

**THE EFFECT OF A TAURINE DEFICIENCY ON
THE CARDIOVASCULAR RESPONSE OF BROOK
CHAR (*SALVELINUS FONTINALIS*) TO HYPOXIA**

BY

Samuel Patrick McGaw

A thesis submitted to the
Department of Chemistry and Biochemistry
Mount Allison University
in partial fulfillment of the requirements for the
Bachelor of Science degree with Honours

April. 12th, 2023

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iv
ABSTRACT	v
LIST OF FIGURES	vii
ABBREVIATIONS	ix
INTRODUCTION	1
<i>Taurine Biosynthesis and Transport</i>	2
<i>Teleost O₂ Transport Cascade</i>	4
<i>Cardiovascular Response of Teleost to Hypoxia</i>	5
<i>Taurine's Contribution to Cardiomyocyte Contraction under Hypoxia</i>	7
<i>Taurine's Maintenance of Cardiomyocyte Osmolality under Hypoxia</i>	9
<i>Taurine's Influence on Cardiomyocyte Metabolism</i>	9
<i>Model Organism and Taurine-Deficiency</i>	10
<i>Research Goals and Hypothesis</i>	10
METHODS	11
<i>Ethics Declaration</i>	11
<i>Fish Diet and Handling</i>	11
<i>Sampling Procedures and Hematology</i>	12
<i>Electrocardiogram Acute Hypoxia and Reoxygenation</i>	12
<i>Ultrasonic Flow Probe Acute Hypoxia and Reoxygenation</i>	14
<i>Molecular Quantifications</i>	15
<i>Plasma and Heart Taurine</i>	15
<i>Plasma and Heart Lactate</i>	16
<i>Statistical Analyses</i>	16
RESULTS	17
<i>Effect of a Taurine Deficiency on Cardiac Parameters</i>	17
<i>ECG f_h and Taurine</i>	17
<i>Ultrasonic Flow Probe f_h, SV, and \dot{Q}</i>	19
<i>Lactate and Hematology</i>	20
DISCUSSION	22
<i>Inhibited hypoxia-induced taurine transport through β-alanine supplementation</i>	22
<i>Effect of a taurine deficiency on O₂ uptake and transport</i>	22
<i>TD fish accumulate less heart lactate under hypoxia</i>	23

<i>Lower resting f_h in TD fish</i>	24
<i>TD fish have a blunted bradycardia and SV increase under hypoxia</i>	25
<i>Conclusion and Future Directions</i>	27
REFERENCES	29

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Tyson MacCormack for your support, patience, and encouragement over the past few years. Your enthusiasm for research has not gone unrecognized and has made this process a true pleasure. My gratitude extends to the courses I was fortunate to have taken under your guidance. You've greatly influenced my interest in biochemistry and my desire for a career in a related field. My appreciation also extends to Dr. Karen Crosby, who kindly agreed to be my second reader, and Dr. Andrea Morash, who consistently offered a helping hand in our shared lab space. I truly appreciate the support of you both.

To my fellow lab mates, Nir El, Micah Lea, Bailey Hatcher, Ryan Wahl, and Charlotte Haché, I appreciate all the laughs and fun times we've shared. I couldn't have asked for a better group to have spent the past few years alongside. I would be remiss if I didn't offer a particular thank you to Nir El. In a challenging program, you've helped make my undergrad a truly enjoyable experience, and it was a pleasure to learn alongside you for the past four years. To Shelley Leblanc and Claire Pabody, our Aqualab technician and lab manager, respectively, I truly appreciate your kind words of encouragement and teachings over the past year. A special thank you to Dr. Sarah McOnie, our Current Advances professor, who has helped provide exposure to the diverse opportunities in the fields of chemistry and biochemistry, along with direction throughout this process.

To my parents, Ray and Stephanie, whom none of this would be possible without. Although this path has been as new to you as it has to me, your continued support has been appreciated immensely. You've always pushed me to be the best version of myself, which I hope the following piece is representative of. I would also like to thank my older brother, Alex. You have always been my biggest motivator, and I take great pride in knowing I have your support.

I would like to sincerely thank the entire Department of Chemistry and Biochemistry here at Mount Allison. These past four years have helped me uncover a true passion for science and a pursuit of knowledge that I will carry forward. I am extremely proud to be a member of the Mount Allison community and I will forever cherish the relationships and memories I'm so lucky to have established.

ABSTRACT

Taurine is a non-proteogenic β -amino acid found in relatively high abundance within the heart of freshwater teleosts. By exhibiting cardioprotective properties through participation in osmoregulation and calcium (Ca^{2+}) homeostasis, taurine has been linked to supporting the cardiovascular physiology under environmental hypoxia. The functional capacity of the heart is defined by cardiac output (\dot{Q}), representing the product of heart rate (f_h) and stroke volume (SV). A taurine deficiency has been shown to impair the ability to increase SV under hypoxia *in vitro* and cause a shorter time to loss of equilibrium (LOE) *in vivo*, indicating cardiovascular disturbances. The knowledge gap addressed in this study is the effect of a taurine deficiency on all three cardiac parameters *in vivo* under hypoxia. These measurements were paired with metrics of the O_2 carrying capacity of the blood and osmotic stress following acute hypoxia and recovery.

Brook char (*Salvelinus fontinalis*) were used as a representative freshwater teleost, for this analysis. A taurine-deficient (TD) model was achieved through 5% dietary β -alanine supplementation, a known competitive inhibitor of taurine transport. f_h , SV, and \dot{Q} *in vivo* were quantified using electrocardiograms (ECG) and ultrasonic flows. Significance was found in lower resting f_h and blunted bradycardia in TD fish. SV was maintained at similar levels to control fish, although TD fish did not elevate SV under hypoxia, likely tied to their lesser bradycardia. \dot{Q} was similar in both models, along with hematological parameters characterizing the capacity to transport O_2 in circulation. TD hearts had decreased lactate levels, an important metabolite to cardiovascular function under a hypoxia stressor.

The main finding of this study is that TD fish have a greater sensitivity to environmental hypoxia, attributed to just a 21% reduction in cardiac taurine. With the typical increase in SV seen under hypoxia attributed to a lower f_h , regulatory disruption of contractile frequency may explain the observed physiological differences. As an important mechanism to mitigate osmotic disturbances under hypoxia, a limited ability to perform taurine efflux may be the underlying cause of cardiac dysfunction. This may additionally lead to alterations to the Ca^{2+} current (I_{Ca}) acting on the heart's pacemaker or autonomic nervous system activation. With the progression of global warming and

associated disturbances to aquatic O₂ availability, ensuring sufficient taurine in the diets of freshwater fish could be a method of supporting cardiovascular function.

LIST OF FIGURES

- Figure 1:** Two- and three-dimensional chemical structure of taurine.....3
- Figure 2:** Tau-T and PAT1 transporters. Tau-T cotransport involves two sodium (Na^+) ions and one chlorine (Cl^-). PAT1 performs cotransport with a single hydrogen ion (H^+).3
- Figure 3:** Anatomy of the teleost heart and directional blood flow from Randall, (1968).4
- Figure 4:** Cardiomyocyte calcium (Ca^{2+}) regulation and transport. Sodium (Na^+) and chlorine (Cl^-) are transported with taurine intracellularly through the taurine transporter (Tau-T). Na^+ is exchanged for Ca^{2+} through the Na^+ - Ca^{2+} exchanger (NCX) in a 3:1 stoichiometric relationship. Ca^{2+} is released from the sarcoplasmic reticulum (SR) through a channel established from ryanodine receptors (RyRs) and pumped back into the SR through the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA2a). Taurine supports SERCA2a activity by promoting phospholamban (PLB) phosphorylation through relieving inhibition of Ca^{2+} /calmodulin dependent protein kinase II (CaMKII) by phosphatase 1.....8
- Figure 5:** Electrode needle and cord placement for electrocardiogram (ECG) measured heart rate (f_h) of brook char (*Salvelinus fontinalis*) during acute hypoxia and reoxygenation. The enlarged portion depicts needles positioned on either side of the heart beneath the skin surfacing the stomach. Black dots represent the locations of the suture placement to prohibit cord tangling.....13
- Figure 6:** Flow probe and cable placement for ultrasonic flow probe measured cardiac output (\dot{Q}) and stroke volume (SV) of brook char (*Salvelinus fontinalis*) during acute hypoxia and reoxygenation. The enlarged portion depicts the location of probe placement on the ventral aorta, oriented to avoid blood flow restriction or dislodgement. Black dots represent the locations of the suture placement.....15
- Figure 7:** Brook char (*Salvelinus fontinalis*) plasma (A) and heart (B) taurine levels of taurine deficient (TD) and control (n = 8 for both groups) fish following 30 min exposures at 12.5 and 8.33 kPa O_2 and 90 minutes of reoxygenation to 20.8 kPa O_2 . Normoxic (20.8 kPa O_2) resting heart rate (f_h) (C), the difference in heart rate (Δf_h) between normoxia and hypoxia (8.33 kPa O_2) (D), and Δf_h from hypoxia and 90 mins of reoxygenation to normoxia (Δf_h reoxygenation) (E) of TD (n = 6) and control (n = 8) brook char. Asterisks represent significant differences between control and TD fish (* p < 0.05, ** p ≤ 0.001).....18
- Figure 8:** Control (n = 6) and taurine-deficient (TD) (n = 7) brook char (*Salvelinus fontinalis*) heart rate (f_h) (A), stroke volume (SV) (B), and cardiac output (\dot{Q}) (C) during normoxia at 20.8 kPa O_2 (O_2), 8.33kPa O_2 (Hypoxia), and reoxygenation to 20.8 kPa O_2 (Reox). Asterisks represent significant differences between control and TD fish (* p < 0.05, ** p ≤ 0.001).....20

Figure 9: Plasma (A) and heart (B) lactate of TD and control (n = 8 for both groups) brook char (*Salvelinus fontinalis*) following 30 min exposures at 12.5 and 8.33 kPa O₂ and 90 minutes of reoxygenation to 20.8 kPa O₂. Asterisks represent significant differences between control and TD fish (* p < 0.05).....21

Table 1: Brook char (*Salvelinus fontinalis*) electrocardiogram (ECG) and ultrasonic flow probe recorded hematological parameters and plasma osmolality following 30 min exposures at 12.5 and 8.33 kPa O₂ before 90 mins of reoxygenation to 20.8 kPa O₂. MCHC represents mean corpuscular hemoglobin concentration.....21

Figure 10: The contractile cycle of the teleost heart and corresponding electrocardiogram (ECG) signal. V represents the ventricle, A represents the atrium, and BA represents the bulbous arteriosus. Depolarization is shown in red, and hyperpolarization is shown in blue. QT interval is labelled and represents ventricle depolarization. Adopted from Arel et al., (2022).....25

ABBREVIATIONS

ATP: Adenosine triphosphate

ANOVA: Analysis of variance

β -alanine: Beta-alanine

BA: Bulbous arteriosus

Ca^{2+} : Calcium

\dot{Q} : Cardiac output

CaMKII: Ca^{2+} /calmodulin-dependent protein kinase II

Cl⁻: Chlorine

Pcrit: Critical oxygen partial pressure

Csad: Cysteine sulfinic acid decarboxylase

ddH₂O: Double distilled water

ECG: Electrocardiogram

f_h : Heart rate

HPLC: High-performance liquid chromatography

LDH: Lactate dehydrogenase

LOE: Loss of equilibrium

MCHC: Mean corpuscular hemoglobin concentration

MtA: Mount Allison University

NaHCO_3 : Na^+ bicarbonate

NCX: Na^+ - Ca^{2+} exchanger

N_2 : Nitrogen

O_2 : Oxygen

OCLTT: Oxygen and capacity limited thermal tolerance

PCA: Perchloric acid

PLB: Phospholamban

PVC: Polyvinyl chloride

K^+ : Potassium

KHCO_3 : Potassium bicarbonate

H^+ : Proton

RBC: Red blood cells

RyRs: Ryanodine receptors

SR: Sarcoplasmic reticulum

SERCA2a: Sarcoendoplasmic reticulum Ca²⁺ ATPase

Na⁺: Sodium

SEM: Standard error of the mean

SV: Stroke volume

TD: Taurine deficient

Tau-T: Taurine transporter

MS222: Tricaine methanesulfonate

V: Ventricle

INTRODUCTION

The survival of any organism depends on the alignment of physiological function and habitat resources. Global warming is an ongoing phenomenon that imposes continual change to the stability of many habitats, necessitating an understanding of novel selective pressures and the physiological stress imposed on specific anatomical compartments. Water breathers are innately predisposed to lower oxygen (O₂) capacitance, making changes to O₂ availability a critical habitat disturbance (Rahn, 1966). A decreased O₂ availability in freshwater environments of elevated temperature is an important burden for many aquatic species (Nikinmaa et al., 2011; Rajwa-Kuligiewicz et al., 2015). The oxygen-and capacity-limited thermal tolerance (OCLTT) hypothesis links temperature and the associated O₂ afforded in an organism's environment as the primary determinant of its performance (Audzijonyte et al., 2019). Freshwater ectotherms are especially susceptible to temperature-related environmental changes based on the thermal mirroring between their core body and habitat (Kalinin et al., 2009). Although seasonal changes induce a degree of acclimation to different temperature-mediated O₂ levels, prolonged exposure to hypoxia is associated with hypoxemia and several metabolic and physiological consequences (Shepard, 1955). The effects of aquatic hypoxia are evident when considering the physiological adjustments aquatic ectotherms make when O₂ becomes scarce. Under hypoxia, freshwater fish increase their breathing rate and branchial perfusion while adjusting metabolic and anatomical machinery to extract more O₂ and direct it to vital internal processes (Shepard, 1955; Van den Thillart & van Waarde, 1985). Additionally, function of the branchial pump is elevated to maintain an equilibrium between the O₂ levels in the surrounding environment and the circulatory system (Holeton & Randall, 1967; Randall & Shelton, 1963).

The cardiovascular system is of fundamental importance when encountering O₂ scarcity, as it is responsible for transporting oxygenated blood to systemic tissues to maintain their performance (Clark, Sandblom, et al., 2008). To sustain this consistent distribution, the heart is itself reliant on O₂ availability to fulfill its metabolic needs (Driedzic & Gesser, 1994). There are remaining uncertainties regarding mechanisms of resiliency to environmental hypoxia as exposure to more severe hypoxic environments

may lead to structural changes in the heart, requiring investigation into the intrinsic processes underlying this sensitivity (Anttila et al., 2015; Audzijonyte et al., 2019).

Freshwater fish face unique environmental pressures under hypoxia predicated on their need to sustain osmotic homeostasis while innately having more internal osmolytes than their surroundings (Matey et al., 2011; Prunet & Bornancin, 1989). At the organismal level, the importance of maintaining an osmotic balance under hypoxia is identified through the osmorepiratory compromise (Matey et al., 2011). A teleost will increase the functional surface area of its gills to maximize environmental O₂ uptake; however, the increased branchial perfusion comes at a cost of the loss of essential ions to the environment (Matey et al., 2011). The β-amino acid taurine was previously characterized as an important osmolyte in mitigating cellular disturbances during this process (Schaffer et al., 2010).

Taurine is non-proteinogenic amino acid that is regarded as an essential dietary nutrient for freshwater fish. It functions to inhibit cell swelling and apoptosis through cellular efflux when an unfavourable osmotic gradient arises (Salze & Davis, 2015; Schaffer et al., 2010). Additionally, gene expression of the taurine transporter and biosynthetic enzymes are upregulated under hyperosmolarity (Bitoun & Tappaz, 2002). With a quantity of ~50 mmol L⁻¹ in the heart of teleost, it is hypothesized that taurine helps mitigate cellular disturbances within the cardiovascular system under hypoxia; however, the mechanistic influence remains largely uncharacterized for freshwater fish (Dixon et al., 2023; Henry & MacCormack, 2018). As such, the contributions of taurine to the cardiovascular response of freshwater fish to hypoxia requires investigation to understand the capacity of these organisms to survive in low-oxygenated habitats.

Taurine Biosynthesis and Transport

Unique in its acidic sulfonate group, taurine or 2-aminoethanesulphonic acid (Figure 1) is an end product in the metabolism of sulphur-bearing amino acids (Allen & Garrett, 1971). Taurine accounts for 25, 50, 53, and 19% of free amino acids within the liver, kidney, muscle, and brain, respectively, representing the most abundant free amino acid (Salze & Davis, 2015). Most abundantly biosynthesized in adipose tissue and the

kidney of teleosts (Betancor et al., 2019), the rate-determining enzyme in taurine biosynthesis is cysteine sulfinic acid decarboxylase (Csd) (Chang et al., 2013).

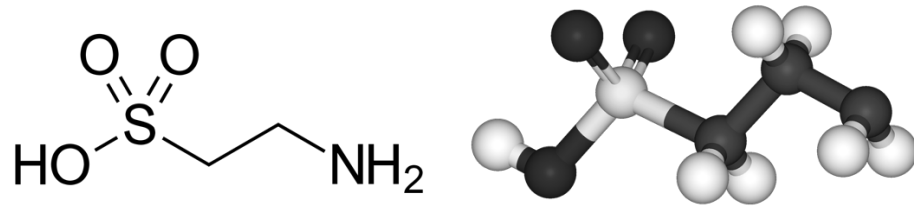


Figure 1: Two- and three-dimensional chemical structures of taurine.

Taurine is predominately found in the intracellular environment, requiring movement against a concentration gradient for cellular uptake through one of the two established taurine transporters, PAT1 and Tau-T (Anderson et al., 2009; Huxtable & Chubb, 1977). Figure 2 describes the cotransport mechanisms for each membrane protein facilitating the taurine influx (Anderson et al., 2009).

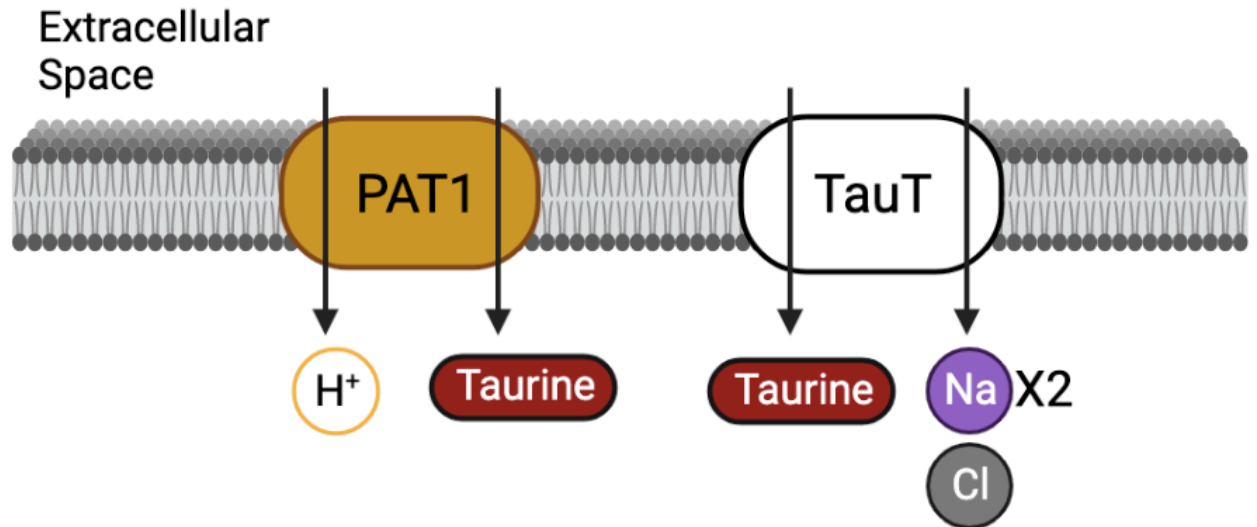


Figure 2: Tau-T and PAT1 transporters. Tau-T cotransport involves two sodium (Na⁺) ions and one chlorine (Cl⁻). PAT1 performs cotransport with a single hydrogen ion (H⁺).

The PAT1 transporter has a low affinity but high capacity for taurine and is pH-dependent, relying on proton (H⁺) availability and a low extracellular pH (Anderson et al., 2009; Bröer, 2008). Tau-T has a high affinity and low capacity for taurine and is dependent on co-transport with sodium (Na⁺) and chlorine (Cl⁻) (Anderson et al., 2009). The secondary active transport of taurine by Tau-T is mediated by the electrochemical

gradient of Na^+ , achieved through the energetic investment associated with the $\text{Na}^+\text{K}^+\text{ATPase}$ transporter (Hastings et al., 1985). Under low taurine levels, the activity of Tau-T predominates while PAT1 activity elevates at higher concentrations of taurine (Anderson et al., 2009). With increased osmotic stress under hypoxia, taurine export occurs through reversible activity of Tau-T, contributing upwards of 50% of changes to intracellular osmolarity in cardiomyocytes (Vislie, 1983). The importance of taurine transport and its contribution to ion distribution requires investigation to understand its influence on cardiovascular function during a hypoxic stressor (Cossins & Gibson, 1997).

Teleost O_2 Transport Cascade

The teleost cardiovascular system has dynamic and static components (Davison, 1989). One ventricle pumps blood through the gills for O_2 uptake before entering the systemic circulation (Gamperl & Driedzic, 2009). Within the blood, red blood cells (RBC) carry O_2 bound to hemoglobin to proximal and distal tissues (Affonso et al., 2002). The single-circuit design in Figure 3 represents an efficient and simplistic model to facilitate the analysis of cardiac parameters and their systemic influences.

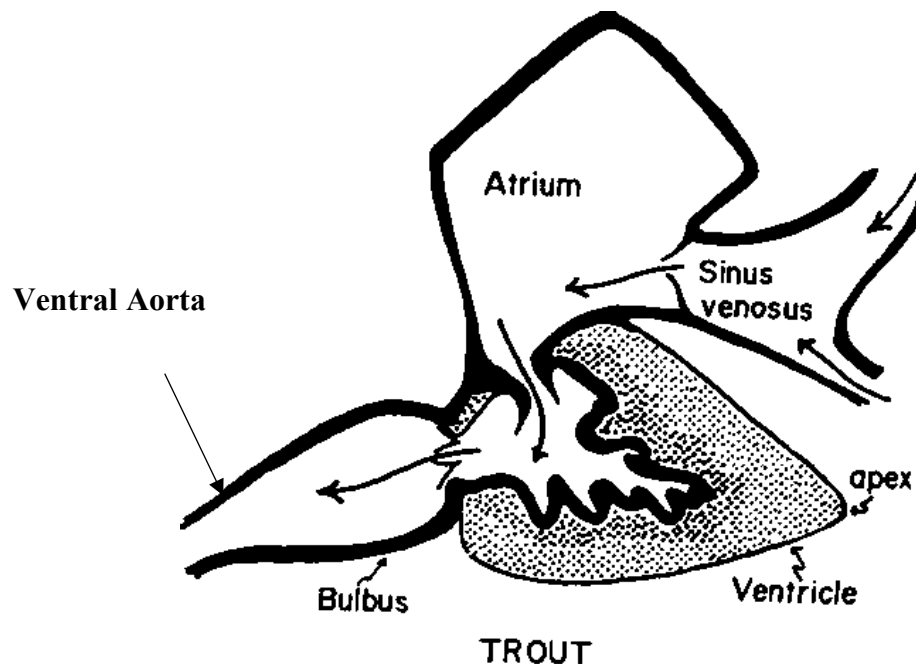


Figure 3: Anatomy of the teleost heart and directional blood flow from Randall, (1968).

The functional capacity of the heart is characterized by cardiac output (\dot{Q}), describing the amount of blood sent out of the heart per unit of time and represented as the product of stroke volume (SV) and heart rate (f_h) (Pörtner et al., 2017). SV equates to the amount of blood pumped with every heartbeat, while f_h represents the frequency of the heart contraction (Vornanen et al., 2002). Additionally, the product of the \dot{Q} and arterial O₂ concentration, a quality dependent on O₂ carrying capacity, is equivalent to the systemic O₂ delivery (Joyce & Wang, 2022).

Aerobic scope presents the gap between maximum and minimum metabolic rates, a quality decreased when under hypoxia (Jordan & Steffensen, 2007). A diminished scope is associated with a lesser ability to transport O₂, a characteristic attributed to cardiovascular function and blood composition, represented by the aforementioned parameters (Jensen et al., 2017). Furthermore, the aerobic scope helps describe energetic capacity and determines the ability of an organism to grow and survive in its environment (Auer et al., 2015). Two responses of the teleost cardiovascular system to a diminished scope under hypoxia are to utilize anaerobic metabolism and diminish power output (Gamperl & Driedzic, 2009). Although some contributions of taurine have been hypothesized toward these cardiac responses, a mechanistic explanation remains uncharacterized.

Cardiovascular Response of Teleost to Hypoxia

A lower environmental O₂ saturation is recognized by external O₂ receptors, manifesting in compensatory reflexes to cardiovascular function (Sundin et al., 1999). These functional alterations under hypoxia are subsequently regulated by the autonomic nervous system (Randall, 1982), with cardiac tissue receiving efferent adrenergic and cholinergic stimulation through the vagus nerve (Gannon, 1971). Adrenergic receptors function as excitatory, while cholinergic receptor activation results in an inhibitory signal cascade (Axelsson et al., 1987). Anatomically, the atrium is hypothesized to receive excitatory and inhibitory signals, while the ventricle is believed to only contain an excitatory innervation (Gannon, 1971). A distinguishing cardiovascular response of teleost to low O₂ is to diminish f_h , termed bradycardia (Holeton & Randall, 1967). Decreasing f_h equates to a lesser cardiac energy demand, helping maintain systemic O₂

levels while sustaining an aerobically driven cardiac metabolism (Farrell, 2007). Additionally, diminishing f_h allows blood to remain in the gills for longer, providing additional uptake of environmental O_2 into circulation (Randall & Shelton, 1963).

Control of f_h is directly tied to vagal tone in a frequency-dependent manner (Cobb & Santer, 1973). Upon recognition of a lower environmental O_2 content by chemoreceptors on the first-gill arch (Sundin et al., 1999), acetylcholine is released at a high-frequency (Cobb & Santer, 1973; Gannon, 1971). Acetylcholine is recognized by muscarinic receptors within the atrium (Holmgren, 1977), leading to hyperpolarization of pacemaker cells and a subsequent decrease in f_h (Saito, 1973).

Sympathetic stimulation of the heart is partially accomplished by increasing circulating levels of catecholamines from chromaffin cells under hypoxia, (Nilsson, 1976; Randall, 1982). This form of activation leads to an increased systemic vascular resistance and \dot{Q} in some fish species under hypoxic conditions (Axelsson & Fritsche, 1991; Stevens & Randall, 1967). An increased \dot{Q} and subsequent blood flow in the gills also functions to increase the pressure and dimensions of the lamellae, providing an elevated area for gas exchange to occur (Farrell et al., 1980).

Teleosts achieve an increased \dot{Q} capable of meeting environmental conditions and metabolic demands through increasing SV to compensate for the bradycardia (Stevens & Randall, 1967.; Wood & Shelton, 1980). This is directly linked to bradycardia establishing a more extensive diastolic period, elevating filling and the quantity pumped, and linked to an increased force of cardiac muscle contraction (Farrell, 2007). Previous studies have found that a taurine deficient (TD)-isolated perfused heart shows a blunted SV under hypoxia when a stable contractile frequency is imposed (Dixon et al., 2023). This may be attributed to the length of muscle contracture (Dixon et al., 2023), and could be associated with taurine-mediated inhibition of ATP-sensitive K^+ channels in the sarcolemma (Park et al., 2004). However, this effect was seen when a continual pacing frequency was administered, so its relevance to function *in vivo* is unclear. Investigation of the influence of taurine on these functional responses of the cardiovascular system *in vivo* is required to understand its implications on hypoxia sensitivity with neuronal connections maintained.

Taurine's Contribution to Cardiomyocyte Contraction under Hypoxia

Calcium (Ca^{2+}) is an essential ion to the contraction and relaxation of the heart muscle (Bers, 2002). Under stressful conditions such as hypoxia, this reliance becomes ever more critical, as shown through intracellular adaptations that elevate Ca^{2+} availability (Cros et al., 2014). Taurine is involved in Ca^{2+} handling and the contractile force development of the heart muscle (Henry & MacCormack, 2018). The co-transport of taurine with Na^+ into heart cells is linked to changes in Ca^{2+} flux (Bkaily et al., 1998). This effect is predicated on exchanging Ca^{2+} from the extracellular environment for Na^+ inside cardiomyocytes by the reversible Na^+ - Ca^{2+} exchanger (NCX) (Mechmann & Pott, 1986; Vornanen et al., 2002). Within the heart of teleosts, the NCX is a predominant mode of obtaining intracellular Ca^{2+} , combining to account for over 80% of intracellular stores with L-type (“long-lasting”) Ca^{2+} channels (Vornanen et al., 2002). Taurine is also believed to help regulate intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) and extracellular Ca^{2+} ($[\text{Ca}^{2+}]_o$) through direct manipulation of the L-type Ca^{2+} channel (Satoh & Sperelakis, 1993). In pacemaker cells, this association results in channel inactivation during an action potential (Satoh & Sperelakis, 1993).

Although lacking a mechanistic explanation, a lower Ca^{2+} sensitivity of cardiomyocytes occurs in TD trout, limiting the frequency of their contraction (Gates et al., 2022). This is hypothesized to be related to sarcoplasmic reticulum (SR) Ca^{2+} handling (Gates et al., 2022). During the absence of an environmental stressor, intracellular Ca^{2+} is mainly derived from transport across the sarcolemma (Cros et al., 2014). However, teleosts can also exploit the SR to manage Ca^{2+} homeostasis, with the SR becoming increasingly important during a stress response (Cros et al., 2014). Under hypoxia, a more pronounced Ca^{2+} dependence may be attributed to the elevated contractile force required to expel a greater quantity of blood from the heart. Taurine is believed to elevate the ability to manage SR Ca^{2+} stores under an O_2 limitation (Henry & MacCormack, 2018). A contributing factor may be the effect of taurine on elevating sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA2a) Ca^{2+} influx, shortening the duration of cardiomyocyte contraction (Dutka et al., 2014). Taurine specifically supports the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) phosphorylation of phospholamban (Ramila et al., 2015) and increases the uptake of Ca^{2+} into the SR by

SERCA2a (Cerra & Imbrogno, 2012). This occurs indirectly by limiting phosphatase 1 activity (Ramila et al., 2015). Figure 4 highlights the contribution of taurine in regulating intracellular Ca^{2+} homeostasis and the binding of myofilaments.

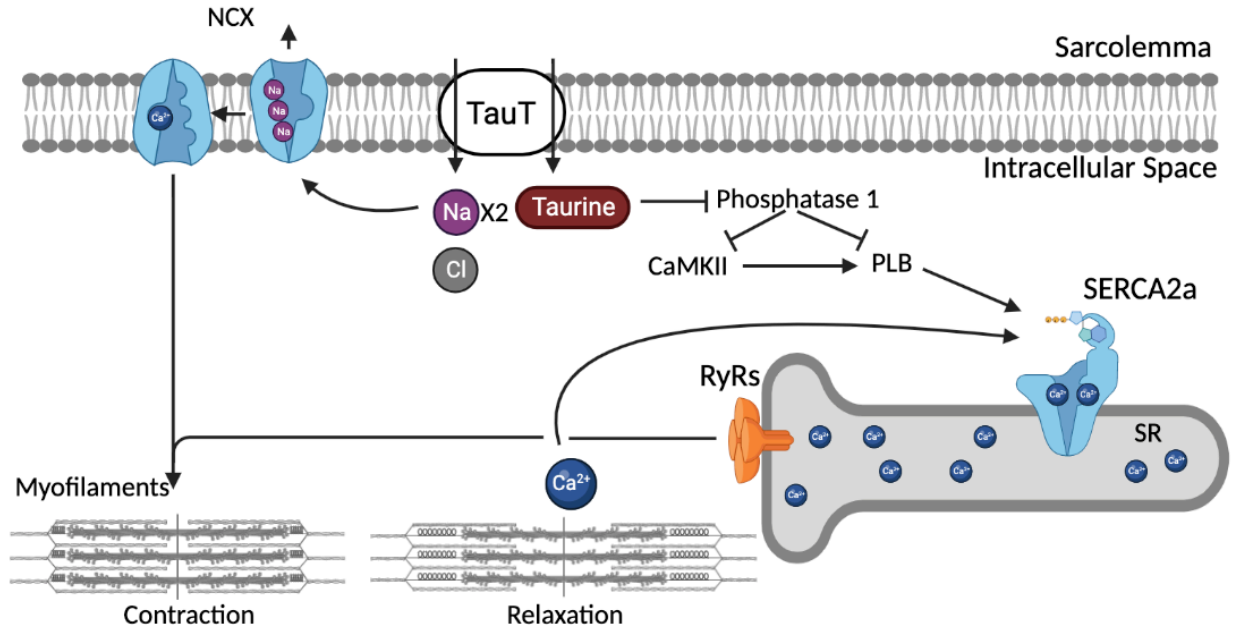


Figure 4: Cardiomyocyte calcium (Ca^{2+}) regulation and transport. Sodium (Na^+) and chlorine (Cl^-) are cotransported with taurine intracellularly through the taurine transporter (Tau-T). Na^+ is exchanged for Ca^{2+} through the Na^+ - Ca^{2+} exchanger (NCX) in a 3:1 stoichiometric relationship. Ca^{2+} is released from the sarcoplasmic reticulum (SR) through a channel established from ryanodine receptors (RyRs) and pumped back into the SR through the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA2a). Taurine supports SERCA2a activity by promoting phospholamban (PLB) phosphorylation through relieving inhibition of Ca^{2+} /calmodulin dependent protein kinase II (CaMKII) by phosphatase 1.

A role of taurine in cardiovascular function is further supported by a taurine deficit in mammalian hearts predisposing to dilated cardiomyopathy (Bélanger et al., 2005). Additionally, taurine enhances the healing of heart cells following ischemia when Ca^{2+} is limited (Öz et al., 1999). However, the mechanistic influence of taurine is not well-established for these impairments (Bkaily et al., 1998). The apparent contributions of taurine to Ca^{2+} homeostasis during O_2 limitation requires further investigation to provide a more thorough description of its contribution to cardiovascular function.

Taurine's Maintenance of Cardiomyocyte Osmolality under Hypoxia

When O₂ availability is insufficient to meet metabolic demands, anaerobic metabolism is often activated to compensate (Van den Thillart & van Waarde, 1985), leading to the buildup of anaerobic end products and osmotic disruption (Rees et al., 2009). This has shown to increase intracellular osmolality by over 50 mM under hypoxia (Steenbergen et al., 1985; Tranum-Jensen et al., 1981). Maintaining an osmotic and ionic balance is key to the viability of these cells (Boutilier & St-Pierre, 2000). As lesser phosphorylation potential is found under anaerobic metabolism due to the redistribution of limited adenosine triphosphate (ATP) to vital cellular processes, the ability of ATPases to overcome an osmotic disequilibrium becomes compromised (Boutilier & St-Pierre, 2000). Taurine efflux from heart cells is hypothesized to be vital to maintaining a proper osmotic gradient and cell volume, contributing to the continued functioning of the heart (Salze & Davis, 2015; Schaffer et al., 2010).

Taurine's Influence on Cardiomyocyte Metabolism

Discrepancies in metabolic substrate preferences during taurine supplementation have been observed in cardiac tissue (MacCormack et al., 2016). More specifically, glucose use increases within the heart muscle when saline perfusing the heart is supplemented with taurine (MacCormack et al., 2016). In mammals, this correlates with a greater insulin sensitivity eliciting further use of glycolysis (Imae et al., 2014; Lampson et al., 1983). Although there have been conflicting findings on the use of glucose within cardiac tissue under hypoxia in aquatic species (Clow et al., 2004; MacCormack & Driedzic, 2007), the implications of taurine on glucose use could impact the metabolic capacity of the hypoxic heart. A common anaerobic end product is lactate, which accumulates to increase osmolarity within cardiomyocytes (Tranum-Jensen et al., 1981). Lactate is also capable of being used as a fuel source in teleost cardiomyocytes (Lanctin et al., 1980). The use of lactate functions to bypass the energy investment phases of glycolysis and is preferentially used over glucose in isolated teleost hearts when O₂ is available (Lanctin et al., 1980). Measuring lactate concentrations in the cardiac tissue of TD and control fish may show discrepancies in metabolic preferences and osmotic

disturbances, two important features to maintaining cardiomyocyte function under O₂ limitation (Salze & Davis, 2015; Schaffer et al., 2010).

Model Organism and Taurine-Deficiency

Brook char (*Salvelinus fontinalis*) is a species of freshwater fish that characteristically live in highly aerated freshwater, hypothesized to explain their preference for low-temperature environments (Creaser, 1930). These fish avoid habitats where O₂ concentrations fall below 4 mg/L, having a critical O₂ partial pressure (P_{crit}) slightly above this mark and showing preference for O₂ levels above 5mg/L (Spoor, 1990; Wahl, unpublished). This level of sensitivity makes this species an excellent model organism to study cardiac mechanisms underlying hypoxia tolerance (Spoor, 1990). Structural analogs may be used to decrease tissue taurine levels by binding and blocking taurine transporters (Jacob et al., 1991). Beta-alanine (β -alanine) is one such analog capable of inhibiting taurine transport in cardiomyocytes by both PAT1 and Tau-T (Anderson et al., 2009; Rasmussen et al., 2016), slowing taurine buildup under hypertonicity (Han et al., 2006). A feed of only 3% β -alanine significantly decreases heart taurine in teleosts (Gates et al., 2022), enabling a TD model to be achieved through a β -alanine-supplemented diet.

Research Goals and Hypothesis

This experiment analyzed the cardiac parameters of TD and control brook trout *in vivo* upon exposure to environmental O₂ conditions above and below their preferred level. f_h , SV, and \dot{Q} were measured to quantify functional differences between the control and TD model. Analyzing the electrical activity of control and TD teleost hearts *in vivo* under hypoxia will provide insight into differences in the cardiac excitability or contractile function attributed to taurine. Coupling this reading with a blood flow measurement will elucidate whether a taurine deficiency compromises the typical cardiovascular response to hypoxia.

Increasing RBC counts and hemoglobin concentrations are hematological changes observed in some teleost species in O₂-deficient environments, correlating to the circulatory system's O₂-carrying capacity (Affonso et al., 2002; Gallagher & Farrell,

1998). These parameters require investigation in control and TD fish to see if hematological compensation is employed in association with functional disruptions to the cardiovascular system. Additionally, a cardiac and plasma lactate analysis will uncover any metabolic disturbances or stress discrepancies attributed to taurine under hypoxia. Altogether, these metrics are important to further understanding taurine's contribution to the functional capacity of the cardiovascular system under hypoxia for freshwater fish.

We hypothesize that the TD fish will have a blunted bradycardia and a smaller SV response to acute hypoxia; however, we predict \dot{Q} to be unchanged. This prediction is based on taurine's support of cardiac contractility and maintenance of osmolality, coupled with the blunted SV found in an *in vitro* TD model upon hypoxia exposure (Dixon et al., 2023). We further hypothesize that the hematological indices will differ between the two groups, with TD fish exhibiting a lesser adjustment. These findings would support TD fish being more sensitive to environmental hypoxia, reinforcing taurine's contribution to proper cardiovascular function.

METHODS

Ethics Declaration

The procedures performed in this study were in accordance with guidelines set by the Canadian Council of Animal Care and Mount Allison University Animal Care and Use Committee (Protocol #101873 and 103144).

Fish Diet and Handling

Brook char (*Salvelinus fontinalis*; 585.9 ± 27.8) of either sex were obtained from the University of New Brunswick Aqualab and held in two recirculating 750L freshwater tanks separated based on diet treatment at the Harold Crabtree Aqualab facility at Mount Allison University (MtA). Both recirculating tanks were filtered, aerated, and maintained at 16.3 ± 3 °C. Fish were fed to satiation once a day with either a control feed composed of 3 mm VITA salmonid chow or a 3 mm VITA chow vacuum coated with 5% β -alanine to be used as a TD model.

Sampling Procedures and Hematology

Following experimentation, fish were anesthetized with a knockout dose of SYCAINE tricaine methanesulfonate (MS 222) (Syndel, Nanaimo, B.C., Canada, Product No. 02168510) at a concentration of 300 mg L⁻¹ combined with 600 mg L⁻¹ sodium bicarbonate (NaHCO₃) to neutralize the acidic MS 222. A heparinized needle was inserted beneath the anal fin to collect approximately 2 mL of blood from the caudal artery. Blood was immediately transferred onto ice in preparation for hematological analyses. Fish were then euthanized by severing the spinal cord before tissue sampling. A mass of each heart's ventricle was recorded, and collected tissues were flash-frozen in liquid nitrogen (N₂). Blood hemoglobin was recorded using an HB201 meter (Hemocue AB, Ängelholm, Sweden) utilizing a fish blood correction as per Clark, Eliason, et al., (2008). A hematocrit reading was obtained in doublet by collecting a portion of the blood sample into a capillary tube before being centrifuged at 500 x g. Measurement of the relative height of RBC to the entire blood sample volume quantified hematocrit. Excess blood was centrifuged at 500 x g for 5 mins in a microcentrifuge tube, with subsequent isolation of plasma and RBC before flash-freezing the samples in liquid N₂. Plasma osmolality was quantified after thawing on ice using a vapor pressure osmometer (VAPRO 5520; Wescor Inc., Logan, UT) and measured in triplicate. Both tissue and blood samples were held at -80°C.

Electrocardiogram Acute Hypoxia and Reoxygenation

A total of 8 control (562.3 ± 58.1g) and 6 TD (521.3 ± 37.8g) brook char were used for f_h analysis. Fish were taken from Aqualab tanks and anesthetized at a knockout dose of MS 222 (300 mg L⁻¹ MS 222, 600 mg L⁻¹ NaHCO₃). Following the loss of equilibrium (LOE), fish were placed onto a foam table equipped with aerated water at 16 ± 0.1°C housing a maintenance dose of MS 222 (83 mg L⁻¹ MS 222, 166 mg L⁻¹ NaHCO₃) flowing over the gills of the fish for continued anesthetization. A platinum subdermal needle electrode (AD Instruments; Product No. MLA1213-DC-19A) was placed beneath the fish's skin rostral to the stomach on either side of the heart, with the electrode leads attached to the lateral side of the fish as shown in Figure 5.

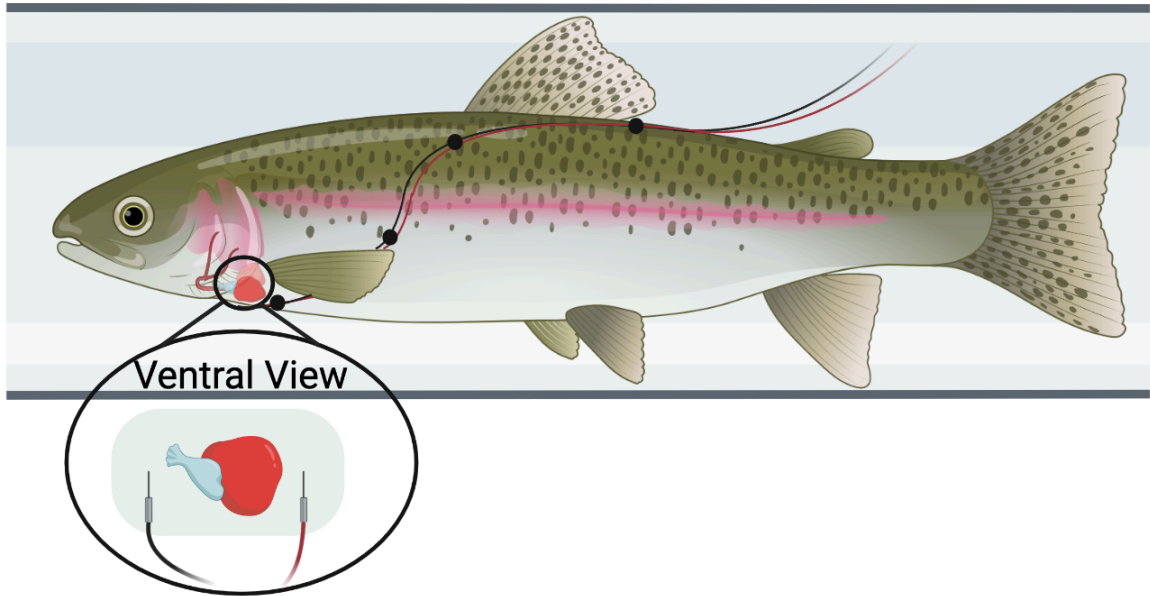


Figure 5: Electrode needle and cord placement for electrocardiogram (ECG) measured heart rate (f_h) of brook char (*Salvelinus fontinalis*) during acute hypoxia and reoxygenation. The enlarged portion depicts needles positioned on either side of the heart beneath the skin surfacing the stomach. Black dots represent the locations of the suture placement to prohibit cord tangling.

Following electrode placement, fish were transferred to a perforated polyvinyl chloride (PVC) pipe in a 30L anesthetic-free freshwater tank maintained at $16 \pm 0.1^\circ\text{C}$ with continuous aeration and filtration. Fish were provided with limited maneuverability, although movement was hypothesized to have negligible confounding effects as previous findings describe the bradycardia response unchanged in response to restraint (Smith & Jones, 1978). This environment prevented electrocardiogram (ECG) cords from becoming intertwined. Fish were left to rest for 18-24 h before acute hypoxia treatments.

The f_h was recorded using a PowerLab 8/35 system (AD Instruments; Australia) linked to a BioAmp amplifier (AD Instruments; Australia) and associated Lab Chart 8 software. N_2 bubbling declined O_2 levels to 12.5 and then 8.33 kPa O_2 , which were measured using a WTW Oxi 3210 dissolved O_2 meter (Weilheim, Germany). Fish were maintained at each level of O_2 for 30 minutes. Reoxygenation was performed for 90 minutes by continual aeration to quantify recovery. An f_h measurement was continuously recorded for recovery, hypoxia, and reoxygenation. Following reoxygenation, fish were euthanized along with tissue sampling, as described above.

Ultrasonic Flow Probe Acute Hypoxia and Reoxygenation

A total of 6 control ($680.9 \pm 68.3\text{g}$) and 7 TD ($568.9 \pm 54.6\text{g}$) brook char were used for analysis of SV and \dot{Q} coinciding with f_h . Fish were taken from housing tanks, exposed to a knockout dose of anesthetic (300 mg L^{-1} MS 222, 600 mg L^{-1} NaHCO_3), and placed on a foam table as described above for the ECG analysis. Temperature-controlled freshwater ($16 \pm 0.1\text{ }^\circ\text{C}$) was administered over the gills supplemented with pure O_2 due to the more extensive procedural length. Fish were placed on their side with a wetted cloth strip fitted through the mouth and gills of the fish and mounted to a retort stand to elevate the branchial tissue for operation. A small incision was made in the skin beneath the ventral branchial tissue, anterior to the pectoral fin, to uncover the ventral aorta. A small or medium-sized ultrasonic flow probe was carefully positioned on the outside of the ventral aorta to avoid circulatory disruption to measure blood flow from the heart (Transonic Systems Inc., Ithaca, New York, USA; Product Nos. MC2PSB-JS-WC90-CRS10-GX and MC1.5PRB-JS-WC60-CRS10-GX). The posterior part of the probe was positioned to face the medial region of the fish to avoid dislodgement, with the probe's cable sutured to the side of the fish to avoid tangling. Following the operation, fish were placed within the same PVC pipe apparatus described in the ECG analysis in temperature-controlled freshwater at $16 \pm 0.1^\circ\text{C}$ with constant aeration and filtration. Figure 6 displays the probe placement and cable securement.

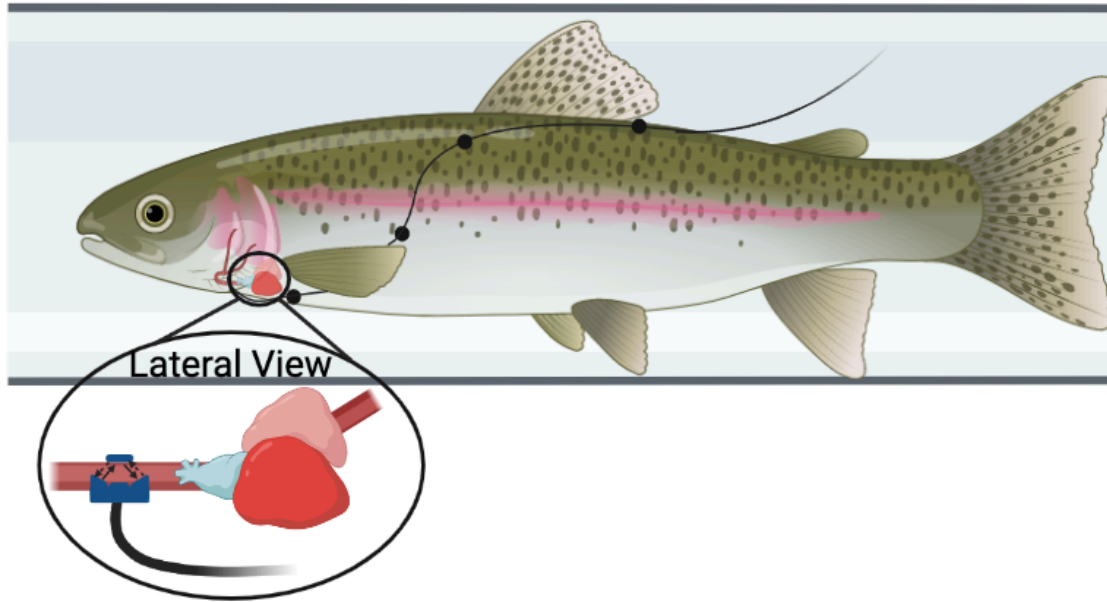


Figure 6: Flow probe and cable placement for ultrasonic flow probe measured cardiac output (\dot{Q}) and stroke volume (SV) of brook char (*Salvelinus fontinalis*) during acute hypoxia and reoxygenation. The enlarged portion depicts the location of probe placement on the ventral aorta, oriented to avoid blood flow restriction or dislodgement. Black dots represent the locations of the suture placement.

Flow data was recorded using a PowerLab 4/26 system (ADInstruments; Australia) linked to a transit-time perivascular flowmeter (Transonic Systems Inc., Ithaca, New York, USA; Product No. T402A91229). The sequence of acute hypoxia and reoxygenation was identical to that performed for the ECG experiment.

Molecular Quantifications

Plasma and Heart Taurine

Flash-frozen heart tissue was homogenized in 1.5 M perchloric acid (PCA) at a volume 10 times greater than the mass, taken with Kimble Kontes Teflon pestles (DWK Life Sciences, NJ, USA) and a wand-type sonicator (F60 Sonic Dismembrator; ThermoFisher Scientific, Waltham, MA, USA). Frozen plasma samples were thawed before a uniform 50 μ L volume was abstracted. Two double distilled water (ddH₂O) PCA volumes were added to the homogenates before neutralization was completed with a 0.5 PCA volume of 3M potassium bicarbonate (KHCO₃). Samples were homogenized at 3000 x g for 5 mins at 4 °C. High-performance liquid chromatography (HPLC) was used

for taurine analysis with a 1200 series HPLC containing a diode array detector (Agilent; Santa Clara, CA, USA). Samples were run through a 150 x 4.6 mm ACE Equivalence C18 column (ACE Equivalence, EQV-5C18-1546). Four taurine standards were used and comprised 0, 1, 10, and 20 mmol L⁻¹ taurine. The mobile phase contained a phosphate buffer at 20 mmol L⁻¹ (pH 7.4) and a 45:45:10 methanol:acetonitrile:ddH₂O solution. Before entry into the column, a 0.4 mol L⁻¹ borate buffer (pH 10.2) was mixed with each sample, along with a 1 mL volume of both phthaldialdehyde reagent and 2-mercaptoethanol in-needle for derivatization.

Plasma and Heart Lactate

Flash-frozen plasma and heart samples were thawed on ice before mixing with 6% PCA at a volume 5 x that of the plasma volume or 9 x that of the heart mass. Heart tissue homogenization was performed using a PowerGen 125 sonicator (Fisher Scientific; Hampton, New Hampshire, USA; Product No. LR60902) in 3 spurts of 15 seconds with 30 seconds in between where the samples were left on ice. All samples were centrifuged at 10 000 x g for 5 mins with supernatant taken and pellets discarded. Standards at concentrations of 0, 0.5, 1, 1.5, and 2 mM L-lactate were made in 6% PCA and used to generate a standard curve. Standards and samples were prepared in 0.2 M glycine buffer during absorbance readings (Sigma Life Sciences; Product No. 1003137489). Triplicate readings were taken at 340nm, quantifying the enzymatic activity of lactate dehydrogenase (LDH) taken from bovine heart (CALZYME Laboratories, Inc.; San Luis Obispo, CA, USA; Product No. 93401) through the reduction of NAD⁺ to NADH in a SpectraMax 190 microplate spectrophotometer (Molecular Devices; Sunnyvale, CA, USA; Product No. 94089).

Statistical Analyses

Prism 5 Software (GraphPad; San Diego, CA, USA) was used in completing the statistical analyses. Grubbs' test was used in detecting outliers. Unpaired t-tests identified significant differences between experimental and control group hematological parameters, taurine, and lactate. An F test verified equal variances, and a Shapiro-Wilk test assessed normality. A non-parametric Mann-Whitney test was used if variances differed. A 2-way repeated measures ANOVA assessed f_h , SV, and \dot{Q} for a significant

effect of taurine. An unpaired t-test was again used to compare the cardiac parameters of TD and control groups to find and specifically identify significant interactions and differences. A *P*-value of 0.05 was used to identify significance. Numerical data are presented as the mean \pm SEM, while box and whisker plots display the median values and the data range.

RESULTS

Morphological discrepancies of the cardiac system attributed to a taurine deficiency were analyzed by comparing relative ventricular mass following exposure to acute hypoxia and reoxygenation. No difference ($p = 0.801$) was found between the control and TD brook char at 0.084 ± 0.003 % and 0.082 ± 0.004 %, respectively ($n = 15$ for control and 16 for TD fish).

Effect of a Taurine Deficiency on Cardiac Parameters

The 12.5 kPa O₂ exposure was insufficient to induce consistent bradycardia in control fish, while a stereotypical bradycardia response was consistently observed at the 8.33 kPa O₂ exposure. As such, the 8.33 kPa O₂ level was chosen as the marker of environmental hypoxia for analysis of cardiac parameters.

ECG f_h and Taurine

Plasma taurine was significantly lower in the TD fish (Fig. 7A; $p = 0.0045$) following the same acute hypoxia and reoxygenation as controls. In contrast, heart taurine was similar between TD and control fish (Fig. 7B; $p = 0.787$). Additionally, control brook char exposed to acute hypoxia and reoxygenation exhibited a 15.5% lesser heart taurine content than control fish not exposed to the environmental stressor (Dixon et al., 2023). In contrast, TD fish displayed a negligible difference in cardiac taurine based on whether they experienced acute hypoxia (Dixon et al., 2023). A significant interaction between treatment and O₂ level was initially found in a 2-way repeated measures ANOVA analyzing absolute f_h during normoxia (20.8 kPa O₂), hypoxia (8.33 kPa O₂), and reoxygenation (20.8 kPa O₂). Unpaired t-tests were subsequently used to analyze the effect of the taurine deficiency at each O₂ level on f_h and the degree of change in f_h between O₂ levels. The TD brook char were found to have a lower resting f_h at initial

normoxia (Fig. 7C; $p = 0.0426$). Additionally, a blunted bradycardia response was observed for the TD fish upon exposure to hypoxia (Fig. 7D; $p = 0.0010$). f_h was similar between control and TD fish during reoxygenation and in the change in f_h between hypoxia and the reoxygenation.

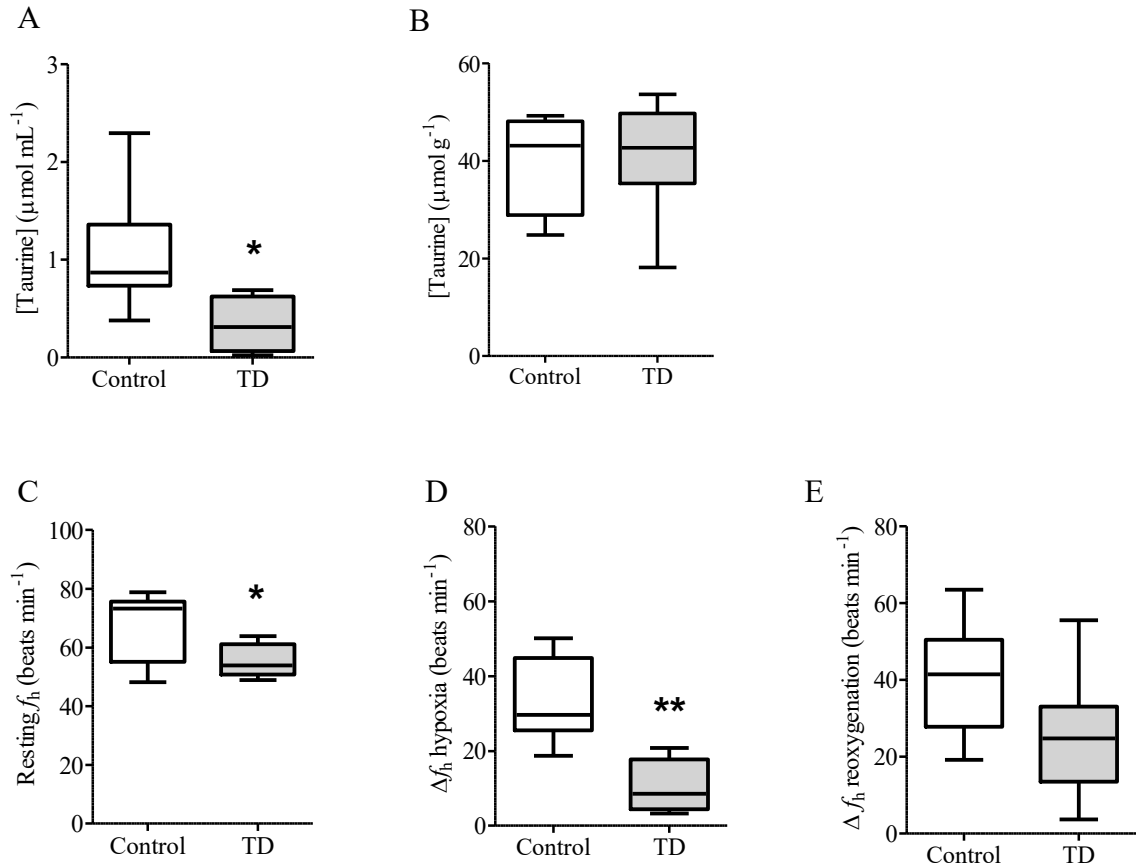


Figure 7: Brook char (*Salvelinus fontinalis*) plasma (A) and heart (B) taurine levels of taurine deficient (TD) and control ($n = 8$ for both groups) fish following 30 min exposures at 12.5 and 8.33 kPa O_2 and 90 minutes of reoxygenation to 20.8 kPa O_2 . Normoxic (20.8 kPa O_2) resting heart rate (f_h) (C), the difference in heart rate (Δf_h) between normoxia and hypoxia (8.33 kPa O_2) (D), and Δf_h from hypoxia and 90 mins of reoxygenation to normoxia (Δf_h reoxygenation) (E) of TD ($n = 6$) and control ($n = 8$) brook char. Asterisks represent significant differences between control and TD fish (* $p < 0.05$, ** $p \leq 0.001$).

The control brook char exhibited a 49% decrease in f_h in response to hypoxia, representing a characteristic bradycardia response, while the TD model reduced f_h by only 21%. For a more comprehensive analysis of cardiac function, potential disturbances in SV and \dot{Q} were subsequently evaluated in association with f_h for a new set of TD and

control brook char using an ultrasonic flow probe followed by an identical sequence of exposures to acute hypoxia and reoxygenation.

Ultrasonic Flow Probe f_h , SV, and \dot{Q}

An outlier in resting f_h of the control group dataset was detected using Grubb's test, and data from that fish was dropped from analysis. Similar trends were again observed for f_h between control and TD fish. Resting f_h was lower in TD fish (Fig. 8A; $p = 0.0008$), along with the f_h following recovery to normoxia (Fig. 8A; $p = 0.0001$). Additionally, there was a trend in control fish experiencing a larger decrease in f_h between normoxia and hypoxia ($p = 0.0778$).

SV represents the amount of blood pumped from the heart with every beat. There was no difference in SV between TD and control fish at any point in the exposure sequence, however control fish exhibited a significant increase in SV during hypoxia (Fig. 8B; $p = 0.0212$) and a decrease following reoxygenation (Fig. 8B; $p = 0.0152$), while TD fish exhibited no change.

Characterizing the amount of blood pumped by the heart per unit of time, \dot{Q} , under hypoxia is determined by the degree of SV increase to compensate for the bradycardia response (Gamperl & Driedzic, 2009). Although \dot{Q} was consistently lower for the TD group at each level of O_2 , there were no significant effects of taurine on \dot{Q} during or between the different O_2 exposures. Additionally, both groups protected \dot{Q} under hypoxia as no changes were found in \dot{Q} between oxygenation states.

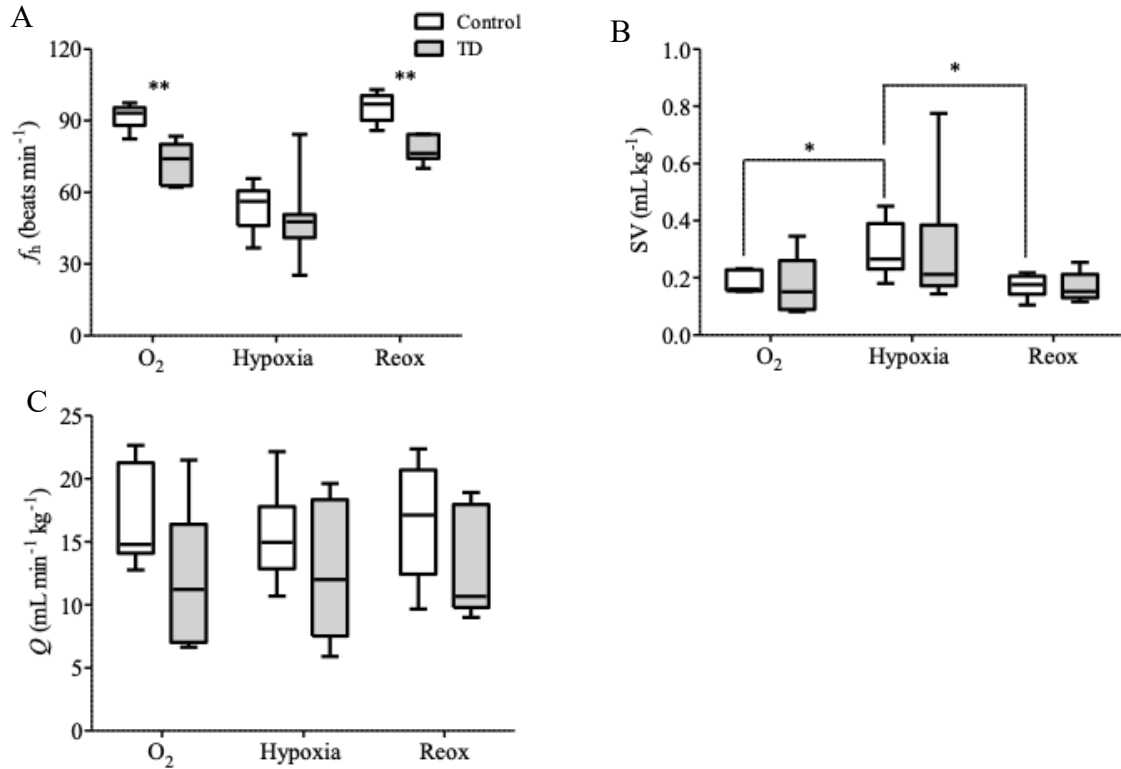


Figure 8: Control ($n = 6$) and taurine-deficient (TD) ($n = 7$) brook char (*Salvelinus fontinalis*) heart rate (f_h) (A), stroke volume (SV) (B), and cardiac output (\dot{Q}) (C) during normoxia at 20.8 kPa O₂ (O₂), 8.33kPa O₂ (Hypoxia), and reoxygenation to 20.8 kPa O₂ (Reox). Asterisks represent significant differences between control and TD fish (* $p < 0.05$, ** $p \leq 0.001$).

Lactate and Hematology

Lactate is a predominant anaerobic end product and stress marker. No difference in plasma lactate was found between the control and TD fish following acute hypoxia and reoxygenation (Fig. 9A; $p = 0.898$). In contrast, TD fish had significantly less heart lactate as compared to the controls (Fig. 9B; $p = 0.0118$).

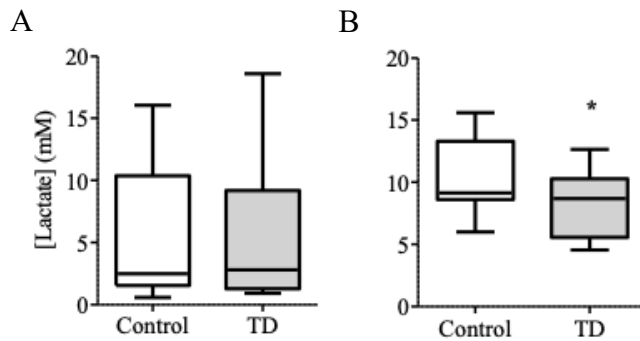


Figure 9: Plasma (A) and heart (B) lactate of TD and control (n = 8 for both groups) brook char (*Salvelinus fontinalis*) following 30 min exposures at 12.5 and 8.33 kPa O₂ and 90 minutes of reoxygenation to 20.8 kPa O₂. Asterisks represent significant differences between control and TD fish (* p < 0.05).

Hematological indices were collected in ECG and flow probe experiments following acute hypoxia and reoxygenation to quantify the O₂-carrying capacity of the blood. Osmolality was also measured to quantify and compare osmotic strength. No differences were found between the control and TD fish for all metrics shown in Table 1.

Table 1: Brook char (*Salvelinus fontinalis*) electrocardiogram (ECG) and ultrasonic flow probe recorded hematological parameters and plasma osmolality following 30 min exposures at 12.5 and 8.33 kPa O₂ before 90 mins of reoxygenation to 20.8 kPa O₂. MCHC represents mean corpuscular hemoglobin concentration.

Experiment	ECG		Flow Probe	
	Control	TD	Control	TD
Plasma Osmolality (mOsmol kg ⁻¹)	296.9 ± 7.7	295.8 ± 3.0	291.7 ± 7.2	284.6 ± 6.3
Hemoglobin (g L ⁻¹)	70.2 ± 4.3	60.0 ± 4.1	59.2 ± 4.0	70.2 ± 3.5
Hematocrit (%)	41.4 ± 2.2	37.8 ± 1.7	34.6 ± 1.6	35.6 ± 2.6
MCHC (g L ⁻¹)	171.0 ± 10.6	169.0 ± 8.6	171.2 ± 14.3	197.1 ± 14.8

With no differences between treatment groups in hemoglobin, hematocrit or mean corpuscular hemoglobin concentration (MCHC), O₂ transport within the circulatory system likely did not differ between treatment groups.

DISCUSSION

Heart complications such as cardiomyopathy, inhibition of excitation-contraction coupling, and a lesser ability to cope with oxidative stress and osmoregulatory challenges have all been attributed to a taurine deficiency (Schaffer et al., 2010). As freshwater fish face a unique susceptibility to osmotic disturbances in their environments, taurine is an important component to their maintenance of cell integrity and function. Furthermore, taurine's specific contribution to maintaining cardiovascular function while exposed to hypoxia in aquatic species has yet to be well described (Satoh & Sperelakis, 1998). This study established a TD model, introduced a hypoxia stressor, and quantified cardiac, hematological, and stress-related parameters. TD fish not exposed to a hypoxia stressor had 21% less taurine than control animals held under similar conditions (Dixon et al., 2023). The decline in taurine is associated with a more pronounced sensitivity to environmental hypoxia in the TD fish. These findings illustrate the contribution of taurine to cardiovascular function in freshwater teleosts, an important consideration given the progression of global warming.

Inhibited hypoxia-induced taurine transport through β -alanine supplementation

Within teleost and elasmobranch fish, cardiac taurine levels are typically between 25-76 $\mu\text{mol g}^{-1}$ and may vary by 20 $\mu\text{mol g}^{-1}$ across closely related species (Gates et al., 2022). Following exposure to acute hypoxia and reoxygenation, taurine levels dropped in control hearts but remained the same in TD hearts, thus making levels similar between them (Fig. 7B). The high plasma taurine in controls is evidence that it was released, and the low plasma taurine in TD fish suggests it was not, representing a 70% difference (Fig. 7A). A lack of taurine export from the intracellular environment upon exposure to the hypoxia-mediated osmotic stress likely explains this disparity. It is reasonable to assume this is attributed to β -alanine supplementation and the disruption of Tau-T mediated taurine efflux during hypoxia (Anderson et al., 2009).

Effect of a taurine deficiency on O_2 uptake and transport

An increase in hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration (MCHC) have been observed in teleosts during hypoxia exposure (Affonso

et al., 2002). This study found no differences between TD and control fish in these hematological parameters (Table. 1), suggesting the O₂ transport capacity of the blood was not affected by taurine deficiency. Additionally, no differences in the morphometrics of the fish gills were observed between TD and control fish upon exposure to environmental hypoxia (Dixon et al., 2023). Cumulatively, these findings indicate that O₂ uptake and transport within circulation aren't likely to be limiting throughout the hypoxia exposure for TD fish.

TD fish accumulate less heart lactate under hypoxia

Blood lactate levels in teleosts are typically between 0.5-2 mM (Wardle, 1978), acting as a metabolic substrate and systemic signaling molecule, specifically promoting ventilation during hypoxia (Thomsen et al., 2017). Following acute hypoxia and reoxygenation, lactate levels rose considerably in both TD and control fish, indicative of anaerobic metabolism and potential osmotic stress. However, this stress is believed to be comparable between groups based on there being no difference in plasma osmolality (Table. 1).

When experiencing O₂ deprivation, lactate provides an efficient fuel for cardiac metabolism (Driedzic, 1978). Control fish were found to have a significantly greater (by 22%) amount of cardiac lactate (Fig. 9B). When used as a substrate in aerobic metabolism, lactate is first enzymatically converted to pyruvate by the reversible lactase dehydrogenase (LDH) enzyme (Gesser & Poupa, 1975) before entering the citric acid cycle and electron transport chain (Lanctin et al., 1980). Lactate can be utilized as a fuel by the teleost heart, exhibiting a greater level of oxidation than glucose when offered separately and preferentially used when provided together (Driedzic & Hart, 1984; Lanctin et al., 1980). A mammalian model has shown a 30% decrease in cardiomyocyte O₂ consumption under a taurine deficiency (Jong et al., 2012). A lesser O₂ uptake may partially explain the diminished production of lactate through an inferior activation of anaerobic metabolism. Furthermore, the ability of the teleost heart to sustain function under hypoxia is believed to be associated with the anaerobic metabolism of the myocardium, of which lactate is an end product (Farrell, 1984). An inability to mitigate

cell swelling through taurine efflux may have additionally led to an accumulation of water within the cardiomyocytes and diluted the cardiac lactate present.

Lower resting f_h in TD fish

The TD fish exhibited lower resting f_h compared to the control fish by a difference of 23% (Fig. 7C, Fig. 8A). This could be related to alterations to the activity of the heart pacemaker and humoral responses potentially associated with taurine's interaction with the neurotransmitter, GABA. GABA is an inhibitory neurotransmitter, acting to diminish the parasympathetic activity of the vagus nerve, leading to an increased f_h (Bentzen & Grunnet, 2011). Previous studies have found that taurine is an agonist of GABA receptors (El Idrissi & L'Amoreaux, 2008), potentially contributing to a lower resting f_h in TD fish. However, the association is described as weak (Schaffer & Kim, 2018), and likely doesn't fully explain the pronounced decrease observed here. As shown in Figure 4, taurine also supports the relaxation phase of the Ca^{2+} transient by increasing SERCA2a activity of the SR (Ramila et al., 2015). As such, a taurine-deficiency may prolong the uptake of Ca^{2+} into the SR and decrease f_h , although the magnitude of this effect is unknown as fish typically don't rely heavily on SR Ca^{2+} stores (Shiels, 2011).

Three currents give rise to the membrane potential of the pacemaker; the calcium (I_{Ca}) and potassium (I_k) currents along with the established inward 'funny' current upon hyperpolarization (I_f) (Satoh, 1995). The I_f is hypothesized to have only a marginal influence, leaving the I_{Ca} and I_k as two main contributors to the hearts contractile frequency (Satoh, 1995). Mammalian studies have discovered different chronotropic effects of taurine based on extracellular Ca^{2+} levels (Satoh, 1995). Fish typically don't rely on the SR-housed Ca^{2+} , making transport of Ca^{2+} across the sarcolemma critical to the contraction of cardiomyocytes (Shiels, 2011). Taurine is believed to partially control the concentration of $[Ca^{2+}]_i$ and $[Ca^{2+}]_o$ through manipulation of Ca^{2+} channels within the sarcolemma in an $[Ca^{2+}]_i$ -dependent manner (Satoh & Sperelakis, 1993). Within cardiac tissue, two predominant forms of Ca^{2+} channels are present, represented by L-type, or "long-lasting," and T-type, or "transient," named for the duration of their activation (Shah et al., 2022). L-type channels represent the principal transporter responsible for generating the I_{Ca} that contributes to membrane depolarization (Shah et al., 2022).

Specifically, unless experiencing excessive $[Ca^{2+}]_i$, taurine shortens the action potential duration (APD) through manipulation of the voltage-dependent component of I_{CaL} inactivation (Sato, 1995; Sato & Sperelakis, 1993). The effect is hypothesized to be mediated through the direct association of taurine with the L-type channels (Sato & Sperelakis, 1993). This has previously been tied to a higher f_h through a shorter QT interval of the contractile cycle relating to ventricle depolarization (Attwell et al., 1981), as represented in Figure 10.

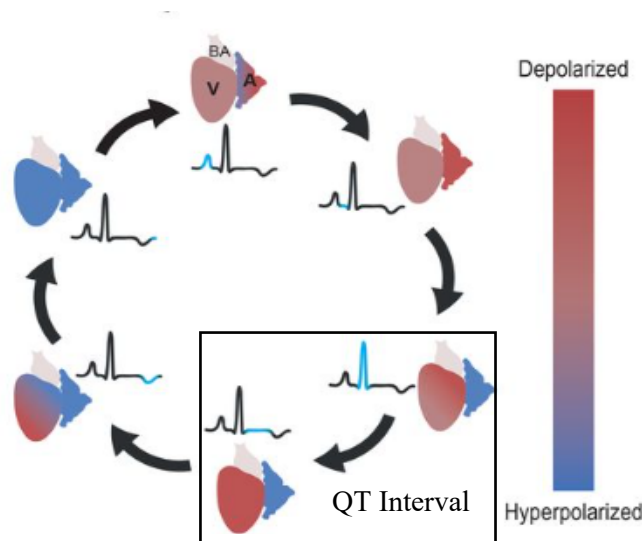


Figure 10: The contractile cycle of the teleost heart and corresponding electrocardiogram (ECG) signal. V represents the ventricle, A represents the atrium, and BA represents the bulbous arteriosus. Depolarization is shown in red, and hyperpolarization is shown in blue. QT interval is labelled and represents ventricle depolarization. Adopted from Arel et al., (2022).

As such, a lower cardiac taurine content could contribute to a diminished resting f_h through I_{CaL} disturbance. However, whether the level of deficiency in cardiac taurine established in this experiment could elicit such a response is unknown and will require further investigation. Future analysis of the ECG signal acquired for the TD fish may reveal changes in the QT interval and clarify the importance of this mechanism.

TD fish have a blunted bradycardia and SV increase under hypoxia

The hypoxia bradycardia was reduced by 93% in TD fish (Fig. 7D). Under normal conditions, the bradycardia in teleosts is mediated by chemoreceptors on the first-gill arch causing an acetylcholine-induced inhibitory tone, due to an elevation in K^+

conductance (Sakmann et al., 1983; Fritsche, 1990; Randall, 1982; Saito, 1973; Smith & Jones, 1978). This culminates in a more negative membrane potential and hyperpolarization of the pacemaker (Saito, 1973). The blunted bradycardia in TD fish may be mediated through increased acetylcholinesterase activity, decreased acetylcholine release by the vagus, or fewer muscarinic receptors on the pacemaker to initiate the downstream signal cascade. The positive inotropic response to circulating catecholamines binding β -adrenergic receptors (Randall, 1982) of the atrium and ventricle (Gannon, 1970) may also overpower the inhibitory acetylcholine signal.

With TD fish having a diminished *in vitro* SV under hypoxia when pacing frequency was held constant (Dixon et al., 2023), we hypothesized a smaller decrease in f_h under hypoxia would function to protect \dot{Q} . Lacking neuronal input, the results of the *in vitro* model point towards a functional disruption at the tissue level. Additionally, a severe reduction in time to LOE under acute hypoxia exposure in TD fish (Dixon et al., 2023) supports the interpretation that the function of the cardiovascular system is compromised *in vivo*. The bradycardia response provides more O₂ to the spongy myocardium and an enlarged SV through increased diastolic filling (Frank-Starling Law) (Farrell, 2007). Additionally, an increased SV determines how \dot{Q} is handled under hypoxia, an important response used by trout to maintain circulatory function and systemic O₂ supply (Wood & Shelton, 1980). Although neither SV, nor \dot{Q} differed between the TD and control fish (Fig. 8B and C), SV increased significantly in response to hypoxia in the control fish, but not in TD fish (Fig.8B). Confirming our hypothesis, this appears related to the diminished bradycardia in TD fish under hypoxia, likely to maintain a consistent \dot{Q} . The pacing frequency of cardiac tissue is directly associated with SV, with elevated frequencies equating to lesser SV through inhibited diastolic filling time (Farrell et al., 1989). However, a lesser power output (PO) was further observed in the isolated perfused heart of TD fish, likely attributed to physiological disruption of the cardiac tissue (Dixon et al., 2023). The protection of \dot{Q} during hypoxia in teleosts is linked to increased nervous adrenergic activity (Farrell et al., 1989). With no significant differences in \dot{Q} found between O₂ levels within or between groups, hormonal discrepancies are unlikely.

As illustrated in Figure 4, the cotransport of taurine with Na^+ is believed to contribute to the I_{Ca} through the reversible function of the NCX (Vornanen et al., 2002). Under O_2 limitation, it is reasonable to assume that achieving maximum energetic efficiency is critical. Utilizing the SR for Ca^{2+} uptake under such conditions would represent the most effective means of Ca^{2+} transport (Vornanen et al., 2002). This phenomenon is attributed to the stoichiometric relationship of one ATP mediating the movement of one Ca^{2+} molecule when using the NCX, attributed to establishing the Na^+ gradient through the $\text{Na}^+\text{K}^+\text{ATPase}$, while two Ca^{2+} may be transported using SERCA2a (Vornanen et al., 2002). Taurine activates $\text{Na}^+\text{K}^+\text{ATPase}$ activity, while β -alanine does not (Hastings et al., 1985). The inability of TD fish to maintain cardiovascular function under hypoxia may be related to disruptions in Ca^{2+} handling through either impaired cotransport of taurine with Na^+ to enhance NCX function or limited activation of SR Ca^{2+} handling. The latter was previously found to be influenced by taurine under hypoxia in a model teleost, the Atlantic killifish (Henry & MacCormack, 2018).

Additionally, previous studies have found that blocking SR-housed Ca^{2+} flux prevented the effect of taurine administration from increasing the maximum tension development of isolated ventricular muscle strips (Gates et al., 2022). Although this phenomenon was observed only under high-pacing frequencies, it emphasizes the need for additional studies on the effects of taurine on SR-mediated Ca^{2+} release. The additional availability of Ca^{2+} from the SR may contribute to the elevation in SV and contractile strength of the cardiac tissue under hypoxia. With the energetic implications of taurine-related transport and contribution to cardiomyocyte contraction, further analysis of intracellular and extracellular Ca^{2+} coupled with characterizing transporter activity may help uncover a mechanistic explanation for the physiological disruption.

Conclusion and Future Directions

The findings of this study uncovered an increased sensitivity to acute environmental hypoxia in TD fish. A lower resting f_h , diminished hypoxic bradycardia, and lack of SV increase under hypoxia all coincided with taurine deficiency. Although these changes did not cause a difference in \dot{Q} , they represent functional disturbances to cardiovascular performance attributed with a lack of sufficient intracellular taurine. These

discoveries further support the contention that taurine is a cardio-protectant and an essential amino acid. Metabolic discrepancies were further found in cardiac tissue, specifically with TD fish accumulating less lactate under hypoxia. Although a mechanistic explanation remains elusive, this highlights the additional importance of taurine in sustaining cardiac energy metabolism in freshwater teleost.

Proteomics data has recently shown a decreased hepatic Csad expression in TD brook char (MacCormack, unpublished), a key rate-determining enzyme to taurine biosynthesis. In some teleost species, lack of Csad inhibits proper cardiovascular development (Chang et al., 2013). An increase in the activity of Csad was expected in the TD model to compensate for the deficiency. Understanding why hepatic Csad expression is lower in TD fish may help elucidate how fish may deal with low taurine diets in the wild and contribute to understanding how freshwater species will interact with their future environments.

With discrepancies in SV found between an *in vitro* TD isolated perfused heart and the *in vivo* model used in this study, it is likely that alterations in neuronal regulation of cardiac function contribute in some way to the phenotype of TD brook char. Analyzing the intrinsic mechanisms underlying f_h and contractile activation is an area of future research that may identify the specific effect of a taurine deficiency and any corresponding compensatory mechanisms. This could be accomplished with injections of atropine and propranolol, which block parasympathetic acetylcholine receptor activation and sympathetic (adrenergic) activity, respectively. Using these blockers during exposure to a similar acute hypoxic stressor may help uncover the specific receptors susceptible to taurine. Quantifying muscarinic receptor density or acetylcholinesterase activity may also help to uncover differences in nervous system regulation. Finally, measuring heart tissue osmolality may help quantify the osmotic challenges imposed on the cardiac tissue under hypoxia and how taurine contributes to the maintenance of heart function.

REFERENCES

- Affonso, E. G., Polez, V. L. P., Corrêa, C. F., Mazon, A. F., Araújo, M. R. R., Moraes, G., & Rantin, F. T. (2002). Blood parameters and metabolites in the teleost fish *Colossoma macropomum* exposed to sulfide or hypoxia. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(3), 375–382. [https://doi.org/10.1016/S1532-0456\(02\)00127-8](https://doi.org/10.1016/S1532-0456(02)00127-8)
- Allen, J. A., & Garrett, M. R. (1971). Taurine in Marine Invertebrates. In *Advances in Marine Biology*, *9*, 205–253. [https://doi.org/10.1016/S0065-2881\(08\)60343-0](https://doi.org/10.1016/S0065-2881(08)60343-0)
- Anderson, C. M. H., Howard, A., Walters, J. R. F., Ganapathy, V., & Thwaites, D. T. (2009). Taurine uptake across the human intestinal brush-border membrane is via two transporters: H⁺-coupled PAT1 (SLC36A1) and Na⁺- and Cl⁻-dependent TauT (SLC6A6). *The Journal of Physiology*, *587*(4), 731–744. <https://doi.org/10.1113/jphysiol.2008.164228>
- Anttila, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppänen, E., Kolari, I., & Nikinmaa, M. (2015). Warm acclimation and oxygen depletion induce species-specific responses in salmonids. *Journal of Experimental Biology*, *218*(10), 1471–1477. <https://doi.org/10.1242/jeb.119115>
- Arel, E., Rolland, L., Thireau, J., Torrente, A., Bechard, E., Bride, J., Jopling, C., Demion, M., & Guennec, J.-Y. L. (2022). *Characterization of the adult zebrafish electrocardiogram* <https://doi.org/10.1101/2022.02.02.478776>
- Attwell, D., Cohen, I., & Eisner, D. A. (1981). The effects of heart rate on the action potential of guinea-pig and human ventricular muscle. *The Journal of Physiology*, *313*(1), 439–461. <https://doi.org/10.1113/jphysiol.1981.sp013675>
- Audzijonyte, A., Barneche, D. R., Baudron, A. R., Belmaker, J., Clark, T. D., Marshall, C. T., Morrongiello, J. R., & van Rijn, I. (2019). Is oxygen limitation in warming waters a valid mechanism to explain decreased body sizes in aquatic ectotherms? *Global Ecology and Biogeography*, *28*(2), 64–77. <https://doi.org/10.1111/geb.12847>
- Auer, S. K., Salin, K., Rudolf, A. M., Anderson, G. J., & Metcalfe, N. B. (2015). The optimal combination of standard metabolic rate and aerobic scope for somatic

- growth depends on food availability. *Functional Ecology*, 29(4), 479–486.
<https://doi.org/10.1111/1365-2435.12396>
- Axelsson, M., Ehrenström, F., & Nilsson, S. (1987). Cholinergic and adrenergic influence on the teleost heart in vivo. *Experimental Biology*, 46(4), 179–186.
- Axelsson, M., & Fritsche, R. (1991). Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. *Journal of Experimental Biology*, 158(1), 181–198. <https://doi.org/10.1242/jeb.158.1.181>
- Bélangier, M. C., Ouellet, M., Queney, G., & Moreau, M. (2005). Taurine-Deficient Dilated Cardiomyopathy in a Family of Golden Retrievers. *Journal of the American Animal Hospital Association*, 41(5), 284–291.
<https://doi.org/10.5326/0410284>
- Bentzen, B. H., & Grunnet, M. (2011). Central and Peripheral GABA_A Receptor Regulation of the Heart Rate Depends on the Conscious State of the Animal. *Advances in Pharmacological Sciences*, 2011.
<https://doi.org/10.1155/2011/578273>
- Bers, D. M. (2002). Cardiac excitation–contraction coupling. *Nature*, 415(6868), 198–205. <https://doi.org/10.1038/415198a>
- Betancor, M. B., Laurent, G. R., Ortega, A., de la Gándara, F., Tocher, D. R., & Mourente, G. (2019). Taurine metabolism and effects of inclusion levels in rotifer (*Brachionus rotundiformis*, Tschugunoff, 1921) on Atlantic bluefin tuna (*Thunnus thynnus*, L.) larvae. *Aquaculture*, 510, 353–363.
<https://doi.org/10.1016/j.aquaculture.2019.05.040>
- Bitoun, M., & Tappaz, M. (2002). Gene Expression of Taurine Transporter and Taurine Biosynthetic Enzymes in Hyperosmotic States. *Advances in Experimental Medicine and Biology*. 483, 239–248. https://doi.org/10.1007/0-306-46838-7_26
- Bkaily, G., Jaalouk, D., Sader, S., Shbaklo, H., Pothier, P., Jacques, D., D’Orléans-Juste, P., Cragoe, E. J., & Bose, R. (1998). Taurine indirectly increases [Ca]ⁱ by inducing Ca²⁺ influx through the Na⁺-Ca²⁺ exchanger. *Molecular and Cellular Effects of Nutrition on Disease Processes*. 26, 187–197.
https://doi.org/10.1007/978-1-4615-5763-0_20

- Boutilier, R. G., & St-Pierre, J. (2000). Surviving hypoxia without really dying. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 126(4), 481–490. [https://doi.org/10.1016/S1095-6433\(00\)00234-8](https://doi.org/10.1016/S1095-6433(00)00234-8)
- Bröer, S. (2008). Amino Acid Transport Across Mammalian Intestinal and Renal Epithelia. *Physiological Reviews*, 88(1), 249–286. <https://doi.org/10.1152/physrev.00018.2006>
- Cerra, M. C., & Imbrogno, S. (2012). Phospholamban and cardiac function: A comparative perspective in vertebrates. *Acta Physiologica*, 205(1), 9–25. <https://doi.org/10.1111/j.1748-1716.2012.02389.x>
- Chang, Y.-C., Ding, S.-T., Lee, Y.-H., Wang, Y.-C., Huang, M.-F., & Liu, I.-H. (2013). Taurine homeostasis requires de novo synthesis via cysteine sulfinic acid decarboxylase during zebrafish early embryogenesis. *Amino Acids*, 44(2), 615–629. <https://doi.org/10.1007/s00726-012-1386-8>
- Clark, T. D., Eliason, E. J., Sandblom, E., Hinch, S. G., & Farrell, A. P. (2008). Calibration of a hand-held haemoglobin analyser for use on fish blood. *Journal of Fish Biology*, 73(10), 2587–2595. <https://doi.org/10.1111/j.1095-8649.2008.02109.x>
- Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G., & Farrell, A. P. (2008). Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 295(5), R1631-1639. <https://doi.org/10.1152/ajpregu.90461.2008>
- Clow, K. A., Rodnick, K. J., MacCormack, T. J., & Driedzic, W. R. (2004). The regulation and importance of glucose uptake in the isolated Atlantic cod heart: Rate-limiting steps and effects of hypoxia. *Journal of Experimental Biology*, 207(11), 1865–1874. <https://doi.org/10.1242/jeb.00965>
- Cobb, J. L. S., & Santer, R. M. (1973). Electrophysiology of cardiac function in teleosts: Cholinergically mediated inhibition and rebound excitation. *The Journal of Physiology*, 230(3), 561–573. <https://doi.org/10.1113/jphysiol.1973.sp010204>

- Cossins, A. R., & Gibson, J. S. (1997). Volume-Sensitive Transport Systems and Volume Homeostasis in Vertebrate Red Blood Cells. *Journal of Experimental Biology*, 200(2), 343–352. <https://doi.org/10.1242/jeb.200.2.343>
- Creaser, C. W. (1930). Relative Importance of Hydrogen-Ion Concentration, Temperature, Dissolved Oxygen, and Carbon-Dioxide Tension, on Habitat Selection by Brook-Trout. *Ecology*, 11(2), 246–262. <https://doi.org/10.2307/1930261>
- Cros, C., Sallé, L., Warren, D. E., Shiels, H. A., & Brette, F. (2014). The calcium stored in the sarcoplasmic reticulum acts as a safety mechanism in rainbow trout heart. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 307(12), R1493–R1501. <https://doi.org/10.1152/ajpregu.00127.2014>
- Davison, W. (1989). Training and its effects on teleost fish. *Comparative Biochemistry and Physiology Part A: Physiology*, 94(1), 1–10. [https://doi.org/10.1016/0300-9629\(89\)90775-5](https://doi.org/10.1016/0300-9629(89)90775-5)
- Dixon, T.-A., Rhyno, E.-L. M., El, N., McGaw, S. P., Otley, N. A., Parker, K. S., Buldo, E. C., Pabody, C. M., Savoie, M., Cockshutt, A., Morash, A. J., Lamarre, S. G., & MacCormack, T. J. (2023). Taurine depletion impairs cardiac function and affects tolerance to hypoxia and high temperatures in brook char (*Salvelinus fontinalis*). *Journal of Experimental Biology*, 226(4), jeb245092. <https://doi.org/10.1242/jeb.245092>
- Driedzic, W. R. (1978). Carbohydrate Metabolism in the Perfused Dogfish Heart. *Physiological Zoology*, 51(1), 42–50. <https://doi.org/10.1086/physzool.51.1.30158663>
- Driedzic, W. R., & Gesser, H. (1994). Energy metabolism and contractility in ectothermic vertebrate hearts: Hypoxia, acidosis, and low temperature. *Physiological Reviews*, 74(1), 221–258. <https://doi.org/10.1152/physrev.1994.74.1.221>
- Driedzic, W. R., & Hart, T. (1984). Relationship between exogenous fuel availability and performance by teleost and elasmobranch hearts. *Journal of Comparative Physiology B*, 154(6), 593–599. <https://doi.org/10.1007/BF00684413>

- Dutka, T. L., Lambolley, C. R., Murphy, R. M., & Lamb, G. D. (2014). Acute effects of taurine on sarcoplasmic reticulum Ca²⁺ accumulation and contractility in human type I and type II skeletal muscle fibers. *Journal of Applied Physiology*, *117*(7), 797–805. <https://doi.org/10.1152/jappphysiol.00494.2014>
- El Idrissi, A., & L'Amoreaux, W. J. (2008). Selective resistance of taurine-fed mice to isoniazide-potentiated seizures: In vivo functional test for the activity of glutamic acid decarboxylase. *Neuroscience*, *156*(3), 693–699. <https://doi.org/10.1016/j.neuroscience.2008.07.055>
- Farrell, A. P. (1984). A review of cardiac performance in the teleost heart: Intrinsic and humoral regulation. *Canadian Journal of Zoology*, *62*(4), 523–536. <https://doi.org/10.1139/z84-079>
- Farrell, A. P. (2007). Tribute to P. L. Lutz: A message from the heart – why hypoxic bradycardia in fishes? *Journal of Experimental Biology*, *210*(10), 1715–1725. <https://doi.org/10.1242/jeb.02781>
- Farrell, A. P., Small, S., & Graham, M. S. (1989). Effect of heart rate and hypoxia on the performance of a perfused trout heart. *Canadian Journal of Zoology*, *67*(2), 274–280. <https://doi.org/10.1139/z89-040>
- Farrell, A. P., Sobin, S. S., Randall, D. J., & Crosby, S. (1980). Intralamellar blood flow patterns in fish gills. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. *239*(5), 428-436. <https://doi.org/10.1152/ajpregu.1980.239.5.R428>
- Fritsche, R. (1990). Effects of hypoxia on blood pressure and heart rate in three marine teleosts. *Fish Physiology and Biochemistry*, *8*(1), 85–92. <https://doi.org/10.1007/BF00004435>
- Gallaugh, P., & Farrell, A. P. (1998). 6—Hematocrit and Blood Oxygen-Carrying Capacity. In S. F. Perry & B. L. Tufts (Eds.), *Fish Physiology*, *17*, 185–227. Academic Press. [https://doi.org/10.1016/S1546-5098\(08\)60262-9](https://doi.org/10.1016/S1546-5098(08)60262-9)
- Gamperl, A. K., & Driedzic, W. R. (2009). Chapter 7 Cardiovascular Function and Cardiac Metabolism. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology*, *27*, 301–360. Academic Press. [https://doi.org/10.1016/S1546-5098\(08\)00007-1](https://doi.org/10.1016/S1546-5098(08)00007-1)

- Gannon, B. J. (1971). A study of the dual innervation of teleost heart by a field stimulation technique. *Comparative and General Pharmacology*, 2(6), 175–183. [https://doi.org/10.1016/0010-4035\(71\)90008-5](https://doi.org/10.1016/0010-4035(71)90008-5)
- Gates, M. A., Morash, A. J., Lamarre, S. G., & MacCormack, T. J. (2022). Intracellular taurine deficiency impairs cardiac contractility in rainbow trout (*Oncorhynchus mykiss*) without affecting aerobic performance. *Journal of Comparative Physiology B*, 192(1), 49–60. <https://doi.org/10.1007/s00360-021-01407-4>
- Gesser, H., & Poupa, O. (1975). Lactate as substrate for force development in hearts with different isoenzyme patterns of lactate dehydrogenase. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 52(2), 311–313. [https://doi.org/10.1016/0305-0491\(75\)90070-x](https://doi.org/10.1016/0305-0491(75)90070-x)
- Han, X., Patters, A. B., Jones, D. P., Zelikovic, I., & Chesney, R. W. (2006). The taurine transporter: Mechanisms of regulation. *Acta Physiologica*, 187(1–2), 61–73. <https://doi.org/10.1111/j.1748-1716.2006.01573.x>
- Hastings, D. F., Welty, J. D., & Rohani, F. (1985). Taurine Stimulation of Isolated Hamster Brain Na⁺, K⁺-ATPase: Activation Kinetics and Chemical Specificity. *Journal of Neurochemistry*, 44(6), 1764–1769. <https://doi.org/10.1111/j.1471-4159.1985.tb07166.x>
- Henry, E. F., & MacCormack, T. J. (2018). Taurine protects cardiac contractility in killifish, *Fundulus heteroclitus*, by enhancing sarcoplasmic reticular Ca²⁺ cycling. *Journal of Comparative Physiology B*, 188(1), 89–99. <https://doi.org/10.1007/s00360-017-1107-4>
- Holeton, G., & Randall, D. (1967). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *The Journal of Experimental Biology*, 46(2), 317–327. <https://doi.org/10.1242/jeb.46.2.317>
- Holmgren, S. (1977). Regulation of the Heart of a Teleost, *Gadus morhua*, by Autonomic Nerves and Circulating Catecholamines. *Acta Physiologica Scandinavica*, 99(1), 62–74. <https://doi.org/10.1111/j.1748-1716.1977.tb10353.x>
- Huxtable, R., & Chubb, J. (1977). Adrenergic Stimulation of Taurine Transport by the Heart. *Science*, 198(4315), 409–411. <https://doi.org/10.1126/science.198879>

- Imae, M., Asano, T., & Murakami, S. (2014). Potential role of taurine in the prevention of diabetes and metabolic syndrome. *Amino Acids*, *46*(1), 81–88. <https://doi.org/10.1007/s00726-012-1434-4>
- Jacob, J. P., Blair, R., Hart, L. E., & Gardiner, E. E. (1991). The Effect of Taurine Transport Antagonists on Cardiac Taurine Concentration and the Incidence of Sudden Death Syndrome in Male Broiler Chickens. *Poultry Science*, *70*(3), 561–567. <https://doi.org/10.3382/ps.0700561>
- Jensen, D. L., Overgaard, J., Wang, T., Gesser, H., & Malte, H. (2017). Temperature effects on aerobic scope and cardiac performance of European perch (*Perca fluviatilis*). *Journal of Thermal Biology*, *68*, 162–169. <https://doi.org/10.1016/j.jtherbio.2017.04.006>
- Jong, C. J., Azuma, J., & Schaffer, S. (2012). Mechanism underlying the antioxidant activity of taurine: Prevention of mitochondrial oxidant production. *Amino Acids*, *42*(6), 2223–2232. <https://doi.org/10.1007/s00726-011-0962-7>
- Jordan, A. D., & Steffensen, J. F. (2007). Effects of Ration Size and Hypoxia on Specific Dynamic Action in the Cod. *Physiological and Biochemical Zoology*, *80*(2), 178–185. <https://doi.org/10.1086/510565>
- Joyce, W., & Wang, T. (2022). Regulation of heart rate in vertebrates during hypoxia: A comparative overview. *Acta Physiologica*, *234*(3), e13779. <https://doi.org/10.1111/apha.13779>
- Kalinin, A. L., Costa, M. J., Rantin, F. T., & Glass, M. L. (2009). Effects of Temperature on Cardiac Function in Teleost Fish. *Cardio-Respiratory Control in Vertebrates: Comparative and Evolutionary Aspects*. 1, 121–160. https://doi.org/10.1007/978-3-540-93985-6_6
- Lampson, W. G., Kramer, J. H., & Schaffer, S. W. (1983). Potentiation of the actions of insulin by taurine. *Canadian Journal of Physiology and Pharmacology*, *61*(5), 457–463. <https://doi.org/10.1139/y83-070>
- Lanctin, H. P., McMorrin, L. E., & Driedzic, W. R. (1980). Rates of glucose and lactate oxidation by the perfused isolated trout (*Salvelinus fontinalis*) heart. *Canadian Journal of Zoology*, *58*(9), 1708–1711. <https://doi.org/10.1139/z80-234>

- MacCormack, T. J., Callaghan, N. I., Sykes, A. V., & Driedzic, W. R. (2016). Taurine depresses cardiac contractility and enhances systemic heart glucose utilization in the cuttlefish, *Sepia officinalis*. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, 186(2), 215–227. <https://doi.org/10.1007/s00360-015-0946-0>
- MacCormack, T. J., & Driedzic, W. R. (2007). The impact of hypoxia on in vivo glucose uptake in a hypoglycemic fish, *Myoxocephalus scorpius*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 292(2), R1033–R1042. <https://doi.org/10.1152/ajpregu.00308.2006>
- Matey, V., Iftikar, F. I., De Boeck, G., Scott, G. R., Sloman, K. A., Almeida-Val, V. M. F., Val, A. L., & Wood, C. M. (2011). Gill morphology and acute hypoxia: Responses of mitochondria-rich, pavement, and mucous cells in the Amazonian oscar (*Astronotus ocellatus*) and the rainbow trout (*Oncorhynchus mykiss*), two species with very different approaches to the osmo-respiratory compromise. *Canadian Journal of Zoology*, 89(4), 307–324. <https://doi.org/10.1139/z11-002>
- Mechmann, S., & Pott, L. (1986). Identification of Na-Ca exchange current in single cardiac myocytes. *Nature*, 319(6054), 597–599. <https://doi.org/10.1038/319597a0>
- Nikinmaa, M., Gassmann, M., & Bogdanova, A. (2011). Oxygen Sensing: The Role of Reactive Oxygen Species. In *Oxidative Stress in Aquatic Ecosystems* 165–177. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781444345988.ch12>
- Nilsson, S. (1976). Fluorescent Histochemistry and Cholinesterase Staining of Sympathetic Ganglia in a Teleost, *Gadus morrhua*. *Acta Zoologica*, 57(2), 69–77. <https://doi.org/10.1111/j.1463-6395.1976.tb00212.x>
- Öz, E., Erbaş, D., Gelir, E., & Arıcıoğlu, A. (1999). Taurine and calcium interaction in protection of myocardium exposed to ischemic reperfusion injury. *General Pharmacology: The Vascular System*, 33(2), 137–141. [https://doi.org/10.1016/S0306-3623\(98\)00284-5](https://doi.org/10.1016/S0306-3623(98)00284-5)
- Park, E.-J., Bae, J. H., Kim, S.-Y., Lim, J.-G., Baek, W.-K., Kwon, T. K., Suh, S., Park, J.-W., Lee, I.-K., Ashcroft, F. M., & Song, D.-K. (2004). Inhibition of ATP-sensitive K⁺ channels by taurine through a benzamido-binding site on

- sulfonylurea receptor 1. *Biochemical Pharmacology*, 67(6), 1089–1096.
<https://doi.org/10.1016/j.bcp.2003.11.003>
- Pörtner, H.-O., Bock, C., & Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: Bridging ecology and physiology. *Journal of Experimental Biology*, 220(15), 2685–2696. <https://doi.org/10.1242/jeb.134585>
- Prunet, P., & Bornancin, M. (1989). Physiology of salinity tolerance in tilapia: An update of basic and applied aspects. *Aquatic Living Resources*, 2(2), 91–97.
<https://doi.org/10.1051/alr:1989011>
- Rahn, H. (1966). Aquatic gas exchange: Theory. *Respiration Physiology*, 1(1), 1–12. [https://doi.org/10.1016/0034-5687\(66\)90024-7](https://doi.org/10.1016/0034-5687(66)90024-7)
- Rajwa-Kuligiewicz, A., Bialik, R., & Rowiński, P. (2015). Dissolved oxygen and water temperature dynamics in lowland rivers over various timescales. *Journal of Hydrology and Hydromechanics*, 63, 353–363. <https://doi.org/10.1515/johh-2015-0041>
- Ramila, K. C., Jong, C. J., Pastukh, V., Ito, T., Azuma, J., & Schaffer, S. W. (2015). Role of protein phosphorylation in excitation-contraction coupling in taurine deficient hearts. *American Journal of Physiology-Heart and Circulatory Physiology*, 308(3), H232–H239. <https://doi.org/10.1152/ajpheart.00497.2014>
- Randall, D. (1982). The Control of Respiration and Circulation in Fish During Exercise and Hypoxia. *Journal of Experimental Biology*, 100(1), 275–288.
<https://doi.org/10.1242/jeb.100.1.275>
- Randall, D. J. (1968). Functional morphology of the heart in fishes. *American Zoologist*, 8(2), 179–189. <https://doi.org/10.1093/icb/8.2.179>
- Randall, D. J., & Shelton, G. (1963). The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. *Comparative Biochemistry and Physiology*, 9(3), 229–239. [https://doi.org/10.1016/0010-406X\(63\)90046-X](https://doi.org/10.1016/0010-406X(63)90046-X)
- Rasmussen, R. N., Lagunas, C., Plum, J., Holm, R., & Nielsen, C. U. (2016). Interaction of GABA-mimetics with the taurine transporter (TauT, Slc6a6) in hyperosmotic treated Caco-2, LLC-PK1 and rat renal SKPT cells. *European Journal of Pharmaceutical Sciences*, 82, 138–146. <https://doi.org/10.1016/j.ejps.2015.11.020>

- Rees, B. B., Boily, P., & Williamson, L. a. C. (2009). Exercise- and hypoxia-induced anaerobic metabolism and recovery: A student laboratory exercise using teleost fish. *Advances in Physiology Education*, 33(1), 72–77.
<https://doi.org/10.1152/advan.90188.2008>
- Sakmann, B., Noma, A., & Trautwein, W. (1983). Acetylcholine activation of single muscarinic K⁺ channels in isolated pacemaker cells of the mammalian heart. *Nature*, 303(5914), 250–253. <https://doi.org/10.1038/303250a0>
- Saito, T. (1973). Effects of vagal stimulation on the pacemaker action potentials of carp heart. *Comparative Biochemistry and Physiology Part A: Physiology*, 44(1), 191–199. [https://doi.org/10.1016/0300-9629\(73\)90381-2](https://doi.org/10.1016/0300-9629(73)90381-2)
- Salze, G. P., & Davis, D. A. (2015). Taurine: A critical nutrient for future fish feeds. *Aquaculture*, 437, 215–229. <https://doi.org/10.1016/j.aquaculture.2014.12.006>
- Satoh, H. (1995). Electrophysiological Actions of Taurine on Spontaneously Beating Rabbit Sino-Atrial Nodal Cells. *The Japanese Journal of Pharmacology*, 67(1), 29–34. <https://doi.org/10.1254/jjp.67.29>
- Satoh, H., & Sperelakis, N. (1993). Effects of taurine on Ca²⁺ currents in young embryonic chick cardiomyocytes. *European Journal of Pharmacology*, 231(3), 443–449. [https://doi.org/10.1016/0014-2999\(93\)90122-X](https://doi.org/10.1016/0014-2999(93)90122-X)
- Satoh, H., & Sperelakis, N. (1998). Review of Some Actions of Taurine on Ion Channels of Cardiac Muscle Cells and Others. *General Pharmacology: The Vascular System*, 30(4), 451–463. [https://doi.org/10.1016/S0306-3623\(97\)00309-1](https://doi.org/10.1016/S0306-3623(97)00309-1)
- Schaffer, S., & Kim, H. W. (2018). Effects and Mechanisms of Taurine as a Therapeutic Agent. *Biomolecules & Therapeutics*, 26(3), 225–241.
<https://doi.org/10.4062/biomolther.2017.251>
- Schaffer, S. W., Ju Jong, C., KC, R., & Azuma, J. (2010). Physiological roles of taurine in heart and muscle. *Journal of Biomedical Science*, 17(1), S2.
<https://doi.org/10.1186/1423-0127-17-S1-S2>
- Shah, K., Seeley, S., Schulz, C., Fisher, J., & Gururaja Rao, S. (2022). Calcium Channels in the Heart: Disease States and Drugs. *Cells*, 11(6), 943.
<https://doi.org/10.3390/cells11060943>

- Shepard, M. P. (1955). Resistance and Tolerance of Young Speckled Trout (*Salvelinus fontinalis*) to Oxygen Lack, with Special Reference to Low Oxygen Acclimation. *Journal of the Fisheries Research Board of Canada*, 12(3), 387–446.
<https://doi.org/10.1139/f55-024>
- Shiels, H. A. (2011). DESIGN AND PHYSIOLOGY OF THE HEART | Cardiac Excitation–Contraction Coupling: Routes of Cellular Calcium Flux. In A. P. Farrell (Ed.), *Encyclopedia of Fish Physiology*, 1045–1053. Academic Press.
<https://doi.org/10.1016/B978-0-12-374553-8.00175-1>
- Smith, F. M., & Jones, D. R. (1978). Localization of receptors causing hypoxic bradycardia in trout (*Salmo gairdneri*). *Canadian Journal of Zoology*, 56(6), 1260–1265. <https://doi.org/10.1139/z78-181>
- Spoor, W. A. (1990). Distribution of fingerling brook trout, *Salvelinus fontinalis* (Mitchill), in dissolved oxygen concentration gradients. *Journal of Fish Biology*, 36(3), 363–373. <https://doi.org/10.1111/j.1095-8649.1990.tb05616.x>
- Steenbergen, C., Hill, M. L., & Jennings, R. B. (1985). Volume regulation and plasma membrane injury in aerobic, anaerobic, and ischemic myocardium in vitro. Effects of osmotic cell swelling on plasma membrane integrity. *Circulation Research*, 57(6), 864–875. <https://doi.org/10.1161/01.RES.57.6.864>
- Stevens, E. D., & Randall, D. J. (1967). Changes of Gas Concentrations in Blood and Water During Moderate Swimming Activity in Rainbow Trout. *Journal of Experimental Biology*, 46(2), 329–337. <https://doi.org/10.1242/jeb.46.2.329>
- Sundin, L. I., Reid, S. G., Kalinin, A. L., Rantin, F. T., & Milsom, W. K. (1999). Cardiovascular and respiratory reflexes: The tropical fish, traira (*Hoplias malabaricus*) O₂ chemoresponses. *Respiration Physiology*, 116(2–3), 181–199.
[https://doi.org/10.1016/s0034-5687\(99\)00041-9](https://doi.org/10.1016/s0034-5687(99)00041-9)
- Thomsen, M. T., Wang, T., Milsom, W. K., & Bayley, M. (2017). Lactate provides a strong pH-independent ventilatory signal in the facultative air-breathing teleost *Pangasianodon hypophthalmus*. *Scientific Reports*, 7(1), 63–78.
<https://doi.org/10.1038/s41598-017-06745-4>
- Tranum-Jensen, J., Janse, M. J., Fiolet, W. T., Krieger, W. J., D’Alnoncourt, C. N., & Durrer, D. (1981). Tissue osmolality, cell swelling, and reperfusion in acute

- regional myocardial ischemia in the isolated porcine heart. *Circulation Research*, 49(2), 364–381. <https://doi.org/10.1161/01.RES.49.2.364>
- Van den Thillart, G., & van Waarde, A. (1985). Teleosts in hypoxia: Aspects of anaerobic metabolism. *Molecular Physiology*, 8, 393–409.
- Vislie, T. (1983). Cell volume regulation in fish heart ventricles with special reference to taurine. *Comparative Biochemistry and Physiology Part A: Physiology*, 76(3), 507–514. [https://doi.org/10.1016/0300-9629\(83\)90453-X](https://doi.org/10.1016/0300-9629(83)90453-X)
- Vornanen, M., Shiels, H. A., & Farrell, A. P. (2002). Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 132(4), 827–846. [https://doi.org/10.1016/S1095-6433\(02\)00051-X](https://doi.org/10.1016/S1095-6433(02)00051-X)
- Wardle, C. S. (1978). Non-Release of Lactic Acid From Anaerobic Swimming Muscle of Plaice *Pleuronectes Platessa* L.: A Stress Reaction. *Journal of Experimental Biology*, 77(1), 141–155. <https://doi.org/10.1242/jeb.77.1.141>
- Wood, C. M., & Shelton, G. (1980). The reflex control of heart rate and cardiac output in the rainbow trout: Interactive influences of hypoxia, haemorrhage, and systemic vasomotor tone. *Journal of Experimental Biology*, 87(1), 271–284. <https://doi.org/10.1242/jeb.87.1.271>