olsson, 2011a; Wilson and Castro, 2011). The gastrointestinal tract is responsible for a diverse range of physiologically important functions such as digestion and absorption of nutrients (Bakke et al., 2011), ionic and osmotic regulation (Grosell, 2011), barrier and immune function (Cain and Swan, 2011), gas exchange and acid-base balance (Taylor et al., 2011), endocrine/neuroendocrine/paracrine functions (Takei and Loretz, 2011), respiration (Nelson and Dehn, 2011), and in some species the mouth even functions as a site for egg incubation (Kuwamura, 1986). However, significant knowledge gaps still remain with respect to the role and importance of motility and blood flow for optimal gastrointestinal function in teleosts.

Gastrointestinal motility and gastrointestinal blood flow (GBF) are essential for the effective digestion and absorption of food, as well as the maintenance of osmotic homeostasis in teleosts. Therefore, this thesis will focus on these processes during the pre-prandial (*i.e.* unfed) or post-prandial (*i.e.* fed) state of two teleost species inhabiting either freshwater (FW) or seawater (SW). However, before delving into the complex motility patterns and GBF responses, a brief overview of the underlying control mechanisms involved in these processes is required.

### *1.2.1. Control of gastrointestinal motility*

Gastrointestinal motility can be described as the contractions and relaxations of circular and longitudinal smooth muscle (or skeletal muscle in the upper two-thirds of the oesophagus), which result in the movement of intraluminal contents (Kunze and Furness, 1999). To achieve an effective level of mixing and propulsive activity, gastrointestinal motility is tightly regulated and coordinated (Fig. 1).

The control of gastrointestinal motility involves multiple regulatory mechanisms. In some circumstances, gastrointestinal smooth muscle cells can generate their own rhythmic activity, but are typically controlled by other specialized cells that surround them (Sanders et al., 2006). Regulation of neurotransmission and the underlying electrical rhythmicity of smooth muscles are controlled by the interstitial cells of Cajal (ICC), which are small fusiform or stellate cells with prominent varicose processes that form networks in gastrointestinal tissues (Cajal, 1911; Sanders et al., 2012). In mammals, myenteric ICC (ICC-MY) are derived from the same mesenchymal precursors as longitudinal smooth muscle cells, whereas intramuscular ICC (ICC-IM) and deep muscular plexus ICC (ICC-DMP) arise postnatally from circular smooth muscle cells (Kondo et al., 2015; Ward and Sanders, 2001). The function of these subpopulations of ICC



differ, as ICC-MY act as electrical pacemakers whilst ICC-IM and ICC-DMP act as modulators of neurotransmission (Sanders et al., 2006).

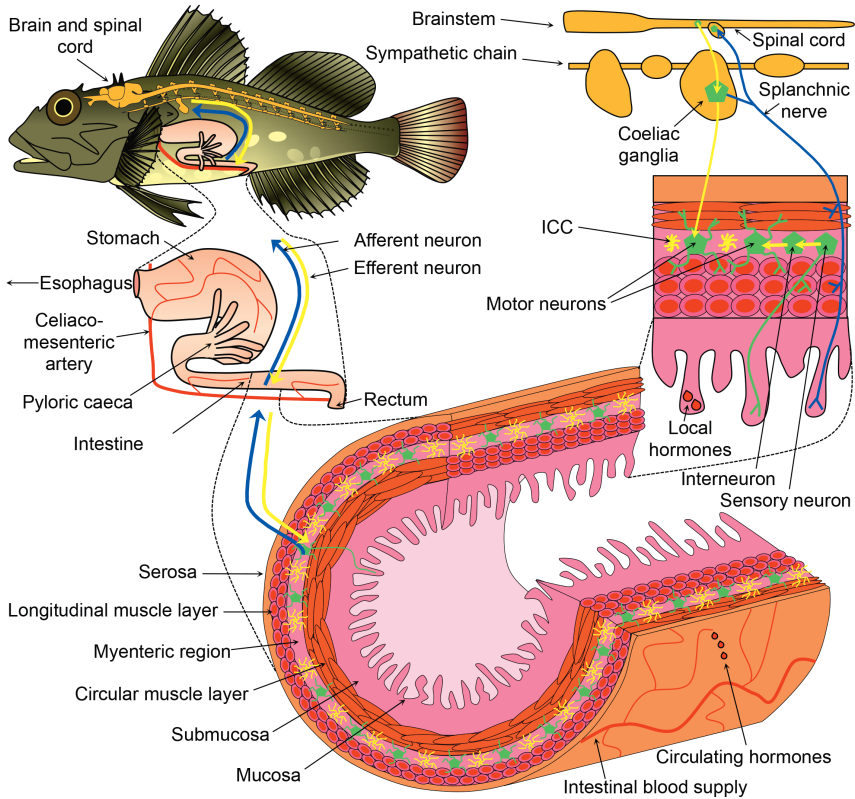
The spontaneous electrical activity or slow waves from the ICC-MY result from the activation of a large inward current carried by  $\text{Cl}^-$  ions (Zheng et al., 2014). Specifically,  $\text{Ca}^{2+}$  is periodically released from intracellular stores and activates Anoctamin 1 (Ano1), which are  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels. This causes an efflux of  $\text{Cl}^-$  and creates spontaneous transient inward currents, which leads to spontaneous transient depolarisations of the membrane. When the threshold potential is reached for the activation of T-type channels,  $\text{Ca}^{2+}$  enters the cell and reinforces the activation of Ano1, which further depolarizes the cell. This leads to the generation of whole cell slow wave currents, which depolarizes adjacent ICC-MY cells via gap junctions (Sanders et al., 2006; Zheng et al., 2014).

Slow waves also conduct to smooth muscle cells via gap junctions and the subsequent depolarization activates voltage-dependent  $\text{Ca}^{2+}$  channels (Sanders et al., 2006). This results in the influx of extracellular  $\text{Ca}^{2+}$  and the release of intracellular  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum, which triggers the excitation-contraction coupling mechanism. Specifically, intracellular  $\text{Ca}^{2+}$  forms a complex with the protein calmodulin that subsequently activates myosin light chain kinase to phosphorylate the light chain of myosin, which enables the molecular interaction of myosin with actin. In the presence of ATP, this allows cross-bridge cycling between myosin and actin (Webb, 2003). Smooth muscle cell relaxation occurs when intracellular  $\text{Ca}^{2+}$  decreases and myosin light chain phosphatase activity increases (Webb, 2003).

However, smooth muscle contractions and relaxations need to be coordinated in order to produce appropriate propulsion and mixing of intraluminal contents (Sanders et al., 2012). Propagation of activity both within and between the smooth muscle layers and pacemaker networks occurs mainly via gap junctions (Gabella and Blundell, 1981). Furthermore, since the electrical slow waves produced by the ICC-MY cannot be regenerated by the smooth muscle cells, continuous networks of ICC are required throughout the gastrointestinal tract to provide a pathway for the active propagation of slow waves (Horowitz et al., 1999; Sanders et al., 2012). This allows the coordination of organ-level propagation of contractions and creates the basis for a wide range of motility patterns.

While the basic rhythmical properties of gastrointestinal contractions are determined by ICC-MY and smooth muscle; the strength, coupling and pattern of these contractions are largely determined by superimposed neural input (Huizinga and Lammers, 2009; Sanders et al., 2012). This is mainly due to the directed release of a wide range of excitatory (e.g. acetylcholine,

substance P, neurokinin A) and inhibitory neurotransmitters (e.g. nitric oxide, vasoactive intestinal polypeptide) from enteric neurons (Olsson, 2011b). Furthermore, locally released and circulating hormones (e.g. cholecystokinin, somatostatin, histamine, ghrelin) that are predominantly controlled by the autonomic nervous system have also been demonstrated to have significant effects on gastrointestinal motility (Olsson, 2011b).



**Figure 1. The multiple layers of regulatory mechanisms involved in the control of intestinal motility in teleosts.** The rhythmic, spontaneous electrical activity generated by myenteric interstitial cells of Cajal (ICC-MY) can depolarise gastrointestinal smooth muscles, which results in low amplitude contractions. Extrinsic and intrinsic autonomic reflexes influence the strength, coupling and patterning of these contractions. Sensory neurons respond to chemical (luminal content or hormones released from mucosal endocrine cells) or mechanical (stretching of muscle layers) stimuli and send information to either intrinsic (enteric) or extrinsic autonomic neurons. The extrinsic reflexes involve afferent (blue arrows) and efferent (yellow arrows) pathways in the splanchnic and to some extent vagal nerves. The efferent nerves then synapse with intrinsic inter- or motor neurons. Hormones reaching the gut via the circulation, as well as those locally produced by mucosal endocrine cells, also influence contractile activity. Illustration by Jeroen Brijs, Albin Gräns and Catharina Olsson.

In summary, the excitability and contractility of gastrointestinal smooth muscle results from the integrated behaviour of multiple regulatory mechanisms that superimpose upon myogenic activity (Sanders et al., 2012).

### 1.2.2. Control of gastrointestinal blood flow

A sufficient perfusion of the gastrointestinal tract is essential to sustain oxygenation and nutritional levels of tissues, as well as to transport metabolic wastes and assimilated nutrients, water and ions from the gastrointestinal tract to other sites for excretion or utilisation. Most of the blood in the gastrointestinal tract of teleosts is supplied via the coelomesenteric artery, which is the first caudal branch of the dorsal aorta. This artery then progressively divides into smaller vessels such as the coeliac and mesenteric arteries to provide blood to the stomach, intestine and liver (Seth et al., 2011).

Regulation of pre-prandial GBF has been suggested to occur mainly via tonic  $\alpha$ -adrenergic constriction of the gastrointestinal vasculature in a range of teleosts (Axelsson and Fritsche, 1991; Axelsson et al., 1989; Axelsson et al., 2000; Holmgren et al., 1992). This mechanism is highly conserved as it has also been reported in reptiles, birds and mammals (Axelsson et al., 1991; Butler et al., 1988; Ross, 1971). The tonic vasoconstriction of vascular beds including the gastrointestinal tract is necessary as cardiac output cannot be increased sufficiently enough to maintain arterial blood pressure if all vascular beds are fully dilated (Farrell et al., 2001). Interestingly, during stress, the  $\alpha$ -adrenergic vascular tone of the red Irish lord (*Hemilepidotus hemilepidotus*) increased to such an extent that blood flow completely ceased in the gastrointestinal tract (Axelsson et al., 2000). Therefore, it is clear that when necessary, mechanisms exist to prioritise circulation to oxygen-sensitive organs such as the heart and brain, whereas less flow is directed to organs such as the gastrointestinal tract that are able to withstand periods of lower blood supply (Farrell et al., 2001).

Mammals generally increase GBF by redistributing blood from other vascular beds to the gastrointestinal tract via alterations in systemic and gastrointestinal vascular resistances with relatively little compensatory modifications in cardiac output (Gallavan et al., 1980; Vatner et al., 1974). In contrast, increased GBF in teleosts mostly occurs via a reduced gastrointestinal vascular resistance, which is accompanied by an equivalent or larger increase in cardiac output (Axelsson and Fritsche, 1991; Axelsson et al., 1989; Axelsson et al., 2000). Changes in GBF are regulated through a combination of inputs from the autonomic nervous system, circulating and locally produced vasoactive substances, and metabolite-induced control (Kågström and Holmgren, 1997; Kågström et al., 1996; Seth et al., 2010; Seth et al., 2011). These control mechanisms allow teleosts to finely tune

GBF when experiencing changes in physical status (*i.e.* stress, exercise and feeding) and/or environmental conditions (*i.e.* temperature, hypoxia and hypercapnia) (Axelsson and Fritsche, 1991; Axelsson et al., 1989; Axelsson et al., 2000; Axelsson et al., 2002; Gräns et al., 2007; Gräns et al., 2009a; Gräns et al., 2009b; Gräns et al., 2010; Seth and Axelsson, 2009).

### **1.3. The gastrointestinal tract and its role in feeding**

The primary function of the gastrointestinal tract is to process food and water, assimilate the vital nutrients and excrete bodily wastes. Food processing begins in the mouth and varies widely depending on the method of capture and feeding strategy of the particular species (Bakke et al., 2011). For example, piranhas initiate the processing of food by mechanically breaking down prey with their sharp teeth prior to swallowing the pieces (Agostinho et al., 1997), cyprinids utilise pharyngeal teeth to break up hard food items (Sibbing, 1982), whereas salmonids generally swallow their prey whole (Bakke et al., 2011). Food is then transported via the oesophagus to the stomach, or directly to the intestine in stomachless species, whereupon further mechanical processing occurs and chemical digestion is initiated.

In teleosts possessing a stomach, the ingested food is temporarily stored in the stomach to undergo an initial physical and enzymatic breakdown through the coordinated secretion of digestive fluids (Bakke et al., 2011). Hydrochloric acid and pepsinogen are released from oxynticopeptic cells, which are generally located in the central fundic and distal pyloric regions of the stomach (Bomgren et al., 1998; Norris et al., 1973). Hydrochloric acid lowers pH, denatures proteins and converts the inactive pepsinogen into active pepsin, which is a proteolytic enzyme (Wu et al., 2009). Simultaneously, mucus is secreted from goblet cells to protect the mucosa from the low pH environment (Krogdahl et al., 2011; Morrison and Wright, 1999). Furthermore, marine species with diets consisting largely of chitinous invertebrates have also been found to secrete chitinase, an enzyme suggested to aid in breaking down the chitin-containing exoskeletons of their prey (Fänge et al., 1979; Gutowska et al., 2004).

Upon entry to the intestine, the food contents or chyme, are mixed with intestinal, pancreatic, hepatic and biliary secretions (Bakke et al., 2011). Electrolytes within these secretions, mainly bicarbonate, play an important role in neutralising the pH of the chyme, which subsequently enhances the chemical environment for pancreatic and intestinal digestive enzymes (Krogdahl et al., 2011). Pancreatic enzymes are essential for the breakdown of fats, proteins and carbohydrates, and the quantity and activity of these enzymes differ depending on the species and/or diet (Krogdahl et al., 2011).

The delivery of nutrients to the intestinal lumen is the primary trigger for the secretion of these enzymes, and the composition of nutrients and digestibility of the diet influences the differential secretion of specific enzymes, *e.g.* high protein diets result in elevated trypsin secretion (Olli et al., 1994; Peres et al., 1998). Bile acids secreted from the liver and gall bladder also play a major role, as they emulsify dietary lipids and fat-soluble vitamins ensuring efficient lipid digestion (Bakke et al., 2011).

Following the breakdown of food, solubilized nutrients are taken up across the apical membrane of enterocytes lining the intestine. Nutrients can enter and exit the enterocytes by either following a concentration gradient or via specialized transporters to eventually enter the circulatory system (Bakke et al., 2011). The remaining indigestible components of the diet and other waste products are excreted via the rectum and anus.

For the effective and efficient digestion of food and absorption of nutrients, the abovementioned mechanisms must coincide with a range of tightly regulated and coordinated gastrointestinal motility and blood flow responses (Gräns and Olsson, 2011; Seth et al., 2011). These responses will be discussed in detail in the following two sections.

### *1.3.1. Pre- and post-prandial motility*

A gastrointestinal motility pattern can be defined as ‘a group of phasic pressure waves with associated inter-wave intervals and spread that can be recognized visually as related’ (Husebye, 1999). Motility patterns are achieved via coordinated contractions and relaxations of circular and/or longitudinal smooth muscle in the gastrointestinal tract, and serve multiple functions such as transporting and breaking down food, as well as preventing bacterial overgrowth and removing wastes (Kunze and Furness, 1999; Nieuwenhuijs et al., 1998; Szurszewski, 1969).

Gastrointestinal motility has been studied for over 100 years, resulting in the description of propagating and non-propagating patterns in a wide range of vertebrates (Bayliss and Starling, 1899; Cannon, 1902; Chang and Leung, 2014; Husebye, 1999). The most intensively studied pre-prandial propagating motility pattern is the neurally regulated migrating motor complex (MMC) (Wingate, 1981). MMCs are comprised of three phases: phase I is a period of quiescence, phase II consists of irregular contractile activity and phase III, which is the most characteristic phase, consists of bands of regular pressure waves propagating along the intestine for relatively long distances (Brierley et al., 2001; Husebye, 1999; Szurszewski, 1969). MMCs are suggested to serve a ‘housekeeping’ function by preventing bacterial overgrowth and propelling indigestible food components and waste products out of the system (Nieuwenhuijs et al., 1998; Vantrappen et al., 1977). In many species, ingestion of food can have a pronounced effect on

the MMC cycle. In the majority of the intermittently feeding mammals studied, feeding switches the MMC cycle from phase III back to phase II (Bueno and Ruckebusch, 1976; Ruckebusch and Bueno, 1976; Szurszewski, 1969), whereas in continuous feeders, MMCs tend to persist after food intake (Galligan et al., 1985; Grivel and Ruckebusch, 1972; Zheng et al., 2009).

Post-prandial motility includes non-propagating (*i.e.* segmentation) and propagating (*i.e.* peristalsis) contractions (Hennig et al., 2010; Huizinga et al., 2011). Intestinal segmentation consists of annular circular muscle contractions that propel contents short distances in a to-and-fro manner, which serves to mix the intestinal chyme (Chang and Leung, 2014). Post-prandial peristalsis is generally defined as contractions that partially or totally occlude the lumen in order to propel intraluminal contents in an anal direction over longer distances (Huizinga and Lammers, 2009; Huizinga et al., 2011). Peristaltic movements occur in all sections of the gastrointestinal tract and involve the synchronous contraction and relaxation of both circular and longitudinal muscle on either side of a bolus in order to propel it in an anal direction (Chang and Leung, 2014).

'Ripples' are another type of propagating contraction found in mammals. They are rhythmic, shallow contractions of the circular muscle, which can propagate equally well in both directions, although they tend to mainly propagate orally (D'Antona et al., 2001). Ripples are predominantly due to myogenic mechanisms, as they persist following neuronal blockade and their occurrence coincides with slow waves generated by the ICC (Bercik et al., 2000). It has been speculated that ripples could promote or optimise absorption by mixing/circulating intestinal contents over the mucosa (Chen et al., 2013; Dinning et al., 2012; Hennig et al., 2010).

In contrast to the mammalian literature, qualitative and quantitative descriptions of *in vivo* motility patterns in teleosts, as well as the underlying control mechanisms, is a field still in its infancy. Propagating and non-propagating contractions have been described *in vitro* or *in situ* in isolated sections of intestine from brown trout (*Salmo trutta*) and Atlantic cod (*Gadus morhua*) (Burnstock, 1958a; Burnstock, 1958b; Karila and Holmgren, 1995). However, these contractions most likely differ to those occurring naturally as the intestine was separated from its blood supply, extrinsic nervous control, and other regions of the gastrointestinal tract (Burakoff and Percy, 1992; Fox et al., 1983; Yin and Chen, 2008). Until now, *in vivo* gastrointestinal motility patterns in teleosts have only been described in larval zebrafish (*Danio rerio*) and halibut (*Hippoglossus hippoglossus*) (Holmberg et al., 2003; Holmberg et al., 2004; Holmberg et al., 2006; Holmberg et al., 2007; Rönnestad et al., 2000). Holmberg et al. (2003) demonstrated that gastrointestinal motility in larval zebrafish progresses from irregular contractile activity into coordinated motility patterns prior to the first feeding event, which coincides

with an increasing development of neuronal control. Furthermore, some contractile activity persists following neuronal blockade, indicating that these contractions are myogenic in origin (Holmberg et al., 2007). These contractions have been suggested to be controlled by ICC (Holmberg et al., 2007; Rich et al., 2007), however relatively little is known about the presence, let alone the physiological function, of these cells in teleosts.

As in mammals, the breakdown and transport of ingested food through the gastrointestinal tract of teleosts would be expected to coincide with an increased gastrointestinal contractile activity. Indeed, following gavage feeding, *in vivo* contractile activity significantly increases in the intestine of rainbow trout (*Oncorhynchus mykiss*) (Gräns et al., 2009a). These findings are consistent with a range of *in vitro* and *in situ* studies performed on rainbow trout, as well as *in vivo* studies performed on Atlantic cod and four species of flatfish (*Pleuronectes platessa*, *Limanda limanda*, *Scophthalmus rhombus* and *S. maximus*), which all demonstrate an increased frequency of gastrointestinal contractions in response to distension (Grove and Holmgren, 1992a; b). Although the abovementioned studies strongly indicate that contractile activity increases post-prandially, it remains unknown if this is due to an up-regulation of pre-prandial motility patterns or whether motility patterns differ according to feeding state, as is observed in a range of intermittently feeding mammals (Bueno and Ruckebusch, 1976; Ruckebusch and Bueno, 1976; Szurszewski, 1969).

In summary, significant knowledge gaps regarding *in vivo* gastrointestinal motility in teleosts remain, namely with respect to qualitative and quantitative descriptions of motility patterns in adults and whether specific motility patterns are related to the feeding state of the animal. Furthermore, a better understanding of the role that ICC play in the control of gastrointestinal motility is needed to provide a framework for explaining the different motility patterns.

### *1.3.2. Pre- and post-prandial circulatory responses*

For the contractile activity of gastrointestinal smooth muscle to occur over a long period of time, a functional and well-regulated blood supply is required. Pre-prandial GBF is regulated to a level sufficient for the housekeeping costs of the gastrointestinal tract prior to the ingestion of a meal (Seth et al., 2011). This level varies amongst the teleost species investigated thus far, ranging from 10% of cardiac output in sea bass (*Dicentrarchus labrax*) to 40% in Atlantic cod (Axelsson and Fritsche, 1991; Dupont-Prinet et al., 2009). Like most other vertebrates, teleosts do not have the cardiovascular capacity to maximally perfuse all their circulatory beds simultaneously (Farrell et al., 2001). Therefore, physiological and/or environmental challenges that place demands on the circulatory system have been demonstrated to substantially

decrease pre-prandial GBF in a wide range of species (Altimiras et al., 2008; Axelsson and Fritsche, 1991; Axelsson et al., 2000; Crocker et al., 2000; Dupont-Prinet et al., 2009; Gräns et al., 2007; Thorarensen et al., 1993).

Following food ingestion, GBF increases to transport absorbed nutrients from the mucosa around the body for modification, storage and use, as well as to supply oxygen to the metabolising gastrointestinal tissues (Farrell et al., 2001). Post-prandial GBF responses vary depending on species, water temperature, meal size, meal type, physiological status and gastric emptying rates (Farrell et al., 2001; Seth et al., 2011). The magnitude of the post-prandial increase ranges from 71% in sea bass to 156% in rainbow trout (Axelsson et al., 2002; Gräns et al., 2009a). The temporal dynamics of this response also varies widely, as GBF in sea bass increases within 1 h of ingesting a meal to reach peak after 6 h, whereas in other species such as red Irish lord, shorthorn sculpin (*Myoxocephalus scorpius*) and rainbow trout, GBF slowly increases over time and is elevated for over 72 h (Altimiras et al., 2008; Axelsson et al., 2000; Eliason et al., 2008; Sandblom et al., 2012; Seth and Axelsson, 2009). In some species, it appears that the temporal dynamics of the post-prandial GBF response corresponds with gastric lag phase and total gastric emptying time (Gräns et al., 2009a; Olsson et al., 1999). Furthermore, examination of coeliac (*i.e.* supplying stomach and liver region) and mesenteric (*i.e.* supplying intestinal region) arterial blood flow in red Irish lord and Atlantic cod demonstrates a post-prandial blood flow response consistent with the time-dependent passage of food, as the increase in blood flow through the mesenteric artery is delayed compared with the coeliac artery (Axelsson et al., 2000; Behrens et al., 2012).

The post-prandial increase in GBF places an increased demand on the cardiovascular system of teleosts. As many species experience fluctuations in environmental conditions such as water temperature, oxygen levels and even water salinity, they must be able to adjust their cardiovascular system or make physiological compromises. Indeed, in the face of environmental challenges such as elevated environmental temperature or hypoxia the magnitude of the post-prandial GBF response in some species decreases (Axelsson et al., 2002; Gräns et al., 2009a). Yet, relatively little information exists concerning the consequences of water salinity on pre- or post-prandial GBF in teleosts. This is surprising as many ecologically and economically important species undergo salinity transitions throughout their lives, which have the potential to affect GBF, as the gastrointestinal tract is an essential osmoregulatory organ (Grosell, 2011).



### 1.3.3. *Metabolic implications of feeding*

Specific dynamic action (SDA) describes the increase in heat production due to the post-prandial increase in metabolism (McCue, 2006). The processes that account for the increased energy expenditure can be grossly categorized into those that occur during the pre-absorptive, absorptive and post-absorptive stages of food processing. The pre-absorptive stage includes processes such as chewing, swallowing, gut peristalsis, enzyme secretion, acid secretion, protein catabolism, intestinal remodelling and blood pH regulation, while the absorptive stage includes intestinal absorption and nutrient transport (for review see McCue, 2006). However, increasing amounts of evidence suggest that post-absorptive processes such as protein synthesis, ketogenesis, amino acid deamination/oxidation, glycogen production, urea production, renal excretion and growth comprise the majority of the SDA response (Brown and Cameron, 1991a; b; Jobling and Davies, 1980; Seth et al., 2011).

Initially, SDA was determined by using direct calorimetry to estimate heat production, but this method has largely been replaced by measuring whole animal oxygen consumption as a proxy for metabolism (Seth et al., 2011). SDA is calculated by subtracting the dynamic pre-prandial metabolic rate from the dynamic post-prandial metabolic rate (Roe et al., 2004). Four variables are typically used to characterise SDA: the peak value of post-prandial metabolism, time to reach peak, duration of elevated metabolism, and the total postprandial metabolic cost (the area between the post-prandial oxygen consumption curve and the pre-prandial baseline) (Chabot et al., 2016).

Investigations of SDA in teleosts have mainly been conducted on species important for commercial (*e.g.* cod, salmon and tuna) and recreational fishing (*e.g.* sunfish, bass and walleye), as well as those used in aquaculture (*e.g.* catfish, trout and tilapia) (for review see Secor, 2009). In aquaculture, research has primarily focused on meal size and composition, water temperature and stocking density, with the central aim of identifying optimal conditions for minimizing SDA so that the maximum amount of absorbed energy is allocated to growth (Chakraborty et al., 1992; Fu and Xie, 2004; LeGrow and Beamish, 1986). Studies have been carried out on a wide range of body masses ranging from 0.5 g zebrafish to 11 kg bluefin tuna (*Thunnus maccoyii*), and reveal substantial differences in SDA ranging from 0.01 to 1.90 kJ (Fitzgibbon et al., 2007; Lucas and Priede, 1992). In teleosts, absolute SDA tends to increase with increasing body mass, meal mass and temperature (Secor, 2009).

On average, feeding results in a rapid two- to three-fold increase in the metabolic rate of teleosts, which peaks within 24 h depending on body temperature (for review see Secor, 2009). This is subsequently followed by a

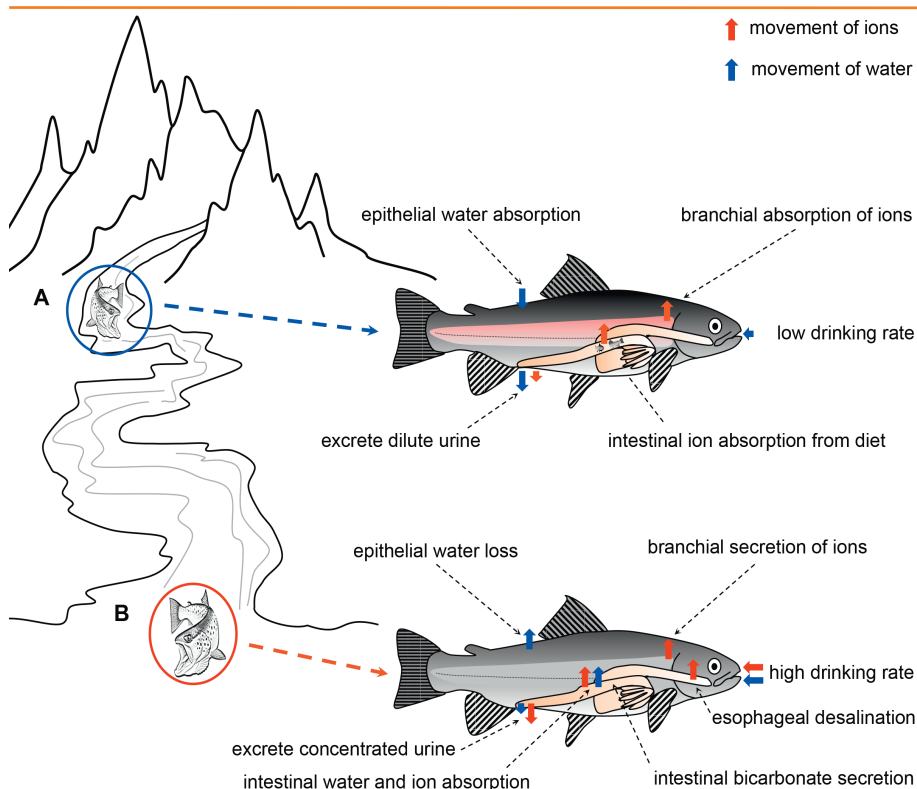
slow return to the pre-prandial level. The duration of the response varies widely, ranging from 6 h in rainbow trout (Peck et al., 2003) to 390 h in Antarctic spiny plunderfish (*Harpagifer antarcticus*) (Boyce and Clarke, 1997). The SDA duration typically increases with meal mass and decreases with increasing body temperature (Secor, 2009). Oxygen levels in the water can also greatly impact the SDA response in teleosts, as Atlantic cod exposed to hypoxic conditions display a smaller post-prandial peak, yet more prolonged response due to the reduction in aerobic capacity (Jordan and Steffensen, 2005). Although salinity has been demonstrated to have effects on the SDA response of aquatic invertebrates (McGaw, 2006), relatively little information exists for teleosts.

The devotion of energy towards SDA has been demonstrated to compromise swimming performance in species such as rainbow trout, Atlantic cod and Chinook salmon (*Oncorhynchus tshawytscha*) (Alsop and Wood, 1997; Jordan and Steffensen, 2005; Thorarensen and Farrell, 2006). Conversely, other studies show that energy devoted to SDA decreases in order to maintain activity levels (Blaikie and Kerr, 1996) or that swimming has no influence on SDA dynamics (Beamish, 1974). The disparity in the above findings is not entirely surprising, as various species have adopted different behavioural foraging strategies, which may influence the relationship between SDA and activity levels. Indeed, the examination of behavioural, digestive and metabolic characteristics of teleosts with different foraging strategies has revealed a range of distinctly different responses (Fu et al., 2009). For example, the southern catfish (*Silurus meridionlis*) classed as a sedentary, ambush predator has a large SDA accompanied with a profound reduction in post-prandial locomotory capacity, whereas more active species such as grass carp (*Ctenopharyngodon idellus*) and crucian carp (*Carassius auratus*) are able to maintain their post-prandial locomotory capacity via either exhibiting a small SDA or increasing their cardiorespiratory capacity, respectively (Fu et al., 2009). These findings highlight that the ecology of different species can have a profound effect on the partitioning of their metabolic capacity for different activities such as movement and digestion.

#### **1.4. The role of the gastrointestinal tract in osmoregulation**

The gastrointestinal tract also plays an integral role in the maintenance of osmotic homeostasis in teleosts. It has been estimated that approximately 95% of teleost species are stenohaline, residing in either FW or SW for their entire lives, whilst the remaining species are euryhaline and have the capacity to withstand large variations in environmental salinity (Evans, 1984). The majority of teleosts are surrounded by water with an osmotic

pressure that significantly differs from their plasma osmolarity (~290-340 mOsmol L<sup>-1</sup>; McCormick and Saunders, 1987). FW and SW habitats pose contrasting osmotic challenges and individuals transitioning between them must be able to switch between hyper- and hypoosmoregulatory mechanisms (McCormick, 2001). In FW, teleosts must counteract the passive gain of water and loss of ions, whereas inhabitants of marine environments must counteract the passive loss of water and gain of ions (Fig. 2).



**Figure 2. The contrasting osmotic challenges that euryhaline teleosts face when transitioning between FW and SW. (A)** In FW, teleosts must hyperosmoregulate to counter the continual loss of salts and entry of water across their permeable body surfaces. **(B)** In SW, teleosts must hypoosmoregulate to counter the diffusional entry of salts and osmotic loss of water. Illustration by Jeroen Brijs and Albin Gräns.

In FW, limnic and euryhaline teleosts hyperosmoregulate to maintain osmotic homeostasis by minimizing drinking rates, increasing glomerular filtration rates, and producing copious amounts of dilute urine (Perry et al., 2003). Furthermore, mitochondrion-rich cells in the gills contain apical proton pumps coupled to Na<sup>+</sup> channels, as well as apical Na<sup>+</sup>/H<sup>+</sup> exchangers

to actively take up ions from the water (Evans, 2008). Another essential source of ions is from the diet, as the quantity of electrolytes absorbed from chyme far exceeds the levels absorbed from the surrounding water (Wood and Bucking, 2011). Furthermore, since many uptake pathways are coupled as  $\text{Na}^+$ /nutrient co-transport systems (Bakke et al., 2011), the costs of gastrointestinal uptake of ions has been suggested to be significantly less than branchial uptake (Wood and Bucking, 2011). The gastrointestinal uptake of ions is even more important in species that inhabit FW environments with extremely low ion concentrations (Wood and Bucking, 2011), or a high concentration of environmental contaminants that prevent branchial uptake mechanisms (Kamunde et al., 2003), or in species lacking specific ion uptake mechanisms at the gills (Patrick et al., 1997; Tomasso and Grosell, 2005).

In SW, marine and euryhaline teleosts must hypoosmoregulate to maintain osmotic homeostasis (Evans, 2008). To maintain water balance, drinking rates in SW are 10 to 50-fold higher than in FW (Grosell, 2006; Perrott et al., 1992; Smith, 1930; Takei and Tsuchida, 2000). Imbibed SW initially undergoes significant desalination in the oesophagus with estimates suggesting that approximately half of the  $\text{NaCl}$  is absorbed in this section of the gastrointestinal tract, which is relatively impermeable to water (Hirano and Mayer-Gostan, 1976; Parmelee and Renfro, 1983). In the stomach, the SW is further diluted before entering the intestine with a markedly reduced osmotic pressure (Grosell, 2006; 2011; Hirano and Mayer-Gostan, 1976).

Despite the absence of net osmotic gradients across the intestinal epithelium, the absorption of water is driven by active  $\text{NaCl}$  absorption via a range of co-transporters, exchangers, ion channels and  $\text{Na}^+/\text{K}^+$ -ATPase (Grosell, 2011; Skadhauge, 1974). Fluids absorbed by the intestinal epithelium create a region of localised hypertonicity within the lateral intercellular space, which draws water from the lumen across the intestine and into the body (Grosell, 2006; Larsen et al., 2009; Skadhauge, 1974). The excess gain of monovalent ions from intestinal absorption, as well as the diffusional gain at the respiratory surfaces, is subsequently secreted via mitochondrion-rich cells in the gills (Evans, 2008), whereas the divalent ions are generally secreted renally (Marshall and Grosell, 2005). Interestingly, secretory processes within the intestine have been demonstrated to minimize the demand for renal excretion and thus urinary fluid loss (Grosell, 2011). High intestinal  $\text{HCO}_3^-$  secretion via apical  $\text{Cl}^-/\text{HCO}_3^-$  exchangers in marine teleosts significantly increases fluid absorption due to the net  $\text{Cl}^-$  gain, as well as causing an increased precipitation of divalent cations such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the lumen (Wilson and Grosell, 2003; Whittamore, 2012; Whittamore et al., 2010). These precipitates are insoluble carbonates and can be rectally excreted, alleviating the divalent ion load on the kidney, which

further facilitates water uptake as the osmolytes in the lumen are effectively removed (Wilson and Grosell, 2003).

The role that the gastrointestinal tract plays in maintaining osmotic homeostasis in teleosts, as well as a wide range of the underlying mechanisms responsible are well established. Yet significant knowledge gaps remain, specifically regarding the effects of contrasting osmotic environments on gastrointestinal motility and circulatory responses in teleosts.

#### 1.4.1. *Gastrointestinal motility in FW and SW*

Teleosts residing in FW tend not to drink, thus the gastrointestinal tract is mainly active with respect to osmoregulation following the ingestion of a meal (Wood and Bucking, 2011). Therefore, one could expect that post-prandial gastrointestinal motility patterns of these individuals are optimised in a manner that incorporates digestive and osmoregulatory processes.

In contrast, teleosts residing in SW need to continuously drink water and subsequently absorb fluid in the intestine to maintain water balance. Drinking rate in the Japanese eel (*Anguilla japonica*) is regulated via mechano- and ionoreceptors responding to the distension of the stomach/intestine and chemical composition of imbibed SW, respectively (Ando et al., 2003; Hirano, 1974). As intestinal water absorption cannot occur when luminal osmotic pressure is too high, the regulation of drinking is crucial to ensure that imbibed SW is sufficiently desalinated in the oesophagus prior to entering the intestine (Grosell, 2011; Skadhauge, 1969).

As optimal mixing and transport of imbibed fluids increase the efficiency of ion and water absorption (Lee, 1983), one could expect that the receptors regulating drinking rate may also be involved in regulating gastrointestinal motility. This line of reasoning is supported by findings demonstrating that gastrointestinal contractions in rainbow trout and a range of marine teleosts are induced in response to balloon distension (Grove and Holmgren, 1992a; b). However, no propagating contractions could be induced in the intestine of Atlantic cod in response to balloon distension, but peristaltic-like contractions could still be evoked via electrical stimulation (Karila and Holmgren, 1995). In light of their findings, Karila and Holmgren (1995) suggested that ionoreceptors might instead form the main sensory link for the observed contractions.

The input from mechano- and/or ionoreceptors within the gastrointestinal tract of teleosts in SW may result in motility patterns that are similar, but not identical to post-prandial motility patterns, as the chemical composition of imbibed water differs from that of ingested food. Furthermore, in order to optimise ion and water absorption across epithelia,

water should be transported through the gastrointestinal system via coordinated opening and closing of sphincters between the different sections of the tract, as well by motility patterns that mix and propel the fluid from one section to the next (Lee, 1983). The motor activity induced by imbibing SW may also prevent the mucosal build-up of insoluble carbonates and induce the rectal outflow of these precipitates (Grosell, 2011). However, currently these are all speculations, as the effect of salinity on gastrointestinal motility in teleosts has not yet been examined and begs investigation.

#### *1.4.2. Cardiovascular status in FW and SW*

As the gastrointestinal tract of teleosts in FW is mainly active with respect to osmoregulation following the ingestion of a meal, pre-prandial blood flow is most likely regulated to a level sufficient for the housekeeping costs of the gastrointestinal tract (Seth et al., 2011). Post-prandial blood flow responses on the other hand, are most likely simultaneously optimised for digestion, absorption and osmoregulation.

In contrast, even in the pre-prandial state of teleosts in SW, the substantial movement of ions and water across the intestinal epithelium into the blood would be expected to coincide with circulatory modifications, as these products need to be transported around the body for excretion or utilisation (Shehadeh and Gordon, 1969). If intestinal contractile activity is elevated in teleosts residing in SW, an increased blood flow may be necessary due to elevations in the metabolic demand of intestinal smooth muscle and/or due to the fact that signalling molecules altering motility, such as acetylcholine and cholecystokinin, can also invoke a circulatory response (for review see Chou, 1982). Furthermore, the dehydrating effect of SW most likely impacts cardiac function as blood volume and blood pressure significantly decreases in a range of teleosts following SW transfer (Hirano, 1974; Olson and Hoagland, 2008; Pedersen et al., 2014). The only study thus far that has investigated changes in blood flow in a euryhaline teleost following SW transfer showed that cardiac output of rainbow trout increased by approximately 30% (Maxime et al., 1991). However, as the circulatory responses of rainbow trout were only examined for 24 h following SW transfer, the effects of prolonged exposure to SW remain unknown.

As GBF represents the only means for transporting ions and water from the gastrointestinal to systemic system, it seems astonishing that GBF responses to varying salinities remains completely unexplored. Circumstantial evidence for the importance of GBF for teleosts in SW can be surmised in studies showing exacerbated dehydration in Chinook salmon exercising in SW (Gallaughier et al., 2001; Thorarensen et al., 1993). Typically this has been interpreted as the ‘osmo-respiratory’ compromise, which is where increased gill blood flow and hypertension during exercise

leads to increased water loss (Nilsson, 1986). However, as GBF of teleosts typically decreases when swimming, it does raise the possibility that impaired intestinal water absorption during exercise may also contribute towards dehydration (Altimiras et al., 2008; Farrell et al., 2001). This line of reasoning is further supported by findings from chronically exercise-trained Chinook salmon that were better able to both preserve GBF, as well as plasma osmolality, during swimming relative to untrained conspecifics (Gallaughier et al., 2001; Thorarensen et al., 1993).

Moreover, another reason to suspect that teleosts in SW may require an elevated GBF is for the maintenance of acid-base balance. Although the secretion of metabolically produced  $\text{HCO}_3^-$  into the intestine is essential for osmoregulation, it has substantial implications on acid-base balance (Cooper et al., 2014; Wilson and Grosell, 2003). Specifically, this process generates excess  $\text{H}^+$ , which must be excreted to avoid acid-base disequilibria of both intracellular and extracellular environments (Taylor et al., 2011). Indeed, increased intestinal  $\text{HCO}_3^-$  secretion in European flounder (*Platichthys flesus*) coincides with increased branchial acid excretion, which demonstrates that transportation of  $\text{H}^+$  from the intestine to the gills is occurring (Cooper et al., 2010; Wilson and Grosell, 2003).

If little was known about pre-prandial levels of blood flow in euryhaline teleosts osmoregulating in SW, then even less is known about their post-prandial responses. Although elevated levels of blood flow may be necessary for maintaining osmotic homeostasis, it may also have substantial consequences on the capacity for further increase following the ingestion of a meal. This in turn may have implications on the transportation of absorbed nutrients, oxygen delivery to gastrointestinal tissues, and acid-base regulation during digestion.

### *1.4.3. Metabolic implications of osmoregulation*

Teleosts that are surrounded by water with an osmotic pressure that significantly differs from their plasma osmolarity require transepithelial transport of ions against their concentration gradient in order to maintain salt and water balance. Processes facilitating this transepithelial transport of ions such as  $\text{Na}^+/\text{K}^+$ -ATPase require energy, and thus it has been suggested that exposure to salinities differing from the animal's plasma osmolarity should impose an increased energetic demand (Ern et al., 2014).

An investigation into the effects of salinity on the oxygen consumption of Nile tilapia (*Tilapia nilotica*) showed that energy expenditure was lowest in the absence of an osmotic gradient (*i.e.* brackish water, 11.6‰) and greatest when the osmotic gradient was highest (*i.e.* SW, 30‰) (Farmer and Beamish, 1969). However, subsequent studies on a wide range of teleosts do not reveal a common trend supporting this hypothesis. Whilst some studies

did indeed reveal that the lowest energy expenditure occurs in isoosmotic waters, others have shown that it occurs in waters with a salinity that the particular species normally experiences. Some studies have even shown that the lowest energy expenditure occurs in water salinities that the particular species never naturally experiences (for review see Ern et al., 2014).

In addition to the conflicting findings mentioned above, estimates of the energetic cost of osmoregulation also vary significantly from a few percent up to approximately one third of the organism's standard metabolic rate (Farmer and Beamish, 1969; Morgan and Iwama, 1999; Rao, 1968). However, a wide range of species, acclimation periods, body size and oxygen consumption measurement techniques have been used, all of which make comparisons among studies difficult (Ern et al., 2014). Knowledge concerning the energetic costs of osmoregulation would greatly benefit from a thorough investigation of whole animal energy expenditure in euryhaline teleosts transitioning from FW to SW. Ideally, these animals would be sourced from the same hatchery and examined under similar experimental conditions using methods that allow continuous, high-resolution and accurate measurements of oxygen consumption.

Although whole animal studies, with regard to the energetic costs of osmoregulation, provide a measure of the total cost that a shift in environmental salinity imposes on an organism, they do not provide information concerning regional differences in metabolism within an animal. Theoretical calculations suggest that the direct cost of branchial ion transport in teleosts contributes towards less than 6% of standard metabolic rate (Eddy, 1982; Kirschner, 1993; 1995). An experimental approach measuring oxygen consumption of isolated gills at different salinities also found that the branchial osmoregulatory cost was less than 4% of standard metabolic rate (Morgan and Iwama, 1999). However, the energetic costs of other osmoregulatory organs, such as the intestine and kidney, remain unknown and the cumulative costs of all the organs may indeed significantly influence the overall energy expenditure of teleosts.



# **AIMS**

The overall aim of this thesis was to increase the knowledge concerning the importance of intestinal motility and gastrointestinal blood flow in teleosts during essential processes such as digestion and osmoregulation. In order to address the overall aim, I have devised the following specific goals:

- Qualitatively and quantitatively describe intestinal motility patterns and their underlying control mechanisms in two different teleost species, rainbow trout and shorthorn sculpin, which display differences in feeding strategies and gastrointestinal morphology.
- Demonstrate the effects that food intake or water salinity has on intestinal motility patterns in the abovementioned teleost species.
- Determine the temporal dynamics of drinking behaviour in euryhaline rainbow trout transitioning from FW to SW, and the consequent effects of drinking on intestinal motility.
- Elucidate the circulatory and metabolic responses of euryhaline rainbow trout during SW acclimation.
- Evaluate the circulatory and metabolic responses of euryhaline rainbow trout simultaneously processing food whilst maintaining osmotic homeostasis in FW and in SW.

Fulfilling these specific goals will enable a greater insight into the importance of the gastrointestinal mechanisms involved in the processing of food and maintenance of osmotic homeostasis, which ultimately underlie the relative fitness of marine and euryhaline teleosts living in the sea.

# **METHODOLOGIES**

This section will provide an overview and discussion of the methodologies used in the studies comprising this thesis. For a more detailed description of the specific surgical procedures and experimental protocols used in each study, the reader should refer to the respective paper indicated by the bold Roman numeral.

## **2.1. Experimental animals**

Two different teleost species were studied in this thesis: shorthorn sculpin (Fig. 1, **I & II**) and rainbow trout (Fig. 2, **III, IV & V**). Both of these species are relatively robust, easily kept in a laboratory setting and are easily sourced from locations near the university.

Shorthorn sculpin is a benthic marine species in the family Cottidae. This species is widespread in the northern hemisphere (Scott and Scott, 1988). Shorthorn sculpin are opportunistic feeders that utilise a wide range of prey items such as gastropods, amphipods, shrimps, chitons, molluscs, polychaetes and other teleosts (Andersson et al., 1984; Brijs, 2013; Dick et al., 2009). Sculpin are ambush predators and their intermittent carnivorous feeding strategy makes this species an excellent model for examining pre- and post-prandial intestinal motility patterns. They can devour relatively large prey items, which is reflected in their gastrointestinal morphology. Sculpin possess a large muscular U-shaped sac-like stomach with pyloric caeca located in a ring at the very proximal end of the intestine that is relatively short when compared to herbivorous teleosts (Buddington et al., 1997; Olsson, 2011b).

Rainbow trout, a member of the Salmonidae family, are also carnivorous but tend to feed on numerous small prey items. This is reflected in the slight differences in gastrointestinal morphology when compared to shorthorn sculpin. In trout, the pyloric caeca are more dispersed along the proximal intestine, which forms almost a straight tube from the stomach to the distal intestine unlike the multiple twists and bends found in the proximal intestine of sculpin (see Fig. 1 in Gräns, 2012). The rainbow trout in Sweden are of European stock and therefore a mixture of different strains. They are largely derived from early German strains, which were most likely obtained from California and so resident strains seem to be part of the mixture (Stankovic et al., 2015). The strains in Europe tend not to go through full morphological smoltification and are therefore technically not steelhead trout. However, they are capable of physiological acclimation to SW, as escapees from aquaculture are commonly found roaming the coasts of Sweden in very good condition. Many of the hyper- and hypoosmoregulatory

adaptations of rainbow trout are well documented in the literature, with the exception of motility and circulatory responses. Therefore, the reason we chose this animal as a model species is that we can discuss our results with regard to what is already known, as well as providing some of the missing pieces in the ‘osmoregulatory puzzle’.

## **2.2. *In vivo* intestinal motility**

The responses of *in vivo* intestinal motility with respect to feeding state and/or osmoregulatory status were investigated using a couple of different methods. This allowed qualitative and quantitative descriptions of intestinal motility during these processes in either anaesthetised or conscious animals, as well as elucidating some underlying control mechanisms.

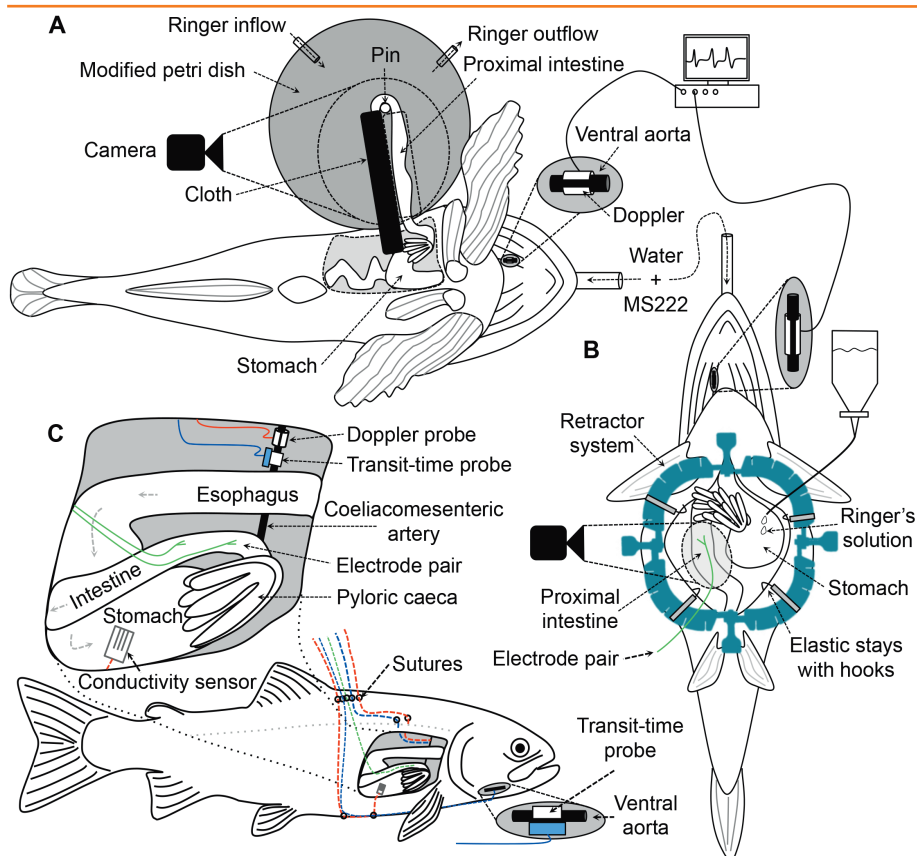
### *2.2.1. Description of motility patterns*

*In vivo* intestinal motility patterns of shorthorn sculpin (**I & II**) and rainbow trout (**III**) were qualitatively and quantitatively described using a method originally used for *in vitro* studies of gastrointestinal motility in small mammals. This method is based on ‘spatio-temporal maps’ (ST maps) generated from video recordings of the gastrointestinal tract (D'Antona et al., 2001; Hennig et al., 2010). Video recordings have also been used to describe gastrointestinal motility *in vivo* in larval teleosts (Holmberg et al., 2003; Holmberg et al., 2007; Rönnestad et al., 2000). However, whereas the gastrointestinal movements of larvae could be directly recorded through their transparent body wall, methodological modifications were required to allow *in vivo* video recordings of motility in adult teleosts.

In the shorthorn sculpin (Fig. 3A), the abdominal cavity was opened via a mid-ventral incision and the intestine was then gently teased out and placed in a modified Petri dish filled with re-circulating Ringer’s solution. This process was done carefully to ensure that the intestine was not severed from its blood supply, extrinsic nervous control systems and other regions of the gastrointestinal tract. The intestine was positioned in the Petri dish in a way that there were no twists, restrictions or damage to any blood vessels, nerves and intestinal tissue, and then submerged in Ringer’s solution. The preparation was viable for over 5 h, as it did not visually deteriorate and intestinal motility patterns were observed to be repeatable over the entire experimental period. This allowed the documentation of intestinal motility patterns over extended periods of time, as well as the effects of successive additions of pharmacological agents.

In rainbow trout, it was necessary to slightly modify the experimental setup used for shorthorn sculpin due to morphological differences of the intestine. Rainbow trout has a relatively short intestine when compared to sculpin and therefore it was not possible to tease the intestine out of the

abdominal cavity in order to place it in a modified Petri dish. Instead, the mid-ventral incision was extended and the abdominal cavity was held open to form a ‘natural Petri dish’, allowing us to video record the movements of the intestine while it remained in its place and was submerged in Ringer’s solution (Fig. 3B).



**Figure 3. Schematic representations of the surgical instrumentation used in the studies comprising this thesis. (A)** Anaesthetized shorthorn sculpin with the proximal intestine exteriorized and bathed in Ringer’s solution in a modified Petri dish awaiting video recording (I & II). **(B)** Anaesthetized rainbow trout with the proximal intestine remaining in the abdominal cavity bathed in Ringer’s solution, but still exposed for video recording (III). Enteric electrical activity (EEA) and intestinal movements around the electrode pair were simultaneously recorded in a few individuals (green line, III). **(C)** Rainbow trout instrumented with pairs of electrodes in the wall of the proximal intestine for recording EEA (green lines, III), or with a pulsed Doppler flow probe around the coeliacomesenteric artery and a conductivity sensor inserted into the stomach (red lines, IV), or transit-time flow probes around the coeliacomesenteric artery and ventral aorta (blue lines, V). Illustration is a modified version of the original figures in papers I, III & IV.

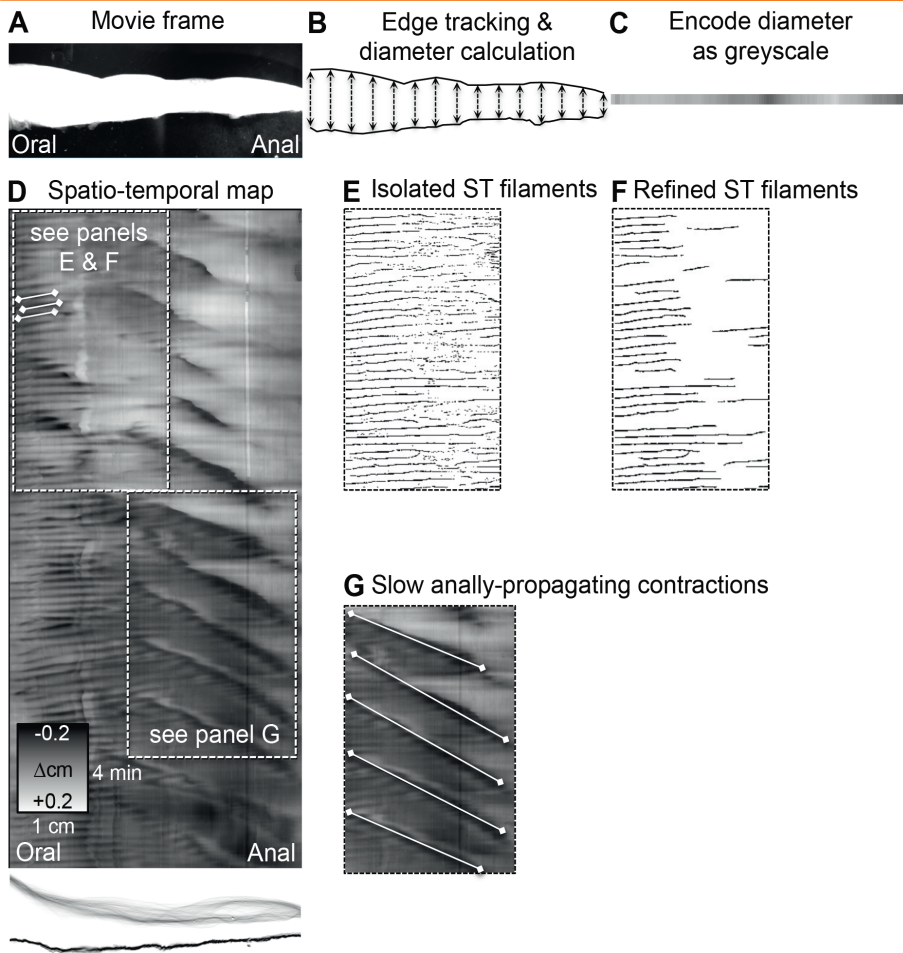
The video recordings were then subsequently transformed to ST maps using a custom-made computer program (VolumetryG8, designed by Dr. Grant Hennig). Essentially, this program transforms each frame of the video-recording into a row of pixels, which corresponds to the diameter of each point along the proximal intestine, and then compiles the rows of pixels into a ST map to allow the visualization, as well as quantification, of complex motility patterns (Fig. 4). The computer software analysis of the ST maps produced calculations of frequency, velocity, duration, propagation distance, amplitude, and interval between consecutive contractions for a wide range of intestinal motility patterns. This allowed the identification of a range of patterns, as well as their respective regional distribution and temporal characteristics. ST maps were used to describe *in vivo* pre- and post-prandial motility patterns, as well as underlying control mechanisms, in shorthorn sculpin (**I & II**), and the effects of salinity on *in vivo* intestinal motility patterns in rainbow trout (**III**).

This approach most likely allows a better approximation of true *in vivo* motility patterns compared to previously used *in vitro* preparations, as the lack of blood supply and extrinsic innervation to the gastrointestinal tract in the latter most likely results in contractile activities different to those occurring naturally (Burakoff and Percy, 1992; Fox et al., 1983; Yin and Chen, 2008). A potential limitation of the current experimental setup is that the animal needs to be anaesthetized during the experiment, which potentially alters the frequency, amplitude and propagation speed of motility patterns (Bueno et al., 1978; Fujimiya and Inui, 2000).

### 2.2.2. Control mechanisms of intestinal motility

The involvement of neurogenic and myogenic mechanisms in the control of *in vivo* intestinal motility in shorthorn sculpin was investigated by applying specific drugs into the modified Petri dish mentioned in the previous section.

Tetrodotoxin (TTX) was used for determining the neural involvement in the control of intestinal motility (**I & II**). TTX is a highly selective blocker of voltage-gated sodium channels in nerve cell membranes, which prevents the production of action potentials (Blankenship, 1976). Interestingly, there is a gradation of sensitivity in excitable fibres to TTX with nerve fibres being the most sensitive (Blankenship, 1976). Therefore, low concentrations can be used as a neural blockade without impacting the overall cardiovascular status of the individual (Holmberg et al., 2007). A concentration of 1  $\mu\text{M}$  TTX was used, as this concentration has been previously shown to diminish/abolish a range of gastrointestinal motility patterns in teleosts (Holmberg et al., 2007; Jensen and Holmgren, 1985; Karila and Holmgren, 1995; Kitazawa et al., 2012). Although TTX is a powerful tool for neural blockade, one must be aware that TTX-resistant voltage-gated sodium channels exist (Blankenship,



**Figure 4. Construction of a spatio-temporal map (ST map).** (A) Movie frames are taken from the video recording and (B) the upper and lower edges of the intestine are identified to calculate the diameter at each pixel along the segment. (C) The diameter is converted to a greyscale value and plotted in a row with black pixels representing maximum contraction and white pixels representing maximum dilation. (D) An ST map constructed from rows of pixels positioned underneath each other, with each row corresponding to diameters from a single video frame. The overall movements of the intestine can be seen in the edge map located below the ST Map in (D). Position along the intestine is on the horizontal axis, time on the vertical axis (30 min) and diameter is represented by the greyscale value (see calibration box in lower left corner). From these maps, motility patterns can be qualitatively and quantitatively described. The velocity, amplitude, frequency and duration of ripples, which are overlaid with white lines in upper left dashed box of (D), were determined via a series of differential filters, thresholds and criterion (E-F). (G) Slow anally propagating contractions were identified manually and linear lines were overlaid on the contractions to calculate motility parameters similar to that of ripples. Illustration originally from figure in paper I.

1976). The majority of these channels are primarily located in cardiac tissue, however they have also been identified in a sub-population of enteric neurons in guinea pigs and rats (Rugiero et al., 2003).

Benzbromarone is a specific and potent blocker of Ano1, which as previously mentioned is a  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channel found to be expressed exclusively in ICC. Benzbromarone was used to determine whether the myogenically driven component of intestinal motility in shorthorn sculpin involves ICC (**II**). The final bath concentration for benzbromarone (100  $\mu\text{M}$ ) was selected after the initial testing of both 10  $\mu\text{M}$  and 100  $\mu\text{M}$ , which were demonstrated to successfully block Ano1 in mammalian studies (Bernstein et al., 2014; Huang et al., 2012).

Furthermore, to validate these findings, the presence and distribution of ICC in this species was determined via immunohistochemistry using Ano1 as a specific biomarker (see **II** for more details).

### 2.2.3. Enteric electrical activity

Electrodes implanted into the wall of a specific section of the gastrointestinal tract enable the detection of the electrical discharge produced by smooth muscle contractions, similar to electromyography. This electrical discharge has recently been defined as 'enteric electrical activity' (EEA) in order to summarise all the electrical activity that occurs in the specific section of the gastrointestinal tract surrounding the implanted electrodes (Gräns, 2012; Gräns et al., 2009a; 2013). The correlation between EEA and smooth muscle contractions has been verified in a number of mammalian and teleost species, and thus this method allows the investigation of *in vivo* gastrointestinal motility in a conscious animal (Aviv et al., 2008; Ferre and Ruckebusch, 1985; Fioramonti and Bueno, 1984; Gräns et al., 2009a; 2013; Gräns and Olsson, 2011; Rodriguez Membrilla et al., 1995).

Electrical spikes observed in EEA recordings have been defined as 'events'. Groups of these events, whereby successive events are separated by periods of less than 1 min, have been defined as 'clusters' (Gräns et al., 2009a). An increasing number of events included in a cluster corresponds to an increasing duration of contraction (Gräns, 2012). Clusters have been demonstrated to occur in a rhythmic fashion in a range of teleosts with little variation in the time between successive clusters (Gräns, 2012; Gräns et al., 2009a; 2013). However, previous studies have only used a single pair of electrodes, which made it impossible to distinguish between propagating and non-propagating contractile activity. By implanting the proximal intestine of an anaesthetised rainbow trout with a pair of electrodes, whilst simultaneously video-recording the intestinal movements around the electrodes, it was possible to correlate EEA with specific motility patterns (**III**).

By combining all of this information and continuously recording EEA in the proximal intestine of euryhaline rainbow trout transitioning from FW to SW, it was possible to document the temporal dynamics of *in vivo* intestinal motility in response to SW exposure. This in turn could be compared with other time dependent processes, such as drinking behaviour and circulatory responses to SW, to provide further insight on the underlying reasons shaping the migration patterns of salmonids (III & IV).

### **2.3. Drinking response**

It is well documented that teleosts living in SW have substantially higher drinking rates than those in FW. The traditional method for evaluating drinking dynamics in teleosts is to dissolve a marker in the water and subsequently measure gastrointestinal uptake (Bath and Eddy, 1979a; Fuentes et al., 1996; Shehadeh and Gordon, 1969). However, this method does not provide the high temporal resolution required for assessing drinking dynamics of a euryhaline teleost acutely transitioning between habitats of different salinity. To our knowledge, the only other method for examining minute-by-minute drinking dynamics utilises a relatively complex and intricate experimental setup including pulse injectors, drop counters, numerous sensors and multiple catheters (Takei et al., 1998). The relatively simple surgical procedure of implanting a conductivity sensor, with an integrated temperature sensor, into the pyloric region of the stomach used in paper IV can also provide minute-by-minute information on drinking dynamics (Fig. 3C). Due to the substantial differences in the conductivity of FW and SW, it was abundantly clear when the animal initiated a drinking response following a transition to SW.

### **2.4. Circulatory responses**

Two different types of blood flow measuring systems were used throughout the studies. The Doppler system measures the speed of blood cells by sending out a well defined sound pulse (20 mHz) and analysing the shift in frequency of the echo. Analysis of these measurements allows the changes in relative blood flow and heart rate to be determined. The transit-time system measures the difference in ultrasound transit time between two transducers and provides absolute values of flow ( $\text{ml min}^{-1}$ ) (Transonic, [www.transonic.com](http://www.transonic.com)).

Pulsed Doppler flow probes were placed on the ventral aorta of anaesthetised shorthorn sculpin and rainbow trout (I, II & III) to determine whether individuals were sufficiently anaesthetised and healthy during the experiments (Fig. 3A & B). This was determined by recording relative blood flow and heart rate of the anaesthetised individual prior to making the mid-



ventral incision and then monitoring these parameters throughout the experiment. If blood flow and heart rate decreased by more than 30% during the experiment, video recording was stopped and the individual was euthanized.

In paper **IV**, pulsed Doppler flow probes were used to measure relative GBF and heart rate to determine the circulatory response of euryhaline rainbow trout during an acute transition from FW to SW (Fig. 3C). These probes were used for the initial investigation instead of transit-time flow probes due to their smaller size and ease of instrumentation. However, to record absolute blood flow and investigate the hemodynamic mechanisms underlying the GBF response observed in paper **IV**, as well as pre- and post-prandial circulatory changes in FW and SW-acclimated rainbow trout in paper **V**, transit time flow probes were placed on the ventral aorta and coeliacomesenteric artery (Fig. 3C). The absolute blood flow values provided by these probes allow comparisons with previously published values in teleosts, as well as to determine the proportion of cardiac output directed towards the gastrointestinal tract during SW acclimation.

## **2.5. Oxygen consumption**

Recent technological advances, such as REDFLASH technology, allow higher resolution oxygen consumption measurements over a broader temporal scale (PyroScience, [www.pyro-science.com](http://www.pyro-science.com)). The measuring principle is based on the quenching of the REDFLASH indicator luminescence caused by the collision between oxygen molecules and the indicators immobilised on the sensor tip. The REDFLASH indicators are excitable with red light and show an oxygen-dependent luminescence in the near infrared range. The external temperature sensor and integrated pressure sensor of the oxygen meter increase the accuracy of long-term oxygen measurements, as fluctuations in these parameters are automatically compensated for.

This opens the door for investigating the aforementioned disparities in the energetic costs of osmoregulation in teleosts. By utilising similar sized animals sourced from the same hatchery under similar experimental conditions, high-resolution recordings of whole animal oxygen consumption allowed us to elucidate the aerobic metabolic costs of rainbow trout osmoregulating in FW and SW (**IV & V**). Additionally, by evaluating whole animal oxygen consumption before and after gavage feeding in FW and SW, the effects of salinity on the SDA of rainbow trout could be determined (**V**).

## 2.6. Statistics

The statistical analyses in all of the studies were performed with SPSS Statistics 21 (IBM Corp., Armonk, NY, USA). The types of statistical analyses varied depending on the study and detailed descriptions can be found in the respective papers. For all the analyses the significance level was adjusted to compensate for multiple comparisons (if required), and a significant difference was assumed when the  $p$ -value adjusted for was  $<0.05$ .

Briefly, in papers **I & II**, significant differences between quantitative parameters of motility patterns in shorthorn sculpin were assessed via independent or dependent samples  $t$ -tests when normally distributed, otherwise Mann-Whitney  $U$ -tests were used. Additionally, a Chi-square test was used in paper **I** to determine whether feeding state was associated with slowly anally propagating contractions.

In paper **II**, the use of normalized contraction values to better identify active contractions prevented comparisons of overall contraction amplitudes between groups using the mean. Therefore, the prevalence of contractions that reduced the diameter by 10% was chosen, as the strength of these contractions are likely to be of biological importance. To determine whether significant differences occurred between the different treatments, a one-way repeated measures ANOVA and Scheffe post-hoc test were used, as Scheffe's method is a single-step multiple comparison procedure which applies to the set of estimates of all possible contrasts among the factor level means, not just the pairwise differences.

In paper **III**, an independent samples  $t$ -test was used to determine whether significant differences occurred between body mass, intestinal diameter, and the mean interval duration between successive MMCs of FW- and SW-acclimated rainbow trout. A one-way repeated measures ANOVA was used to determine whether there were any statistically significant differences in EEA variables following a transition from FW to SW.

Linear mixed models were used in papers **IV & V** and the covariance structure with the lowest Akaike's information criterion was selected for the respective models. In paper **IV**, the cardiorespiratory variables were repeatedly measured over time and so a first-order autoregressive repeated covariance structure was determined as the best fit. This model used individuals as a subject, measured data as dependent variables, time following a transfer to either FW or SW as a within-subject factor, salinity as a between-subject factor. The interaction between factors, as well as time and intercept were used as random factors to account for individual differences in the time taken to initiate the increase in GBF, as well as the magnitude of the response.

In paper V, pre- and post-prandial responses of rainbow trout acclimated to FW or SW were investigated using a model with an unstructured covariance structure. This model used individuals as a subject, measured data as dependent variables, time (day or night for pre-prandial comparisons of FW and SW acclimated trout) or feeding status (pre- and post-prandial for comparing GBF response to feeding in FW and SW acclimated trout) as a within-subject factor, salinity as a between-subject factor, and the interaction between factors. Differences in SDA responses of FW- and SW-acclimated rainbow trout were analysed using an independent samples t-test.

# **RESULTS AND DISCUSSION**

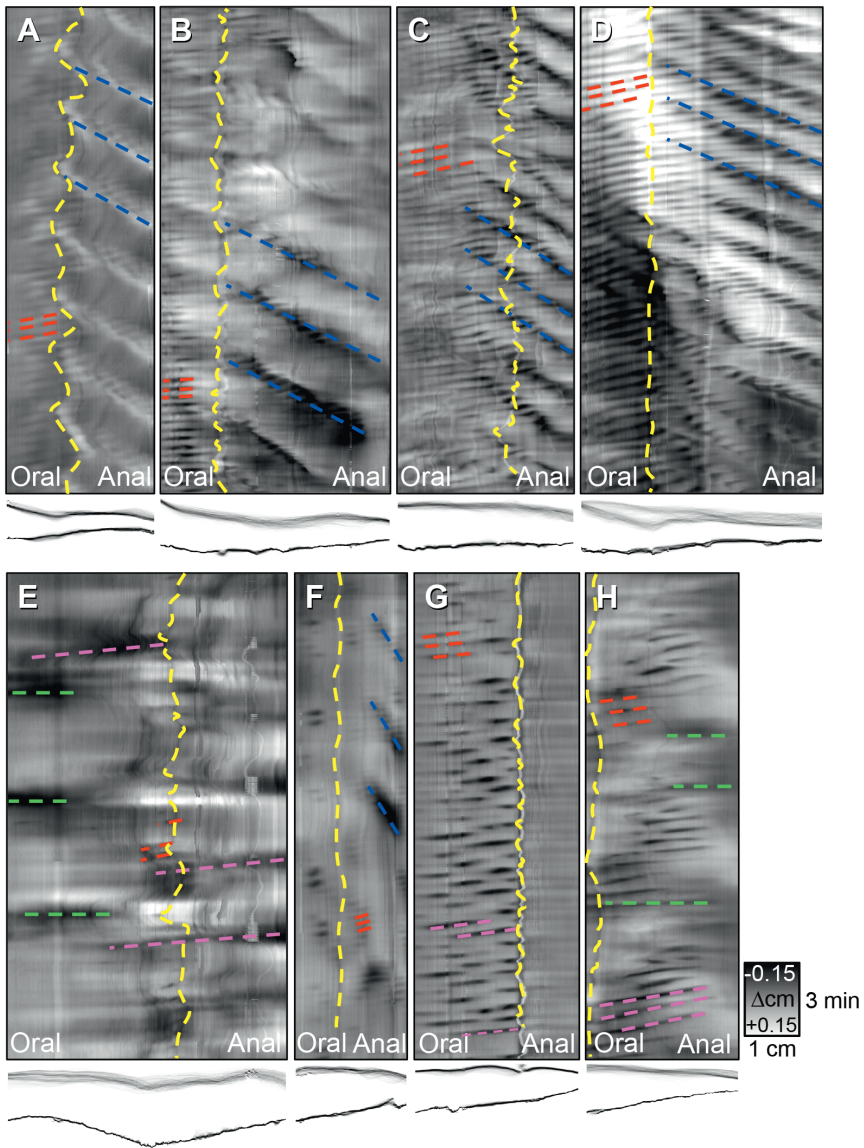
## **3.1. *In vivo* intestinal motility patterns**

A diverse array of *in vivo* intestinal motility patterns was qualitatively and quantitatively described in adult shorthorn sculpin and rainbow trout (**I, II & III**). Some of the patterns displayed in these studies were reminiscent of, or may have similar functions to, mammalian motor patterns and included both propagating and non-propagating contractions. Although patterns potentially sharing similar functions were observed between the species, it was clear that characteristics of these patterns differed significantly, which most likely reflects the differences in feeding strategy and gastrointestinal morphology (Gräns and Olsson, 2011). The most characteristic motility patterns of both species, as well as the influence of food intake and water salinity on these patterns, are discussed below (for detailed descriptions of patterns not discussed refer to **I, II & III**).

Rhythmic anally propagating motility patterns could be observed preprandially in shorthorn sculpin and rainbow trout. The rhythmicity, frequency and propagation direction suggest that these patterns are analogous to mammalian MMCs, and most likely serve a similar housekeeping function (Husebye, 1999; Husebye and Engedal, 1992; Nieuwenhuijs et al., 1998; Szurszewski, 1969; Vantrappen et al., 1977). However, clear differences in the characteristics of this pattern could be observed between the two species.

In shorthorn sculpin, the MMC-like pattern consisted primarily of prolonged, circular muscle contractions (see blue dashed lines in Fig. 5, **I & II**). However, in contrast to neurally mediated mammalian MMCs (Brierley et al., 2001; Spencer et al., 2000), the generation of these circular muscle contractions in shorthorn sculpin was mainly dependent on underlying slow waves, as blockade of neural activity only modulated the frequency and amplitude of these contractions but did not abolish them (**II**). Because of the differences between these contractions and the mammalian MMC, we used the term ‘slow anally-propagating contractions’ to define this motility pattern.

In rainbow trout, detailed analysis of the rhythmic anally propagating contractions revealed many similarities with the mammalian MMCs, and thus to avoid unnecessary additional terminology they were referred to as MMCs (**III**). The MMCs in rainbow trout involve the synchronous contractions and relaxations of both circular and longitudinal muscles, which is then followed by a period of quiescence till the next contractile front (Fig 6, **III**). Furthermore, from the analyses of the simultaneous recordings of intestinal movements and EEA in anaesthetised rainbow trout, it could be determined that MMCs directly correspond to rhythmic EEA clusters (**III**).



**Figure 5. Spatio-temporal (ST) maps displaying a range of motility patterns in the proximal intestine of shorthorn sculpin.** In fasted sculpin (**A–D**), motility patterns predominantly consist of ongoing, slow anally propagating contractions (blue dashed lines) and orally propagating ripples (red dashed lines). Fed sculpin (**E–H**) displayed a similar frequency of ripples, a significantly reduced frequency and rhythmicity of slow anally propagating contractions, a slightly reduced frequency of longitudinal contractions (yellow dashed lines), and an increased frequency of standing contractions (green dashed lines), as well as long duration, orally propagating contractions (pink dashed lines). Outlines of the intestines are displayed below each ST map. Illustration is a modified version of the original figure in paper I.

This information will allow a better interpretation of changes that occur in the intestinal motility of a conscious individual in response to changing environmental conditions, such as those faced by euryhaline teleosts transitioning from FW to SW (see 3.2.2). Unfortunately, it was not possible to determine the underlying control mechanisms of motility patterns in rainbow trout, such as MMC and ripples, as the addition of TTX or benzbramarone into the abdominal cavity resulted in a drastic deterioration of the cardiovascular status and survivorship of rainbow trout (personal observation).

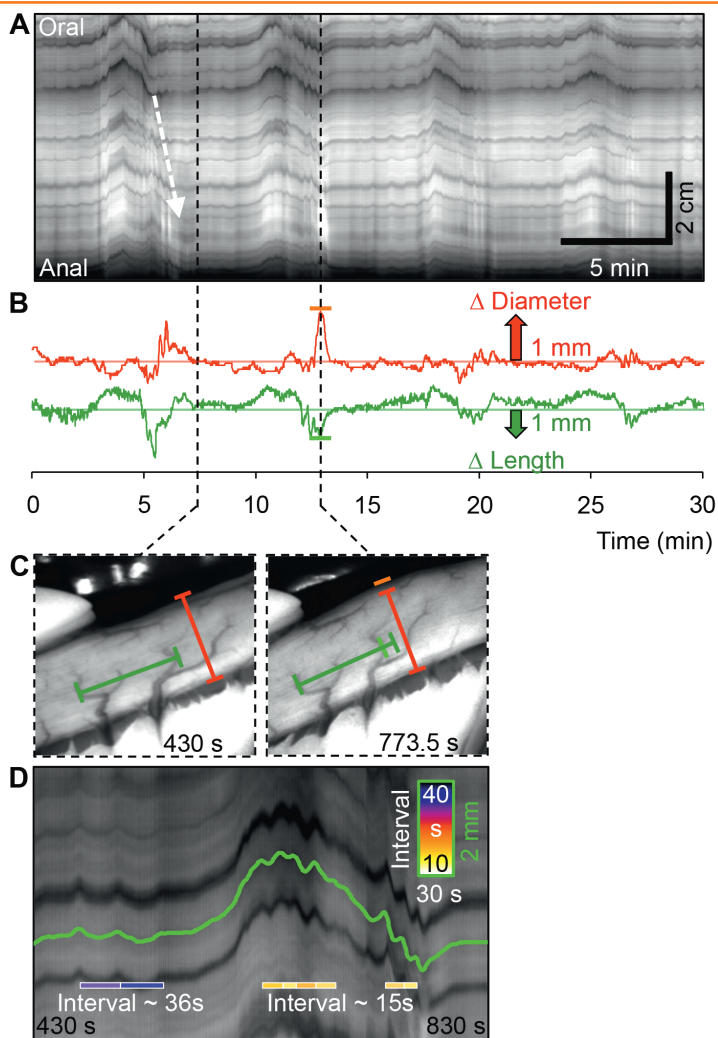
Myogenic ripples were observed, albeit with some variability of prevalence, in all shorthorn sculpin and are best described as rhythmic, shallow circular muscle contractions that generally propagate in an oral direction (see red dashed lines in Fig. 5, **I & II**). Ripples are most likely caused by underlying slow waves in shorthorn sculpin (**II**), as the blockade of channels involved with generating pacemaker transients in the ICC resulted in a complete loss of this pattern (Hwang et al., 2009; Hwang et al., 2016; Zhu et al., 2009). Myogenic ripples in mammals have been suggested to promote or optimise absorption by mixing and circulating the intestinal contents over the mucosal surface of the gastrointestinal tract (Chen et al., 2013; Hennig et al., 2010; Lee, 1983). Although this seems a likely function in teleosts as well, similar frequencies of ripples in shorthorn sculpin regardless of feeding state, as well as an increased presence of ripples in SW-acclimated rainbow trout suggests that this motility pattern may have additional functions (see 3.1.2).

In summary, the methods used in papers **I**, **II & III** proved to be successful for describing *in vivo* intestinal motility patterns in two teleost species, and could easily be applied on a wide range of vertebrates under varying environmental and/or physiological conditions.

### *3.1.1. Food intake and intestinal motility*

By comparing the findings from the intermittently feeding shorthorn sculpin with the continuously feeding rainbow trout (Gräns et al., 2009a; **I & III**), it seems that a similar relationship between feeding strategy and post-prandial motility patterns, as observed in mammals, may also exist in teleosts.

Similar to intermittently feeding dogs and humans (Bueno and Ruckebusch, 1976; Ruckebusch and Bueno, 1976; Szurszewski, 1969; Yin et al., 2004), food intake significantly altered intestinal motility of shorthorn sculpin, as the frequency of MMC-like contractions were reduced while irregular standing contractions increased (compare Fig. 5A-D with E-H). Post-prandial motility patterns of sculpin reflect the change that is occurring in gastrointestinal function, as the large amplitude and long duration of the



**Figure 6. The migrating motor complex in rainbow trout.** (A) ST map demonstrating four successive migrating motor complexes (MMCs) in a FW-acclimated rainbow trout. After the contractile front (dashed arrow) had ceased, motor activity was subdued with only small longitudinal movements occurring for ~2 min. The amplitude and regularity of the longitudinal movements increased as time progressed, which was sometimes accompanied by an increasing degree of sustained shortening. This continued for ~5 min until it transitioned into rapid longitudinal shortening, which was accompanied by sustained or high frequency circular contractions (B & C). During this period, the frequency of phasic contractions almost doubled as seen in the magnified view in (D) with intervals between successive contractions decreasing from ~36 to ~15 s. Horizontal red and green lines in (B) represent the diameter of the intestine (4.4 mm, red line in C) and distance between two blood vessels (5.5 mm, green line in C), respectively, after the completion of a complex. Illustration originally from figure in paper III.

standing contractions most likely serve to mix and grind the chyme, as well as propelling contents out of the contracting segment and over the mucosa of adjacent regions for absorption (Husebye, 1999; Kunze and Furness, 1999).

Interestingly, in contrast to mammals (Hennig et al., 1999; Tonini et al., 1996), peristaltic contractions propelling intraluminal contents along the tract were not often observed in fed sculpin (I). Since gastric emptying rates, post-prandial GBF responses and SDA duration in teleosts is typically longer than in mammals (*e.g.* days vs. hours; McCue, 2006; Seth et al., 2011), the relatively low frequency of these contractions may be advantageous as this would increase the contact time between chyme and mucosa and therefore promote absorption (Husebye, 1999). Another possibility is that the propulsive activity from the standing contractions may be enough to slowly transport the chyme in an anal direction, which would negate the need for peristaltic contractions in this species (Nguyen et al., 1995).

Pre- and post-prandial intestinal motility of FW-acclimated rainbow trout has previously been investigated using recordings of EEA (Gräns et al., 2009a). Pre-prandial motility of trout was characterised by the rhythmic occurrence of EEA clusters, at a frequency similar to the clusters observed in FW-acclimated trout in paper III, which persisted following the ingestion of a meal. However, Gräns et al. (2009) only measured EEA at one location in the intestine and were therefore unable to determine whether clusters corresponded to propagating contractions. This prevented a comparison with mammalian MMCs, as migration is a key prerequisite for phase III of the MMC (Husebye, 1999). When combining the findings from paper III where EEA clusters represent MMCs, with the results in Gräns et al. (2009), it seems that similar to continuously feeding mammals, MMCs are present in the pre-prandial state of rainbow trout and persist following food intake (Galligan et al., 1985; Gräns et al., 2009a; Grivel and Ruckebusch, 1972; Zheng et al., 2009). The post-prandial persistence of clusters was also accompanied by a significant increase in EEA events and events per cluster, which corresponds to an elevation in post-prandial contractile activity associated with the active processing of chyme (Gräns et al., 2009a).

In summary, these findings provide an example that the distinction between post-prandial motility patterns and feeding strategy holds true in at least two species of teleosts. Both species displayed a rhythmically occurring pre-prandial motility pattern analogous to mammalian MMCs and depending on their respective feeding strategy, the ingestion of food can influence the presence of this motility pattern. However, further studies utilising different species of teleosts are warranted in order to determine whether the above findings are applicable to a wider range of species.

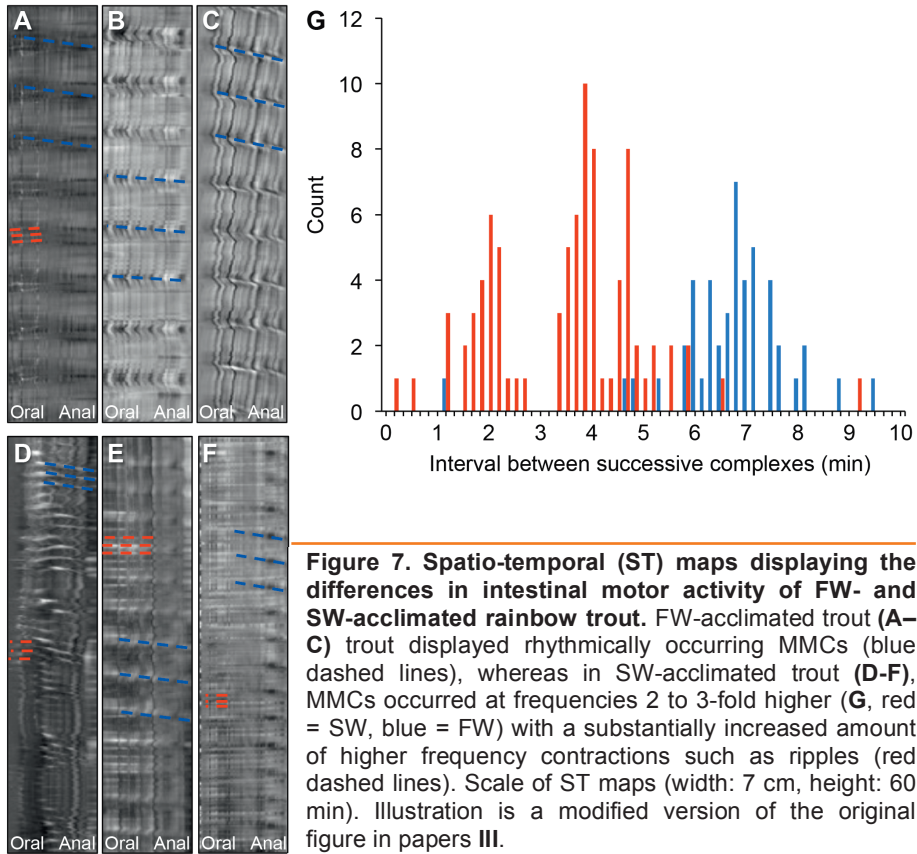


### 3.1.2. Water salinity and intestinal motility

Acclimation to FW or SW resulted in substantial differences in intestinal motility of rainbow trout (III). In FW-acclimated individuals, intestinal motility was best described by the rhythmic occurrence of MMCs approximately every 7 min (Fig. 7A-C). In SW-acclimated individuals, there was a substantial increase in the excitatory input and/or decrease in the overall inhibition of intestinal motor activity (Fig. 7D-F). The frequency of MMCs increased two to three-fold (Fig. 7G) and the periods of quiescence were replaced by high frequency contractions. This included shallow circular muscle contractions, which are most likely similar to the myogenic ripples observed in shorthorn sculpin (I & II). The increased intestinal contractile activity in SW-acclimated rainbow trout may shed some light on the prevalent occurrence of ripples, regardless of feeding state, in the marine shorthorn sculpin. By mixing and circulating imbibed SW over the intestinal mucosa, ripples may promote or optimise intestinal ion and water absorption necessary for maintaining osmotic homeostasis (Lee, 1983).

Intestinal diameters of FW-acclimated trout were relatively similar and ranged between 3.3 and 4.8 mm, while SW-acclimated trout ranged between 4.0 and 10.1 mm. As all individuals were fasted and similarly sized, these findings indicate that drinking can substantially distend the intestine, and that individual variability in drinking rates and subsequent intestinal distension exists in SW-acclimated individuals (III). Furthermore, the level of intestinal distension within SW-acclimated trout had a substantial effect on intestinal motility, as high levels of distension coincided with a higher frequency and strength of contractions when compared to lower levels of distension (III). These findings strongly indicate that the increased intestinal motility of SW-acclimated trout is, at least in part, due to the input from mechanoreceptors responding to intestinal distension. However, it is well documented that euryhaline teleosts acclimated to SW display elevated levels of ions such as  $\text{Na}^+$  and  $\text{Cl}^-$  in the tissue and cells when compared to the levels displayed in FW (Houston, 1959; Eddy, 1982; Bath and Eddy, 1979a; b). Therefore, in addition to potential input from ionoreceptors, the direct effects of ions on intestinal smooth muscle and/or ICC can not be discounted and warrant further investigation.

In summary, these findings demonstrate that *in vivo* intestinal motility of euryhaline rainbow trout is upregulated during SW acclimation. As intestinal water absorption is essential for the survival of teleosts in marine environments, the increased contractile activity and modifications to intestinal motility patterns is most likely necessary to transport and mix the imbibed SW in an optimal manner to increase the efficiency of ion and water absorption, as well as to prevent the mucosal accumulation of carbonate precipitates (Grosell, 2011; Wilson et al., 2002).

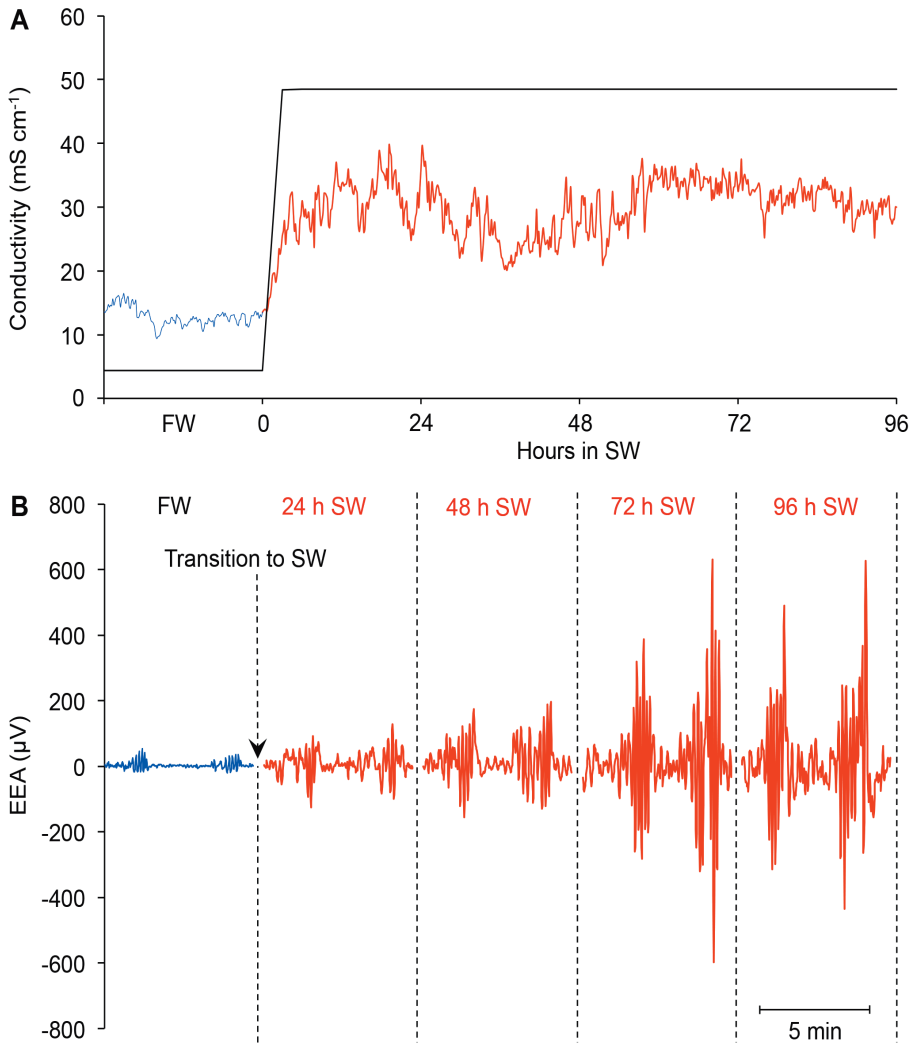


## 3.2. Crossing the freshwater-ocean boundary

### 3.2.1. The drinking response

The high-resolution recordings of stomach fluid conductivity confirmed that rainbow trout tend not to drink whilst in FW (Fuentes et al., 1996; Shehadeh and Gordon, 1969), as conductivity of stomach fluids was stable and remained approximately three-fold higher than the surrounding water (Fig. 8A, IV). Rainbow trout initiated a drinking response when exposed to increasing salinity, as seen by an increase in stomach fluid conductivity. All individuals had begun drinking long before the surrounding water had changed from FW to full strength SW, which took approximately 2.5 h. These recordings also demonstrate that substantial desalination of imbibed SW occurs in the oesophagus, as conductivity of fluids entering the stomach was always less than 60% of the conductivity of SW (Fig. 8A). As

previously mentioned, oesophageal desalination of imbibed SW is essential for facilitating subsequent intestinal water absorption (Hirano and Mayer-Gostan, 1976; Kirsch and Meister, 1982; Parmelee and Renfro, 1983).



**Figure 8. Temporal dynamics of the drinking response and changes in the intestinal motility of rainbow trout transitioning from FW to SW. (A)** The changes in stomach fluid conductivity of an individual rainbow trout transitioning from FW to SW (the black line represents the conductivity of the surrounding water). **(B)** A raw trace demonstrating the typical changes that occur in EEA, a proxy for intestinal contractile activity, of a rainbow trout undergoing a similar transition from FW to SW. This trace demonstrates the typical increase in the frequency and amplitude of EEA events, as well as the increase in the frequency in EEA clusters and events per cluster. Illustration is a modified version of the original figures in papers III & IV.

### 3.2.2. *Acute changes in intestinal motility*

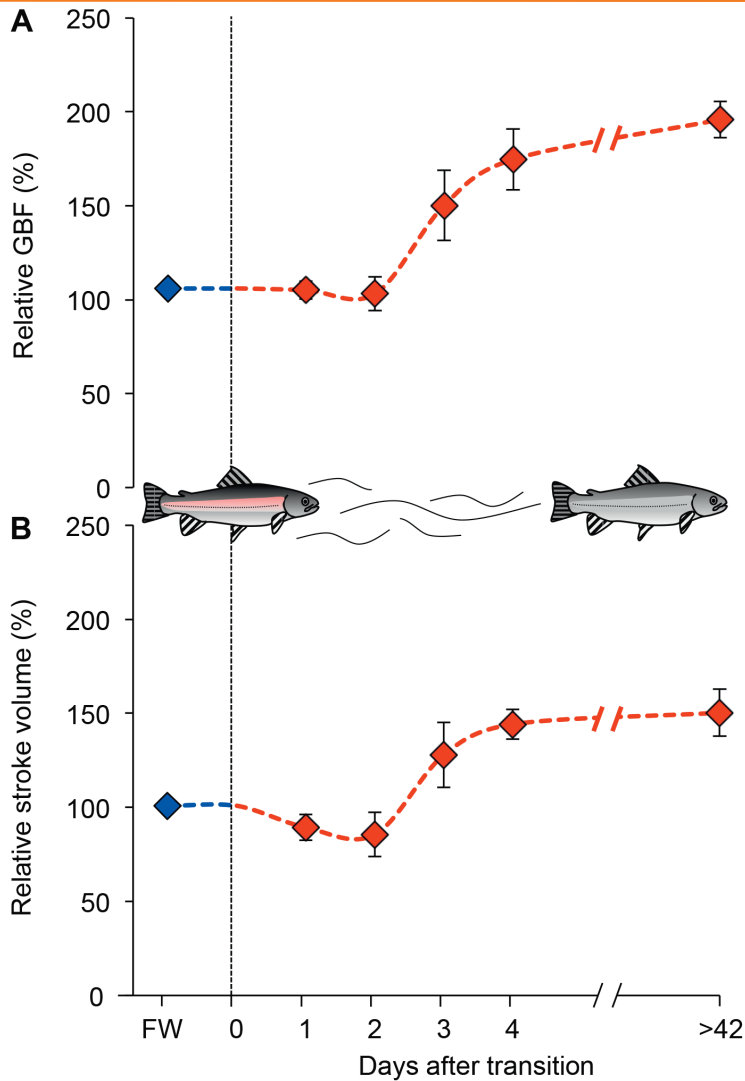
In FW, the EEA of unfed rainbow trout was characterised by rhythmic clusters or MMCs occurring every 4-5 minutes, which was higher than the frequency observed in the anaesthetised individuals discussed in the previous section. However, this can most likely be attributed to the effects of anaesthesia, which has previously been demonstrated to alter the frequency of motility patterns in a range of mammals (Bueno et al., 1978; Fujimiya and Inui, 2000). Based on aforementioned findings, the acute transition to SW would be expected to coincide with an increased intestinal motor activity in this euryhaline teleost species. Indeed, visual and statistical inspection of the EEA traces revealed a gradual increase in the motor activity of the intestine (Fig. 8B). After approximately two days, the contractile activity of the intestine typically seemed to plateau at a significantly elevated level with an increased frequency of EEA clusters/MMCs and events, which corresponds well to the findings from the video recordings of the intestine in SW-acclimated animals (III).

Collectively, these findings clearly demonstrate that upon exposure to SW, drinking and intestinal contractile activity of rainbow trout significantly increases, and remains at an elevated level in fully acclimated animals.

### 3.2.3. *Hypoosmoregulatory circulatory responses*

The acute transition (IV) and long-term acclimation (V) of rainbow trout to SW was closely linked with several circulatory modifications. Following the rapid drinking response and subsequent changes in intestinal motility, GBF began to increase after approximately 2 days in SW. After 4 days in SW, GBF was two-fold higher than that observed in FW (IV). This substantially elevated level was maintained in fully acclimated individuals (Fig. 9A, V).

Studies on a range of salmonid species have demonstrated that branchial and intestinal  $\text{Na}^+/\text{K}^+$ -ATPase begins to increase after ~24-72 h in SW (Genz et al., 2011; Madsen and Naamansen, 1989; Seidelin et al., 2000; Tipsmark and Madsen, 2001). This approximately coincides with the temporal changes in intestinal motility (III) and GBF (IV), indicating that whilst simultaneously increasing motility and GBF to optimise water uptake and transportation of ions and metabolic waste products for subsequent excretion, it is also necessary for the gills and the gastrointestinal system to transform into a hypoosmoregulatory functional state. Furthermore, the timing of these physiological modifications roughly coincides with the gradual return of osmotic balance after an initial period of significantly elevated plasma osmolality in salmonids transitioning from FW to SW (Bath and Eddy, 1979a; Fuentes et al., 1996; Seidelin et al., 2000). Collectively,



**Figure 9. Circulatory responses of rainbow trout during SW acclimation. (A)** Relative gastrointestinal blood flow (GBF) and **(B)** relative stroke volume of rainbow trout transitioning from FW (blue diamond) to SW (red diamonds). Relative GBF and stroke volume of trout increased during an acute transition to SW ( $p=0.008$  and  $<0.001$ , respectively, **IV**), and remained elevated at these levels after  $>42$  days of SW acclimation ( $p<0.001$  and  $0.079$ , respectively, **V**). Heart rate was not significantly affected (data not shown). Black dashed line represents a transition to SW at 0800 h. Absolute blood flow data from trout  $>42$  days in SW has been converted to a relative value using the mean FW value as 100% in order to plot both data sets on one graph, however, the statistical analyses are based on the original data. Samples sizes are reported in the specific papers. Data are presented as means $\pm$ S.E.M. Illustration is a modified version of the original figures in papers **IV & V**.

these findings suggest that an increased intestinal motility and GBF is important for euryhaline teleosts maintaining osmotic homeostasis in SW.

In FW-acclimated rainbow trout, 30% of cardiac output was directed towards the gastrointestinal tract (V). The elevated GBF of trout in SW was most likely due to local vasodilation of the gastrointestinal vasculature, as this would allow an increased proportion of blood flow to be directed to the gastrointestinal tract. In fact, almost half of the cardiac output was directed to the gastrointestinal tract of SW-acclimated trout (V), which is higher than any previously documented values for unfed teleosts (Seth et al., 2011). In order to maintain arterial blood pressure, the increased proportion of blood directed from the systemic to gastrointestinal vasculature was compensated for by an increase in cardiac output (Seth et al., 2011). The elevation in cardiac output of SW-acclimated trout closely matched the increase in GBF (V). This finding further highlights the importance of GBF for trout in SW, as the gastrointestinal tract received virtually all of the increase in cardiac output.

The increase in cardiac output was primarily mediated via an elevated stroke volume, as heart rate was not significantly different to FW-acclimated individuals (V, Brijs et al., 2016a). Although cardiac output was not determined in trout in paper IV, the combination of an unchanged heart rate and a response profile for relative intestinal pulse volume closely mirroring that of GBF during the acute transition to SW (Fig. 9B), strongly indicates that the underlying hemodynamic mechanisms responsible for the elevated GBF are similar to those observed in rainbow trout acclimated to SW (V).

### 3.2.4. *Underlying hemodynamic mechanisms*

Exposure to SW could be expected to have a volume-depleting effect on trout, which would subsequently result in a reduced circulating blood volume and central venous pressure, unless there are active reductions in venous capacitance (Olson, 1992; Sandblom and Axelsson, 2007a). Central venous pressure represents the cardiac filling pressure and is the main determinant of the end-diastolic volume *in vivo*, which subsequently determines stroke volume (Farrell, 1991; Farrell et al., 1988; Farrell and Jones, 1992). Therefore, in order to achieve an elevated stroke volume in SW (V), euryhaline rainbow trout must possess the capacity for active venous compensation.

The only study comparing *in vivo* cardiac filling pressures of teleosts acclimated to either FW or SW reported lower central venous pressures in SW-acclimated rainbow trout, which indicates a very limited capacity for active venous compensation (Olson and Hoagland, 2008). However, it should be noted, that the surgical procedures used in that study involved opening the pericardium, which has been demonstrated to compromise

cardiac and venous function (Farrell, 1991; Farrell et al., 1988; Sandblom and Axelsson, 2006; Sandblom and Axelsson, 2007b).

Central venous pressure measured in trout with an intact pericardium revealed that FW-acclimated rainbow trout utilized a *vis-à-fronte* cardiac filling mechanism, whereby sub-ambient central venous pressures created by cardiac suction sustained resting cardiac output (Brijs et al., 2016a). This is consistent with other studies on FW-acclimated trout (Altimiras and Axelsson, 2004; Sandblom and Axelsson, 2005; Sandblom and Axelsson, 2007b). In contrast, SW-acclimated trout exhibited a greater reliance on *vis-à-tergo* cardiac filling where central venous pressure attained positive values (Brijs et al., 2016a). This increase in cardiac filling pressure could explain the elevated stroke volume in SW-acclimated trout, which resulted in a resting cardiac output ~50% higher than FW-acclimated trout (V, Brijs et al., 2016a). Furthermore, these hemodynamic modifications coincided with a significant increase in the proportion of compact myocardium (Brijs et al., 2016a), which may be necessary to compensate for the increased wall tension associated with a larger stroke volume (Farrell, 1991).

### 3.2.5. Energetic costs of osmoregulation

It has been suggested that maintaining osmotic homeostasis in SW could be energetically more costly than in FW, due to the larger ionic gradient between the internal and external medium (Boeuf and Payan, 2001; Ern et al., 2014). Yet, the acute transition to SW did not significantly affect the standard metabolic rate of trout (IV). This suggests that the abovementioned physiological and behavioural modifications that occur during the first 96 h in SW do not incur an additional aerobic metabolic cost. In contrast, after 6 weeks of acclimation to SW, rainbow trout exhibited standard metabolic rates ~20% higher than FW-acclimated trout (V), suggesting that the more permanent regulatory changes associated with hypoosmoregulation such as increased intestinal and branchial  $\text{Na}^+/\text{K}^+$ -ATPase impose an additional cost.

However, a recent in-depth investigation into the metabolism of rainbow trout acclimating to SW contradicts the metabolic findings of paper V. By investigating the temporal dynamics of osmotic balance, intestinal  $\text{Na}^+/\text{K}^+$ -ATPase, whole animal oxygen consumption, intestinal mitochondrial respiration, as well as the activities of aerobic and anaerobic metabolic enzymes in uninstrumented rainbow trout during a 35 day SW acclimation period (Brijs et al., 2016b), we were better able to characterize and understand the energy metabolism of this species during the transition from a hyper- to hypoosmoregulatory state. Briefly, the simultaneous upregulation of  $\text{Na}^+/\text{K}^+$ -ATPase activity in the gills, pyloric caeca and intestine coincide with the return of osmotic homeostasis in rainbow trout ~5 days after a transition to SW (Brijs et al., 2016b; Madsen et al., 1995; Seidelin et al.,

2000). The increased ATP demand of  $\text{Na}^+/\text{K}^+$ -ATPase did not incur significant aerobic costs at the whole animal level, as the standard metabolic rate of trout during SW acclimation period did not differ (Brijs et al., 2016b). However, a recent review has highlighted that measures of whole animal oxygen consumption rate may only be a partial proxy for energy metabolism as the amount of ATP generated per unit of oxygen consumed can vary significantly, within and between individuals, as well as between populations and environments (Salin et al., 2015). Indeed, the underlying metabolic mechanisms that fuel the osmoregulatory machinery in the intestine of rainbow trout were modulated in SW, as mitochondrial coupling efficiency and glycolytic ATP production were increased to satisfy the elevated energetic demands (Brijs et al., 2016b). These findings highlight the importance of integrating measurements of metabolism at different levels of biological organization when investigating the link between energy metabolism and animal performance.

The lack of an elevated standard metabolic rate in uninstrumented SW-acclimated rainbow trout in Brijs et al. (2016b) made me question the underlying reason for the elevations in pre-prandial standard metabolic rate of instrumented SW-acclimated rainbow trout in paper V. Closer examination of individual whole animal oxygen consumption rates indicates that SW-acclimated rainbow trout may require a longer recovery time from surgery than FW-acclimated individuals. After 24 h of recovery, the oxygen consumption of all FW-acclimated trout had stabilised, whereas in SW-acclimated trout these values were still tending to decrease. Further indications that 24 h may not be a sufficiently long enough recovery period following surgery for SW-acclimated rainbow trout can be seen in their slightly elevated plasma osmolality ( $\sim 350 \text{ mOsmol kg}^{-1} \text{ H}_2\text{O}$ ) compared with the average plasma osmolality of uninstrumented SW-acclimated individuals ( $\sim 315 \text{ mOsmol kg}^{-1} \text{ H}_2\text{O}$ ) (Brijs et al., 2016a; b). Collectively, this emphasizes that the time required for recovery from surgery is variable and situation dependent, even within species, and thus needs to be considered when designing new experiments and interpreting data in the future.

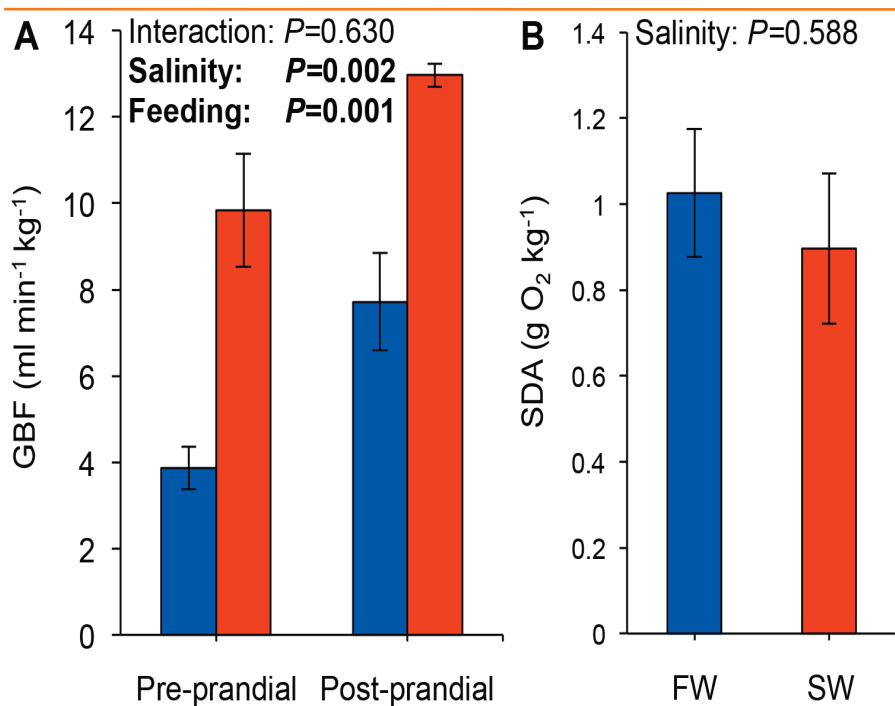
### **3.3. Circulatory scope of a euryhaline teleost**

The elevated pre-prandial circulatory demands imposed on rainbow trout maintaining osmotic homeostasis in SW may have implications on the capacity for this species to further increase GBF during the processing and digesting of a meal. If SW-acclimated trout cannot further increase GBF in response to a meal, it may negatively impact the transportation of absorbed nutrients, oxygen delivery to gastrointestinal tissues, as well as acid-base regulation during digestion (Seth et al., 2011; Wilson and Grosell, 2003).



Following the introduction of a meal corresponding to 1% body mass by gavage, FW- and SW-acclimated rainbow trout displayed similar increases in absolute GBF (Fig. 10A, V). However, there are indications that SW-acclimated trout are approaching their maximum capacity for GBF, as individual variation in post-prandial GBF was much less than that observed pre-prandially, as well as the variation generally observed in FW-acclimated individuals (compare error bars in Fig. 10A).

The elevated post-prandial GBF was due to an increased proportion of cardiac output directed towards the gastrointestinal system, with increases of  $7.1\pm 4.6\%$  and  $5.7\pm 4.4\%$  in FW and SW respectively, which was compensated for by an elevated cardiac output (Fig. 11, V). SW-acclimated trout exhibit a relatively smaller post-prandial increase in cardiac output than



**Figure 10. Post-prandial circulatory and metabolic responses of rainbow trout in FW and SW. (A)** Gastrointestinal blood flow (GBF) and **(B)** specific dynamic action (SDA) of rainbow trout in FW (blue bars) or SW (red bars). GBF increases by similar amounts following feeding in both acclimated groups, however, trout in SW maintain a consistently higher GBF than individuals in FW (V). The SDA, determined by integrating the area between the post-prandial oxygen consumption curve and pre-prandial oxygen consumption baseline, did not significantly differ between acclimation groups (V). Samples sizes are reported in V. Significance is defined as  $P<0.05$ . Data are presented as means $\pm$ S.E.M. Illustration is a modified version of the original figure in paper V.

FW-acclimated individuals. This may indicate that the gastrointestinal system cannot receive any more blood as it has reached a maximum level at ~55% of cardiac output, which is greater than any documented values for teleosts (for review see Seth et al., 2011), or that these individuals only require a smaller additional circulatory response to aid in the process of digestion due to their elevated pre-prandial level.

Rainbow trout acclimated to SW generally exhibited higher pre- and post-prandial oxygen consumption rates when compared to FW-acclimated individuals (V). However, the total postprandial metabolic cost or SDA, which was determined by integrating the area between the post-prandial oxygen consumption curve and the pre-prandial baseline, did not significantly differ between the groups (Fig. 10B). The duration of the SDA in rainbow trout, regardless of salinity, lasted longer than 72 h, which is typical of an ectothermic species digesting a meal at low temperatures (McCue, 2006; Wang et al., 2002). These findings suggest that environmental salinity does not significantly affect the metabolic costs associated with digestion (V). However, a limitation of this study was the high mortality rate in SW-acclimated trout following gavage feeding (50% compared to <8% in FW). All individuals in both acclimation groups were voluntarily feeding without any mortality prior to the experiments, which indicates that gavage feeding had a greater negative impact on the SW-acclimated trout. This may be related to a pronounced disruption in the acid-base balance of SW-acclimated trout, as not only do SW-acclimated trout display a greater post-prandial alkaline tide than FW-acclimated individuals (Bucking and Wood, 2008; Bucking et al., 2009;), but that the process of gavage feeding further exacerbates this metabolic alkalosis (Cooper and Wilson, 2008). This may have resulted in a post-prandial alkaline tide that half of the SW-acclimated individuals were unable to recover from. Ideally, future investigations should use biotelemetric techniques, which would allow the investigation of postprandial metabolic and circulatory responses of freely swimming and voluntarily feeding euryhaline teleosts acclimated to different salinities (Gräns et al., 2009; 2010).

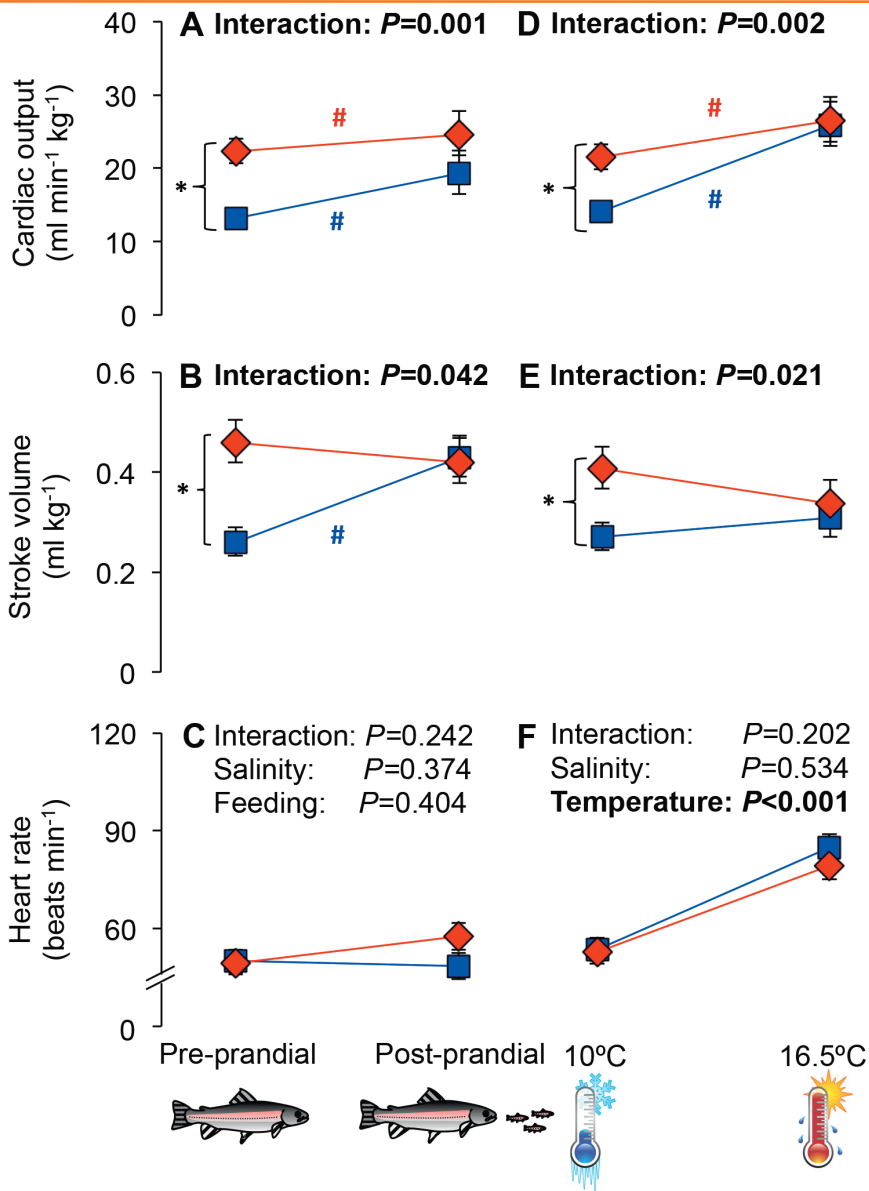
Although SW-acclimated trout had the circulatory capacity required to elevate arterial blood flow post-prandially in paper V, another study carried out on similar sized conspecifics from the same hatchery revealed that they may have a limited capacity for further elevations in blood flow when exposed to an acute temperature increase (Brijs et al., 2016a). In this study (Fig. 11D-F), cardiac output increased in response to acute warming through a similar tachycardia in both FW- and SW-acclimated trout, consistent with previous studies (Clark et al., 2008; Ekström et al., 2014; Gamperl et al., 2011; Sandblom and Axelsson, 2007b). However, the magnitude of the

temperature-induced increase in cardiac output differed between the groups due to a tendency for a reduction in stroke volume of SW-acclimated trout.

It has been suggested that a reduction in stroke volume due to increasing heart rate is caused by a reduction in cardiac filling time (Altimiras and Axelsson, 2004). However, this mechanism does not explain the tendency for stroke volume to decrease only in SW-acclimated trout, as both acclimation groups exhibited a similar heart rate response to acute warming. Moreover, as the central venous pressure remained higher in the SW-acclimated group, this should have buffered the negative effect of the tachycardia on stroke volume to a greater extent than in the FW-acclimated group where central venous pressure remained sub-ambient (Brijs et al., 2016a). This suggests that factors other than a reduced cardiac filling time are constraining stroke volume and therefore limiting the increase in cardiac output of SW-acclimated trout following an acute temperature increase.

Again, this may be related to a pronounced disruption in the acid-base balance of SW-acclimated trout, as the hypoosmoregulatory mechanism involving intestinal precipitation of carbonates is associated with a net H<sup>+</sup> gain, which must then be transported to the gills to be excreted (Genz et al., 2008; Grosell, 2011). This blood acidosis may be more pronounced following an acute temperature increase due to a direct temperature effect on intestinal carbonate precipitation (Wilson et al., 2002; Wilson et al., 2009), and/or an increase in osmoregulatory activity at higher temperatures in order to maintain osmotic homeostasis in the face of exacerbated dehydration due to a greater 'osmo-respiratory compromise' (Nilsson, 1986). The pronounced blood acidosis in SW-acclimated trout may negatively affect cardiac contractility (Driedzic and Gesser, 1994), which could explain the tendency for stroke volume to decrease during acute warming (Brijs et al., 2016a).

Although the abovementioned studies demonstrate the complex interacting effects of water salinity with either feeding or temperature on the cardiovascular function of rainbow trout, one must be wary interpreting the ecological implications of these findings. It is possible that circulatory responses were either exaggerated or constrained due to a range of factors: i) gavage feeding has been suggested to bypass crucial neuroendocrine mechanisms for initiating preparatory compensatory acid-base responses (Cooper and Wilson, 2008), ii) the rate of heating in Brijs et al. (2016a) was relatively rapid compared to natural events such as heat waves (Meehl and Tebaldi, 2004), and iii) although rainbow trout are capable of physiological acclimation to SW, they are a farmed FW resident strain as opposed to wild migratory steelhead strains. Nevertheless, my findings provides a solid physiological platform for further investigations on the ecological implications of interacting abiotic and biotic factors on euryhaline and migrating teleosts.



**Figure 11. Circulatory responses of rainbow trout following the ingestion of a meal or an acute temperature increase.** Cardiac output, stroke volume and heart rate of FW- (blue squares and lines) and SW-acclimated rainbow trout (red diamonds and lines) following the ingestion of a meal (**A-C**) or an acute temperature increase (**D-F**). Statistical analyses generated via a two-way mixed ANOVA are summarized in each figure panel with statistically significant responses highlighted in bold. Significance is defined as  $P<0.05$ . Data are presented as means $\pm$ S.E.M. Illustration is a modified version of the original figures in paper **V** and Brijs et al., 2016a.

# **SUMMARY AND CONCLUSIONS**

The overall aim of this thesis was to increase the knowledge concerning the importance of intestinal motility and gastrointestinal blood flow in teleosts during essential processes such as digestion and osmoregulation. In order to accomplish this, a range of specific goals needed to be achieved.

By further developing and adapting video recording and spatio-temporal map analyses, it was possible to qualitatively and quantitatively describe a range of propagating and non-propagating intestinal motility patterns *in vivo* in adult teleost species for the first time (**I, II & III**). Many of the motility patterns observed in shorthorn sculpin and rainbow trout are reminiscent of well-documented mammalian motor patterns, which allow further insight on their respective functions. However, unlike mammals, the majority of these rhythmic contractile patterns are generated and propagated by ICC, while neural activity only modulates the frequency and amplitude of these contractions (**II**).

Intestinal motility patterns differ between fasted and fed individuals, which reflects the differences in gastrointestinal function that occurs during the two different feeding states (**I**). Pre-prandially, both species of teleost display a rhythmic anally propagating motility pattern that resembles, and most likely shares a similar housekeeping function as, mammalian MMCs (**I-III**). Following the ingestion of food, the rhythmic anally propagating motility pattern is reduced and replaced by irregular motor activity in the intermittently feeding shorthorn sculpin (**I**), whereas this pattern persists in the continuously feeding rainbow trout. These findings demonstrate that the distinction between post-prandial motility patterns and feeding strategy in mammals also holds true in at least two species of teleosts.

By examining a range of behavioural and physiological parameters in rainbow trout transitioning from FW to SW, it was possible to elucidate additional processes that are involved in the maintenance of osmotic homeostasis (**III, IV & V**). Although it is already well documented that drinking and oesophageal desalination of imbibed SW is essential for subsequent fluid absorption in the gastrointestinal tract, our findings demonstrate the relatively immediate nature of these responses in a euryhaline teleost transitioning from FW to SW (**IV**). When rainbow trout imbibe SW, it results in a substantial distension of the intestine and a significant increase in intestinal contractile activity, which is maintained in fully SW-acclimated trout (**III**). The observed relationship between levels of intestinal distension and motor activity in SW-acclimated trout suggests that mechanoreceptors are involved in the upregulation of intestinal motility in SW, although the influence of ionoreceptors and the direct effects of elevated ions on gastrointestinal motility cannot be discounted (**III**). Increased

intestinal motor activity of euryhaline teleosts in SW is likely part of the overall response to maintain osmotic homeostasis, as increased drinking and mechanical perturbation of fluids is necessary to optimize intestinal ion and water absorption, as well as to prevent the mucosal accumulation of carbonate precipitates.

The increased intestinal motility of rainbow trout in response to SW also coincides with essential circulatory modifications (**IV**). GBF of rainbow trout transitioning from FW to SW began to increase after ~2 days and reached a level two-fold higher than in FW after 4 days (**IV**). The elevated level of GBF was maintained in fully SW-acclimated trout (**V**). The increase in GBF is most likely due to a decrease in the vascular resistance of the gastrointestinal tract, which is compensated for by significant increases in cardiac output via an elevated stroke volume (**V**). The elevation in blood flow in euryhaline teleosts residing in SW is important for the transportation of ions, water and metabolic wastes from the gastrointestinal tract to their respective sites around the body. In uninstrumented rainbow trout, the abovementioned osmoregulatory modifications do not increase standard metabolic rate, instead mitochondrial coupling efficiency and glycolytic ATP production is increased to satisfy the elevated energetic demands. As the temporal dynamics of the abovementioned elevations in intestinal motility and blood flow closely match the gradual return of osmotic homeostasis in rainbow trout following a transition to SW, my findings strongly suggest that these processes are integral for the maintenance of osmotic homeostasis in euryhaline teleosts (**III, IV & V**).

Finally, despite elevated pre-prandial levels of GBF, SW-acclimated trout were still able to further increase gastrointestinal perfusion following a meal (**V**). Furthermore, the similar SDA response of FW- and SW-acclimated trout in the present study suggests that environmental salinity does not significantly affect the metabolic costs associated with digestion. Although SW-acclimated trout had the circulatory scope required to elevate arterial blood flow post-prandially (**V**), there are indications that cardiovascular scope is reduced in these individuals, especially during acute environmental temperature increases.

In conclusion, this thesis clearly demonstrates the importance of gastrointestinal motility and blood flow in teleosts during digestion and osmoregulation, as well as providing a physiological platform for future investigations into processes underlying the relative fitness of teleosts in the marine environment. Due to the potential limitations in circulatory scope of euryhaline rainbow trout, a pressing issue requiring attention are the effects of interacting environmental factors on gastrointestinal function in teleosts and the ecological implications of these effects, especially when one considers the rate at which the planet is currently changing.

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