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Cardiorespiratory Function of Euryhaline Teleosts

Regulatory Mechanisms, Effects of Warming and Costs of
Osmoregulation

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CARDIORESPIRATORY FUNCTION OF EURYHALINE TELEOSTS –
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OF OSMOREGULATION

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Dissertation abstract

Teleost fishes have colonized almost every aquatic habitat on earth and exhibit adaptations that allow them to thrive in environments with very diverse physico-chemical properties. One fundamental environmental variable in aquatic ecosystems is salinity, as most fish species are only able to tolerate a narrow range of salinities. Nevertheless, a small proportion of fish species are euryhaline, meaning that they can live in a wide range of salinities including freshwater and full strength seawater. While there is substantial knowledge about some aspects of the physiological mechanisms underlying euryhalinity, the cardiovascular mechanisms in different salinities have so far been surprisingly overlooked. Furthermore, human-induced climate change is severely increasing the frequency and magnitude of extreme weather events including draughts, floods and heatwaves, which may severely alter temperature and salinity conditions of many aquatic habitats. Thus, temperature and salinity are two environmental drivers that can interact and may have unexpected physiological consequences for fish, but this has so far received relatively limited attention. The aim of this thesis is to increase our knowledge on the cardiovascular adjustments and their regulatory mechanisms in euryhaline fishes at different salinities. I also examined the linkages between cardiorespiratory function across salinities and aerobic and growth performance, as well as the interacting effects of environmental warming on cardiovascular function.

Rainbow trout acclimated to seawater typically display stroke-volume mediated elevations in cardiac output and reduced dorsal aortic blood pressure. First, using *in vivo* recordings of cardiovascular function and pharmacological tools, I assessed the role of α -adrenergic control systems in eliciting these cardiovascular responses. I found that systemic vascular resistance was lower in seawater-acclimated rainbow trout partly explaining the arterial hypotension. Additionally, the reduced vascular resistance was partly due to a lowered α -adrenergic tone on the vasculature.

Marine fishes drink to compensate for the dehydrating effects of living in seawater. This is associated with elevated gastrointestinal blood flow likely to provide oxygen and transport water, ions and waste. In a second study, I tested whether gastric perfusion of a hyperosmotic fluid in freshwater elicits some of the cardiovascular changes observed in fish transitioning to seawater. Gastric perfusion resulted in stroke volume-mediated elevations in cardiac output, elevations in gastrointestinal blood flow and reduced vascular resistances. This suggests that intestinal osmo- or mechanoreceptors mediate in the cardiovascular responses observed in seawater.

Cardiovascular responses to warming may differ depending on the environmental salinity as increased water temperature exacerbates dehydration in seawater and fluid gain in freshwater. Rainbow trout in freshwater and seawater exposed to acute warming increased gastrointestinal blood flow as well as cardiac output via elevations in heart rate. Nevertheless, the increase in cardiac output and gastrointestinal blood flow in seawater-acclimated trout was larger. This suggests enhanced compensatory elevations in gastrointestinal water processing, requiring improved gastrointestinal oxygenation and convective transport of nutrients, water and ions. The larger elevations in cardiac output also suggest that scope for increasing cardiac output may be compromised at higher temperatures in seawater.

Optimization of gas exchange across the gills also increases the exchange of water and ions. Due to this, high-energy demand fishes can be hypothesized to have large costs of osmoregulation, which may be reduced in brackish water. Here I show that the yellowtail kingfish, an aerobically active pelagic species, acutely and long-term exposed to brackish water maintained metabolic rate and cardiac function similar to kingfish in seawater, although acclimation to brackish water was associated with cardiac remodeling. Growth performance was also unaffected by acclimation to different salinities.

Collectively, I show that α -adrenergic stimulation mediates some of the cardiovascular responses observed in trout at different salinities and that hyperosmotic fluid ingestion likely regulates cardiovascular function via internal gastrointestinal sensing mechanisms. Furthermore, the magnitude of the cardiovascular responses elicited by warming depend on the environmental salinity, reflecting the osmoregulatory role of the gastrointestinal tract. Finally, acute and long-term reductions in salinity had few cardiorespiratory effects on kingfish suggesting that this high-energy demand species has lower costs of osmoregulation than initially hypothesized.

Keywords: salinity, temperature, cardiac, vascular, gastrointestinal, acclimation, growth

Svensk sammanfattning

Tack vare olika artanpassningar har fiskar koloniserat nästan jordens alla akvatiska miljöer. Anpassningarna har gjort att olika arter kan tolerera miljöer med vitt skilda fysikaliska och kemiska egenskaper. För vattenlevande organismer har det omgivande vattnets salthalt en avgörande betydelse, och de flesta fiskarter är beroende av att salthalten håller sig på en relativt stabil nivå. Vissa fiskarter är däremot anpassade för att klara av stora variationer i salthalt vilket gör att de kan överleva i både söt- och saltvatten, så kallade euryhalina fiskar. Vissa av de fysiologiska mekanismerna bakom salthalt tolerans är välkända, men överraskande nog har de kardiovaskulära funktionerna bakom anpassningen när fisken förflyttar sig mellan olika salthalter förbisetts. Dessutom ökar mänskligt inducerade klimatförändringar frekvensen och omfattningen av extrema väderhändelser, inklusive torka, översvämningar och värmeböljor, som allvarligt kan förändra temperatur- och salthaltsförhållandena i många vattenlevande livsmiljöer. Temperatur och salthalt är alltså två variabler i den omgivande miljön som kan interagera och orsaka fysiologiska konsekvenser för fisken, men effekten av interaktionen mellan två variabler har hittills fått relativt lite uppmärksamhet. Syftet med denna avhandling är att öka vår kunskap om de kardiovaskulära regleringsmekanismerna när euryhalina fiskar exponeras för, och anpassar sig till, olika salthalter. Sambanden mellan kardiorespiratorisk funktion på olika salthalter med avseende på tillväxt och aerobprestanda, samt de interagerande effekterna av uppvärmning, undersöktes också.

Regnbåge i havsvatten uppvisar vanligtvis högre slagvolym, vilket förhöjer hjärtminutvolymen och ett minskat dorsal aortablodtryck jämfört med regnbåge i sötvatten. Med hjälp av *in-vivo*-inspelningar undersökte jag vilken roll det α -adrenerga kontrollsystemet har för att framkalla dessa kardiovaskulära anpassningar genom att administrera olika läkemedel och undersöka hur det påverkar den kardiovaskulära funktionen. Jag fann att det systemiska vaskulära motståndet var lägre hos regnbåge i havsvatten, vilket delvis förklarar varför fisken uppvisar arteriell hypotoni (lågt blodtryck). Dessutom berodde det minskade kärlmotståndet delvis på ett sänkt α -adrenerg tonus på kärlsystemet.

Marina fiskar dricker för att kompensera för de uttorkande effekterna av att leva i havsvatten. Detta är förknippat med förhöjt blodflöde till mag-tarmorganen vilket transporterar syre, vatten, joner och avfall. I den andra undersökningen testade jag om magperfusion av en hyperosmotisk vätska i sötvatten framkallar några av de kardiovaskulära förändringarna som observerats hos fisk som flyttat till havsvatten. Gastrisk perfusion resulterade i att blodflödet från hjärtat ökade genom en ökad slagvolym, förhöjda blodflöden till mag-tarmorganen och minskat vaskulärt motstånd. Detta tyder på att osmo- eller mekanoreceptorer i tarmen förmedlar de kardiovaskulära anpassningarna som observeras när fisken befinner sig i havsvatten.

Kardiovaskulära anpassningar till uppvärmning kan skilja sig åt beroende på salthalten i den omgivande miljön eftersom högre vattentemperaturer ökar fiskens uttorkning när den befinner sig i havsvatten och, tvärtom, ökar vätskemängden i sötvatten. Regnbåge i sötvatten och havsvatten utsatt för akut uppvärmning ökade det gastrointestinala blodflödet samthjärtminutvolymen genom höja hjärtfrekvensen. Ökningen av hjärtminutvolym och gastrointestinalt blodflöde var större i havsvatten jämfört med när regnbåge var i sötvatten. Detta tyder på förbättrade kompensatoriska funktioner i gastrointestinal vattenhantering i havsvatten, vilket kräver högre gastrointestinal syresättning och konvektiv transport av näringsämnen, vatten och joner. De större ökningarna av hjärtminutvolymen tyder också på att utrymmet för att öka hjärtminutvolymen kan vara begränsad vid högre temperaturer i havsvatten.

När gasutbytet över gälarna ökar sker också en ökning i utbytet av vatten och joner. På grund av detta kan fiskar med högt energibehov antas ha stora kostnader för osmoregulering, vilket kan minska i bräckt vatten. Här visar jag att sydseriolan, en aerobt aktiv pelagisk art, bibehåller ämnesomsättning och hjärtfunktion när den kort- eller långvarigt exponeras för bräckt vatten jämfört med när den befinner sig i saltvatten, även om fiskar som långvarigt har exponerats för bräckt vatten uppvisar förändringar i hjärtmorfologi. Tillväxten var också opåverkad av olika salthalter.

Sammanfattningsvis, visar jag att α -adrenerg stimulering medierar några av de kardiovaskulära anpassningarsom observeras hos regnbåge vid olika salthalter, samt att intag av saltvattensannolikt reglerar kardiovaskulär funktion via receptorer i tarmen. Vidare beror omfattningen av de kardiovaskulära anpassningarna som framkallas av uppvärmning på miljöns salthalt, vilket belyser magtarm-kanalens roll för osmoregulering. Slutligen hade akuta och långvariga minskningar av salthalten få kardiorespiratoriska effekter på sydseriola, vilket tyder på att denna art med hög energibehov har lägre kostnader för osmoregulering än vad som ursprungligen antagits.

List of abbreviations

A-VO₂: Arteriovenous O₂ content difference

CO: Cardiac output

EPOC: Excess post-exercise O₂ consumption rate

FCR: Feed conversion ratio

GBF: Gastrointestinal blood flow

HR: Heart rate

MO₂: O₂ consumption rate

P_{DA}: Dorsal aortic blood pressure

PO₂: Partial pressure of O₂

R_{GI}: Gastrointestinal vascular resistance

R_{Sys}: Systemic vascular resistance

SMR: Standard metabolic rate

SV: Stroke volume

List of papers

This thesis is based on the following papers, referenced throughout the text according to their Roman numerals in bold.

Paper I Sundell, E., **Morgenroth, D.**, Brijs, J., Ekström, A., Gräns, A. and Sandblom, E. (2018). Seawater acclimation affects cardiac output and adrenergic control of blood pressure in rainbow trout (*Oncorhynchus mykiss*) - implications for salinity variations now and in the future. *Conserv. Physiol.* 6, coy061-coy061.

Paper II **Morgenroth, D.**, McArley T., Ekström, A., Gräns, A., Axelsson M. and Sandblom E. (2021). Continuous gastric saline perfusion elicits cardiovascular responses in freshwater rainbow trout (*Oncorhynchus mykiss*). *J. Comp. Physiol. B.* 10.1007/s00360-021-01408-3.

Paper III **Morgenroth, D.**, Ekström, A., Hjelmstedt, P., Gräns, A., Axelsson, M. and Sandblom, E. (2019). Hemodynamic responses to warming in euryhaline rainbow trout: implications of the osmo-respiratory compromise. *J. Exp. Biol.* 222, jeb207522.

Paper IV **Morgenroth D.**, McArley T., Danielo, Q., Harford, A., Hickey, A. J. R., Khan, J. and Sandblom E. Manuscript. Kingfish (*Seriola lalandi*) adjust to low salinity in aquaculture with only subtle effects to cardiorespiratory and growth performance.

Additional papers

The following studies were performed during the course of the doctoral studies but are not included in the thesis:

- Gong N., Lundin J., **Morgenroth D.**, Sheridan M. A. and Björnsson B. Th. Submitted. Roles of leptin in initiation of acquired growth hormone resistance and control of metabolism in rainbow trout.
- Kallstenius N., Sandblom E., McArley T., Gräns A., Axelsson M., Zena L., Ekström A. and **Morgenroth D.** Manuscript. Increased reliance on coronary perfusion for cardiorespiratory performance in seawater-acclimated rainbow trout.
- McArley, T. J., **Morgenroth, D.**, Zena, L. A., Ekström, A. E. and Sandblom, E. (2021). Normoxic limitation of maximal oxygen consumption rate, aerobic scope and cardiac performance in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* 224.
- **Morgenroth D.**, McArley T., Gräns A., Axelsson M., Sandblom E. and Ekström A. (2021). Coronary blood flow influences tolerance to environmental extremes in fish. *J. Exp. Biol.* 224.
- Sundell, E., **Morgenroth, D.**, Ekström, A., Brijs, J., Axelsson, M., Gräns, A. and Sandblom, E. (2021). Energetic savings and cardiovascular dynamics of a marine euryhaline fish (*Myoxocephalus scorpius*) in reduced salinity. *J. Comp. Physiol. B* **191**, 301-311.
- Ekström, A., Sundell, E., **Morgenroth, D.**, McArley, T., Gårdmark, A., Huss, M. and Sandblom, E. (2021). Cardiorespiratory adjustments to chronic environmental warming improve hypoxia tolerance in European perch (*Perca fluviatilis*). *J. Exp. Biol.* 224.
- Ekström, A., Sundell, E., **Morgenroth, D.** and Sandblom, E. (2021). Adrenergic tone benefits cardiac performance and warming tolerance in two teleost fishes that lack a coronary circulation. *J. Comp. Physiol. B.* **191**(4):701-709.
- Hjelmstedt, P., Sundh, H., Brijs, J., Ekstrom, A., Sundell, K. S., Berg, C., Sandblom, E., Bowman, J., **Morgenroth, D.** and Grans, A. (2020). Effects of prophylactic antibiotic-treatment on post-surgical recovery following intraperitoneal bio-logger implantation in rainbow trout. *Sci. Rep.* 10, 5583.

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1. Introduction

Fish, like most other organisms, require a relatively stable internal environment in which the combination of biological processes that make up a living being can take place. Being aquatic organisms, fish are generally immersed in water that essentially represents a solution with often-variable physicochemical characteristics that starkly contrast with that of their body fluids. For this reason, they require a complex repertoire of coordinated adaptive processes to maintain internal stability. This state of internal stability is known as homeostasis (Cannon, 1932). Still, all organisms are adapted to live in environments with a range of characteristics beyond which their performance and survival declines. That is because beyond that range of characteristics, homeostasis can no longer be maintained and thus, the functioning of their biological processes deteriorates. One environmental driver that gives aquatic habitats its characteristic chemistry is salinity.

1.1. Maintaining homeostasis in a changing world

For most fish, which are water-breathing, the contact between the environment and their bodily fluids/internal milieu is particularly intimate as the exchange of respiratory gases, such as O_2 and CO_2 occurs across permeable membranes, primarily the gills. Through these permeable membranes, fish also often excrete waste products and exchange water and ions with the environment. This exchange of water and ions frequently occurs passively following the concentration gradient across the permeable membranes that separates their internal milieu from the environment. If left uncompensated, this would result in altered composition of internal fluids, followed by a collapse of physiological functions and death. Therefore, a key homeostatic process is osmoregulation, which allows fish to maintain internal fluid concentrations within certain boundaries even if ambient conditions change. Thus, for many fish, these osmoregulatory mechanisms are dynamically regulated in response to environmental salinity changes. This is the case for fish species subjected to different salinities throughout their life cycle (*e.g.* salmonids and eels) and for species living in habitats prone to naturally occurring fluctuations in salinity. For example, the salinity of estuaries, lagoons, tide pools, salt marshes, may vary considerably depending on the tides, evaporation and mixing from freshwater sources, such as rainfall and ice melt (Brockmann, 1974; Goss-Custard et al., 1979; Potter et al., 2010).

The consequences of anthropogenic emissions of greenhouse gases already have noticeable global effects with elevated average environmental temperatures, shifts of climatic zones, altered precipitation patterns and an increased frequency and severity of extreme weather events including droughts, floods and heat waves (Arnell et al., 2019; IPCC, 2021). This has significant ecological consequences with impacts on species distribution, local extinctions and changes in the overall composition of biological communities (Collie et al., 2008; Hastings et al., 2020; Ummenhofer and Meehl, 2017; Wernberg et al., 2016). While some of these ecological changes may stem from biotic interactions such as alterations of the food-web structure, the combined effect of all abiotic factors ultimately determines whether a habitat is suitable for an organism and may drive some these biotic changes (Casini et al., 2010; Zhang et al., 2017). For example, abovementioned climate-change related global effects are predicted to intensify the global water cycle and alter the salinity of aquatic environments (Durack et al., 2012; Gräwe et al., 2013).

When multiple environmental drivers that affect the physiology of an organism co-occur, they may interact with each other such that the response of an organism to one driver may be modulated by another driver (McBryan et al., 2016; Todgham and Stillman, 2013; Velasco et al., 2019). Thus, when examining the physiological responses to environmental drivers, one needs to keep in mind the complexity and multifactorial essence of the natural world (Boyd et al., 2018; Todgham and Stillman, 2013). Given that current climatic trends will likely continue and even be exacerbated in the future (Cheung and Frölicher, 2020; Hastings et al., 2020; IPCC, 2021; Vuorinen et al., 2015), understanding the physiological mechanisms that allow fish to endure environmental changes, and their interactions, is imperative to understand current and future responses of fish populations. For example, the life cycle of some fishes includes stages of residency in contrasting salinities. Thus, the effect warming has on their physiological performance, may vary considerably depending on the salinity in which they experience it.

The circulatory system transports gases, nutrients and waste products to and from every tissue in the body, including those that make up the osmoregulatory organs; therefore, cardiovascular function is a fundamental aspect of osmoregulation. In this thesis, I investigate the cardiovascular function of euryhaline teleosts at different environmental salinities. The main goal is to increase our understanding on the cardiovascular responses that allow these fishes to maintain osmotic homeostasis in different salinities and the underlying control mechanisms behind these responses. Moreover, it aims to increase our knowledge on the effects salinity changes have on some aspects

of fish performance, such as aerobic capacity and growth, as well as the cardiovascular changes fish in different salinities experience when faced with warming events.

Before expanding on the effects of salinity on different aspects of fish performance and cardiovascular function, I will introduce the concept of euryhalinity and give a brief overview of osmoregulatory mechanisms in freshwater and seawater.

1.2. What is a euryhaline fish?

The term fish refers to a highly diverse paraphyletic group of aquatic craniates with gills and fins containing more than 33 000 species, 96% of which belong to the infraclass teleost (Helfman et al., 2009). From a likely marine origin, fish now thrive in virtually all aquatic ecosystems with salinities ranging from 0 to well over three times the average salinity of seawater (Gonzalez, 2012; Nordlie and Haney, 1998; Schultz and McCormick, 2013). Euryhaline is a loosely defined term, indicating the ability to survive in a wide range of salinities. Some euryhaline fishes can even perform remarkable transitions between freshwater and seawater without any apparent long-term negative effects on their physiology. Rather, this ability may confer unique advantages such as increased food availability and reproductive success (Gross et al., 1988), reduced competitive interactions (Betancur et al., 2012) and reduced predator avoidance-related costs (Bell et al., 2004). Despite these potential benefits, euryhalinity is a relatively rare trait and only 3-5% of fish species are estimated to be able to tolerate wide salinity fluctuations (McCormick et al., 2013). Instead, most fish species are stenohaline, which means that they are unable to tolerate wide variations in salinity, with approximately half of all fish species being restricted to freshwater and the other half to seawater (Bloom et al., 2013).

The reason for the low incidence of euryhalinity is probably multifaceted, but one important reason is likely that the bodily processes utilized by fish to maintain osmotic homeostasis and acid-base balance differ considerably between environmental salinities (Fig.1). Thus, fish transitioning between salinities must undergo numerous behavioral, morphological, physiological and biochemical adjustments to cope with the changes in the osmotic gradient across their biological membranes (Edwards and Marshall, 2013; Kültz, 2015).

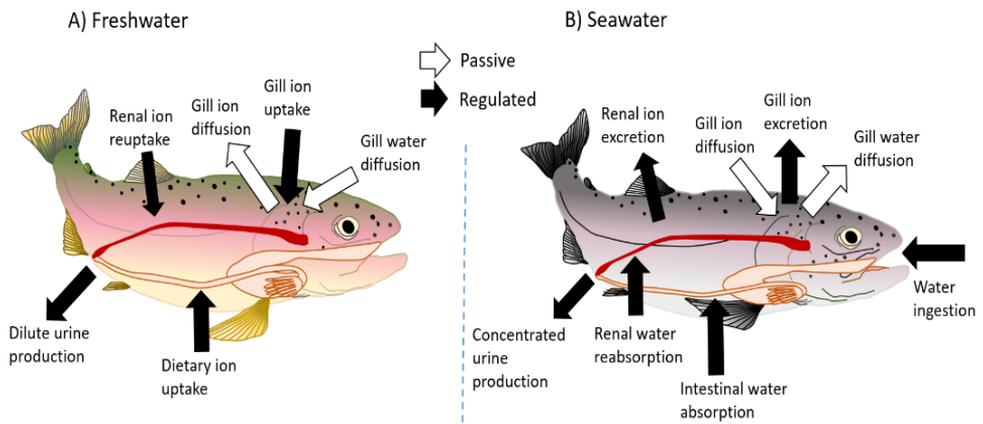


Figure 1. Schematic drawing of the ionic and osmotic challenges posed by different salinities and the compensatory mechanisms employed by fish. A) Fish in freshwater are hyperosmotic relative to the environment and must absorb ions through the gills and gut and produce large amounts of dilute urine to compensate for the passive loss of ions and osmotic gain of water. B) Fish in seawater are hypoosmotic relative to the surroundings and, therefore, drink to absorb water through the intestine, while they excrete ions through the gills and kidneys to compensate for the passive loss of water and diffusive entry of ions.

1.2.1. Mechanisms of hyper- and hypoosmoregulation

The majority of marine and freshwater fish (*e.g.*, most teleosts), maintain a constant internal osmolality of $\sim 300\text{--}400\text{ mOsm kg}^{-1}$ by regulating their plasma ion composition regardless of the surrounding osmolality (Edwards and Marshall, 2013; Takei, 2021). In seawater, teleosts have a lower plasma osmolality than the surrounding environment; therefore, they must hypoosmoregulate to compensate for the passive gain of ions and osmotic loss of water. On the other hand, in freshwater, they must hyperosmoregulate to compensate for loss of ions and gain of water. Thus, freshwater fish produce large amounts of dilute urine by having high glomerular filtration rates and renal urinary ion reabsorption (Larsen et al., 2014). They also actively take up ions via Na^+/K^+ -ATPases located in ionocytes in the branchial epithelium. In contrast, marine teleosts face chronic dehydration, and therefore actively drink to replenish the water lost to the hyperosmotic environment. Water is then processed and absorbed through the gastrointestinal tract (see below). In marine fish, the gills actively secrete monovalent ions (H^+ , Na^+ and Cl^-), while the kidneys produce reduced amounts of urine isosmotic to the plasma in

which divalent ions such as SO_4^{2-} , Ca^{2+} and Mg^{2+} are excreted (Fig. 1) (Evans et al., 2005; Marshall and Grosell, 2005; Takvam et al., 2021).

The osmoregulatory role of the gastrointestinal tract of freshwater fish mainly involves uptake of ions from food during digestion (Bucking and Wood, 2006a, b; Wood and Bucking, 2011). Drinking is generally considered very low in freshwater fish, although during feeding events fish ingest some water (Perrott et al., 1992; Shehadeh and Gordon, 1969). On the other hand, fish in seawater continuously imbibe and process water to compensate for the water passively lost to the environment (Fig. 1). The drinking response starts immediately after transition from freshwater to increasing salinities (Brijs et al., 2015; Fuentes and Eddy, 1997). The regulation of drinking is rather complex and appears to vary among species (Rash and Lillywhite, 2019). Overall, drinking seems to be regulated partly by the renin-angiotensin system (Fuentes and Eddy, 1996; Takei et al., 1979; Takei and McCormick, 2013), which at the same time is activated in response to blood hypovolemia (Ando et al., 2000; Beasley et al., 1986; Fuentes et al., 1996), as well as by externally located sensors such as Cl^- receptors in the gills or buccal cavity (Hirano, 1974; Mayer-Gostan and Hirano, 1976).

The imbibed fluid first passes through the esophagus, which is impermeable to water, but permeable to Na^+ and Cl^- . However, absorption of these ions occurs both passively and actively, such as via Na^+/K^+ -ATPases (Takei et al., 2017), and because of these absorptive processes, the fluid reaching the stomach is approximately half the osmolality of seawater (Brijs et al., 2015; Nagashima and Ando, 1994; Parmelee and Renfro, 1983). The fluid is further diluted through minor fluxes of water and ions in the stomach, following which it enters the intestine. Most of the water absorption occurs in the anterior intestine with both paracellular and transcellular pathways, which are largely driven by the osmotic gradient produced in the lateral interspace between the enterocytes. Na^+/Cl^- and $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ co-transporters located on the apical membrane of the enterocytes transport ions into the cell, while Na^+/K^+ -ATPases and Cl^- channels transfer Na^+ and Cl^- into the lateral interspace, which becomes a hyperosmotic compartment that draws luminal water into the plasma (Grosell, 2006; Grosell, 2011). Additionally, HCO_3^- produced endogenously in the cytosol of the enterocytes or brought exogenously from the plasma is secreted into the intestinal lumen in exchange for Cl^- (Grosell and Genz, 2006; Grosell et al., 2009). HCO_3^- can either react with Mg^{2+} and Ca^{2+} and form the insoluble carbonate compounds MgCO_3 and CaCO_3 that precipitate and further reduce the luminal osmolality allowing for further water absorption, or be rehydrated thus consuming H^+ and contributing

to the alkalization of the intestinal lumen and promote further carbonate formation (Grosell, 2011; Whittamore et al., 2010; Wilson and Grosell, 2003). The precipitated MgCO_3 and CaCO_3 is ultimately excreted with the feces (Wilson and Grosell, 2003).

1.3. Energy metabolism and osmoregulation

The processes utilized by fish to maintain osmotic balance (summarized above in *section 1.2.1*) involve the uptake or secretion of ions by means of processes that are either directly fueled by ATP (*e.g.*, ATP driven proton pumps, Na^+/K^+ -ATPases) or indirectly, fueled by electrochemical gradients generated by ATPases (Edwards and Marshall, 2013). Thus, the energetic costs associated with maintaining osmotic homeostasis are likely specific to each salinity as the mechanisms employed by the different osmoregulatory organs, such as the gills, gastrointestinal tract and kidney, differ depending on the direction of the osmotic gradient between the environment and the internal milieu of the fish. For example, Na^+/K^+ -ATPase activity in different segments of the intestine increases upon transfer to seawater and remains elevated following acclimation, clearly reflecting the active osmoregulatory role of the gastrointestinal tract in seawater (Barany et al., 2020; Brijs et al., 2017c; Colin et al., 1985; Ruiz-Jarabo et al., 2017; Seidelin et al., 2000). Such elevated gastrointestinal ATPase activity suggests a higher metabolism, and indeed, it is generally associated with an enhanced blood perfusion and possibly O_2 delivery to these tissues (Brijs et al., 2015; Brijs et al., 2016). Despite this, it remains unknown whether the metabolic rate of the gastrointestinal tract is elevated in seawater and there is conflicting evidence on whether the collective metabolic costs of the different osmoregulatory organs at different salinities affect overall energy expenditure.

Hypothetically, the costs of osmoregulation should be reduced at salinities that are isosmotic to the internal milieu of the fish, as there is no osmotic gradient and thus no need to allocate energy to compensate for passive fluxes of water or ions between the fish and its environment (Boeuf and Payan, 2001). Nevertheless, as shown in a review by Ern et al. (2014) this is often unsupported by the data and the effects of salinity on maintenance O_2 consumption rate (expressed as standard metabolic rate, SMR) in fish are highly variable and no universal trends can be found. The disparity on the effects of salinity on SMR highlight that the effects on overall aerobic metabolism need to be assessed on a species-specific basis.

1.3.1. The osmo-respiratory compromise

The gills are a complex and multi-faceted organ involved in numerous fundamental physiological processes including gas exchange and osmoregulation (Evans et al., 2005). An important aspect of this is that characteristics that benefit gas exchange such as elevated functional gill surface area, reduced lamellar diffusion distances, and elevated branchial ventilation and blood perfusion, will also favor the diffusion of water and ions. Thus, fish must continuously balance these conflicting needs of exchanging gases with that of preventing osmotic imbalances (Randall et al., 1972), a phenomenon called the “osmo-respiratory compromise” (Gonzalez and McDonald, 1992). Depending on the environmental salinity, metabolically demanding situations with increased gas exchange at the gills will exacerbate the osmotic gain or loss. For example, freshwater has a volume-loading effect while ions are lost to the environment and these effects are exacerbated during exercise (Gonzalez and McDonald, 1992; Onukwufor and Wood, 2018; Wood and Randall, 1973). Similarly, the gain of ions and loss of water across the gills in seawater is exacerbated during exercise (Gallaughner et al., 2001; Thorarensen et al., 1993). Thus, in exercising fish, the energetic costs of mitigating osmotic imbalances resulting from the need to elevate gas exchange, adds to the direct costs of swimming. For this reason, the specific costs of osmoregulation may dramatically increase during these metabolically challenging events. For example, when fish exercise exhaustively, they develop a large O₂ debt, as the glycolytic muscle fibers largely involved in burst swimming (*i.e.* ‘white muscle’) obtain ATP from the anaerobic breakdown of glycogen resulting in an accumulation of lactate in blood and tissues. The additional O₂ required to replenish the glycogen, ATP and creatine phosphate stores, clear lactate and re-establish acid-base and osmotic and ionic balance following such exhaustive exercise is known as the excess-post exercise O₂ consumption rate (EPOC) (Kieffer, 2000; Scarabello et al., 1991; Wang et al., 1994). Thus, EPOC is expected to be higher at salinities where osmotic and ionic disturbances following exercise are larger. Accordingly, the only previous study investigating the effect of salinity on EPOC, showed in two populations of killifish (*Fundulus heteroclitus*), one native to freshwater and another to brackish water, that EPOC was lowest for both populations when exercised in isosmotic conditions (Brennan et al., 2016). However, studies on more species are clearly needed to clarify if this is a general pattern among euryhaline fishes.

Similar to exercise, the trade-off between enhanced gas exchange and osmotic diffusion during warming results in increased water influx in

freshwater and a net water efflux in seawater (Isaia, 1972; Onukwufor and Wood, 2018). In seawater, warming elicits compensatory elevations in drinking rate, likely to compensate for the loss of water (Maetz and Evans, 1972; Motais and Isaia, 1972; Skadhauge and Lotan, 1974). These elevated drinking and accelerated processing of seawater should be accompanied by changes in blood perfusion to the gastrointestinal tract. The cardiovascular changes associated with metabolically demanding events such as warming will also be explored throughout the thesis.

1.3.2. Salinity and growth performance

Growth can theoretically be improved at salinities where metabolic costs are lower, as the “excess energy” that would otherwise be used for osmoregulatory purposes, can be allocated to growth (Boeuf and Payan, 2001). Indeed, previous studies have documented elevated growth rate in some species at salinities where O₂ consumption rate is reduced (Gaumet et al., 1995; Morgan and Iwama, 1991; Woo and Kelly, 1995). Changes in salinity may also affect aerobic scope (difference between maximum MO₂ and SMR), which represents the aerobic power budget available for allocating into processes beyond basic metabolic needs. Thus, fish at salinities where costs of osmoregulation are reduced resulting in decreased SMR and/or elevated maximum MO₂, could have an elevated aerobic scope, which may be allocated to fuel anabolism and enhance growth. Additionally, decreased costs of osmoregulation following metabolically demanding events, as indicated by a reduced EPOC, may also allow for energetic savings and enhanced growth.

Physically active fishes tend to have larger branchial surface areas and reduced diffusion distances to sustain blood oxygenation and a high metabolism compared with more sedentary species (Hughes and Morgan, 2008). Given the potential trade-offs between branchial gas exchange and ionic and osmotic balance, it can be hypothesized that the costs of osmoregulation in highly physically active teleosts must be unusually high, and that these costs should be greatly reduced at salinities approaching isosmotic conditions. Any resulting energetic savings at low salinities can be potentially allocated in growth. The yellowtail kingfish is an example of a physically active marine teleost. Its active lifestyle is clearly reflected in its aerobic performance, as they have high SMR and aerobic scope (Clark and Seymour, 2006). Juvenile kingfish acclimated to reduced salinity have an improved growth performance, mainly linked to an increased feed intake (Blanco Garcia et al., 2014). They also display a reduced branchial Na⁺/K⁺-ATPase activity and a trend for improved feed conversion ratio (FCR) (Blanco

Garcia et al., 2014), which may reflect a reduced energetic cost of osmoregulation at lower salinities.

1.4. The circulatory system of teleost fishes

Given that many of the processes related to osmoregulation mentioned above are active (*see sections 1.2. and 1.3.*), they likely involve changes in tissue energy turnover that require adjustments in O₂ and metabolic substrate supply. Consequently, fish must increase the blood supply to ensure that the metabolic needs of osmoregulatory tissues are met. The blood supply must also be adjusted to serve convectional purposes as absorbed water and ions must be circulated throughout the body. At the same time, metabolic byproducts resulting from the osmoregulatory processes need to be removed from the osmoregulatory tissues and excreted and/or processed. For this, adequate circulatory responses to changes in environmental salinity are likely to be a pivotal aspect of osmoregulation. However, this has been less studied than most of the other processes of osmoregulation mentioned above and there are still substantial knowledge gaps pertaining to the cardiovascular function of fishes at different salinities. However, before exploring the current knowledge and knowledge gaps related to the circulatory responses to changes in salinity, I will give a brief overview on the anatomy of the circulatory system of teleosts and the control of their cardiovascular function.

1.4.1. Anatomical arrangement of the circulatory system of teleosts

The typical circulatory arrangement in water breathing fishes is a one-way closed circulatory system with the heart generating the pressure that drives the movement of blood throughout the vasculature. The fish heart is composed of four chambers: the sinus venosus, atrium, ventricle and the bulbus arteriosus, all encased in the pericardial membranes (Fig. 2) (Farrell et al., 1988). The first cardiac chamber, the sinus venosus, drains into the atrium, a thin-walled contractile chamber (Olson, 1998a). From the atrium, blood enters the ventricle, which generates the arterial blood pressure that drives the blood flow through the body (Farrell and Smith, 2017). The ventricular myocardium of approximately two thirds of all teleost species is entirely spongy while the rest have an outer layer tightly packed with cardiomyocytes referred to as compact myocardium (Farrell et al., 2012; Santer et al., 1983; Tota et al., 1983). The relative proportion of compact and spongy myocardium varies within species and is influenced by multiple internal and external factors, for example, life-

stage, sexual maturation and environmental cues (Agnisola and Tota, 1994; Cerra et al., 2004; Gamperl and Farrell, 2004; Klaiman et al., 2011).

Upon ventricular contraction, deoxygenated blood is pumped into the bulbus arteriosus, followed by the ventral aorta. The ventral aorta divides into the afferent gill arteries which supply the gill circulation. Within the gill circulation, blood can be diverted through two different pathways: the arterio-arterial circulation, where the majority of gas exchange occurs and directs oxygenated blood into the systemic vasculature, and the arterio-venous circulation, which returns blood to the central venous vasculature after branching from the gills (Olson, 2011; Olson, 1998b). Efferent branchial arteries converge in the dorsal aorta, from which all of the blood within the systemic circulation originates (Olson, 2011; Olson, 1998b). The systemic circulation is further divided into the somatic circulation and the gastrointestinal circulation, which starts with the celiacomesenteric artery branching from the dorsal aorta.

The celiacomesenteric artery is the first main arterial branch of the dorsal aorta (Farrell et al., 2001). In salmonids, the celiacomesenteric artery splits into the gastric artery, which supplies the gonads, stomach, spleen and parts of the anterior intestine, and the intestinal artery, which supplies part of the stomach and middle and posterior intestine (Seth et al., 2009; Thorarensen et al., 1991).

Arteries branch into arterioles and further into capillaries. It is across the one-cell layer thick capillary walls where the exchange of gases, nutrients, ions, water and waste products between the blood and the tissues occurs. Following its passage through the capillaries, the blood is collected in the venous system. Veins are a highly compliant (*i.e.* small changes in blood pressure elicit large changes in contained blood volume) and generally have low pressure. Blood coming from the gastrointestinal tract drains into the hepatic portal vein, which connects with the liver. After passing through the liver, blood enters the hepatic vein, which converges with the sinus venosus. Another main pathway for blood return are the cardinal veins, which fuse with the ductus of Cuvier that also drain into the sinus venosus (Figure 2) (Olson, 1998b).

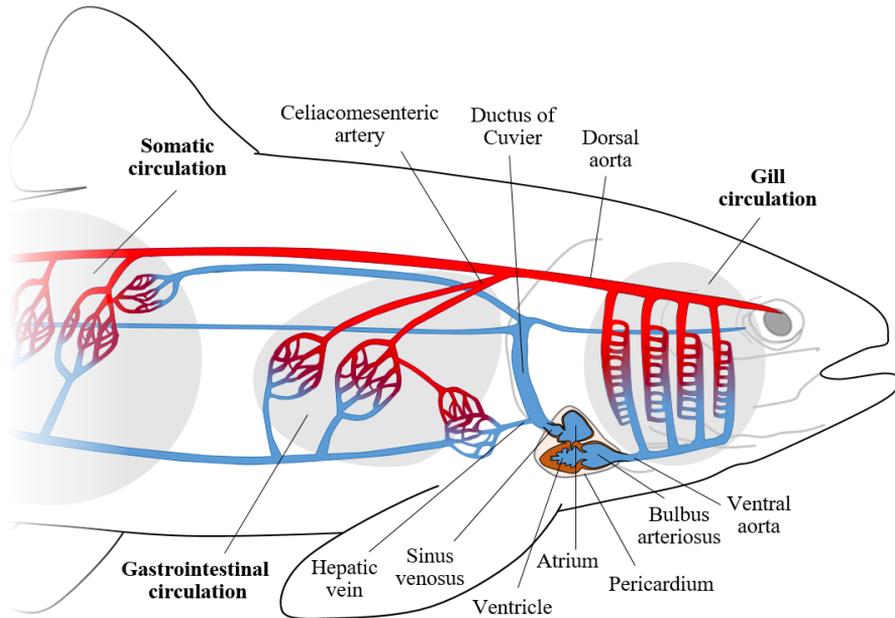


Figure 2. Schematic diagram of the circulatory system of a salmonid. The heart is composed of the sinus venosus, atrium, ventricle and bulbus arteriosus, all encased in the pericardium. The heart ventricle pumps deoxygenated blood into the gill circulation, which is composed of four paired gill arches where the exchange of gases between the blood and the environment occurs. The oxygenated blood then enters the systemic circulation via the dorsal aorta, which branches into smaller vessels. The systemic circulation can be divided into the gastrointestinal and somatic circulations. The gastrointestinal circulation in teleosts is supplied by an early branching from the dorsal aorta, the celiacomesenteric artery. After blood has passed through the capillary networks of the systemic circulation, the blood is collected in the venous vasculature (*e.g.*, hepatic portal vein, hepatic vein, ductus of Cuvier), which ultimately converges into the sinus venosus.

1.4.2. The control of regional blood flow

Tissue O_2 delivery is perhaps the most essential role of the circulatory system. At a whole-organism level, the total tissue blood flow corresponds to cardiac output (CO), which is the total amount of blood pumped by the heart per unit of time. The relationship between CO and aerobic metabolic rate (often approximated as O_2 consumption rate, MO_2) is given by the following equation based on the Fick principle:

$$MO_2 = CO \times (A - VO_2) \quad (1)$$

where $A\text{-VO}_2$ is the difference in O_2 content between the arterial and venous blood and represents the amount of O_2 extracted by the tissues per volume of blood (Clark et al., 2008). Consequently, to be able to elevate its aerobic metabolism, an organism can elevate either CO or $A\text{-VO}_2$, or as it is often the case, both. Various mechanisms are in place to elevate $A\text{-VO}_2$ such as improving blood O_2 carrying capacity by increasing the concentration of the O_2 -carrying pigment hemoglobin in the blood, or improving blood oxygenation via changes in ventilation patterns or optimizing gas exchange in the respiratory surfaces. CO is a product of heart rate (HR) and stroke volume (SV) and sets the maximum blood flow going through the circulation; yet, other factors outside of the heart play the most prominent role in determining the distribution of blood within the circulatory system (*see below*) (Joyce and Wang, 2020).

Flow through a vessel is determined by the following formula:

$$\text{Flow} = \frac{\Delta P}{R} \quad (2)$$

where ΔP is the blood pressure difference along the vessel, with the flow always following the pressure gradient, and R is the vascular resistance. As already mentioned, at the whole organism level, flow is equivalent to CO. With adequate techniques, blood pressure can be measured in many different vessels. Nevertheless, if we are interested in the driving pressure for the systemic circulation of the fish, the most representative is the dorsal aortic blood pressure (P_{DA}), which can be measured from the dorsal aorta. Technically, to accurately determine the systemic ΔP , central venous pressure must also be measured and subtracted from P_{DA} . However, given the low blood pressure in the venous vasculature of fishes, central venous pressure is generally considered negligible (Sandblom and Axelsson, 2007). If we then reorganize Eq. 2 to calculate R as the quotient of P_{DA} divided by CO, we will obtain the systemic vascular resistance (R_{sys}). Thus, blood perfusion of the different tissues result from the interplay between blood pressure and vascular resistance, where the pressure generated by the heart drives overall blood flow and vascular resistance regulates its regional distribution via changes in arterial and arteriolar vascular tone. Vascular tone is dependent on the diameter of the vessels, that is, how contracted or dilated (*i.e.* relaxed) the smooth muscle within the vessels is. Multiple factors regulate vascular resistance, including paracrine and metabolite-induced factors, gaso-transmitters, hormones and neural control via the autonomic nervous system, which is covered in more detail below (Axelsson and Fritsche, 1991; Sandblom and Gräns, 2017).

While peripheral vascular resistance is mainly regulated by the vascular tone of the arterial system, the venous vascular tone also plays an important role in the dynamic regulation of CO. For example, elevated venous tone may increase central venous pressure, and thus end-diastolic volume, resulting in increased myocardial stretch and force contraction (Sandblom and Axelsson, 2007; Shiels and White, 2008).

1.4.3. Autonomic control of the vasculature

Most systemic resistance vessels of fish are innervated by neurons from the autonomic nervous system. These neurons can be broadly divided into sympathetic and parasympathetic pathways, although only the gill vasculature has both sympathetic and parasympathetic innervation (Nilsson and Sundin, 1998). Another subdivision of the autonomic nervous system is the enteric nervous system, a network of neurons embedded in the walls of the gastrointestinal tract, which can function independently from central nervous control (Nilsson and Sundin, 1998). The enteric nervous system plays a key role in regulating local vascular resistance of the gastrointestinal tract (Seth and Axelsson, 2010). Moreover, venous tone and compliance are also regulated by the sympathetic nervous system (Sandblom and Axelsson, 2006; Zhang et al., 1998).

The sympathetic nervous system of all extant vertebrates, with the exception of the agnathans, share a common basic design. Presynaptic neurons originating from the central nervous system synapse with neurons outside of the central nervous system in neuronal clusters, some of which form the sympathetic chain (Nilsson, 2011). Postganglionic neurons release the catecholamines adrenaline and noradrenaline which, when bound to α -adrenoreceptors typically produce vasoconstriction and vasodilation when bound to β -adrenoreceptors (Patel, 2015; Sandblom and Axelsson, 2011). Even though both α - and β -adrenoreceptors can be found in the systemic resistance vessels of teleosts, generally the α -adrenergic response dominates (Sandblom and Axelsson, 2011; Smith et al., 1985). Additionally, the venous vasculature is mainly responsive to α -adrenergic stimulation (Conklin and Olson, 1994).

Adrenaline and noradrenaline can also be secreted from chromaffin cells, located in the head kidney, into the blood stream, although the role of these circulating catecholamines in regulating R_{sys} seems to be contentious (Perry and Bernier, 1999). While some studies suggest a generally lesser role of circulating catecholamines at rest and during exercise (Axelsson and

Nilsson, 1986; Smith et al., 1985; Xu and Olson, 1993), results from Bernier and Perry (1999) indicate that noradrenaline can affect R_{Sys} at physiological concentrations above resting levels.

1.4.4. Control of gastrointestinal blood flow at rest

The gastrointestinal tract of fish is heavily innervated by the autonomic nervous system and can be divided into extrinsic (mainly sympathetic fibers in the splanchnic nerve, and both parasympathetic and sympathetic fibers in the vagus nerve), as well as intrinsic (enteric) innervation (Furness et al., 2014; Furness and Costa, 1980; Holmgren and Nilsson, 1982). These nerves integrate a series of stimuli that contribute to the regulation of intestinal processes such as intestinal circular and longitudinal muscle contractility and rhythmicity, as well as blood flow (Olsson and Holmgren, 2011). The gastrointestinal tract is extensively vascularized and, like in any other vascular bed, the blood flow in the gastrointestinal vasculature is tightly regulated to meet the dynamic tissue demands throughout a whole range of physiological processes. In resting unfed fish, gastrointestinal blood flow (GBF) ranges from 10% of CO in seabass (*Dicentrarchus labrax*) (Dupont-Prinet et al., 2009) to 48% in seawater-acclimated rainbow trout (Brijs et al., 2016). Pharmacological α -adrenergic blockade significantly reduces overall gastrointestinal vascular resistance (R_{GI}) at rest in sea raven (*Hemitripterus americanus*) (Axelsson et al., 1989) and the red Irish lord (*Hemilepidotus hemilepidotus*) (Axelsson et al., 2000), as well the resistance of the mesenteric vasculature in Atlantic cod (*Gadus morhua*) (Axelsson and Fritsche, 1991). Similarly, sectioning of the splanchnic nerve (*i.e.* splanchnic denervation) results in reduced R_{GI} and increased GBF of resting rainbow trout (Seth and Axelsson, 2010). Combined, this indicates that neurally mediated α -adrenergic tone is an important determinant of R_{GI} in various teleost species.

1.5. Regulation of cardiovascular function with changing salinity

Cardiovascular function is dynamically regulated under diverse environmental conditions to match the requirements of the different tissues. For example, during exercise, CO increases, while blood perfusion to the gastrointestinal tract decreases, likely to prioritize O₂ supply to the muscle tissues that are essential for swimming (Axelsson et al., 2002; Axelsson and Fritsche, 1991; Dupont-Prinet et al., 2009; Farrell et al., 2001; Gräns et al., 2009b;

Thorarensen and Farrell, 2006; Thorarensen et al., 1993). Similarly, GBF is generally markedly reduced during hypoxia, while CO may either increase, decrease or remain stable. This is likely to reduce gastrointestinal O₂ consumption and prioritize/protect tissues that are more sensitive to O₂ deprivation such as the brain (Axelsson et al., 2002; Axelsson and Fritsche, 1991; Dupont-Prinet et al., 2009; Gräns et al., 2009b). Cardiovascular function is also regulated in response to changes in salinity and this, in part reflects the blood perfusion needs of the osmoregulatory organs. For example, in freshwater, the gastrointestinal blood perfusion requirements of an unfed fish are those linked to basic housekeeping processes. On the other hand, in seawater, even in a fasted state, the gastrointestinal tract is continuously processing the water imbibed to prevent dehydration. Accordingly, euryhaline fishes elevate GBF when exposed to increased salinities (Brijs et al., 2015), although very little is known regarding the mechanisms behind these cardiovascular adjustments.

Freshwater-acclimated rainbow trout acutely exposed to seawater increase CO via elevations in SV within a couple of hours of being transferred (Maxime et al., 1991). These elevations in CO are associated with decreases in R_{sys} and P_{DA} (Maxime et al., 1991). Concomitantly, blood flow directed to the gastrointestinal tract gradually increases, resulting in a doubling of GBF within days following exposure to seawater (Brijs et al., 2015). This gradual increase in GBF is hypothesized to match the changes in gill and intestinal Na⁺/K⁺-ATPase activity (Brijs et al., 2015; Madsen et al., 1996; Seidelin et al., 2000), and is thought to provide additional oxygenation to the more metabolically active gut tissues in seawater and increase the convective transport of absorbed water, ions and metabolites (Brijs et al., 2015).

In rainbow trout acclimated to seawater, SV and CO remain elevated and this is associated with increased central venous pressure, which likely elevates the end-diastolic volume explaining the elevated SV (Brijs et al., 2017b). The exclusively SV-mediated elevation in routine CO in seawater means that the scope for HR increase is maintained (Brijs et al., 2017b). The heart also undergoes plastic changes in response to long-term salinity changes. Notably, seawater-acclimated rainbow trout develop a greater proportion of compact myocardium (Brijs et al., 2017b). This likely contributes to elevating or maintaining the pressure generating capacity of the ventricle as the end-diastolic volume becomes larger (Farrell et al., 2009).

Seawater-acclimated rainbow trout and European eel (*Anguilla anguilla*) display a reduced P_{DA} compared to freshwater-acclimated

conspecifics (Jones et al., 1969; Olson and Hoagland, 2008; Tierney et al., 1995). In rainbow trout, some evidence indicates that this partly due to an inability to fully compensate for the passive water loss, which results in hypovolemia in seawater (Olson and Hoagland, 2008). Still, it is likely that that a reduced R_{Sys} , driven by a reduced R_{GI} , contributes to such reductions in P_{DA} . This is because rainbow trout acclimated to seawater still maintain an elevated GBF (Brijs et al., 2016), which is only possible if either celiacomesenteric artery pressure (which is equivalent to P_{DA} , Axelsson et al., 1989) increases or R_{GI} decreases. Given the reductions in P_{DA} observed in seawater, the most likely explanation is a reduced R_{GI} . This is supported by the observed redistribution of blood in seawater, where half of all CO is distributed to the gastrointestinal tract compared to approximately one third in freshwater-acclimated rainbow trout (Brijs et al., 2016). To confirm this, however, a more detailed analysis of cardiovascular function at different salinities is required.

As can be inferred from the information above, most information on the cardiovascular changes to altered environmental salinity relates to migratory euryhaline fishes, particularly salmonids. Additionally, most of the available literature explores cardiovascular dynamics of freshwater-acclimated fish acutely exposed to seawater, or compares cardiovascular status of freshwater- vs seawater-acclimated fish, while very few studies have explored the cardiovascular changes occurring in euryhaline marine fishes exposed to reductions in salinity. Importantly, this knowledge gap is not driven by a lack of ecological relevance as transitions from higher to lower salinities is a crucial step in the life cycle of many migratory fishes. It is also a frequent endeavor carried out by many euryhaline fishes inhabiting estuarine and intertidal habitats as they often experience large salinity differences during, for example, feeding incursions from or into brackish waters (Craig, 1984). Despite this, to the best of my knowledge, cardiovascular effects of reduced salinity have only been analyzed comprehensively in one recent study on the shorthorn sculpin (*Myoxocephalus scorpius*) (Sundell et al., 2021). Seawater-acclimated sculpin acutely exposed to a reduced salinity of 15 ppt displays a clearly reduced CO, which is consistent with an opposite response observed in fish transferred to elevated salinity. However, in contrast to the consistently increased GBF with seawater exposure, there is no change in GBF between brackish- and seawater-acclimated sculpin (Sundell et al., 2021). Given the scarcity of studies on the cardiovascular responses of marine fishes to reduced salinity, it is still not possible to infer whether this is a general response of marine fish to reduced salinity.

1.5.1. Mechanisms of cardiovascular control at different salinities

Successful reversal of osmoregulatory strategies upon exposure to elevated salinity comprises a complex interplay among several organ systems in fish, which is regulated by multiple neural, hormonal and local changes (Kültz, 2013; Takei and McCormick, 2013). These include mechanisms that ultimately result in the cardiovascular responses explored above. As introduced in *section 1.4.3.*, the α -adrenergic control system has a major role in regulating vascular tone. Thus, it can be hypothesized that the cardiovascular alterations observed in seawater-acclimated rainbow trout are caused by reductions in α -adrenergic tone. This may either be due to a reduced sympathetic nerve activity or by a blunted vascular response to α -adrenergic stimulation, potentially due to a reduction in vascular α -adrenoceptor density. The latter has been observed in rainbow trout fed a high salt diet, which exhibits elevated P_{DA} , a blunted pressor response to catecholamines and a reduced α -adrenoceptor mRNA expression in parts of the vasculature (Chen et al., 2007). This shows that α -adrenoceptor density is plastic in rainbow trout and potentially affected by salinity.

Furthermore, the processes resulting in cardiovascular responses at different salinities likely involve the ability to perceive both internal and external changes, such as osmosensors gauging osmotic and ionic status (Kültz, 2013; Kültz, 2015). Yet, although GBF is dynamically regulated at different salinities and increases in seawater, it is still unknown what the mechanisms are whereby elevated environmental salinity elicits these changes. In fact, most information regarding stimuli and mechanisms eliciting elevations in GBF in fish has so far mainly been in relation to GBF increases in response to feeding (*i.e.* postprandial hyperemia). Given that it is possible that some of the mechanisms responsible for regulating GBF following feeding are common to the elevations in GBF with seawater acclimation, I will briefly introduce some important mechanisms and stimuli known to elicit gastrointestinal hyperemia in vertebrates that may be of relevance to changes in GBF during osmoregulation in fish.

In mammals, important stimuli known to induce gastrointestinal vasodilation include intestinal hyperosmolality (Bohlen, 1982; Bohlen, 1998; Levine et al., 1978; VanHeerden et al., 1968), mechanical stimulation of the intestinal mucosa (Biber et al., 1971; Eklund et al., 1980; Fahrenkrug et al., 1978) and intestinal wall distension (Chou and Grassmick, 1978; Fahrenkrug et al., 1978). It is possible that some of these stimuli also elicit cardiovascular responses in fish. For example, given that fish in seawater drink large amounts

of water, it is possible that internal stimuli from elevated osmolality of the lumen of the gastrointestinal tract or mechanical stimulation from the imbibed water, either directly or via release of vasoactive agents result in cardiovascular responses. In shorthorn sculpin (*Myoxocephalus scorpius*), mechanical stomach distension using an inflatable balloon increases P_{DA} , R_{Sys} and R_{GI} without changes in CO or GBF (Seth and Axelsson, 2009). In rainbow trout, similar stomach distension results in elevations in P_{DA} as well as R_{Sys} and R_{GI} , an effect that is inhibited following pharmacological pre-treatment with an α -adrenergic antagonist (Seth et al., 2008). Nevertheless, these vascular effects were not associated with changes in CO or GBF (Seth et al., 2008). Although these results suggest that gastric distention is not a mechanism involved in elevating CO or GBF, it is still possible that distension of more distal sections of the gastrointestinal tract is involved.

1.6. Interacting effects of salinity and warming on cardiovascular function

Another important environmental driver that has profound effects on the physiology of fish is temperature. Most fish are ectotherms, meaning that they lack mechanisms to produce and preserve substantial internal body heat (Helfman et al., 2009). Thus, their body temperature is directly dependent on the temperature of the environment. This means that their rates of biochemical interactions and metabolic processes are also directly dependent on the environment. Thus, environmental warming has a direct stimulatory effect on the cellular metabolism of fish, resulting in elevated O_2 consumption rate sustained by increases in CO and/or A- VO_2 content difference (Eliason and Anttila, 2017). These temperature-induced elevations in CO are mainly driven by increases in HR, while SV generally remains relatively unchanged or decreases (Eliason and Anttila, 2017; Gollock et al., 2006; Morgenroth et al., 2021). The elevated CO combined with increased branchial ventilation during warming increases osmotic imbalances according to the osmo-respiratory compromise (see *section 1.3.1.*), thus, sensitivity to warming may be influenced by acclimation salinity. As warming has a dehydrating effect in fish in seawater and given the role of the gastrointestinal tract in maintaining osmotic balance at this salinity, it is likely that cardiovascular responses to temperature vary between salinities. Indeed, a previous study on rainbow trout shows that, even though CO is elevated in seawater-acclimated trout at 10.5°C, cardiac function appears to be compromised following acute warming, as CO increased less in seawater- than in freshwater-acclimated fish mainly due to a

tendency for a reduction in SV of seawater-acclimated trout (Brijs et al., 2017b). Additionally, despite having an important osmoregulatory role, GBF responses during warming in fish acclimated to different salinities and its relationship with osmotic balance has not been analyzed.

1.7. Research aims

The overarching goal of this thesis is to expand knowledge on the cardiovascular and respiratory function of euryhaline fish following changes in environmental salinity, and evaluate potential linkages to osmoregulatory function, temperature responses and growth performance. Towards that end, I have focused on four specific objectives:

- 1) Identify specific mechanisms underlying cardiovascular changes in fish acclimating from freshwater to seawater, with particular focus on α -adrenergic control of the vasculature in rainbow trout (**Paper I**).
- 2) Examine whether internal gastrointestinal mechanisms responsive to osmotic changes mediate the cardiovascular changes observed in seawater (**Paper II**).
- 3) Evaluate interacting effects of two important environmental drivers; salinity and temperature, on cardiovascular and osmoregulatory function in rainbow trout (**Paper III**).
- 4) Analyze acute cardiorespiratory responses and long-term plasticity to salinity reductions, and the consequences for growth performance, in a highly physically active marine euryhaline teleost (**Paper IV**).

2. Methodological considerations

The following section provides a general overview of experimental animals and methods utilized in the studies included in this thesis, as well as brief discussion complementing the information available in the *Materials and methods* section of the different studies.

2.1. Animals

The studies included in this thesis were performed using two different euryhaline teleosts. In **paper I-III**, I used rainbow trout, while yellowtail kingfish was used in **paper IV**.

Although the native range of rainbow trout is along the North American Pacific Coast and East Asia, it has been introduced in many countries worldwide due to its increasing importance as an aquaculture and recreational sport fishing species. It was first introduced into Europe in the late 1800s (see Stanković et al., 2015). Many salmonids are anadromous, meaning they are born in freshwater, and as juveniles, undergo the process known as smoltification, which allows them to migrate to seawater. There, they grow until they migrate back to freshwater as sexually mature adults to spawn. In their native environment, the steelhead trout is the anadromous form of rainbow trout and migrates to seawater as part of its natural life-cycle. On the other hand, the rainbow trout found in Europe are the freshwater resident form. Nevertheless, they are still euryhaline and are often reared in marine cages and escapees from aquaculture facilities are often found along the coast (Stanković et al., 2015). This shows that rainbow trout is well suited to studies of salinity tolerance and mechanisms of euryhalinity, since they are equipped with the physiological mechanisms necessary to acclimate to both freshwater and seawater.

The yellowtail kingfish is a large marine pelagic fish species found in subtropical and temperate waters across the southern hemisphere (Bray, 2018). It is generally found in coastal areas and is known to venture into estuaries where water salinity may be significantly reduced. In fact, kingfish can successfully acclimate to salinities down to 14 ppt with no apparent negative consequences to its physiology (Blanco Garcia et al., 2014).

Kingfish are athletic swimmers and, as many carangids, are carnivorous during their adult stage, preying on smaller fish in powerful bouts of burst swimming (Hixon, 2009). Due to its environmental robustness and fast

growth, along with great commercial value and feasibility to breed and rear it in captivity, kingfish has recently experienced a rapid expansion as aquaculture species (Sicuro and Luzzana, 2016; Stuart and Drawbridge, 2013; Symonds et al., 2014). As a result, there is considerable research interest on multiple performance aspects, including the effects of environmental variables on physiology and growth across life-stages (Abbink et al., 2012; Larios-Soriano et al., 2021; Palstra et al., 2014; Pan et al., 2020; Watson et al., 2018; Woolley et al., 2014). Thus, yellowtail kingfish is an interesting species, not just due to its remarkable physiology and ecological importance, but also its economic value.

2.2. Techniques for *in vivo* recordings of cardiovascular function

Blood flow was recorded in two different vessels: the ventral aorta to obtain CO and the celiacomesenteric artery to obtain GBF. To do this, two different approaches were used. Transit-time ultrasonic flow probes (Transonic Systems, Ithaca, NY, USA) were used to estimate CO in **papers I-IV** and GBF in **paper II**, while ultrasonic pulsed Doppler flow meters (Iowa Doppler products, Iowa City, IA, USA) were utilized to estimate GBF in **paper III**.

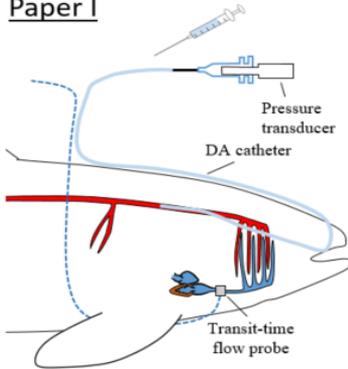
The transit-time flow probe is composed of two transducers and a reflector. Ultrasonic sound waves are emitted from one transducer, crosses the vessel, bounces on the reflector and are captured by the other transducer. This is performed in both directions. The sound wave slows or speeds up depending on the direction and speed of the blood flow. The difference in time between the signals sent by both transducers can then be used to calculate the absolute flow through the vessel.

Doppler probes also contain a transducer that emits an ultrasonic signal, which bounces on the particles contained in the fluid, such as the erythrocytes, and return at an altered frequency. This change in frequency is known as the Doppler shift. The change in frequency from the emission frequency of the sound and the received frequency is then used to determine the relative speed of the fluid, which is linearly proportional to volume flow (Axelsson and Fritsche, 1991). This method only provides relative changes in blood flow, without subsequent *in situ* calibration of the probe with known flow rates, the flow in the vessel can only be expressed in relative terms.

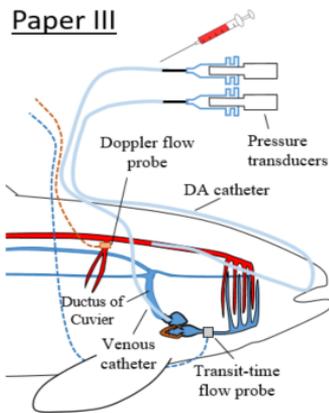
To record blood pressure two different intra-vascular catheterization techniques were used. The first method, employed in **papers I-III**, was catheterization of the dorsal aorta, which allowed me to continuously measure dorsal aortic blood pressure. The second method was the catheterization of the sinus venosus, allowing for continuous measurements of central venous pressure in **paper III**. Additionally, implantation of intravascular catheters allowed me to sequentially sample blood and administrate pharmacological substances into the blood stream with minimal disturbance to the animal. In **papers I-III**, polyethylene catheters were implanted for one or several of these purposes (Fig. 3). Both methods have been repeatedly validated in rainbow trout and other fish species, *i.e.* dorsal aortic catheterization (Gamperl et al., 1995; Lee et al., 2003; Seth et al., 2010) and sinus venosus catheterization (Brijs et al., 2017b; Sandblom et al., 2006; Sandblom et al., 2005).

One of the most challenging aspects of intra-vascular catheterizations is maintaining their continuous patency, particularly to maintain a stable and clean pressure signal and to be able to draw blood. In **papers I and III**, this was achieved simply by maintaining the catheter filled with heparinized saline (0.9% NaCl) and flushing the catheter at least once a day, and sometimes more frequently if the pressure signal deteriorated or easy withdrawal of blood was no longer possible. These studies were relatively short, as they required the intra-vascular catheters to remain patent for >40 hours during the post-surgical recovery and the subsequent experimental protocol. Maintaining the catheter patency in **paper II** was significantly more challenging, given that the total experimental period spanned over 7 days (see detailed description in **paper II**). Following initial efforts to maintain patency via regular flushes with heparinized saline, we opted for maintaining a small, yet continuous flow of heparinized saline through the dorsal aortic catheter using a peristaltic pump (Fig. 3). This was a very successful method and, although it has potential caveats, such as continuously volume loading the fish, the flow was estimated to be lower than the equivalent of flushing the catheter a couple of times a day.

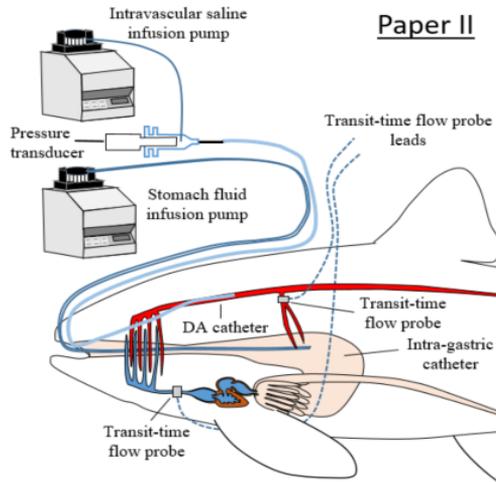
Paper I



Paper III



Paper II



Paper IV

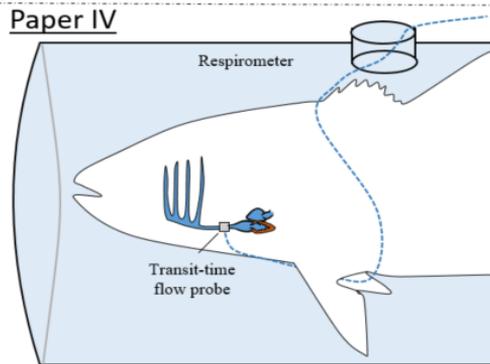


Figure 3. Schematic depiction of the instrumentation used in the different studies of this thesis. In **paper I**, rainbow trout were fitted with a dorsal aortic (DA) catheter, allowing for measurements of blood pressure and injection of pharmacological compounds, and a transit-time flow probe around the ventral aorta to measure cardiac output. In **paper II**, rainbow trout were instrumented with a DA catheter, an intra-gastric catheter and a transit-time flow probe. The DA catheter allowed for the continuous measurement of DA blood pressure, as well as blood sampling. To prevent thrombus formation, the DA catheter had a side-arm connected to a peristaltic pump, which provided a low ($7.5 \mu\text{l h}^{-1}$) constant flow of heparinized (25 IU ml^{-1}) saline. The intra-gastric catheter allowed for the continuous infusion of fluids into the stomach of the fish. In **paper III**, two differently instrumented groups of rainbow trout were included. The first group was fitted with a transit-time flow probe around the ventral aorta and a Doppler flow probe around the celiacomesenteric artery to measure gastrointestinal blood flow. The second group had a DA catheter and a venous catheter, both of which were used to measure blood pressure and to obtain blood samples during a warming protocol. In **paper IV**, kingfish were instrumented with a transit-time flow probe around the ventral aorta

to measure cardiac output and placed in a respirometer to measure O₂ consumption rate.

2.3. Measurements of whole-animal O₂ consumption rate

Whole-animal O₂ consumption rate was measured in **paper IV** using intermittent flow respirometry. Each respirometer was connected to two pumps. One pump formed a closed system with the respirometer, ensuring adequate mixing of the water within the respirometer. The O₂ level within the respirometer was measured continuously using a fiber optic optode (PyroScience, Aachen, Germany), which consists of a photosensitive optical fiber covered with a luminescent coating at the tip. The luminescent coating is excited via pulses of light at a certain wavelength. Yet, the luminescence intensity and lifetime is reduced in the presence of O₂, a process called quenching. Using this O₂-luminescence relationship, PO₂ in the water can be determined (Bittig et al., 2018). The second pump (flush pump) introduced continuously fully air-saturated water from the experimental tank into the respirometer and was connected to a switch that allowed me to program automated ON/OFF flush cycles of any desired length. During the OFF cycles, the flush pump would stop and the oxygen consumed by the fish in the now closed off chamber was continuously recorded by optode O₂ probes for later calculation of the O₂ consumption rate, during the ON cycles the pump would refresh the chamber with fully oxygenated water, returning water O₂ levels to ~100% air saturation.

2.4. Experimental protocols

Below, I provide a brief discussion regarding the experimental protocols used in **papers I-IV**.

2.4.1. Administration of pharmacological compounds

In **paper I**, two pharmacological substances, an α -adrenergic agonist (phenylephrine) and a competitive α -adrenergic antagonist (prazosin), were injected via the dorsal aortic catheter to evaluate the role of adrenergic tone in determining the cardiovascular changes observed with seawater-acclimation. First, to test whether vascular α -adrenergic sensitivity is reduced in seawater-acclimated fish, the α -adrenergic agonist was injected at different concentrations (10, 30, 60 and 100 $\mu\text{g kg}^{-1}$) one after another in a randomized

order, allowing cardiovascular variables to return to resting values between each injection. A single dose of prazosin (1 mg kg^{-1}) was injected to induce a complete α -adrenergic blockade to elucidate whether the observed cardiovascular responses at different salinities are due to differences in the intrinsic α -adrenergic tone. Prazosin was injected last due to its prolonged duration of action. Any observed changes in cardiovascular function are likely to stem from effects on the vasculature, as the trout heart is not directly responsive to α -adrenergic stimulation (Farrell et al., 1986).

2.4.2. Intra-gastric fluid perfusion

In **paper II**, I placed an intra-gastric catheter to be able to mimic drinking via administration of a continuous flow of hyperosmotic fluid into the stomach and determine whether gastrointestinal perfusion elicits cardiovascular responses observed in trout transferred to seawater. To isolate that the cardiovascular responses are elicited by a luminal gastrointestinal sensing mechanism, and not some other stimuli associated with seawater transfer, the perfused fish were kept in freshwater. The polyethylene catheter was inserted through the mouth, thus removing the need for further surgeries or incisions in the wall of the stomach. The catheter had a bubble 8 cm from the tip, thus locking the catheter and ensuring that the placement of the opening of the catheter within the stomach. The intra-gastric catheter was connected to a peristaltic pump, allowing for a constant perfusion of fluid into the stomach. It is not entirely clear from the available literature how the relationship between drinking rate and body mass is characterized in salmonids (Fig. 4). Thus, it is unknown whether drinking rate increases proportionally with mass or not. Still, the volume perfused was adjusted to the mass of each fish and was kept as close as possible to $5.4 \text{ ml kg}^{-1} \text{ h}^{-1}$ as this rate is equivalent to the drinking rate determined for reasonably similar sized rainbow trout (150-250 g) in an earlier study (Shehadeh and Gordon, 1969).

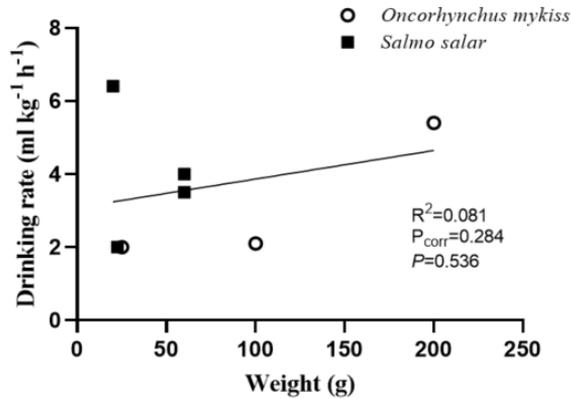


Figure 4. Relationship between mass-specific drinking rates and body mass of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Linear regression between published values for drinking rate and body mass in rainbow trout (n=3, open circles) and Atlantic salmon (n=4, filled squares). Pearson's correlations (P_{corr}) indicates the strength and direction of the association between the two variables. The P-value indicates statistically significant correlations ($P < 0.05$) between body mass and drinking rate. All values are obtained at salinities ranging between 28-33 ppt and temperatures between 7-17°C following an acclimation period in seawater of at least 4 days. References are reported in Table 2 of **paper II**.

2.4.3. Thermal ramping

Water temperature in **paper III** was elevated at a rate of 2°C h^{-1} , which is similar to previously used heating rates in other salmonid species of similar size (Ekström et al., 2014; Gilbert et al., 2020; Steinhausen et al., 2008). While this is a relatively quick rate of warming, it is ecologically relevant, as salmonids often perform vertical migrations in thermally stratified waters in their natural habitats (Ruggerone et al., 2011; Tanaka et al., 2000). Furthermore, at this rate of warming, the core temperature of the fish has enough time to equilibrate with the environment. For example, brook char (average size 419 g) equipped with internal temperature loggers acutely transferred to 4-13°C warmer waters, reached a core temperature equal to the environmental temperature within 20 minutes (Pepino et al., 2015).

2.4.4. Exhaustive exercise protocol and feeding trial

Maximum cardiorespiratory responses of kingfish in **paper IV** were elicited by opening the end of the respirometer where the tail of the fish was directed, opposite to the inlet flows from the recirculation and flush pumps. To induce

a maximal cardiorespiratory response, the tail of the fish was repeatedly touched which induced vigorous swimming movements until exhaustion. Thus, I refer to this method as ‘exhaustive protocol’ throughout the paper. Kingfish is a highly active and fast swimming pelagic fish, which commences ram ventilating at moderate swimming speeds (Clark and Seymour, 2006), thus, an important caveat of this protocol is that fish rely on opercular ventilation. The most suitable method to elicit and record maximum MO_2 in this species would likely be a swimming respirometer (Norin and Clark, 2016; Rummer et al., 2016), but this was not logistically feasible for this study. However, a commonly used alternative method is chasing the fish (Larios-Soriano et al., 2021; Sandblom et al., 2016). Nevertheless, given the relatively large size of the individual fish (~1000 g) and the athleticism of this species combined with the expensive and sensitive nature of the recording instrumentation, it was decided that removing the fish from the respirometer to be chased in a separate tank was logistically difficult and constituted a high risk of damage to the fish and the equipment. Still, preliminary tests on uninstrumented kingfish subjected to a chase protocol, as well as comparisons with literature values for maximum MO_2 values following swimming in a swim tunnel, suggest that the maximum metabolic responses elicited by the exhaustive protocol used in paper IV are highly comparable to these other methods (see Table 6 in **paper IV**). Following 3 minutes of the exhaustive protocol, the respirometers were immediately closed and continuous MO_2 measurements were performed for 6 hours. The magnitude of EPOC and the time to return to SMR following the maximum respiratory response also depends on the method used, with exhaustive protocols resulting in MO_2 remaining elevated for longer compared to exercise (Zhang et al., 2018). Nevertheless, all of the fish in **paper IV**, except for one fish in the acutely transferred treatment, returned to $SMR + 10\%$ within 6 hours.

In **paper IV**, growth performance rate was also assessed in a separate group of kingfish during a two-month period at salinities of 12, 24 and 35 ppt. Previously, an improved growth performance at lower salinities had been shown in juvenile kingfish (Blanco Garcia et al., 2014). Here, a similar feeding trial was performed on older fish to confirm these results, although with one key difference. Blanco Garcia et al. (2014) showed that increased appetite, rather than improved FCR, seemed to drive the improved growth. To discern potential effects of salinity on growth due to potential osmoregulatory costs, kingfish were fed below satiation, which also better reflects the conditions experienced by these fish in aquaculture.

3. Results and discussion

3.1. Cardiovascular changes associated with seawater acclimation and the role of α -adrenergic control

Simultaneous measurements of CO and P_{DA} in **paper I** allowed me to estimate R_{Sys} , showing for the first time that R_{Sys} is reduced in seawater-acclimated fish. One previous study had analyzed the effect of acute seawater transfer on R_{Sys} , although in that study CO was estimated using the Fick Equation and the instrumentation involved opening the pericardium (Maxime et al., 1991), which is known to alter cardiac function (Sandblom and Axelsson, 2006). Still, results by Maxime et al. (1991) indicate that P_{DA} and R_{Sys} in rainbow trout gradually decrease 24 hours following transfer to seawater. The results obtained in **papers I** and **III**, indicate that these reductions in P_{DA} and R_{Sys} remain in seawater-acclimated fish. Thus, the reduced P_{DA} and R_{Sys} reported in **papers I** and **paper III** combined with the elevated GBF observed previously in trout in seawater (Brijs et al., 2016), clearly point towards a reduced R_{GI} in seawater. In fact, given that the somatic and gastrointestinal circulations are arranged in parallel, the reciprocal of R_{Sys} (*i.e.* $1/R_{Sys}$) equals the sum of the reciprocal of the somatic vascular resistance and the reciprocal of R_{GI} (Axelsson and Seth, 2011). Thus, it appears that the observed reductions in R_{Sys} in seawater are mainly driven by vasodilation within the gastrointestinal vasculature.

In **paper I**, it is shown that the reduced P_{DA} and R_{Sys} observed in seawater-acclimated rainbow trout are partly due to a reduced α -adrenergic tone, since the α -adrenergic blockade with prazosin reduced P_{DA} and R_{Sys} in both freshwater and seawater as expected, yet, the reduction was smaller in seawater. Moreover, administration of the α -adrenergic agonist phenylephrine exerted similar dose-dependent elevations in pressure and resistance in freshwater and seawater, leading to the conclusion that α -adrenoreceptor density and sensitivity of the vasculature to catecholamines is comparable across salinities. Combined, this indicates that the tonic release of catecholamines in seawater-acclimated trout is lower compared to freshwater fish. The catecholamines elevating vascular tone in freshwater are likely of neural origin, as circulating catecholamines have little effect on R_{Sys} at rest (Axelsson and Nilsson, 1986; Smith et al., 1985). Furthermore, levels of circulating catecholamines do not differ between fresh- and seawater-acclimated rainbow trout (Tang and Boutilier, 1988).

Still, other factors unrelated to adrenergic control must also be involved because full α -adrenergic blockade with prazosin in **paper I** did not completely abolish the differences in R_{Sys} between freshwater- and seawater-acclimated trout and R_{Sys} remained lower in seawater. Numerous neural, humoral or locally released vasoactive substances could explain the remaining differences in R_{Sys} . A possibility of particular interest and which warrants further study is that gasotransmitters affecting smooth muscle tone in the gastrointestinal resistance vasculature cause the remaining vasodilatory effect in seawater-acclimated trout following prazosin treatment. For example, nitric oxide has a strong vasodilatory effect across vertebrate classes (see Joyce and Wang, 2020; Sandblom and Gräns, 2017) and, in mammals, intestinal hyperosmolarity induces arteriolar vasodilation (Bohlen, 1998; Steenbergen and Bohlen, 1993), which can be abolished by blocking nitric oxide synthase (Steenbergen and Bohlen, 1993). This idea is also supported by the finding that nitric oxide synthase activity in the anterior intestine increases with seawater transfer in rainbow trout (Gerber et al., 2018).

It is worth to point out that a reduced P_{DA} observed in seawater-acclimated fish in **papers I** and **III** could also, at least in part, result from an increased gill vascular resistance. For example, fast-acting vasoconstrictory hormones such as angiotensin II are quickly secreted following transfer to seawater, which might induce branchial vasoconstriction (Olson, 2002; Takei and McCormick, 2013). Nevertheless, the status of gill vascular resistance in fish acclimated to different salinities remains, to the best of my knowledge, unexplored.

In summary, the results from **papers I** and **III** show for the first time that R_{Sys} is reduced in seawater-acclimated trout and that this is in part due to a lowered α -adrenergic tone. This vascular response is likely fundamental for osmoregulation, as reduced resistance in the gastrointestinal vasculature drives the elevations in blood flow to the gastrointestinal tract. Additionally, the resulting reduction in P_{DA} might also drive the reduced glomerular filtration rate characteristic of seawater-acclimated fish (Olson and Hoagland, 2008; Takvam et al., 2021).

3.2. A potential role of gastrointestinal luminal sensing mechanisms

Fish in seawater continuously drink hypersaline water and this is associated with elevations in GBF (Brijs et al., 2015; Brijs et al., 2016). In **paper II**, I

show that continuous gastric perfusion with half-strength seawater elicits cardiac responses that are largely similar to those found in seawater-acclimated trout. This suggests that receptors sensitive to hyperosmolality or mechanical stimuli along the gastrointestinal tract are stimulated, ultimately resulting in cardiovascular responses. This includes SV-mediated increments in CO without significant changes in HR. Trout perfused with half-strength seawater also have a tendency towards a reduced R_{Sys} ($P=0.068$) and a relatively larger decrease in R_{Sys} compared to control fish, which were instrumented, but received no gastric perfusion. This was caused, in part, by more pronounced decreases in R_{GI} . The reductions in R_{GI} combined with the elevated CO likely drive the doubling of GBF observed by day 4 compared to day 0 in the half-strength seawater perfused treatment. Again, these responses are similar to those observed with seawater-acclimation (**papers I and III**).

Brijs et al. (2015) showed that GBF in rainbow trout starts to increase 36 hours following acute transfer to seawater and continues to increase gradually so that by the last day of recording (*i.e.* day 4 post transfer) GBF has doubled. This response is somewhat different from that observed with direct gastric perfusion with half-strength seawater in **paper II**, where GBF already starts to increase during day 1 and has plateaued by day 2. There are several possible reasons for these differences in responses. Salmonids typically commence drinking immediately after transfer to seawater (Brijs et al., 2015; Usher et al., 1988), but the increase in drinking rate is generally slower and more gradual than the immediate $5.4 \text{ ml kg}^{-1} \text{ h}^{-1}$ utilized in **paper II**. Additionally, the increase in gastric conductivity in trout acutely transferred to seawater is gradual and spans over 12 hours, again indicating a gradual increase in drinking rate following transfer to seawater (Brijs et al., 2015). After that, intra-gastric conductivity stabilizes around 60% the conductivity of seawater for the rest of the exposure. Yet, as the increase in GBF is even more gradual and prolonged (Brijs et al., 2015), this might indicate that elevated gastric osmolality is not the main stimuli eliciting the GBF responses in seawater. Instead, stimuli that would likely take longer to occur such as elevated osmolality in more distal sections of the gastrointestinal tract or intestinal distention may be responsible. The latter might be of particular importance, given that seawater acclimation results in an almost doubling in the intestinal diameter of rainbow trout (Brijs et al., 2017a). Given the comparatively fast perfusion rate employed in **paper II**, intestinal hyperosmolality or distention would occur quicker compared to the gradual elevations in drinking rate in seawater. This explains why GBF increases faster in the gastric perfused fish in **paper II** compared to trout acutely transferred

to seawater (Brijs et al., 2015). Similarly, post-prandial peak GBF in rainbow trout coincides with the entry of chyme into the intestine (Gräns et al., 2009a; Seth and Axelsson, 2009). Although nutrient-induced hyperemia is an important mechanism for this response, it is likely that luminal hyperosmolality and mechanical stimulation also contribute, as seen in mammals (Matheson et al., 2000).

While intestinal receptors likely initiate the mechanism responsible for the cardiovascular responses following gastric perfusion with half-strength seawater, the underlying vasoactive neuro-humoral mechanisms contributing to these responses are unknown. Although, as seen in **paper I**, α -adrenergic regulation may be involved. On the other hand, the mechanisms responsible for eliciting postprandial gastrointestinal hyperemia appear to be mostly independent of sympathetic tone. For example, vagal and splanchnic denervation has negligible effect on postprandial gastrointestinal hyperemia in rainbow trout, while the enteric nervous system appears to be essential for this process (Seth and Axelsson, 2010). Similarly, changes in adrenergic tone could not fully account for the postprandial hyperemia in the red Irish lord (Axelsson et al., 2000). Thus, change in α -adrenergic tone appears to have a greater role in regulating GBF at different salinities compared to during digestion.

Several differences between the cardiovascular responses to seawater-acclimation and intra-gastric half-strength seawater perfusion stand out. For instance, in contrast to seawater acclimation, P_{DA} is not affected by the intra-gastric perfusion. Another important difference is that some of the cardiovascular responses elicited in **paper II**, such as the elevations in GBF, are not as pronounced as those observed in fish transferred to seawater. It is possible that to fully elicit cardiovascular responses typical of seawater-acclimation, additional stimuli are required. A wide array of brain, systemic and epithelial receptors have been described to detect changes in fluid osmolality, ion composition and changes in blood pressure (Kültz, 2013), the stimulation of which might be needed to fully elicit the responses observed in seawater. For example, plasma osmolality of the Japanese eel (*Anguilla japonica*) and rainbow trout typically increases following transfer to seawater, which stimulates the secretion of atrial natriuretic peptide (Kaiya and Takei, 1996a, b; Smith et al., 1991). Atrial natriuretic peptide is a potent vasodilatory hormone and relaxes celiacomesenteric vascular smooth muscle of rainbow trout (Olson and Meishi, 1989; Smith et al., 2000). Dilation of the gastrointestinal vasculature and the consequent reductions in R_{GI} could indeed result in reductions in R_{Sys} and P_{DA} and elevations in GBF. Thus, one

possibility is that, as rainbow trout in **paper II** were maintained in freshwater, plasma osmolality did not increase and there was no secretion of atrial natriuretic peptide, explaining the unchanged P_{DA} and reduced elevations in GBF.

While the SV-mediated elevation in CO in **paper II** was more pronounced in the half-strength seawater perfusion treatment, changes in CO and GBF also occurred in the control fish. In cannulated fish, it is not uncommon to observe gradual reductions in haematocrit for days and even weeks following cannulation (Wells and Weber, 1991; Wood and Shelton, 1980). These reductions in haematocrit could be due to bleeding, although the maintenance of a normal P_{DA} and a relative spleen mass within normal range (Pearson and Stevens, 1991) in **paper II** suggest that bleeding was not substantial. Instead, it is possible that the reduction in haematocrit was, at least in part, due to splenic uptake of erythrocytes. A drop in haematocrit/haemoglobin and a consequent loss of blood O_2 carrying capacity may be compensated for via elevations in CO (Gold et al., 2015; Wang et al., 2014) and explain the gradual increase in both treatments.

3.3. Interacting effects of salinity and warming

Although GBF has been previously reported to increase in response to warming in both freshwater- (Brijs et al., 2018; Gräns et al., 2009b) and seawater-acclimated fish (Gräns et al., 2013), no study has compared the effect of warming across salinities. **Paper III** shows that GBF increases with warming at both salinities, although the relative increase in GBF is larger in the seawater-acclimated trout (75% increase vs. 31%). Thus, fish in seawater are able to respond to warming with larger increases in GBF, despite a larger initial GBF.

Elevations in gill ventilation and branchial blood perfusion during warming exacerbates loss of water across the gills in seawater-acclimated trout (Isaia, 1972; Onukwufor and Wood, 2018). To compensate for the exacerbated dehydration, marine fish elevate drinking rate (Maetz and Evans, 1972; Motais and Isaia, 1972; Skadhauge and Lotan, 1974). This larger fluid intake and subsequent processing requires an elevated GBF to meet the O_2 demand and/or convective needs of the gastrointestinal tract. As shown in **paper II**, increased gastrointestinal luminal osmolality or intestinal mechanical stimulation are associated with elevations in GBF. Thus, in seawater, increased drinking rate with warming can result in greater stimulation of intestinal osmo- and/or

mechanoreceptors along the intestine, which explains the larger elevations in GBF observed in seawater-acclimated trout.

In **paper III**, the larger increases in GBF in seawater-acclimated rainbow trout, are sustained by more pronounced elevations in CO, resulting in the difference in CO between treatments increasing from 34% at 11°C to 55% at 17°C. The elevations in CO were mediated by increases in HR as SV remained stable in both salinity treatments and the lack of change in P_{DA} throughout the warming protocol indicates that reductions in R_{GI} in combination with the elevations in CO drive the increases in GBF observed in both salinities.

The elevated baseline CO at 10-11°C (**papers I and III**) combined with the larger increases in CO in response to warming in seawater (**paper III**), highlight that the scope for increasing CO further may be reduced in seawater-acclimated trout unless compensatory increases in maximum CO take place (Fig. 5). To the best of my knowledge, comparisons of maximum CO across salinities at different temperatures have not been performed in rainbow trout or any other euryhaline species, although, in a follow-up study, freshwater- and seawater-acclimated rainbow trout displayed no differences in maximum CO at 10°C (Kallstenius et al., Unpublished data). If maximum CO of seawater-acclimated trout is not elevated at higher temperatures, a reduced CO scope will limit the ability to increase GBF, which will negatively affect osmoregulatory function at higher temperatures. Indeed, studies on chinook salmon (*O. tshawytscha*) acclimated to seawater showed that, although intestinal blood flow decreased with exercise, fish reared under a high intensity-training regime had a greater CO scope and were able to maintain a larger intestinal blood flow and a better osmoregulatory function compared to low intensity-trained fish (Gallaughier et al., 2001; Thorarensen et al., 1993).

Geographical distribution of fish populations is already affected by changes in the temperature of the ocean, with many populations having shifted their distribution towards the poles as temperatures increase (Hastings et al., 2020; Perry et al., 2005). The underlying cause for some of these shifts might involve cardiovascular limitations since, as seen in Fig. 5, the scope for increasing CO should become limited faster in seawater with warming, unless maximum CO is elevated at higher temperatures. This will constrain the ability of seawater fish to sustain elevated O_2 consumption (e.g., postprandially or during exercise) and maintain an adequate osmoregulatory function.

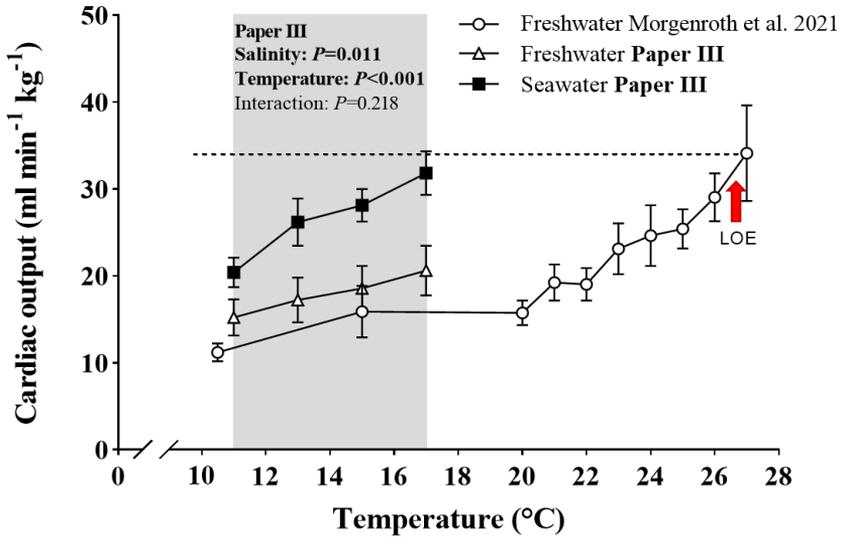


Figure 5. Effects of warming on cardiac output in rainbow trout acclimated to different salinities. Cardiac output of fresh- and seawater-acclimated rainbow trout obtained in two separate studies, **paper III** (open triangles and filled squares for fresh- and seawater-acclimated fish, respectively) and Morgenroth et al. (2021) (open circles, freshwater). The panel is a modified version of the original papers. In Morgenroth et al. (2021) upper thermal tolerance of freshwater-acclimated trout was assessed by warming the fish step-wise until reach of loss of equilibrium (LOE, red arrow). The discontinuous line is aligned with the highest cardiac averages for freshwater and is used as a reference for scope of increase in heart rate and cardiac output in seawater if maximum cardiac output remains equivalent across salinities and temperatures. The results from a mixed model for the respective variables from **paper III** are presented in each panel and comprise the 11 to 17°C temperature range (greyed area). Statistical significance was accepted at $P<0.05$ and indicated in bold. Values are means \pm SEM.

3.4. Effects of reduced salinity on cardiorespiratory and growth performance

SMR is reduced by $\sim 10\%$ in kingfish acutely exposed to brackish water (**paper IV**). Nevertheless, SMR does not differ between fish acclimated to seawater and brackish water, which indicates that the reduced SMR in acutely exposed fish is not due to changes in costs of osmoregulation, as these differences should be present in both acute and long-term treatments. On the other hand, both kingfish acutely and long-term exposed to brackish water had a reduced

EPOC compared to kingfish maintained in seawater. Although SMR do not differ between salinities, this indicates that the costs of osmoregulation are reduced in brackish water during and following exhaustion. A potential mechanism for this is that fish in brackish water require less O₂ to fuel metabolic processes reestablishing any osmotic disturbances resulting following exhaustion. Alternatively, anaerobic ATP production may be reduced in brackish water, thus reducing the O₂ required to clear lactate following exhaustion.

A reduced EPOC can be beneficial to fish as it reduces the recovery time following stressful events or events of elevated activity, increasing how frequently the animal can engage in burst swimming activity, reducing the risk of predation or enhancing the ability to acquire food (Milligan, 1996). Additionally, energy savings resulting from reduced costs of reestablishing homeostasis following energetically demanding events can be allocated to growth. Yet, in **paper IV**, kingfish acclimated to 24 and 12 ppt have similar growth rates than kingfish maintained in full-strength seawater. Environmental salinity affects a variety of biological processes beyond osmoregulation. This is partly due to a considerable overlap in endocrine regulation among these processes, best illustrated by the pluripotent growth hormone-insulin-like growth factor system, which also regulates development, appetite and behavior, all of which may affect growth (Boeuf and Payan, 2001; Jönsson and Björnsson, 2002; Zydlewski and Wilkie, 2013). Thus, instead of fueling growth, energetic savings following bouts of activity might have been allocated to increased activity. Blanco Garcia et al. (2014) showed that kingfish acclimated to lower salinities have an elevated feed intake. This is likely the result of an elevated activity, such as food-seeking behavior. In **paper IV**, we fed kingfish a restrictive diet below satiation, while Blanco Garcia et al. (2014) maintained their fish under an *ad lib* feeding regime. Thus, in the study by Blanco Garcia et al. (2014) the costs associated with an enhanced activity are compensated by the elevated feed intake, resulting in an improved growth rate. On the other hand, kingfish given a restrictive diet in **paper IV** cannot increase their caloric intake and the energetic savings from reduced costs of osmoregulation and the elevated energy expenditure resulting from enhanced activity cancel each other out, resulting in unaltered growth rate across salinities.

Nevertheless, kingfish acclimated to the lowest salinity (12 ppt), have an elevated FCR, which means that they need to consume more food for an equivalent increase in body mass. When we divide our analysis into the two monthly growth measurement intervals, we observe that the elevated FCR at

the lowest salinity occurs exclusively during the first month of acclimation and has negative consequences for growth rate during that month. This difference disappeared during the second month of the feeding trial. Thus, it appears that a severe imbalance between energy expenditure and caloric intake occurs only during the first month, and energy intake and expenditure reaches a balance after a longer acclimation period.

Besides SMR and EPOC, there are no differences in any other cardiorespiratory variables between salinities (**paper IV**). Only one other study has previously reported routine cardiovascular function in a marine fish exposed to reduced salinity. Sundell et al. (2021) exposed shorthorn sculpin, a sedentary benthic species, to reductions in salinity of similar magnitude as in **paper IV** (*i.e.* 15 ppt). This results in 24 and 21% reductions of CO and MO₂, respectively (Sundell et al., 2021). On the other hand, the European seabass, an active marine species, shows no differences in resting and maximum MO₂, aerobic scope or maximum CO (routine cardiovascular function was not reported) when transferred to lower salinity (Chatelier et al., 2005). Thus, it is possible that for some active marine fishes, such as kingfish and seabass, which perform frequent incursions into areas of variable salinity, the costs of osmoregulation are lower than initially hypothesized. Similarly, resting metabolic rate of another active euryhaline species, the coho salmon (*O. kisutch*), does not seem to differ among salinities ranging from 2.5 to 30 ppt (Brijs et al., 2017c). Still, as seen in **papers I** and **III**, cardiac function of the closely related rainbow trout differs between freshwater and seawater although, to the best of my knowledge, cardiac function at intermediate salinities has not been analyzed in this species. Nevertheless, the limited available data suggest pronounced differences in cardiorespiratory responses to altered salinities among euryhaline fishes.

Despite the lack of differences in cardiac function, cardiac morphology is significantly affected by reduced salinity in kingfish (**paper IV**). Kingfish acclimated to brackish water had a reduced proportion of ventricular compact myocardium compared to kingfish maintained in seawater. This is similar to the remodeling observed in rainbow trout, where seawater acclimation results in elevations in the proportion of compact myocardium (Brijs et al., 2017b). Although the mechanisms by which seawater-acclimated fish maintain or develop larger compact myocardium are unknown, it can be speculated that hormones involved in maintaining salt and water balance are involved. For example, angiotensin II, which is a dipsogenic hormone associated with seawater acclimation (Beasley et al., 1986; Rash and Lillywhite, 2019), is

associated with increases in the proportion of compact myocardium (Imbrogno et al., 2013).

4. Conclusions and future perspectives

The overall goal of this thesis was to gain a deeper understanding of the cardiorespiratory changes that determines the resilience of euryhaline fishes to different environmental salinities and temperatures. Combined, the findings from **paper I** and **II** demonstrate that cardiovascular function at different salinities is regulated by a complex interplay between neural factors and intestinal receptors in combination with other unidentified vasoactive factors. The mechanisms eliciting cardiovascular changes in response to luminal hyperosmolality or mechanical stimulation along the gastrointestinal tract resemble those observed in mammals. Thus, an interesting possibility is that, independently of the environmental salinity, these mechanisms also contribute to coordinating and fine-tuning blood flow dynamics during other processes such as digestion.

Still, further studies in other species are required to elucidate whether these cardiovascular responses and their regulatory mechanisms are common to other euryhaline fishes. For instance, in contrast with the differences in cardiac function observed in rainbow trout acclimated to freshwater and seawater (**papers I** and **III**), cardiac function remains similar between kingfish acclimated to 17 ppt and kingfish maintained seawater (**paper IV**). It is possible that the large effects of salinity on cardiovascular function of rainbow trout reflect a switch between hyper- and hypoosmeregulatory strategies, while kingfish are hypoosmoregulating at all salinities tested and thus, the cardiovascular changes are minor. Nevertheless, studies on other euryhaline fishes reveal significant cardiac differences at salinity ranges close to those tested in **paper IV**, which might instead reflect differences in osmoregulatory strategies between species. Therefore, further analyses of cardiovascular plasticity to altered salinity in other euryhaline fishes are required to gain a better insight into potential differences in the regulatory patterns across fish ecotypes and life strategies, as well as to shed light onto the ecological implications of these differences.

Long-term exposure to reduced salinity resulted in significant reductions in the proportion of compact myocardium in kingfish (**paper IV**), which, combined with previous results on other fish species, show that cardiac remodeling in response to changes in salinity might be a response common in

fish. The mechanisms whereby changes in environmental salinity ultimately result in cardiac remodeling warrants further research.

Paper III is the first study comparing GBF dynamics during warming in fish acclimated to different salinities. It shows that seawater-acclimated trout respond to warming with larger elevations in GBF and these are sustained by larger elevations in CO. Given that warming elevates drinking rate in marine fish, the larger increases in GBF suggest an increased O₂ demand or need for convective transport of nutrients, wastes, water and ions. Despite the gastrointestinal tract of euryhaline fishes having upregulated activity of osmoregulation-related ATP-consuming enzymes, it is currently unknown whether the gastrointestinal tract actually consumes more O₂ in seawater than in freshwater. To answer this question, studies comparing the MO₂ of intact gastrointestinal tract (*i.e. in vivo*) of fishes of the same species acclimated to different salinities are required.

The results from **paper III** also show that cardiovascular function might be constrained at high temperatures in seawater. Global average temperatures have increased by over 1°C above pre-industrial levels and, despite worldwide pledges to reduce rates of emission of greenhouse gases, global average temperatures are predicted to increase by ~2.7°C by the end of the century (<https://climateactiontracker.org/>). This, in combination with other aspects of climate change, such as altered precipitation patterns and increased frequency of extreme weather events, will alter the salinity and temperature of aquatic environments, particularly along estuaries and shallow coastal areas. Thus, future studies focusing on the cardiovascular responses to fluctuations in environmental salinity in combination with interacting environmental drivers, such as warming, are required to improve our understanding on the capacity of euryhaline fishes to respond to climate change.

Collectively, the results of this thesis provide a better understanding of the cardiovascular responses of migratory and estuarine fishes. These cardiovascular responses are fundamental for osmoregulatory function, which is a key driver of animal performance and governs the ability of euryhaline fishes to survive broad environmental salinity changes.

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