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The Effects of Temperature on Gut Blood Flow and Gut Motility in Fish

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DISSERTATION ABSTRACT

The Effects of Temperature on Gut Blood Flow and Gut Motility in Fish

Albin Gräns, 2012

In order for a fish to grow and thrive, the gut must function efficiently. This is achieved through a range of processes, including controlled patterns of gut motility and modifications in gut blood flow. How these processes are affected by long and short-term changes in ambient temperature was the main focus of this thesis.

After acclimation to a higher temperature, the interdigestive motility of rainbow trout was higher. This indicates an increased demand for movements involved in the housekeeping functions of the gut. Temperature acclimation did not affect the postprandial response, in neither gut blood flow nor gut motility. These results indicate that thermal compensation processes work to neutralize acute thermal effects, so that these two functions are maintained at a certain rate. Also, *in vitro* preparations of isolated intestine showed signs of thermal compensation processes, as the acute temperature effects on the response to the cholinergic agonist carbachol were abolished after a temperature acclimation period.

Fish species studied responded very differently to an acute change in temperature. A relatively linear correlation with temperature was seen in, both gut blood flow and gut motility, in two species of sculpins (shorthorn and Arctic sculpin). The gut blood flow, in both green and white sturgeon, was unaffected by moderate fluctuations in water temperature during the interdigestive state. White sturgeon were also studied after feeding and a correlation between gut blood flow and temperature was observed, in a similar fashion as for the unfed sculpins.

Thermoregulatory behaviours observed in white sturgeon, show that moving between different temperatures, after a meal, will have a great influence on the volume of blood distributed to the gut. An increase in gut blood flow is probably an important factor explaining why it can be beneficial to migrate into warmer waters after feeding. However, if combining the presented data with data from previous studies, it shows that perfusion of the gut can also be a limiting factor when the environmental temperature changes. This is probably the reason why some fish species forage in warm waters and then move into colder areas when digesting the meal.

A leading hypothesis suggests that the temperature limitations for a fish are mainly set by a reduction in metabolic scope. However, experimental data has shown that temperature can have significant effects on both appetite and growth before metabolic scope is reduced. The findings in this thesis suggest that oxygen-limited thermal tolerance in the gut of fish could also be a significant variable in determining the temperature range that fish can tolerate.

Keywords: Fish, thermoregulatory behaviours, electrical activity, biotelemetry

INCLUDED PAPERS

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

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

II. **Gräns A, Seth H, Axelsson M, Sandblom E, Albertsson F, Wiklander K, Olsson C (2012)** Effects of acute temperature changes on gut physiology in two species of sculpin from the west coast of Greenland (manuscript)

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

IV. **Gräns A, Olsson C, Pitsillides K, Nelson H, Cech J, Axelsson M (2010)** Effects of feeding on thermoregulatory behaviours and gut blood flow in white sturgeon (*Acipenser transmontanus)* using biotelemetry in combination with standard techniques. Journal of Experimental Biology, 213, 3198-3206

TABLE OF CONTENT

INTRODUCTION

What is a fish?

This thesis is based on four studies (**papers I-IV**) that include five species of fish: rainbow trout *Oncorhynchus mykiss*; shorthorn sculpin *Myoxocephalus scorpius;* Arctic sculpin *M. scorpioide;* green sturgeon *Acipenser medirostris* and white sturgeon *A. transmontanus*. Although fish is a well-established term and most people would agree on what is, or what is not a fish, certain points concerning the taxonomic classification should be noted. Rather than being a monophyletic group, like mammals, fish are a paraphyletic group. Thus, fish are principally defined by the exclusion of tetrapods, rather than a unifying group where all who descend from the same ancestry are included (Nelson 2006). Due to this broad definition of fish, the group includes species that have been separated from other fishes for more than 500 million years (the hagfishes). All five species used in this thesis come from the same subgroup, namely the class of chordates called Actinopterygii (ray-finned fishes). The ray-finned fishes constitute over 95% of all extant species of fish and were separated from the other chordates around 420 million years ago (Nelson 2006).

Where do fish live?

Today, there are over 30,000 described extant species of fish in the world, and probably thousands left to identify (www.fishbase.org). They inhabit almost all the world's aquatic ecosystems, regardless of significant changes in environmental factors such as altitude and temperature. Species of fish are found from lakes in the Himalayan mountains at elevations of approximately 4,600 meters, down to the deepest ocean trenches at 11,000 meters (Nelson 2006). They inhabit areas with temperatures ranging from 45°C in the African alkaline hot-springs down to almost -2°C under the ice in the Arctic and Antarctic regions (Axelsson et al. 1992; Johnston et al. 1994). Fish are able to survive and be successful in this wide range of habitats and temperatures due to an enormous scope in morphology, physiology and behavioural adaptations.

Not only can different species of fish be found at different temperatures, but also within species various adaptations have made it possible for individuals to survive a wide range of temperatures both long- and short-term. When individuals of various species have been tracked in the wild, results show that every day involves exposure to a range of temperatures. Fish living in temperate areas are subjected to large variations in temperature, both seasonally and annually (Claireaux et al. 1995; Matern et al. 2000; Sims et al. 2006; Wallman and Bennett 2006). All physiological processes are either directly or indirectly affected by body temperature, and maintaining physiological function as the body temperature changes is critical for the survival of an animal living in a fluctuating environment (Pörtner and Farrell 2008; Farrell 2009). These processes include respiration, reproduction, excretion and uptake of nutrients. In order to absorb nutrients and deliver them to all cells in the body of complex animals such as fish, a functional gastrointestinal system is needed. As most fish are ectothermic, that is they control their body temperature through external means, the high thermal tolerance on an individual level is somewhat remarkable. The main focus of this thesis was how a few of these gastrointestinal functions are affected by long and short-term changes in ambient temperature.

The gut of fish

Functions of the gut

The gut is the organ where food is digested and nutrients are absorbed into the bloodstream, whilst indigestible food ingredients are transported out of the body. This is achieved through controlled digestive secretions, complex motility patterns and regulated perfusion. Secretions of digestive enzymes are essential for the breakdown of food as well as preventing the digestion of gut tissues. Gut motility helps to mix, break down and transport the food through the gut. The vasculature system of the gut supplies the gut tissues with oxygenated blood and facilitates the transport of nutrients to other parts of the body. The gut also has an important function as a barrier, separating the internal environment from the external, and preventing unwanted bacteria and viruses entering the bloodstream (Jutfelt 2011). These functions are similar in most vertebrates. In addition to these more common functions of the gut, some fish species can also use the gut as a supplementary respiratory organ (McMahon and Burggren 1987).

Anatomy of the gut

The definition of what constitutes the gut varies somewhat between authors. Sometimes it is defined as only the gastrointestinal canal (i.e. the tube-like structure from the mouth to the anus) whereas other definitions state that the gut also includes the accessory organs (i.e. liver, salivary glands, biliary system, and pancreas). Throughout this thesis, I will use both the term gut and gastrointestinal systems, which both include the accessory organs. The gut in fish can generally be divided into pharynx, oesophagus, stomach, pyloric caeca, intestine and rectum, see Olsson (2011). Among different species and groups of fishes, large differences in diets and feeding strategies impose different demands on the digestive mechanisms. This has led to specialisations in both the anatomy of the gut and the nature and activity of its digestive enzymes (Olsson 2011). **Figure 1** shows differences in general gut anatomy of species from the three families of fish used in the thesis, and of three additional families that all diverge from the common structures, mentioned above. The arrangement of the gut wall in fish, is similar to most other vertebrates consisting of four tissue layers: the mucosa, the submucosa, the muscular layers and the serosa (Olsson and Holmgren 2001; Olsson 2011) (**See Figure 2**).

The perfusion of the fish gut is achieved through a well-developed vascular network. The anatomy of the vasculature system varies among species, but in most investigated ray-finned fishes, the gut is mainly supplied with blood via one large vessel, often referred to as the celiacomesenteric artery (Thorarensen et al. 1991; Farrell et al. 2001; Seth and Axelsson 2009). The celiacomesenteric artery divides into the intestinal artery (mesenteric artery) and the gastric artery (celiac artery), which are then further divided into increasingly smaller vessels ending with the capillaries. In most fish investigated, additional blood supplies are found towards the rear end of the gut (Thorarensen et al. 1991). Unfortunately, blood flow through these small vessels cannot be measured with techniques available today, and thus it is unknown how much they contribute to the total gut blood flow in fish.

It is also important to note that even though the liver is also perfused via the hepatic artery, the majority of the blood delivered to the liver is

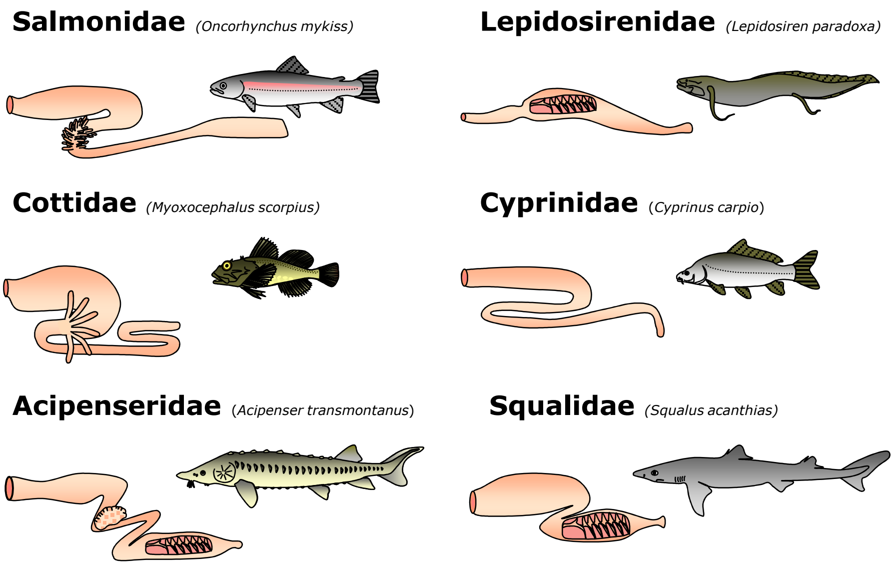

Figure 1. Differences in general anatomy of the gut in six fishes. The three to the left, are species from the families included in the thesis. The main differences between these three families are seen in the appearance of the pyloric ceaca and the intestine. In Cottidae the pyloric ceaca sit in a ring at the very proximal end of the intestine, in Salmonidae they are more dispersed, and in Acipenseridae they are fused together as one structure. The sturgeons have a spiral intestine. On the right, are three families that diverge in different aspects from the common structure: Squalidae, like other elasmobranchs, lack pyloric ceaca and have a spiral intestine;. Cyprinidae is the largest group of fish lacking a stomach; and Lepidosirenidae lack both pyloric ceca and stomach, and have a spiral intestine. Modified from Olsson 2011.

venous blood via the portal circulation. Therefore, most of the blood supply in the liver originates from the celiacomesenteric artery (Thorarensen et al. 1991).

The enteric nervous system

The gut is a densely innervated organ in all vertebrates including fish. The autonomic nerves are involved in the control of most gut functions. The autonomic innervation includes both extrinsic (from outside) sympathetic and parasympathetic nerves and intrinsic nerves (the enteric nervous system) (Kunze and Furness 1999; Olsson 2010; Gräns and Olsson 2011; Holmgren and Olsson 2011). The enteric nervous system consists of all nerves with their cell bodies present in the gut wall. In fish, most of these

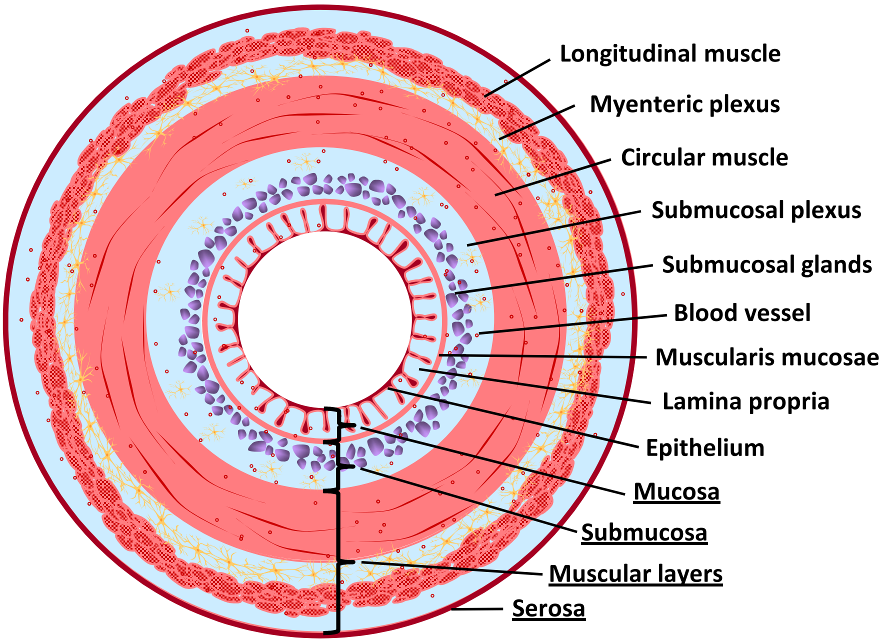

Figure 2. A cross section of the gut, showing the four main tissue layers: the mucosa, the submucosa, the muscular layers and the serosa, as well as structures contained within these layers.

cell bodies are found within the myenteric plexus and the processes of the cell bodies extend to all the other layers in the gut wall, innervating blood vessels, glands, muscle cells etc. In other vertebrates, there is also a submucosal plexus, but in fish this is either absent or very restricted (Olsson 2010; Holmgren and Olsson 2011).

Nerves and endocrine cells within the gut secrete an array of signal substances. Many of these signal substances act in a paracrine manner (influencing neighbouring cells) or in an endocrine manner (transported throughout the circulation and acting as hormones). Also, hormones released elsewhere in the body can affect the functions of the gut. The presence of signal substances in the fish gut has been studied intensively in numerous species, and has recently been compiled into a comprehensive table see Holmgren and Olsson (2011). However, there is limited knowledge on the integrated effects of these signal substances *in vivo* on the gastrointestinal system of fish. Factors such as nutrient composition, distension of the gut wall, pH, and temperature affect the functions of the enteric nervous system, which in turn alter the functions of the gut. The work presented in this thesis has focused on how two of these gut functions, namely gut motility and gut blood flow are affected by temperature.

Gut motility

What is gut motility?

Gut motility depends on the motor activity of the smooth muscle cells in the gut wall and occurs when the muscles contract and relax. In mammals, this has been shown to be essential for mixing and breaking down food particles, as well as transporting ingested food from the mouth to the stomach, and through the intestine, where nutrients are absorbed (Szurszewski 1969; Kunze and Furness 1999). To optimize these functions, and thus the processing of the food, the contractions and relaxations of the smooth muscles need to be tightly regulated and coordinated (Kunze and Furness 1999). The smooth muscle cells in the gut are connected via gap junctions, which make it possible for electrical signals to spread between cells, thus, enabling sections of the muscle layers to act as a single unit (Gabella and Blundell 1981; Webb 2003).

Depolarisation of a smooth muscle cell opens voltage-gated Ca^{2+}-channels, which initiate an increase in intracellular levels of Ca^{2+}. Once the cell reaches a polarisation threshold, achieved by increasing depolarisation of the cell, an action potential is generated and the muscle contracts (Horowitz et al. 1999). In smooth muscles, an action potential is not required to open voltage-gated Ca^{2+} channels, but graded potentials can open a few channels allowing small amounts of Ca^{2+} into the cell (Horowitz et al. 1999). Relaxation occurs when the intracellular Ca^{2+} levels decrease (Webb 2003). The influx and efflux of Ca^{2+} can be seen as cyclic depolarisation and repolarisation of the membrane. These rhythmic electrical oscillations are often referred to as slow wave activity (Koh et al. 1998; Horowitz et al. 1999). Cells responsible for this rhythmic activity are thought to be the interstitial cells of Cajal (ICCs) (Garcia-Lopez et al. 2009). ICCs are found in the proximity of smooth muscle cells throughout the gut and serve as a pacemaker cells (Koh et al. 1998; Garcia-Lopez et al. 2009).

The electrical slow waves spread from the ICCs to the smooth muscle (Garcia-Lopez et al. 2009). Mammalian studies have shown that slow waves often need additional stimuli from nerves or other signal substances in order to initiate a contraction. ICCs are also thought to be present in fish, although little is known of their function (Kirtisinghe 1940; Rich et al. 2007). The motility patterns of the gut, most likely depend on intrinsic properties of the ICCs and smooth muscles, as well as the effects of intrinsic and extrinsic nerves and hormones (Olsson and Holmgren 2001).

In mammals, there are two basic modes of muscular contractions involved in gut motility. Local standing contractions mix the gut contents, whilst propagating contractions are responsible for movement of gut contents (**Figure 3**). There are different types of oral-to-anal propagating contractions in fed and unfed animals. In the interdigestive state, the propagating contractions are slow and travel long distances along the intestine (Szurszewski 1969; Husebye and Engedal 1992; Grzesiuk et al. 2001). Postprandially, the most pronounced change in the gut is an increase in mixing movements, as well as faster propagating contractions that spread throughout shorter segments of the gut (Szurszewski 1969; Husebye and

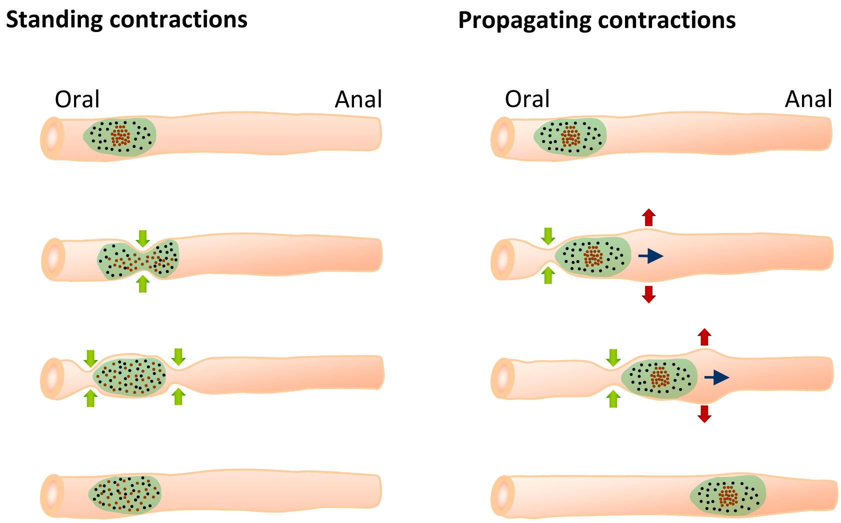

Figure 3. The two main motility patterns of the gut. Local standing contractions mix the gut contents, while propagating contractions move the content in a direction. Green arrows indicate contractions, red arrows relaxation. From Gräns & Olsson 2011, reprinted with permission from Elsevier Inc.

Engedal 1992). Interdigestive motility patterns and secretion are believed to have important housekeeping functions, preventing accumulation of unwanted debris and bacteria (Stotzer et al. 1996; Grzesiuk et al. 2001; Spencer et al. 2003; Lesniewska et al. 2006; Sjövall 2011).

Gut motility in fish

Data on gut motility in fish, mainly stem from *in vitro* studies, in which the effects of different signal substances on isolated smooth muscle strips have been elucidated, see recent review by Gräns and Olsson (2011). However, very little is known about, which motility patterns exist and the timing of these patterns *in vivo* in fish. Regardless of variations in morphology and diets, food is digested and transported through the system (Elliott 1972; Jobling and Davies 1979; Persson 1979; Jobling 1980; Persson 1981; Elliott 1991). The transport of food through the gut is strongly influenced by gut motility. Gut passage time and gastric evacuation rate have both been studied extensively in fish (Elliott 1972; Jobling 1980; Temming et al. 2002; Behrens et al. 2011). Although transportation time of gut contents does not inform us of motility patterns, it can still be used as an estimate of motility (Carlos and Diefenbach 1975; Harwood 1979).

Propagating contractions *in vivo* have been studied with video-microscopy in transparent larvae of zebrafish *Danio rerio* and Atlantic halibut *Hippoglossus hippoglossus* (Rönnestad et al. 2000; Holmberg et al. 2003; Holmberg et al. 2007). Also, *in vitro* and *in situ* studies looking at larger sections of intestine of brown trout *Salmo trutta*, lesser-spotted dogfish *Scyliorhinus canicula*, and Atlantic cod *Gadus morhua*, have identified both propagating and standing contraction patterns (Burnstock 1958; Andrews and Young 1993; Karila and Holmgren 1995). Propagating contractions with both an oral-to-anal (anterograde) and anal-to-oral (retrograde) direction have been reported *in vivo* in fish larvae and *in situ* in lesser-spotted dogfish (Andrews and Young 1993; Rönnestad et al. 2000; Holmberg et al. 2003). The anterograde propagating contractions appear to be relatively regular contractions, spreading along large parts of the intestine (Holmberg et al. 2003). Whether fish, like mammals, change their motility patterns after feeding is still unclear.

Gut blood flow

What is gut blood flow?

Perfusion of the gut is a basic physiological process that is necessary to sustain oxygenation and nutritional levels in all cells. A sufficient blood flow is also required to transport absorbed nutrients from the mucosa to other parts of the body. In an undisturbed and unfed animal during the interdigestive state, the gut blood flow is maintained at levels sufficient for necessary housekeeping functions. These include maintaining interdigestive motility activities, basal secretion, mucosal regeneration, osmotic regulation, and the basal metabolism of the cells (Gallavan et al. 1980; Takala 1996; Seth 2010). In mammals, 20-30% of the total blood flow is allocated to the gut. As the oxygen consumption of the gut at rest is also approximately 20-35% of whole body consumption, it seems that the gut blood flow is finely tuned based on demand and availability (Granger and Norris 1980; Wilmore et al. 1980; Brundin and Wahren 1991). In mammals, the oxygen extraction fraction from the blood in the gut at rest is 22-35%, which is similar to the overall 22-30% extraction of the systemic blood flow (Granger and Norris 1980; Wilmore et al. 1980; Brundin and Wahren 1991).

Gut blood flow is regulated by both intrinsic and extrinsic mechanisms. The extrinsic mechanisms include sympathetic innervation, circulating vasoactive substances and systemic haemodynamic changes. The intrinsic mechanisms include locally produced vasoactive substances, myogenic control, local reflexes and local metabolic control. In mammals, when the metabolic demand of the gut increases e.g. after a meal, gut blood flow also increases so that the oxygen extraction ratio can be maintained (Granger and Norris 1980; Wilmore et al. 1980; Brundin and Wahren 1991). When possible, this increase in gut blood flow is almost entirely achieved by redistributing blood to the gut without any change in total cardiac output (Fronek and Stahlgren 1968; Gallavan et al. 1980). The actual signals leading to vasodilatation when the metabolic demand increases in the gut is unknown, but both tissue PO_2 and the by-products of cell metabolism are believed to be important. In an extreme situation (i.e. during intensive exercise), blood supply to the gut can decrease up to 80%. During such extremes, oxygen extraction in the gut may increase up to 90% (Rowell et al. 1984). However, this is only a short-term solution as it can severely injure the gut over a longer time span (ter Steege and Kolkman 2012).

Gut blood flow in fish

An inactive, undisturbed fish in the interdigestive state, distributes approximately 1/3 of its cardiac output to the gut although variations among species occurs (Axelsson et al. 1989, 2000; Axelsson and Fritsche 1991; Thorarensen et al. 1993; Crocker et al. 2000; Altimiras et al. 2008; Dupont-Prinet et al. 2009). After ingesting a meal, gut blood flow in fish increases between 70 and 160% (Axelsson et al. 1989, 2000, 2002; Axelsson and Fritsche 1991; Thorarensen and Farrell 2006; Altimiras et al. 2008; Eliason et al. 2008; Seth and Axelsson 2009; Seth et al. 2009). A postprandial (i.e. after feeding) increase in gut blood flow can be accomplished through either redistribution of the blood flow from other systemic vascular beds, or through an increase in cardiac output. Most fish increase cardiac output and reduce the vascular resistance enough to sustain a postprandial increase in gut blood flow, with no signs of redistribution, as is the case in mammals.

Even though redistribution of blood flow seems to be of limited importance for fish, there are indications that it does occur under certain conditions. In rainbow trout, when pre-digested food is introduced straight into the intestine, gut blood flow initially increases exclusively via redistribution of blood flow, and only thereafter through an increase in cardiac output (Seth et al. 2009). This pattern can perhaps be explained by how the postprandial increase in metabolic demand starts in the gut tissues and then, as nutrients are absorbed and distributed away, disperses throughout the body. In fish, as much as 70-80% of the postprandial increase in oxygen consumption is thought to be due to processes that actually occur outside the gut (Brown and Cameron 1991a, b).

Relationship between gut blood flow and gut motility

In general, the contraction of a muscle affects blood flow in two ways: by evoking metabolic hyperaemia, and by causing extravascular compression. The situation in the gut is somewhat more complex, as a contraction in one area is often associated with a relaxation in another (Chou 1982). Unless blood flow is measured at the exact area of contraction, the increase in blood flow induced by the contraction may be counteracted by a relaxation in an adjacent area, leaving the overall effect on gut blood flow unaffected (Chou 1982). In dogs, closely corresponding cyclic variations appear postprandially in both gut blood flow and gut motility, when these are recorded in a segment of the gut (Fioramonti and Bueno 1984; Cowles et al. 1999). When the intestine was drained of its content, the cyclic variations in the blood flow were abolished while the variations in gut motility patterns remained (Fioramonti and Bueno 1984). This suggests that smooth muscle activity does not regulate blood flow, but processes induced in the mucosa and submucosa, such as secretion and absorption (Fioramonti and Bueno 1984; Cowles et al. 1999). It is also the mucosal and submucosal tissues which consume the most oxygen and to which most of the blood is allocated (Chou and Grassmick 1978; Gallavan et al. 1980; Walus and Jacobson 1981).

Prior to this thesis, gut blood flow and gut motility had never been recorded simultaneously in fish. It was therefore unknown if the relationship described in mammals was also true in fish. Also, mammalian studies give little information on possible effects of temperature on gut blood flow and gut motility, but it is likely that this is of major importance for ectothermic animals.

Environmental temperature and thermoregulation

Most environments vary in temperature diurnally, seasonally, annually, and over longer terms as seen during the current global warming. This has significant effects on ectothermic animals living in these environments, as they have to cope with the effects of fluctuating temperatures on physiological processes. Biochemical reactions are temperature-sensitive, as all enzymes have an optimal temperature for their function. At temperatures above or below this optimum, enzyme function is impaired. Therefore, fluctuations in temperature constitute a challenge for animals, and they can only survive within a more or less restricted range of temperatures, their so-called ‘thermal window’. The width of this ‘thermal window’ differs substantially among species.

The main aim of the thesis was to study the effects of temperature changes on the cardiovascular and gastrointestinal system. When considering changes in temperature it is important to distinguish between short- or long-term changes. Acute effects of temperature are by far the most studied, but if the animal is given sufficient amount of time, the acute effects can vanish through acclimation processes. Temperature acclimation allows animals to maintain nearly the same activity in a new thermal environment. The acclimation processes are believed to involve complex biochemical and cellular adjustments, but largely this remains to be elucidated (Chown et al. 2010; Dawson et al. 2011). Effects of temperature were studied in all four papers included in this thesis, but in somewhat different contexts.

What is thermoregulation?

In order to optimise physiological processes and avoid harmful body temperatures, many organisms try to keep their body temperature within certain thermal boundaries. This is a process called thermoregulation and is an important aspect of how an animal accomplishes to maintain internal homeostasis when living in a fluctuating environment.

The overwhelming majority of animals are not capable of producing enough heat to maintain a constant body temperature, thus their body temperature changes with the ambient temperature. These animals are termed ectotherms and their means of temperature control are mostly indirect through behaviour, such as migrating to areas of a suitable temperature. However, thermoregulation in ectotherms is found to be quite complex. Besides avoiding harmful temperatures, many animals are able to keep their body temperature more stable than the environment (Nelson et al. 1984). During larger, gradual changes (i.e. seasonal changes) many ectotherms voluntarily expose themselves briefly to extreme temperatures, enabling them to acclimate quicker to the increase in temperature (Hutchison and Maness 1979). For many species of fish, these physiological adjustments are a continuous process, as they encounter relatively large temperature fluctuations over short and long time spans. These temperature changes necessitate both appropriate physiological and behavioural adjustments.

Behavioural thermoregulation is always associated with costs, such as energetic costs of movement and increased predation risks. Only when the benefits of the behaviour outweigh the costs are these behaviour changes expected to occur (Huey and Slatkin 1976). Thermoregulatory behaviours are most pronounced in terrestrial species and have mostly been studied in reptiles. For reptiles, a common behaviour is basking in the sun or avoiding it, in order to achieve or maintain an optimal body temperature (Angilletta et al. 2010). The behaviour may vary with the physiological status of the animal. A common reptilian behaviour is to move into a warmer area postprandially. This has been shown to increase the gut passage rate, increasing absorptive efficiency, and decreasing the duration of the postprandial metabolic response (Hailey and Davies 1987; Dorcas et al. 1997; Secor and Faulkner 2002).

In addition to thermoregulatory behaviours, some reptiles have developed physiological adjustments that facilitate thermoregulation. Changes in heart rate and peripheral circulation of two lizards, the Eastern bearded dragon, *Pogona barbata*, and the Lace monitor, *Varanus varius,* as well as the salt-water crocodile, *Crocodylus porosus,* can increase or decrease their heat transfer to the environment (Seebacher 2000; Seebacher and Franklin 2007). These adjustments increase the rate at which the body is heated when moving into the sun, and conserve heat when moving out of the sun. In addition to maintaining a beneficial body temperature, it also minimizes time spent in the open and thus exposure to predators (Seebacher 2000).

Thermoregulation in fish

Most fish are in thermal equilibrium with the surrounding water due to efficient counter-current heat exchange between the water and blood at the gills (Stevens and Sutterlin 1976). Temperature is sensed in fish both in the brain and peripherally in the skin, and thermoregulatory behaviours are influenced by both (Crawshaw et al. 1985). If the environmental temperature is unfavourable, fish cope by either seeking out areas with more optimal temperatures, or by making the appropriate physiological adjustments to optimize physiological functions at the new temperature (acclimation).

A fish, however, cannot acclimate to any temperature, but is restricted by a 'thermal window'. The width of the 'thermal window' is influenced by factors such as life stage and food availability, but is also highly species-specific with some species tolerating a wide range of temperatures while others being much more temperature sensitive (Brett 1956, 1971). **Figure 4** shows the thermal window and requirements of brown trout, which is

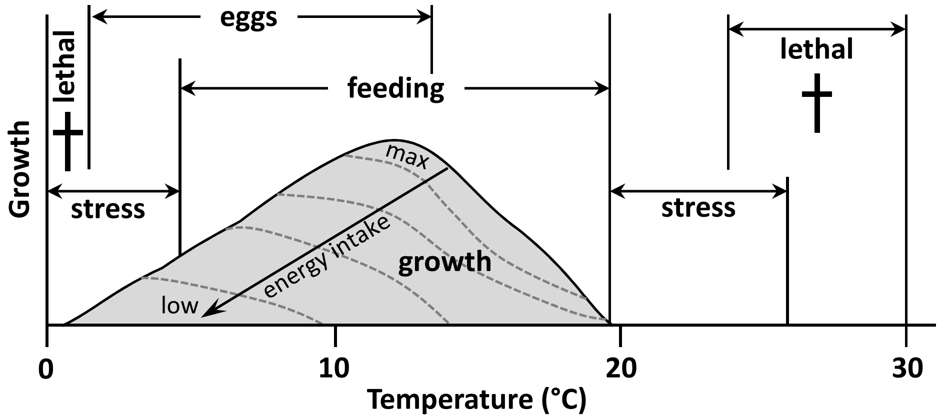

Figure 4. The thermal window and requirements of brown trout *Salmo trutta.* The dashed lines and the arrow shows how both growth rate and optimal growth temperature decrease with decreasing energy intake. Modified from Elliott 1994.

one of the most studied species in this context, and is based on the pioneering work of John M. Elliott (Elliott 1975b, d, c, a, 1976a, c, b). It is important to keep in mind that at any given point in time, only a small section of the thermal window is available, and depending on which temperatures are available, the fish may behave differently.

The behaviour of many reptiles to seek warmer temperatures after ingesting a meal has also been observed in fish. The Bear Lake sculpin, *Cottus extensus*, by postprandially moving from colder (5°C) to warmer water (15°C), can triple their growth rate (Wurtsbaugh and Neverman 1988; Neverman and Wurtsbaugh 1994).

Evidence that warmer temperatures may be beneficial for gut function is also found in studies on warm-bodied fishes. Two groups of fishes, the tuna and the lamnid sharks, have evolved counter-current heat exchange mechanisms for conserving metabolic heat and raising their body temperatures above the ambient temperature (Carey et al. 1971). In both the bluefin tuna, *Thunnus thynnus,* and the white shark, *Carcharodon carcharias*, the gut is thermally isolated by a circulatory heat exchange system, allowing it to maintain gut temperatures as much as 10-15°C above ambient temperature (Carey et al. 1984; Stevens and McLeese 1984; Goldman 1997). In the bluefin tuna, the elevated gut temperatures allow the fish to digest a meal up to three times quicker than normal, enabling them to consume and process about three times as much food per day (Stevens and McLeese 1984). The bluefin tuna are also able to slowly increase the temperature of the stomach after a meal, and slowly decrease it when unfed (Stevens and McLeese 1984). Such postprandial thermoregulation in most other fishes is only possible through behavioural means.

Individual species have evolved the capacity to function within a more-or-less narrow species-specific ‘thermal window’. Consequently, there is great concern over the ability of fish to acclimate and adapt to current increases in ocean temperatures (Haugan and Drange 1996; Levitus et al. 2000; Caldeira and Wickett 2003; Turley et al. 2010). In order for any animal to survive, the functions of the gut must work efficiently, yet still nothing is known about the effects of changing temperature on important functions such as gut blood flow and gut motility. Average ocean temperature is slowly increasing, and temperature fluctuations and heat spells will increase in magnitude and frequency as a consequence of changes in climate (Folland and Karl 2001). Thus one of the aims of this thesis was to study the effects of both long-term and acute changes in temperature on important gastrointestinal functions such as gut blood flow and gut motility.

AIMS

The overall aim of this thesis was to increase the knowledge of how gut functions in fish are affected by ambient temperature, and thereby to better understand how current changes in climate may affect fish populations. In order to accomplish this I first needed to understand how gut blood flow and gut motility are affected by acute and long-term changes in temperature.

As gut motility had not been recorded *in vivo* in adult fish, a method to record gut motility needed to be developed. With the use of such a technique, I aimed to determine the relationship between gut blood flow and gut motility in fish.

Using the newly developed method to record gut motility in combination with traditional gut blood flow recordings, I aimed to determine the effects of temperature acclimation on gut blood flow and gut motility in rainbow trout, in both the postprandial and the interdigestive state (**paper I**).

The acute thermal sensitivity of a thermal generalist was compared to a more strictly Arctic species, during an unusually warm month on the west coast of Greenland. By using the same methodology as in **paper I,** I aimed to determine how acute shifts in temperature affect gut blood flow and gut motility in shorthorn sculpin and Arctic sculpin (**paper II**).

To better understand how fish respond to environmental challenges, when they are not restrained by the recording techniques, I aimed to evaluate the possibility of using biotelemetry in fish, with particular focus on the use of a fully implantable multi-channel biotelemetry system for measurement of blood flow and temperature, in green sturgeon (**paper III**). Using this system**,** I aimed to determine how gut blood flow is modified voluntarily by fish using behavioural thermoregulation, in the postprandial and interdigestive states in free-swimming white sturgeon (**paper IV**).

When the thermal dependence of gut motility and gut blood flow was better understood, I aimed to evaluate how these functions are associated with thermoregulatory behaviours seen in the wild, and, the possibility that these physiological factors set the limits for the thermal window of fish.

METHODOLOGICAL CONSIDERATIONS

Basic terminology

In the literature, there are often inconsistencies in the nomenclature and therefore, a few words and concepts need to be clarified to better understand the ensuing discussion.

In this thesis, the terms gut and gastrointestinal systems generally encompass the gastrointestinal tract and the accessory organs. However, the definition of gut differs somewhat when discussing "gut motility" and "gut blood flow". In gut motility, the word refers to the tract between mouth and anus, whereas in gut blood flow, the word also includes accessory organs, supplied with blood via the celiacomesenteric artery. In the thesis, two different terms are used for the blood via the celiacomesenteric artery, "gut blood flow" is used in **papers I, II & IV** while "gastrointestinal blood flow" is used in **paper III.** Another term frequently used in the literature is "splanchnic blood flow" which is the blood flow to the gut, with the accessory organs included (Takala 1996).

The status of an animal before any kind of treatment can be described with several synonyms. In the thesis, a resting, undisturbed, and unfed fish will be referred to as being in an interdigestive state. In the papers included in this thesis, however, the synonyms used to describe this state include basal (**papers I & II**) resting (**paper I**), control (**papers I & II**), baseline (**paper III**), and routine (**paper IV**). In the literature, sometimes also the term "standard" is used to describe this state.

Experimental animals

Five different species of fish were studied in this thesis: rainbow trout in **paper I**, shorthorn sculpin and Arctic sculpin in **paper II**, green sturgeon in **paper III** and white sturgeon in **paper IV**. These five species all belong to the group of ray-finned fishes, but there are still significant differences in physiology and behaviour, which I have tried to, considered when discussing the results.

Rainbow trout are easy to keep and were acquired from local hatcheries. They belong to a domesticated, freshwater living strain. Rainbow trout are carnivorous, their natural diet are a mixture of insects, crustaceans and other invertebrates as well as fish, with choice of prey changing with size and season. In the hatchery, these fish are fed formulated, pelleted diets. The gut morphology reflects their carnivorous diet of relatively small preys, and includes a medium-sized muscular stomach, an abundance of pyloric caeca and a short intestine (**see Figure 1**) (Buddington et al. 1997). Rainbow trout is also one of the most studied species of fish. As one of the primary goals of **paper I** was method development, it was beneficial to use a species where cardiovascular variables had been described earlier, making comparisons with previous literature possible.

Shorthorn sculpin and Arctic sculpin are two closely related marine species in the family Cottidae. Both are benthic ambush predators, inhabiting shallow waters. Instead of feeding on numerous small prey items as the rainbow trout, the sculpins devour relatively large prey items in a single bite (sometimes as much as 50% of their own weight). Again, their feeding habits are reflected in the morphology of the gut with a large muscular stomach, an abundance of pyloric caeca and a short intestine (**see Figure 1**) (Buddington et al. 1997). One part of **paper II** aimed at investigating if there were differences in acute thermal sensitivity between a thermal generalist and a more strictly Arctic species. These two species differ in their geographical distribution, but could both be caught on the west coast of Greenland.

Green and white sturgeon are two closely related species of the Acipenseridae family. Both are anadromous and spend part of their lives in the sea. They are bottom-feeders, scouring the bottom for living or dead fish or invertebrates. Although their feeding habit, with slow nibbling for smaller prey, resembles that of the rainbow trout, their gut morphology differs substantially. Sturgeons have a straight, rather small, muscular stomach, and a single pyloric caeca. The ceaca consists of several separate ducts joining into one wide which opens into the intestine. The intestine is formed as an open canal, but takes the form of a spiral intestine in its posterior part (**see Figure 1**). Green and white sturgeon were used in **papers III & IV** respectively. They can be acquired in sizes large enough to fit the blood flow biotelemetry system and also have a large easily accessible celiacomesenteric artery that facilitates gut blood flow measurements.

Gut motility measurements

When a muscle cell contracts, it will produce an action potential and this electrical discharge can be detected by measuring electrodes. *In vivo* studies of gut motility in mammals are often performed using electrodes implanted in the gut wall, measuring these electrical activities (Bueno et al. 1975; Fioramonti and Bueno 1984; Ferre and Ruckebusch 1985; Rodriguez-Membrilla et al. 1995; Aviv et al. 2008). In **papers I & II** such a technique was successfully used for the first time to measure gut motility in fish.

Because of inconsistencies in the definitions in previous mammalian studies on the electrical activity of the gut, we suggested the term enteric electrical activity (EEA) as a summarizing term for all electrical activity derived from any part of the gut.

The correlation between EEA and smooth muscule contractions in the gut has been described in various mammalian species (Perkins 1971; Sanmiguel et al. 2007; Aviv et al. 2008). A similar correlation can also be shown through simultaneous measurements of EEA and force development in a contracting fish gut. **Figure 5** shows one of these recordings were muscular contractions are measured with strain gauge force transducers attached to the stomach wall of a shorthorn sculpin.

From analyses of the simultaneous recordings of EEA and muscular contractions, some new insights into how to interpret the EEA recordings were obtained. In a more or less rhythmic fashion, one or several events (spikes) appear in clusters. The more events included in a cluster, the longer the contraction seem to last. The amplitude of the EEA recordings correlates poorly with the amplitude of the contraction and also varies substantially between individuals.

The most pronounced postprandial effects in rainbow trout were that both the frequency of the recorded electrical events and number of events included in a cluster increased (**paper I**). A postprandial increase in electrical events is similar to the results previously reported for various

Figure 5. (A) Enteric electrical activity (EEA) signal trace, filtered with 0.5 Hz Low-pass digital filter. For EEA recordings, a pair of electrodes was inserted into the stomach wall of shorthorn sculpin. (B) Raw signal trace of force development when the stomach contracts, recorded by a strain gauge moulded in silicone and firmly sewn to the stomach wall.

endotherms (Ruckenbusch and Bueno 1976; Fioramonti and Bueno 1984; Rodriguez-Membrilla et al. 1995; Yin et al. 2004).

The interdigestive state of rainbow trout was characterized by rhythmic clusters of events. The rhythmical recordings appeared with around one contraction every four to five minutes (**paper I**). Postprandially, the duration of these events increased until they sometimes were inseparable. These rhythmic clusters, although appearing with a lower frequency, are probably analogues to the oral-to-anal propagating contractions described in zebrafish larvae and the rhythmic spontaneous contractions described in isolated segments of intestine *in vitro,* in Atlantic cod (Karila and Holmgren 1995; Holmberg et al. 2003). The recorded traces in **paper I** strongly resemble that of the propagating contractions recorded in pig (Ruckenbusch and Bueno 1976).

Today, we know little of how factors such as temperature, thickness of the tissue and number of cells in contact with the electrodes affect the signal, and the method can certainly be standardized and refined further to minimize variation possibly caused by these factors.

In **papers I & II**, EEA were only recorded at one site in the gut. Lately, we have worked to develop the method further, and thus maximising the data obtained. By using several electrodes, it is possible to separate standing and propagating contractions, and to determine the speed and direction of the propagating contractions. Future studies will determine if the shift between interdigestive and postprandial motility patterns seen in mammals is also present in fish. Through filtering the EEA signal, we have also obtained data believed to be the first recordings of slow waves in gut wall of fish. A method to record slow wave frequency in fish can be of importance for understanding the mechanisms behind how environmental factors affect gut motility.

In **paper II**, the EEA recordings were complemented by *in vitro* recordings of isolated smooth muscular strips. This is a useful method when trying to separate the integrative effects of temperature from the specific effect on the level of specific organs or tissues.

Blood flow measurements

Two different methods of measuring blood flow have been used in the thesis work. One uses the transit time flow probes, which give absolute values of flow (ml min^{-1}). These probes are bulky, limiting the range of use in fish. The other uses the pulse Doppler flow probes, which are the most commonly used flow probes in fish research. This technique was also used in the biotelemetry system included in both **papers III & IV**. In **paper IV** the telemetry recordings was supplemented with a control group where transit time measurements were made. In **papers I & II** blood flow was measured with traditional pulse Doppler systems.

The first direct measurements of blood flow in intact fish were conducted by Kjell Johansen (1962). Using an electromagnetic blood flow meter technique, he recorded blood flow in the ventral aorta of Atlantic cod. The technique used was rather rough, and during the experiments the cod were restrained and ventilated artificially (Johansen 1962). In his paper, Johansen emphasized the drawback of his measurements and stated that "cardiac function in a physiological preparation is almost inevitably distorted. Ideally, meaningful data on cardiovascular function in fishes should be acquired from freeswimming undisturbed animals". Four years later, he teamed up with two innovators of electronic instrumentation, Robert L. van Citters and Dean L. Franklin, and together they developed the first blood flow telemetry system to be used in fish. Using a Doppler ultrasonic telemetry blood flow meter system, they were able to directly measure the blood flow in the dorsal aorta in three species of elasmobranchs (Johansen et al. 1966). The experiments were carried out while the animals swam in a large aquarium, and fine wires led from the implant to the flow-meter standing alongside the aquarium (Johansen et al. 1966). Just how innovative these men were, may best be highlighted by the fact that it took 43 years until a new system capable of measuring blood flow with telemetry was developed. This is the system used in **papers III & IV.**

Temperature preference

In **paper IV,** the temperature preference was examined in white sturgeon that was allowed to move voluntarily between temperatures in an annular shaped chamber. When working with thermoregulatory behaviours, some important things need to be considered. One is to distinguish between short- and long-term responses. The temperature a fish selects within a short time after being introduced in a device to measure temperature preference, is strongly affected by the acclimation temperature (the temperature the fish has been kept in prior to the experiment). If the fish is left in the measuring device for a longer period (usually 24h but as much as 6 days may be needed), the fish is suggested to select a temperature that is species-specific and independent of acclimation temperature (Reynolds and Casterlin 1979; Golovanov 2006). This is commonly called the final thermal preferendum (Fry 1947).

In a stable environment, when food is abundant, fish tend to select a temperature, which provides the optimal conditions for physiological activity (in this context, physiological activity being equivalent to maximal growth rate) (Magnuson et al. 1979; Reynolds and Casterlin 1979; Jobling 1981). The concept of a species-specific final thermal preferendum has support from most authors, but its importance for fish in their natural habitats has been questioned and over the years a range of different internal and external factors, affecting both short and long-time temperature preference, has been introduced (Mortensen et al. 2007). The affecting factors seem to be species-specific, and thus generalization and extrapolations to ecology should be done with great caution.

Statistics

Various statistical approaches and analysis software were used in the different studies. A more detailed description is found in the respectively paper, but they are briefly described below.

In **paper I**, a repeated measures ANOVA, followed by a Dunnet post-hoc test for comparison between acclimation temperatures was used, and a two-tailed *t*-test assuming unequal variances was used for comparisons within treatments. Analyses were conducted in GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA). In **paper II**, the experiments were designed such that each fish or muscle strip preparation was exposed to all temperature treatments. This resulted in repeated measurements and dependent observations that had to be modeled. Several models for the dependencies were checked, and the best fit was found to be compound symmetry. An improvement of the statistical calculations was made using sandwich option. Analyses were conducted in SAS (Statistical Analysis Software, SAS Institute Inc. Cary, North Carolina, USA), The data obtained in **paper III** did not require statistical analysis. In **paper IV**, a linear mixed model was used for comparisons between and within the experimental groups. As in **paper II**, compound symmetry was used as a type of covariance. The factors and the interactions between variables were compared using a Sidak confidence-interval adjustment. Analyses were conducted in SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

Finally, for all analyses the significance level was adjusted to compensate for multiple comparisons, and a significant difference was assumed when the p-value adjusted for was <0.05.

RESULTS AND DISCUSSION

The relationship between gut motility and blood flow

In **papers I & II**, both gut blood flow and gut motility were measured. It is the first two studies that combine these variables *in vivo* in fish. Although the studies were performed on three different species, the results show some similar trends and contribute to answering the question of how gut blood flow and gut motility relate to each other in fish. From the results, it is clear that there is a relationship between changes in gut blood flow and in gut motility in response to both feeding (**paper I**) and temperature (**paper II**).

Distending a balloon in the stomach of a rainbow trout induces muscular contractions of the stomach (Grove and Holmgren 1992). However, a similar distension did not cause an increase in gut blood flow (Seth and Axelsson 2009). These results suggest that increased muscular contraction frequency, without any food present in the stomach, is not enough to increase net blood flow of the celiacomesenteric artery. Hence, an increased metabolic demand in the mucosa and submucosa is needed to increase gut blood flow. This indicates that the relationship between gut blood flow and gut motility seen after feeding (**paper I**), as well as when the temperature is acutely changed (**paper II**), is an indirect relationship and that the direct factor stimulating an increase in gut blood flow is probably an increased metabolic demand in the mucosa and submucosa layers.

The relationship between blood flow and motility in the rainbow trout gut was also clear during periods of burst swimming, when rapid reductions in gut blood flow as well as in EEA were seen (**Figure 6 in paper I**). During the recovery from this event, there was a lag phase, as gut motility was resumed before the perfusion of the gut was completely restored. The processes involved in digestion, including gut motility, are however dependent on a sufficient oxygen supply. To avoid hypoxia in the gut tissue during this lag phase it is likely that the oxygen extraction of the blood increases. At least in mammals, a change in oxygen extraction may precede an increase in perfusion (Takala 1996).

The reduction of gut motility and gut blood flow is similar to what have been observed in mammals as a response to exercise (Udassin et al. 1995; Takahashi et al. 2001; ter Steege and Kolkman 2012). Similar burst swimming events did also affect gut blood flow in both of both green and white sturgeon (**papers III & IV**), and have been reported for various species including both rainbow trout and white sturgeon (Randall 1982; Crocker et al. 2000). As shown in **Figure 4 in paper IV**, the cardiovascular system of the white sturgeon responds to burst swimming with an increase in cardiac output in combination with the extra blood allocated from the gastrointestinal system. A potential benefit of this response would be to increase the volume of blood distributed to the swimming muscles used in a fight-or-flight response. When it is safe, digestion would recommence by first resuming gut motility patterns and then reperfusion the gut, as seen in **paper I**.

Effects of temperature acclimation

In **paper I**, the effects on postprandial changes in gut motility and gut blood flow were studied in rainbow trout acclimated to either 10 or 16°C. Both groups were kept at their holding temperature for a minimum of four weeks, which is as long or longer than the time used to temperature acclimate rainbow trout in many previous studies (McCaule et al. 1977; McLeese and Don Stevens 1982; Fauconneau et al. 1983; Haverinen and Vornanen 2004).

The interdigestive activity in the group acclimated to 16°C was significantly higher compared with the group acclimated to 10°C (**paper I**). Interdigestive gut motility has proven to be important in order to prevent bacterial overgrowth in the gut of mammals (Stotzer et al. 1996; Grzesiuk et al. 2001; Kruszewska et al. 2005). As an increased intestinal temperature in the puffer fish *Fugu niphobles,* initiates a massive increase in bacterial growth, it might be suggested that increased interdigestive gut motility is essential for successfully “housekeeping” the gut at higher temperatures (Sugita et al. 1989).

No previous studies have directly focused on the effects of temperature acclimation on gut motility in fish or in other ectotherms. If using gut passage time or gastric evacuation rate as rough estimates of gut motility, there are lots of data on the effects of different acclimation temperatures. In various species of fish, including rainbow trout, it has been shown that the food passes through the gut faster when the animals are acclimated to a higher temperature (Windell et al. 1976; Persson 1979; Dossantos and Jobling 1991; Elliott 1991). In rainbow trout, the gastric evacuation rate increased with increased temperature, and it took three to four times as long to empty the stomach at 5°C as it did at 20°C (Windell et al. 1976). In a similar study, it was shown that passage time was shorter in rainbow trout acclimated to 18°C compared to those acclimated to 9-10°C (Fauconneau et al. 1983).

The postprandial EEA response was similar in the two temperature acclimation groups while there was a difference in the interdigestive stage (**paper I**). This is somewhat contradictory to the above-mentioned results indicating that increased acclimation temperature leads to faster gut passage rate. The answer might lie in the increased event amplitude seen, both before and after feeding, in fish acclimated to 16°C compared to fish acclimated to 10°C. However, as mentioned in the methodological considerations, there is no direct correlation between contraction amplitude and EEA amplitude, and hence no conclusions can be drawn from the amplitude of the EEA signal.

Cardiac output, stroke volume and gut blood flow increased postprandially and the responses were similar in both acclimation groups (**paper I**). Consequently, a lower acclimation temperature did not increase the duration of the postprandial blood flow response, which has otherwise been suggested as a general trend when comparing different species at different acclimation temperatures see Seth (2010). Such interspecific comparisons are, however, difficult to make and probably say little of the effects of temperature acclimation.

The postprandial increase in heart rate was significantly higher in the group acclimated to 16°C compared with the group acclimated to 10°C (**paper I**). This could perhaps be explained with that the fish acclimated to lower temperature had a relatively larger heart and thus could pump more blood in each hear beat. This phenomenon has previously been described in various species, including rainbow trout (Goolish 1987; Aho and Vornanen 2001).

That postprandial response in gut motility and gut blood flow appeared independent of acclimation temperature in **paper I** may seem unexpected from the discussion above. However, if considering that the purpose of temperature acclimation is to trigger responses which serve to offset the passive effects of a changed temperature, these are exactly the results one would expect (Johnston and Dunn 1987). Both 10°C and 16°C are temperatures that rainbow trout would normally encounter during a year and the results of **paper I** indicate that rainbow trout can successfully acclimate to these temperatures.

In addition to the *in vivo* study on the temperature-acclimated rainbow trout, an *in vitro* study was conducted looking at the force developed by isolated circular smooth muscle rings in response to different concentration of the cholinergic agonist carbachol (**see Figure 6**). There was no significant difference in force development between rings from fish acclimated to 10°C and 16°C when incubated at the corresponding temperature, while the force at 10°C was significantly higher in preperations from both acclimation temperatures (**see Figure 6**). These results indicate that during temperature acclimation there are processes that offset the passive temperature effects.

In the *in vitro* part of **paper II** were there also indications of thermal compensation processes. Using a similar setup as in the study discussed above, circular smooth muscle strip preparations from fish acclimated to 9°C were exposed to acute changes in the temperature of the organ bath (±5°C). The results show that the potency of carbachol was highest at the acclimation temperature, which suggest that also the drug-receptor interaction is optimized during acclimation (**paper II**).

Another thing worth noting when discussing the effects of temperature on gastric evacuation time is the similarities between species living in at a range of different temperatures. European sea bass *Dicentrarchus labrax* acclimated to 16°C, shorthorn sculpin acclimated to 10°C, and bald notothen *Pagothenia borchgrevinki* acclimated to –1.86°C, all empty ~60% of their stomach content into the intestine within 24 h after ingesting the food (Axelsson et al. 2002; Seth and Axelsson 2009; Sandblom et al. 2012). This could indicate that a half emptying time of 24 h is somehow beneficial. This is probably associated with a diurnal foraging pattern as have been described in several different species of fish

(Wurtsbaugh and Neverman 1988; Matern et al. 2000; Sims et al. 2006). That individuals of one species in a similar way make the physiological adjustments needed to maintain the rate of digestion, is suggested by the results in **paper I** and in **Figure 6**.

Effects of acute temperature changes

In **paper II**, the same methodology as in **paper I** was used to study the effects of acute changes in temperature on gut motility and gut blood flow. Here, we investigated the effects initiated by acute changes in temperature (±5°C from acclimation temperature) during the interdigestive stage of Arctic sculpin and shorthorn sculpin. Also in **papers III & IV**, the effects of acute temperature changes were studied, but then only on gut blood flow.

Enteric electrical activity increase with increasing temperature in both sculpin species (**paper II**). This is consistent with the results from the *in vitro* recordings, where the frequency of spontaneous contractions

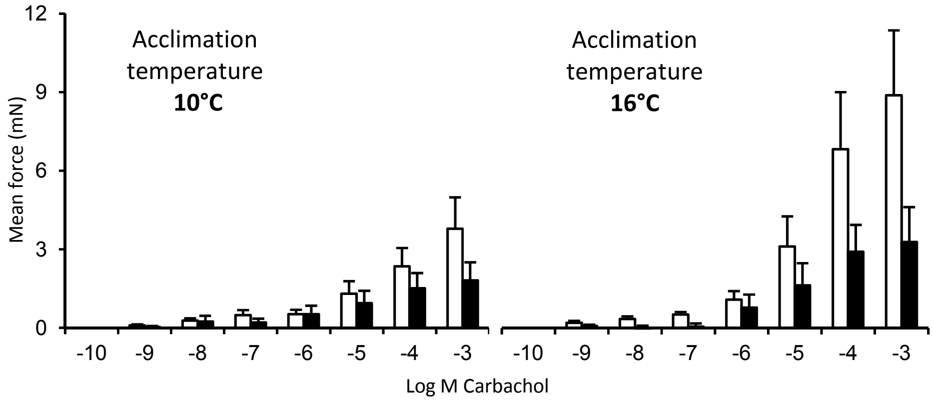

Figure 6. Concentration-dependent increase in mean force in response to carbachol recorded from isolated smooth muscles rings from the intestine of rainbow trout, acclimatized to 10°C or 16°C. The incubation temperature is indicated by the white (10°C) and black (16°C) bars. There was a significantly higher mean force development in the rings incubated in 10°C at both acclimation temperatures. There was no significant difference in force development between rings from fish acclimated to 10°C or 16°C when incubated at the corresponding temperature. A linear mixed model was used for comparisons between and within the experimental groups.

increased with temperature (**paper II**). No differences in temperature sensitivity were found between the two sculpin species despite a large difference in geographical dispersal.

No previous studies have looked at temperature effects on gut motility *in vivo* in fish. Studies on gastric motility in spectacle caiman *Caiman crocodilus* as well as on gastrointestinal pressure fluctuations in the yellow monitor *Varanus flavescens* and the Mexican spiny-tailed iguana *Ctenosaura pectinata* have also shown that the motility increases with increasing temperature (Mackay 1968; Carlos and Diefenbach 1975). Similar temperature effects have been shown in gut preparations from brown trout, brown bullhead *Ameiurus nebulosus,* and rainbow trout *in vitro* (Studier et al. 1977) (Burnstock 1958; Burka et al. 1993). Another indirect indication that contractile activity is increased when the temperature is increased is the faster passage rate through the gut of rainbow trout when temperature was acutely increased from 10°C to 18°C is (Fauconneau et al. 1983).

In general, the temperature sensitivity of isolated intestinal smooth muscle strips *in vitro* is much larger for mammals than for ectotherms (Studier et al. 1977). The results, however, also show that the motility of these ectothermic fish is heavily affected by changes in temperature (**paper II**). The increase in the EEA frequency at higher temperatures may depend on a higher slow wave frequency. Such a temperature effect on slow waves frequency has been described in mammals (Ohba et al. 1975), and in our first attempts to measure slow waves in fish, we have observed the same phenomenon (Gräns et al. unpublished data).

Heart rate, gut blood flow and cardiac output all increased with increasing temperature in both the Arctic sculpin and shorthorn sculpin (**paper II**). The increase in cardiac output was achieved exclusively through an increase in heart rate, as the stroke volume decreased with increasing temperature. The increase in gut blood flow was met through the increase in cardiac output rather than through a redistribution of blood (**paper II**).

In a related study, the basal metabolic rate in these two species was studied (Gräns et al. unpublished data). The interdigestive metabolic rate showed similar response in the two species with a decrease of ~40% when the temperature was decreased from 9°C to 4°C and an increase of ~50% when the temperature was increased from 9°C to 14°. The temperature dependence seen in the interdigestive metabolic rate correlates well with the effects we observed in cardiac output (**paper II**). The relative change in gut blood flow initiated by the different temperature exposures also relates well with changes in both the cardiac output and the metabolic rate (**paper II**). If the amount of blood distributed to the gut in fish reflects the metabolic demand of the gut, then the temperature effects on gut blood flow seen in **paper II** indicates that the metabolic rate in an interdigestive gut is equally affected by temperature, as is the metabolic rate of the whole body.

Also in **papers III & IV**, acute effects of temperate were studied. In these studies, gut blood flow was measured using a novel biotelemetry system in green and white sturgeon, and the results were somewhat surprising. Instead of a close to linear correlation between gut blood flow and temperature, as seen in sculpins between 4°C and 14°C, the gut blood flow in unfed green sturgeon did not increase at temperatures between 19°C and 23°C, but as the temperature reached above ~23°C, a drastic increase (150%) occurred (**paper III**).

In the white sturgeon, no effect on gut blood flow was observed when the fish were moving voluntarily between different temperatures before a meal (**paper IV**). After feeding, however, the pattern changed and gut blood flow correlated with temperature in a similar fashion as shown in the unfed sculpins (**papers II & IV**). The voluntary movements created fluctuations in gut blood flow that far exceeded the flow induced by feeding only (**paper IV**).

The results from **papers III & IV** suggest the presence of a control mechanism, that suppresses gut blood fluctuations when the gut is empty. If the metabolic rate of the sturgeon gut in the interdigestive stage is less sensitive to temperature changes compared with the whole body, such a mechanism could be advantageous. It may also be that as the temperature change during the interdigestive stage, the sturgeon increase the oxygen extraction in the blood distributed to the gut, instead of increasing the flow. Why this would be advantageous only in the interdigestive state, and why the response differs between species is, however, unclear. It may be that species from the Cottidae family (the sculpins) respond to temperature in one way and species from the Acipenseridae family (the sturgeons) in another.

Biotelemetry in fish physiology

Another reason why the response in gut blood flow differs between species might be that while the sculpins were restrained the sturgeons were free-swimming. This is because, biotelemetry were used in the sturgeon studies (**papers III & IV**) and "hardwired" methods was used in the sculpin study (**paper II**). New techniques and experimental protocols are constantly altering our understanding of physiology. One such example is how it was previously believed that fish primarily increase their stroke volume instead of their hear rate to increase cardiac output during exercise (Farrell 1991; Farrell and Jones 1992). Later it has been shown that these assumptions were based on studies on heavily instrumented fish, with abnormal high basal heart rate and thus with only very limited scope for further increase in heart rate when exercised. It is now clear that changes in heart rate are of major importance when modifying cardiac output in most fish species studied (Axelsson et al. 1992; Korsmeyer et al. 1997; Altimiras and Larsen 2000; Cooke et al. 2003). It was also long believed that another primitive fish group, namely the lungfish, more or less lacked cholinergic tone on the heart. A recent study on minimally instrumented animals, has, owever, demonstrated that cholinergic tone in these fish has probably been underestimated (Sandblom et al. 2010). Responses obtained from studies using heavily instrumented animals are always potentially biased by stress, and this is one of the reasons why biotelemetry should be used as much as possible in future studies.

In **paper IV**, the white sturgeon instrumented with the telemetry system had a significantly lower basal heart rate, compared to the "hard wired" group. It may well be that when using traditional restraining techniques to measure blood flow, the studied animals will not respond to treatments as they would if they were not confined. This is especially important to bear in mind when looking at effects on gut blood flow and gut motility, as both are shown to be extremely sensitive to interference and stress. A recent study showed that two days after instrumenting fish with dummy implants, they had a longer gastric evacuation time compared with the control group, but that this difference was abolished ten days after surgery (Behrens et al. 2011). Similar effects of heavy instrumentation have also been described in sea bass, and indicate that gut motility patterns are not fully recovered when the studies were conducted (Altimiras et al. 2008).

It would be interesting to conduct a similar study as in **paper IV**, but with a longer recovery period, to see if the results differ when gut functions are more normal.

It would also be valuable if EEA recordings could be incorporated in the biotelemetry system, since getting normal motility patterns in a stressed and operated animal is difficult. When a biotelemetry system was used to measure gastrointestinal pressure fluctuations in yellow monitor, it was noted that gut muscular contractions ceased when a person entered the room (Mackay 1968). Also in humans, it has been shown that both stress and anaesthesia inhibits normal gut motility patterns, (Ducerf et al. 1992; Yin et al. 2004). Also in **papers I & II**, it was clear that anaesthesia abolished gut motility. Whether the normal patterns were reinstated, and how the "hard wired" restraining techniques affected the recordings is unknown and can only be answered by using biotelemetry in future studies.

Water temperature and gut blood flow

By using the new insights on the strong effects of body temperature on the gastrointestinal system, it is tempting to speculate on how the gut functions of fish in the wild could be affected by voluntary and forced shifts in temperature. In order to estimate the consequences of exposure to a rage of temperatures, I have combined data from **paper II** with other available cardiovascular data on shorthorn sculpin.

An unfed shorthorn sculpin, acclimated to 9°C, has the capacity to increase its cardiac output from 38 ml min^{-1} kg^{-1} to 63 ml min^{-1} kg^{-1} during encouraged exercise (Gräns et al. unpublished data). This is the maximal cardiac output (100%) for these fish, and constitutes the upper limit of how much they can increase it in case of a predator attack or a situation of inter or intra-specific competition. The heart of a resting unfed shorthorn sculpin, at 9°C, is consequently, pumping at 60% of its maximal capacity, and from this approximately 1/3 of the blood is distributes to the gut. If faced with a challenge, the sculpin has the capacity to further increase cardiac output with 40%. When ingesting a meal, 10% of their body weight, the cardiac output increases to 90% of its maximal capacity while the ratio of ~1/3 of the blood still distributed to the gut remains (Seth and Axelsson 2009; Seth

2010). Now the scope available to further increase cardiac output if e.g. attacked by a shark is as little as 10% (**Figure 7**).

If we include the effect seen when the temperature was increased to 14°C in **paper II**, the cardiac output in the resting unfed sculpin increases to around 85% of its maximal capacity. If adding on the postprandial increase in cardiac output needed to digest the meal successfully, it becomes obvious that the maximum cardiac output is violated (**Figure 7**). If cardiac output is increased up to its maximal capacity, this would mean that the gut would receive ~20% less blood than needed. This ischemia could potentially affect the whole cascade of functions necessary for assimilating the meal.

This is most likely a simplification of what would happen to shorthorn sculpin if the temperature increased to 14°C in nature. Still, the calculations indicate that the perfusion of the gut is possibly a limiting factor when the environmental temperature changes. If this is a problem for fish in nature, one would expect to find species that feed in warm water and migrate into colder water in order to successfully digest the meal, without causing

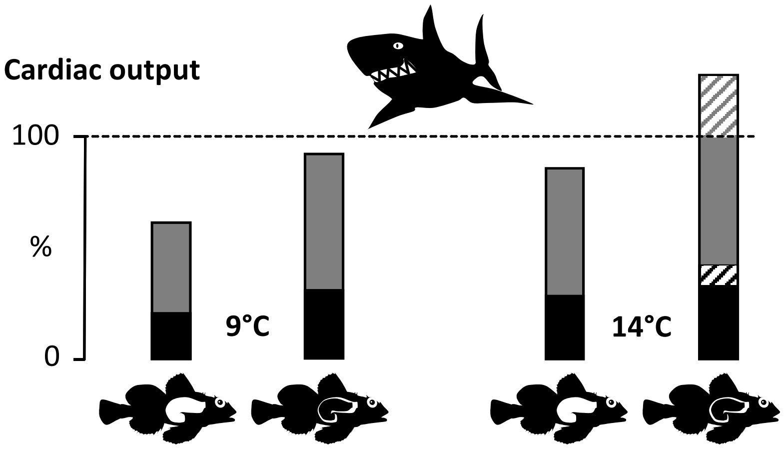

Figure 7. Cardiac output (grey bars) in fed (filled stomach) and unfed (empty stomach) shorthorn sculpin at 9 or 14°C. Black bars indicate the proportion of cardiac output distributed to the gut and dotted line indicated maximum possible cardiac output. Postprandially at 9°C, the scope available to further increase cardiac output is minimal and when increasing the temperature to 14°C the fish may not be able to increase cardiac output enough to successfully assimilate the meal. The hatched areas indicate the difference between the predicted increase in flows and the possible increase in flows.

gastrointestinal ischemia. The behaviour, of fish venturing into warmer waters to feed and then selecting cooler temperatures when satiated, has been described for several species including, lesser spotted dogfish, smallmouth bass *Micropterus dolomieu*, Atlantic stingray *Dasyatis Sabina,* bluegill *Lepomis macrochirus*, yellow perch *Perca flavescens,* and bat rays *Myliobatis californica* (Neill and Magnuson 1974; Matern et al. 2000; Sims et al. 2006; Wallman and Bennett 2006). The fact that several species lose their appetite, despite increased metabolic demands, when they are forced to stay in warm temperatures, further support that high temperatures may limit factors involved in the gut functions (Brett 1971; Elliott 1975b, d, c; Morgan et al. 2001; Handeland et al. 2008).

Ecophysiological relevance

In the previous section, it was suggested that, from a cardiovascular perspective, it would be beneficial for a fish seek out colder water after feeding. However, this is clearly not the case for e.g. the Bear Lake sculpin, or the tunas. To facilitate digestion, these species increase their gut temperature postprandially, by either behavioral or physiological means, (Stevens and McLeese 1984; Neverman and Wurtsbaugh 1994). What is the physiological reason why these differences occur?

As described in the introduction, the thermal window spans an interval of temperatures. This interval can be subdivided into smaller segments. Depending on in what temperature segment the food can be found, it could be potentially beneficial for an animal either to feed at colder temperatures and digest at warmer temperatures, or feed at warmer temperatures and digest at colder temperatures (**see Figure 4**). This suggestion is supported by the Bear Lake Sculpin that fed in relatively cold water (5°C) and moved into warmer water while many of the “hunt warm and rest cool” behaviours are reported from relatively warm waters, including fish feeding in shallow bays or in the heated effluent from a power plant (Neill and Magnuson 1974; Wurtsbaugh and Neverman 1988; Neverman and Wurtsbaugh 1994; Sims et al. 2006). It may also be that neither higher nor lower temperatures are better than the present temperature and this might be the reason why the white sturgeon in **paper IV** did not change its temperature preference after feeding.

The next question is which physiological factors set the limits for the thermal window of a fish? A leading hypothesis suggests that the temperature limitations in ectotherms are mainly set by a reduction in metabolic scope, i.e. the difference between standard and maximal oxygen consumption rates (Pörtner and Farrell 2008; Farrell et al. 2009). The hypothesis states that metabolic scope is closely related with other fitness-related performances, such as growth, locomotion and ultimately reproductive success. Consequently, it can be used as a unifying physiological principle to explain reduced fitness and abundance of ectotherms living outside their thermal window (Fry 1947; Brett 1971; Pörtner and Knust 2007; Pörtner and Farrell 2008; Farrell et al. 2009; Melzner et al. 2009). Lately, metabolic scope has proven useful to explain limitations in swimming performance and survival in anadromous salmonids that experience acute temperature changes when migrating to their spawning grounds (Farrell 2009; Eliason et al. 2011).

Experimental data supporting this hypothesis, derived from animals given sufficient time to acclimate to the new temperature, are still sparse. However there are indications that the concept of metabolic scope as a unifying physiological principle has been somewhat oversimplified. Frederick E. J. Fry, one of the pioneers of environmental biology of fishes, was the first to describe the concept of metabolic scope. Already in 1947 he presented data on brown bullhead, which is capable of increasing its metabolic scope to the upper lethal temperature (35-40°C) even if their optimum temperature for growth is, much lower, around 26°C (Fry 1947; Keast 1985). A similar situation is evident in European sea bass, were metabolic scope and swimming performance increased up to 30°C, even though growth is reduced already at 23°C (Lefebvre et al. 2001; Claireaux et al. 2006).

It is clear that growth in the brown bullhead and the European sea bass is not limited by metabolic scope. Can it instead be local oxygen constraints, within the gut, that limits growth at high temperatures? The calculations presented in the previous section indicate that when the temperature increases, the only way to sustain a cardiac output postprandially is through limiting the amount of blood distributed to the gut. This can lead to local hypoxia in the gut which causes a disturbance in gut functions, and may be the reason why appetite is lost and growth is compromised, when the temperature is high.

As the temperatures of the oceans are increasing as a part of the climate change, there are great concerns regarding the marine ecosystems. Biological effects such as reduced growth rate and shifts in distribution and abundance has already been reported for various marine fishes (Southward et al. 1995; Perry et al. 2005; Pörtner and Knust 2007). Lately, the scientific discussion, regarding the physiological effects of climate change on fish, have been focused on the hypothesis suggesting that the temperature limitations are mainly set by a reduction in metabolic scope (Claireaux and Lefrancois 2007; Pörtner and Knust 2007; Pörtner et al. 2007; Wang and Overgaard 2007; Pörtner and Farrell 2008; Farrell 2009; Farrell et al. 2009). As experimental evidence supporting this hypothesis is still sparse, and it is known how much variation there is among species, I suggest that more data is gathered. Data, which may either, support the leading hypothesis, or constitute the basis for alternative hypothesis. My guess is that the problems associated with high temperatures, start in the gut.

SUMMARY & CONCLUSIONS

In order for a fish to grow and thrive, the gut must function efficiently. This is achieved through a range of processes including controlled patterns of gut motility and modifications in gut blood flow. The overall aim of this thesis work was to increase the knowledge of how gastrointestinal functions in fish are affected by ambient temperature, and thereby to better understand how current changes in climate may affect fish populations. In order to accomplish this, several objectives concerning relationships, methodological development, physiological function, and behavioural influences, needed to be achieved.

The method developed to measure gut motility *in vivo* in adult fish, records the electrical discharges produced by contracting muscle cells. These recordings, termed enteric electrical activity, correlate well with the muscular contractions of the gut wall (**figure 5**).

When combining the EEA measurements with simultaneous measurements of gut blood flow, it is clear that these two variables correlates well and both are significantly affected by feeding (in rainbow trout, **paper I**) and acute shifts in temperature (in shorthorn sculpin and Arctic sculpin, **paper II**).

The interdigestive motility of rainbow trout was higher after acclimation to a higher temperature. This indicates an increased demand for gut movements involved in the "housekeeping" of the gut, preventing accumulation of unwanted debris and bacteria. Temperature acclimation did not affect the postprandial response *in vivo* in neither gut blood flow nor gut motility. These results indicate that thermal compensation processes work to neutralize acute thermal effects and to maintain the rate of gut blood flow and gut motility. A similar pattern was seen *in vitro* when the acute temperature effects on the response to the cholinergic agonist carbachol were abolished after a temperature acclimation period (**figure 6**). A similar sign of thermal compensation processes was seen *in vitro* in **paper II**, as the potency of carbachol was highest at the acclimation temperature, suggesting that the drug-receptor interaction is optimized during acclimation.

The other main finding in **paper II** was that the enteric electrical activity and gut blood flow both increased with increasing temperature, in both Arctic sculpin and shorthorn sculpin. No differences in temperature sensitivity were found between the two sculpin species despite a large difference in geographical dispersal

Also in **papers III & IV**, was the effects of acute temperature changes studied, but only on gut blood flow. The stugeons responded somewhat differently to an acute change in temperature compared with the sculpins in **paper II**. While a close to linear correlation between temperature and both gut blood flow and gut motility was seen in the two sculpins (shorthorn and Arctic sculpin), the gut blood flow, in both green and white sturgeon, was unaffected by moderate fluctuations in water temperature during the interdigestive state. White sturgeon was tested also after feeding and then the pattern had changed so that gut blood flow correlated with temperature in a similar fashion as shown in the unfed sculpins.

If the differences in the acute effects of temperature on gut blood flow observed in these studies are due to species-specific differences or due to the use of flood flow telemetry can be discussed. In **paper IV,** the white sturgeon instrumented with the telemetry system had a significantly lower basal heart rate compare with a control group of “hard wired” fish. It may very well be that when using restraining techniques to measure blood flow, the studied animals will not respond to treatments as they would if they not were confined.

Thermoregulatory behaviours observed in white sturgeon show that moving between different temperatures after a meal will have great influence on the amount of blood distributed to the gut. An increase in gut blood flow is probably essential for the beneficial digestive effects seen in some species that migrate into warmer waters after feeding. However, if combining the presented data with data from previous studies, it seems that gut blood flow may also be a limiting factor at high water temperatures. This is probably a key reason why, in other situations, it can be beneficial to forage in warm waters and then moving into colder areas for digestion. The reason why white sturgeon did not change temperature preference postprandially is unclear, but the effect of surgery on the behaviour is a potential limitation when trying to combine behavioural observations such as thermoregulation with physiological measurements. How surgical procedures affect fish behaviour needs to evaluated further.

Papers I-IV shows that the effect of temperature on the activity of the gastrointestinal system was high in both the interdigestive and the postprandial state. The results suggest that dysfunctions in gut blood flow and motility may have been overlooked when discussing physiological constraints related to temperature, as it seems as sustaining adequate functionality in the gastrointestinal system may be a large challenge to fish living in a fluctuating environment.

A leading hypothesis suggests that the temperature limitations are mainly set by a reduction in metabolic scope. Experimental data however show that both appetite and growth can be affected before metabolic scope is reduced. The findings of this thesis indicate that oxygen-limited thermal tolerance, in these fish may instead be local within the gut.

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