Quantifying biological responses of catch-and-release angling in understudied fish species and practices

By

Jamie T. Card

A thesis submitted to the Faculty of Graduate Studies

in partial fulfilment of the requirements for the

Master of Science Degree

Department of Biology

Master of Science in Bioscience, Technology, and Public Policy

University of Winnipeg

Winnipeg, Manitoba, Canada

August 20, 2021

Table of Contents

Dedication

Grandpa Alex Kowalchuk,

I'm not sure you were fully aware of what I was studying in school, but I do know that you would have been proud of me for pursuing what I love, following through on it and working damn hard while doing it. I wish you could be here to celebrate with us.

In lieu of a celebration together, this thesis is dedicated to you.

Acknowledgements

Caleb – thank you for your constant guidance and support. From statistics questions to craft beer recommendations, you always made yourself available to discuss any issues I was having and gave useful feedback that I am certain helped improve my writing skills. Further, you made me feel more like a colleague which helped build my confidence as a researcher (I'm not sure that this was intentional… perhaps our shared interest in classic comedies and a similar sense of humor fostered this). It was an absolute honor to be one of the first grad students that you brought into your lab and I sincerely value your mentorship.

Mum – you have been my absolute lifeline throughout grad school. When I say that I couldn't have done this without you I wholeheartedly mean it! Moving away from home was difficult but you made sure that I knew you were always just a phone call away to share a laugh together. Your love and support are everything to me, thank you for all that you do.

Dad – thank you for your endless love and encouragement. You have taught me how to be kind and compassionate in all aspects of life.

Barb, Paul, Chloe, Anne and Philip – thank you for your unwavering support and kindness since I moved to Winnipeg and for essentially taking me into your family unit. My favourite activity quickly became hanging out at Gull Lake with all of you. Barb, you are easily the coolest person I've had the privilege to get to know and sharing meals with you has been one of the biggest highlights of my time in grad school. Paul, thank you for the late-night trips to the gravel pit and the garage chats – your advice surrounding grad school and life means the world to me. Chloe, Anne and Philip, it has been such an honor watching you all grow into the incredible humans that you are today.

Dan – thank you for cheering me on and for reminding me to laugh whenever things got stressful. I feel very lucky to be a part of your life and your support has carried me throughout my time in graduate school.

Grad cohort – you all made this experience so wonderful. Working on assignments at the goodwill, undertaking field work together, attending local festivals and concerts in Winnipeg and continuing to connect throughout the pandemic through video calls and discussions surrounding global events and activism are experiences that I will forever hold close to my heart.

Thank you to my committee members Dr. Craig Willis and Dr. Scott Forbes for your time and helpful feedback during committee meetings.

This research was funded by a Natural Sciences and Engineering Research Council (NSERC) Canada Graduate Scholarship held by J. T. Card, an NSERC Discovery Grant held by C. T. Hasler and a University of Winnipeg start-up grant held by C. T. Hasler.

Co-Authorship

Chapter 2: Physiological effects of catch-and-release angling on freshwater drum (*Aplodinotus grunniens*)

Card, J. T., and C. T. Hasler. 2021. Fisheries Research [online serial] 237:105881. DOI: 10.1016/j.fishres.2021.105881.

Jamie T. Card: methodology, investigation, formal analysis, writing – original draft, writing – review and editing, funding acquisition, project administration, visualization.

Caleb T. Hasler: conceptualization, supervision, investigation, writing – review and editing, funding acquisition, resources.

Chapter 3: Examination of potential histological, thermal and reflex effects of ice and air exposure on yellow perch (*Perca flavescens*) following ice-angling

Card, J. T., M. J. Louison, J. F. Bieber, C. D. Suski, and C. T. Hasler. Submitting to Transactions of the American Fisheries Society.

Jamie T. Card: methodology, investigation, formal analysis, writing – original draft, writing – review and editing, funding acquisition, project administration, visualization.

Michael J. Louison: conceptualization, investigation, writing – review and editing.

John F. Bieber: investigation, writing – review and editing.

Cory D. Suski: conceptualization, writing – review and editing.

Caleb T. Hasler: conceptualization, supervision, investigation, writing – review and editing, funding acquisition, resources.

List of Tables

List of Figures

Chapter 1: General Introduction

Recreational Angling: A Widespread Activity

Recreational angling is widely practiced across the globe. Fishing for recreational purposes (with a rod and fishing line) has occurred since at least the early fifteenth century (Policansky 2002) and best estimates predict that 11.5 % of the global population participates in recreational angling (Cooke and Cowx 2004; Davie and Kopf 2006). The act of recreational fishing contributes substantially to local economies, demonstrated by Canadian recreational anglers contributing an average of $8.8 billion per year to the Canadian economy through major purchases and direct expenditures surrounding recreational angling (Brownscombe et al. 2014). Recreational angling differs from commercial harvest in that its primary purpose is only for recreation and it is not executed with the consumptive intention of keeping the fish to sell for profit. Many of the fish captured through recreational angling are then released (Policansky 2002; Cooke and Cowx 2004). Catch-and-release (C&R) angling is defined as "the process of capturing fish by using hook and line, mostly assisted by rods and reels, and then releasing live fish back to the waters where they were captured, presumably to survive unharmed" (Arlinghaus et al. 2007). Reasons for partaking in C&R angling differ depending on the individual, and sometimes release may be required by local regulations, but it is often practiced under the assumption that all fish released will survive to then be re-captured. Catch-and-release angling can be considered an effective conservation tool that supports the desire to fish recreationally while also conserving fish populations (Arlinghaus et al. 2007).

Consequences of Angling: The "Invisible Collapse"

Despite its growing popularity, recreational angling is not without consequences on aquatic ecosystems. It is broadly understood that overfishing for commercial purposes has decimated fish populations across the globe (Jackson et al. 2001; Watson et al. 2003; Hilborn et al. 2003; Pauly et al. 2003; Cooke and Cowx 2004), however, less is known about the impact of recreational angling. Post et al. (2002) describe that a recreational fishery should be self-regulating, meaning that as abundance decreases one would also expect angler pressure to decrease. This is because when catch per unit effort decreases, anglers will likely be deterred from fishing that waterbody as they catch less fish despite spending more time and effort. However, Post et al. (2002) highlight several case studies where this has not been the case and define this as the "invisible collapse" that recreational fisheries in Canada are experiencing, as population declines are not easily noticeable. One of the examples highlighted by Post et al. (2002) is the collapse of 21 out of 27 walleye (*Sander vitreus*) populations in Alberta as a result of overfishing, with the evidence being a severe decline in catch rates. This invisible collapse may be attributed to the widespread popularity of recreational angling coupled with fisheries managers' lack of understanding of the full extent of the recreational angler's impact on fish populations (Post et al. 2002). It is now understood that recreational angling has played a part in the decline of fish populations on a global scale (Coleman et al. 2004; Cooke and Cowx 2004).

Motivations for angling differ among individuals, although anglers can broadly be categorized into three groups: those that harvest every fish that they catch, those that harvest some of their catch and release the rest, and those that strictly release every fish they catch. Additionally, C&R can be either regulatory or voluntary (Arlinghaus et al. 2007). Regulatory C&R is when anglers release fish that they are not able to keep due to local fishing regulations (e.g., when anglers are only allowed to harvest certain species within specific size classes), whereas voluntary C&R is when anglers decide to release the fish they catch based on a variety of reasons, such as personal ethics or morals (Arlinghaus et al. 2007). There are also different levels of understanding amongst C&R anglers as well as differing attitudes. For example, some believe that all fish released will survive, whereas some are not concerned of the status of the fish they release and simply release the fish because they are mandated to do it (Hasler et al. 2011).

C&R programs for conservation are only successful when angler attitudes support them (Anderson and Nehring 1984; Hasler et al. 2011). Thankfully, management suggestions that result from C&R literature, such as better handling practices, are often effective as most anglers are supportive of minimizing their impact on fish populations to ensure the fishery is as productive as possible (Cooke and Sneddon 2007). Management suggestions and regulations are usually aimed at maximizing survival of the fish post-release. Some of the negative implications of releasing angled fish include the activation of the hypothalamic-pituitary-interrenal (HPI) stress axis, decompression issues when caught at depths causing barotrauma, altered feeding patterns, stunted growth, reproduction issues, disease and mortality (Davie and Kopf 2006); some of these implications are explored further throughout this thesis. Additionally, there are population-level changes that may result from C&R as implied by Post et al. (2002), however, this is seldom studied as it is difficult to expand the results of an angling event to an entire ecosystem or population (Arlinghaus et al. 2007).

Differences in Angler Practices

In addition to the differences among angler motivations and attitudes, there are also a variety of skillsets and differing experience levels among anglers. Some studies even focus on differing skill-level and its impact on C&R outcomes, as it was found that anglers with more experience hooked smallmouth bass (*Micropterus dolomieu*) significantly deeper in the mouth than less experienced anglers (Dunmall et al. 2001), and that angling for spotted seatrout (*Cynoscion nebulosus*) by novice anglers resulted in higher levels of mortality than angling by experienced anglers (Stunz and McKee 2006). Anglers vary widely in multiple ways from the type of tackle used to skill level and procedures for dehooking fish (Cooke et al. 2013a), and these behaviours have consequences on the fish that are released. For example, using the wrong type of fishing gear for the species targeted can lead to longer fight times, which has been correlated with higher levels of stress in fish through the continued elevated presence of stress hormones (Gustaveson et al. 1991; Kieffer et al. 1995; Tomasso et al. 1996; Gallman et al. 1999; Thompson et al. 2002; Meka and McCormick 2005; Arlinghaus et al. 2007). Another example is the amount of air exposure that fish are subjected to while either removing a hook, taking photographs, or measuring the fish's length or weight (Arlinghaus et al. 2007; Cooke et al. 2013a). Longer air exposure can lead to collapsed gill lamellae which inhibits gas exchange leading to lower blood oxygen levels (Ferguson and Tufts 1992; Arlinghaus et al. 2007). During an angling event, this period of air exposure is coupled with the exhaustive exercise experienced during the fight of landing the fish and will induce a stress response (Wood et al. 1983; Ferguson and Tufts 1992). In some cases, this may also lead to delayed mortality (Ferguson and Tufts 1992). These examples display how angler skill-level can directly impact the health of captured fish, which must be understood if these individuals will be released for conservation purposes.

History of Catch-and-Release Literature

As it is now broadly understood that recreational angling has an impact on fish that will be released after capture, research has vastly expanded our knowledge on the specific impacts of C&R angling on fish (Arlinghaus et al. 2007). The first C&R angling study was published as early as 1932, when Westerman found that there is an insignificant loss of hooked immature trout once they are returned to the water (Arlinghaus et al. 2007). C&R studies continued moderately over the years until the 1980s when research in this area substantially increased (Arlinghaus et al. 2007). The early C&R literature primarily focused on hooking mortality, which is defined as the proportion of fish caught via angling that die upon release (Muoneke and Childress 1994). It was then realized that hooking mortality should not be the sole focus of these studies because the sublethal effects of C&R angling have been implicated to cause delayed mortality, thus research expanded in this new direction (Muoneke and Childress 1994). Ferguson and Tufts (1992) assessed sublethal effects by looking at the stress physiology of rainbow trout (*Oncorhynchus mykiss*) that were both exhaustively exercised and exposed to air to simulate a C&R angling event, and concluded that this period of air exposure is a significant additional stressor that may lead to death in released fish. As of current time, there have been ample studies that focus on the sublethal effects of C&R angling to such a great extent that it now seems to be the primary focus of the literature. However, there are still noticeable knowledge gaps in this field of study as Cooke and Suski (2005) highlight that there is only a suitable knowledge base regarding the effects of C&R angling on five North American species of fish: largemouth bass (*Micropterus salmoides*), walleye, rainbow trout, striped bass (*Morone saxatilis*), and Atlantic salmon (*Salmo salar*). Although this review was completed in 2005, this indicates that anglers' favourite sportfish are the species that are primarily being studied and that there is a need to broaden the scope to other species of fish, ultimately developing species-specific C&R guidelines (Cooke and Suski 2005). Additionally, Gingerich and Suski (2012) show that results cannot be generalized across species that fill different ecological niches and cannot even be generalized across different fish sizes. As experts in the field have suggested that guidelines and research need to be species-specific, it is crucial that the literature broadens its scope to include understudied species (Cooke and Suski 2005; Davie and Kopf 2006).

Themes Observed in Catch-and-Release Literature

There are multiple themes of study that can be observed in C&R literature, which seem to focus on hooking mortality, rates of injury, behavioural responses and stress physiology. Each theme focuses on the effect that certain aspects of the angling event have on the fish. Hooking mortality is a theme that researchers focused heavily on in the early literature and from that research it is clear that different species undergo different levels of hooking mortality (Muoneke and Childress 1994), however, this depends on a variety of factors (Bartholomew and Bohnsack 2005). Davie and Kopf (2006) highlighted that the amount of time spent reeling in the fish (Meka and McCormick 2005), air exposure and length of time handled (Ferguson and Tufts 1992), hooking location (Millard et al. 2003) and the extent of the hooking injury (Domeier et al. 2003) are all important factors that contribute to the degree of hooking mortality experienced. The type of tackle used also has an impact on hooking mortality (e.g., higher mortality with J-hooks than with circle hooks; Grover et al. 2002; Bartholomew and Bohnsack 2005), as well as whether or not the hooks are barbed (e.g., lower mortality with barbless hooks; Taylor and White 1992). Further, fish with higher tolerance for change in the environment have been found to experience lower hooking mortality (Raat et al. 1997; Davie and Kopf 2006), indicating that hooking mortality is indeed species-specific. Hooking mortality and injury assessments have been studied extensively, and yet, mortality rates are still high despite a greater understanding of the factors that contribute to delayed mortality (Bartholomew and Bohnsack 2005; Arlinghaus et al. 2007). Additionally, it is important to consider that the stressors that fish experience during an angling event are not stand-alone and that they have interactive effects, which should be the focus of future C&R research (Arlinghaus et al. 2007).

Rates of injury are commonly assessed by quantifying hooking location, severity of bleeding at the hooking location and any other obvious signs of injury to the fish through handling (e.g., loss of scales or other abrasions; Bettoli et al. 2000; Bartholomew and Bohnsack 2005). Hooking location is important as it can determine the degree of tissue damage the fish endures, and specific tackle can be used to minimize damage as well as minimize handling time while removing the hook (Muoneke and Childress 1994; Cooke et al. 2001; Cooke and Suski 2005). For example, there is an increased probability of mortality if a fish is hooked in the esophagus or gills as opposed to the jaw (Pelzman 1978; Aalbers et al. 2004; Arlinghaus et al. 2007). Additionally, the severity of bleeding from the hook wound can predict post-release mortality (Cooke et al. 2013a). It has been determined that if fish experience "pulsatile blood flow from a wound" (Arlinghaus et al. 2007), it is likely that the injury is serious and could potentially result in delayed mortality.

Assessing behavioural responses following an angling event has become an increasingly common method of assessing fish vitality. Examples of behavioural responses to C&R angling include impaired swimming capability (i.e., changes in speed or movement), altered feeding behaviour, altered reproductive behaviour, using habitat differently and impaired predator avoidance (Cooke and Suski 2005; Davie and Kopf 2006; Arlinghaus et al. 2007). As changes in behaviour can impact many different life history processes, tools such as reflex action mortality predictors (RAMP) have been developed to assess behavioural impairment post-angling event (Davis 2007; Raby et al. 2012; McLean et al. 2016). RAMP assessments have been shown to accurately reflect fish vitality (McLean et al. 2016), predict delayed mortality (Davis 2007, 2010; Raby et al. 2012) and are also much simpler and cheaper to execute than other analyses (Brownscombe et al. 2017). Because RAMP assessments are so practical, anglers can learn how to perform these assessments on the fish that they capture and use them to determine the condition of the fish, ultimately empowering them to make informed harvest or C&R decisions surrounding their caught fish (Brownscombe et al. 2017).

Measuring changes in stress physiology indicators has become a common method of assessing the impacts of environmental stressors on different organisms (Wikelski and Cooke 2006). When fish are exposed to stressors such as angling events, the individual will undergo changes in their physiology through an active process known as allostasis, in which they are attempting to maintain or re-establish homeostasis when faced with environmental challenges (Romero et al. 2009; McEwen and Wingfield 2010; Gorissen and Flik 2016). When homeostasis is disrupted by the experience of an angling event, stress hormones are released and the ionic/osmotic balance within the blood of the fish is disturbed (Arlinghaus et al. 2007). Once a stressor is perceived in the central nervous system, catecholamines are rapidly released into the bloodstream to instantly mobilize glucose to be used as energy and simultaneously the HPI axis is activated (Wendelaar Bonga 1997; Barton 2002; Schreck and Tort 2016). Corticotropin releasing hormone (CRH) is released from the hypothalamus which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary gland into the blood stream, ultimately controlling cortisol production and release in the interrenal cells of the head kidney (Barton

2002; Gorissen and Flik 2016; Schreck and Tort 2016). Cortisol then maintains high blood glucose levels that can be used as energy to respond to the stressor by preventing glucose from turning into glycogen stores in the liver through the downregulation of glyconeogenic processes (Gorissen and Flik 2016; Schreck and Tort 2016; Lawrence et al. 2017). Rises in cortisol have commonly been used to quantify the stress response in fishes (Pickering and Pottinger 1989; Rotllant and Tort 1997; McLean et al. 2016; Louison et al. 2017b, 2017a). As previously mentioned, blood glucose will rise after angling events occur as glycogen stores are converted to glucose so that glucose can be readily available to be used by tissues such as skeletal muscles (Wendelaar Bonga 1997; Arlinghaus et al. 2007; Schreck and Tort 2016). As energy sources are used up, lactic acid accumulates in the muscle tissue increasing levels of tissue lactate as well as blood lactate, and it has been found that the amount of lactate stored in the muscle or released into the blood is dependent on the type of stress, where hypoxia can lead to higher concentrations of lactate in the blood (Dalla Via et al. 1997; Wells and Pankhurst 1999). Blood pH may also change following the pH decrease in white muscle that results from the dissociation of lactic acid, as this can cause the acidification of plasma as protons may flow out of the muscle tissue into the bloodstream (Wood 1991; Wang et al. 1994). The production of stress hormones following stress events can also impair immune function, thus injury and stress physiology may work together to further negatively impact the fish, indicating that repeated C&R events may have serious consequences (Arlinghaus et al. 2007). Instead of devoting energy to growth, energy is focused on recovering from the stress event which may have long-term implications for the growth and fitness of the individual if prolonged exposure to stressors occurs (Wendelaar Bonga 1997; Arlinghaus et al. 2007).

Knowledge Gap #1: Understudied Species

Despite the many studies that look at the effects of C&R angling on different fish species, the focus is primarily on anglers' favourite sportfish species and major gaps in the literature exist, as several authors have highlighted that research must focus on other species (Cooke and Suski 2005; Arlinghaus et al. 2007; Cooke and O'Connor 2010). The species that have been the leading focus of C&R research typically grow large, thus the angler experiences a thrilling fight and exciting photograph opportunities present themselves for the angler. Even when targeting a specific species, often times other species will be unintentionally hooked and are considered by-catch (Davis 2002). It is important to understand how these species respond to C&R stress events as they are almost always released post-capture. Additionally, some species are only targeted by specialized anglers and the literature is also lacking regarding how these species respond to C&R (Landsman et al. 2011; Card and Hasler 2021).

An example of an understudied species that is absent from C&R literature is freshwater drum (*Aplodinotus grunniens*). To date, there have been no C&R studies performed on this species, and only few studies that focus on their diet and biology (Daiber 1952; Bodensteiner and Lewis 1992; French III and Love 1995; French III and Bur 1996; Jacquemin et al. 2014, 2015; Wong et al. 2021) and the use of their otoliths as aging structures (Pereira et al. 1995). Freshwater drum is an example of a species that is targeted by specialized anglers primarily for C&R, as Manitoba's Hooked Magazine recently published an article highlighting the thrills of angling for large trophy drum (Wood 2015). Most anglers that fish for freshwater drum practice "extreme C&R" (North 2002) which is when almost all fish that are caught are released (Arlinghaus et al. 2007), thus highlighting the importance of studying the impact of these angling events on this species. Further, it is possible that this species is actually more sensitive to C&R

events than others previously studied due to their biological characteristics. Freshwater drum have a large, uniquely round body shape, and teeth in many rows including well-developed pharyngeal teeth (Scott and Crossman 1973). These characteristics pose challenges for hook removal leading to longer air exposure and injury from prolonged handling. Freshwater drum also have large scales when compared to other species that may fall off during an angling event (Scott and Crossman 1973). This could put the individual at greater risk of injury once released as scales provide protection from infection. Additionally, freshwater drum are a long-lived species (maximum age of 71 years; Pereira et al. 1995; Jacquemin et al. 2015) so there is an increased likelihood that mortality could have negative population-level consequences. Should these consequences influence survival or have long term effects, freshwater drum populations may be negatively impacted as a result of overfishing from recreational anglers (Post et al. 2002). Although freshwater drum are currently ranked as Secure (G5; S5) by NatureServe both globally and in Manitoba (the northern periphery of their range), their conservation status varies across regions as they are considered Critically Imperiled (S1) in North Carolina, Imperiled (S2) in Virginia and Presumed Extirpated (SX) in New Mexico (NatureServe 2020). To my knowledge, abundance data are not collected in Manitoba because the population is deemed secure, and no major threats are currently known for this species.

Knowledge Gap #2: Understudied Practices

Another significant knowledge gap in the literature exists surrounding the study of C&R angling in colder temperatures during ice-angling, as this has rarely been studied. Ice-angling is a popular recreational activity in colder climates, and experts in the field have acknowledged the need for future study in this area (Arlinghaus et al. 2007). Reasons for this lack of study include challenges of winter field biology such as cold temperatures, malfunctioning field equipment and higher costs associated with winter field research (Lavery 2015). To date only a handful of ice-angling studies that have been published, focusing on hooking location and delayed mortality (Dextrase and Ball 1991; Persons and Hirsch 1994; DuBois et al. 1994; Twardek et al. 2018; Althoff et al. 2020; Somers et al. 2021) as well as physiological and behavioural effects of ice-angling on fish (Louison et al. 2017b, 2017a; Logan et al. 2019; Bieber et al. 2019). These physiological assessments highlight that stress responses exhibited in fish following a C&R event during ice-angling are different than those angled in warmer temperatures (Arlinghaus et al. 2009; Louison et al. 2017b, 2017a; Twardek et al. 2018). Specifically, there is a delay in recovery time during cold conditions which may result from colder water temperatures decelerating enzyme activity (Louison et al. 2017b, 2017a; Logan et al. 2019). This attenuated stress response is exemplified by physiological stress indicators peaking much later on than in studies performed in warmer water temperatures (Louison et al. 2017b, 2017a). The general conclusion is that ice-angled fish will take longer to recover than fish caught in warmer water temperatures, and further studies are required to determine how long the recovery period is during ice-angling conditions as well as how different species are impacted by ice-angling events (Louison et al. 2017a).

To date, ice-angling research has only scratched the surface of how these events might impact fish welfare, so there is a need to continue to build on these studies to further understand the entire effect of the ice-angling event on fish that will be released. For example, there is a lack of focus on the freezing of tissues during ice-angling. Depending on the tissue, when exposed to air (as commonly occurs during ice-angling events) the tissue may freeze and ultimately cause damage to the fish once released. This potential damage to tissues has infrequently been quantified in previous studies (Bieber et al. 2019). Specifically focusing on the gill, if substantial damage to the delicate gill tissue occurs during ice-angling events with prolonged periods of sub-zero air exposure, oxygen uptake and gas exchange may be impaired upon release. Finally, the low air and water temperatures could induce cold shock and lead to delayed mortality that physiological variables could not predict (Louison et al. 2017a). Given the widespread practice of ice-angling in northern climates, it is essential that this knowledge gap is filled through the continued pursuit of C&R ice-angling studies on a variety of species.

Management Implications of Catch-and-Release Literature

The focus of fisheries research is often to develop recommendations for fisheries managers, thus the management implications that result from these studies are important, if not the focus of the entire study itself. Physiological C&R studies are usually "solutions-based" (Cooke et al. 2013a) meaning the results of these studies often include a suggestion of best practices that anglers should adopt. These best practices often include limiting air exposure, decreasing hook removal time, using proper gear depending on the species targeted and landing fish as quickly as possible (Ferguson and Tufts 1992; Wilkie et al. 1996; Cooke and Suski 2005; Arlinghaus et al. 2007; Cooke et al. 2013a; Brownscombe et al. 2017). In addition to these established best practices, the literature often supports the use of C&R for fish conservation. Examples of this include studies that have shown hooking mortality is relatively low for sauger (*Sander canadensis*; Bettoli et al. 2000), walleye (Payer et al. 1989) and lake trout (*Salvelinus namaycush*; Loftus et al. 1988).

As multiple studies have connected the angler's impact on aquatic ecosystems with community-level changes of fish populations (Post et al. 2002; Almodovar and Nicola 2004), it is important that adequate management strategies and policies are applied to avoid population declines and biodiversity loss (Arlinghaus et al. 2007). The need for species-specific recommendations is fundamental to management, as fisheries managers are often required to make decisions for understudied species based on the results of studies that focus on well understood species (Cooke and Suski 2005). However, these species are diverse and may differ in a variety of means such as behaviour, morphology or ecology (Cooke and Suski 2005). This could mean that extrapolations from more conventionally studied species are inappropriate yet may still be used to make management decisions for other species where knowledge is lacking. As it is known that recreational angling is widespread, it is likely that populations of fish are undergoing C&R events on a continuous basis (Cooke and O'Connor 2010). Fisheries managers cannot manage populations effectively if there is a lack of knowledge regarding an event that is happening to these populations continuously and communicating that recreational anglers should adopt certain best practices can be challenging (Danylchuk et al. 2018). The previously identified gaps in the literature must be adequately filled to better understand the implications of C&R angling on all species and for all practices, and importantly, to develop best angling practices that recreational anglers can adopt to sustain fisheries.

Chapter 2: Physiological effects of catch-and-release angling on freshwater drum (*Aplodinotus grunniens)*

This chapter has been published as: Card, J. T., and C. T. Hasler. 2021. Physiological effects of catch-and-release angling on freshwater drum (*Aplodinotus grunniens*). Fisheries Research [online serial] 237:105881. DOI: 10.1016/j.fishres.2021.105881.

Abstract

Many catch-and-release angling events involve air exposure and exhaustive exercise that elicit a physiological stress response, and depending on a variety of factors, delayed mortality is a possible outcome. There have been ample studies in this area, however, significant gaps exist in the literature for species that are targeted by more specialized anglers, such as freshwater drum (*Aplodinotus grunniens*). I quantified physiological and reflex responses in freshwater drum following angling, across seasons. Once a fish was on the line, the fight duration and time exposed to air were varied to account for differences in angler skill level (fight time range: 5 s to 2 min; air exposure range: 20 s to 3 min). Location and severity of injury were determined, blood biopsies were taken to quantify physiological stress, and reflex impairment was assessed. Thirty-one percent of fish captured were deeply hooked in the esophagus tissue. Freshwater drum experienced a disruption in homeostasis as blood glucose, plasma cortisol and plasma lactate increased significantly from baseline values following angling. Additionally, seasonal differences were observed for blood glucose and plasma cortisol as higher values were observed in the summer when compared to the spring. The 'orientation' reflex was the most frequently impaired (29 % of fish lacked this reflex), but impairment did not differ seasonally. Because

freshwater drum have the largest latitudinal range of any North American freshwater fish and are being targeted more frequently by anglers as of late, it is important to fill this knowledge gap regarding their responses to angling events to develop best practices for anglers to promote conservation. The wide distribution of freshwater drum may also make them a candidate model species for addressing the convergence between assessing the impacts of catch-and-release angling and other environmental issues facing freshwater fishes, such as climate change.

Introduction

Recreational fisheries occur when fish are captured but not used as a primary source of nutrition for the fisher and are not sold or traded in any markets (EIFAC 2008; FAO 2011). Practiced across the world, an estimated 11.5 % of the global population participates in recreational fisheries (Cooke and Cowx 2004; Davie and Kopf 2006). Because many partake in recreational angling, it is also economically important; for example, Canadian resident anglers spent $2.5 billion on direct fishing expenditures in 2015 (Fisheries and Oceans Canada 2019). Due to its popularity, recreational fisheries can be at risk of collapse, particularly if fisheries stakeholders lack research into the social and ecological processes that are involved with their fishery (Post et al. 2002, 2008; Cooke and Cowx 2004; Post 2013).

Catch-and-release (C&R) angling is a practice that has been adopted by anglers to prevent population declines in recreational fisheries (Cowx 2002; Cooke and Cowx 2006; Brownscombe et al. 2014). Either because of a conservation ethic, morals, or regulations, anglers release fish back to the waterbody of capture with the intention that the fish will survive and contribute to recruitment (Arlinghaus et al. 2007). Despite its conservation goals, research is still lacking to support C&R angling as a sustainable practice for all recreationally caught fishes (Cooke and Suski 2005; Brownscombe et al. 2017). In particular, studies that focus on the individual-level impacts of C&R angling are needed to provide species-specific recommendations (termed ‘best practices’) for how anglers may improve upon their angling and handling techniques to promote the long-term viability of populations (Cooke and Suski 2005; Madliger et al. 2016; Brownscombe et al. 2017).

There are several ways that C&R angling events impact fish, and acquiring this knowledge for different species can assist with the development of best practices (see

Brownscombe et al. 2017). The hooking of a fish causes tissue damage and bleeding, and infection is possible, especially when a fish is hooked in a location other than its jaws (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005). The retrieval of the fish (i.e., ‘the fight’) causes energetic exhaustion (Wood et al. 1983; Ferguson and Tufts 1992) leading to physiological changes and an increase in oxygen demand that may lead to delayed mortality upon release (Muoneke and Childress 1994; Davis 2002; Davie and Kopf 2006). Netting and handling of the fish (i.e., de-hooking and ‘the photo op’) leads to further tissue damage and removal of slime that can cause infection (Barthel et al. 2003; Brownscombe et al. 2017), and air exposure causes gill lamellae to collapse decreasing the amount of gas exchange possible (Ferguson and Tufts 1992). Ultimately the air exposure during handling can lead to lower levels of oxygen in the blood and causes additional physiological changes (Ferguson and Tufts 1992; LeBlanc et al. 2010). Furthermore, the entire angling process can culminate in whole-body changes to behaviour (e.g., reflex impairment; Raby et al. 2012) and the degree to which a fish is affected by the stressors can be interactive with other factors (e.g., body length, water temperature; Meka and McCormick 2005). Many researchers have focused on studying how C&R events impact fishes regarding hooking mortality, injury, reflex impairment and physiology (e.g., Ferguson and Tufts 1992; Muoneke and Childress 1994; Arlinghaus et al. 2007; Raby et al. 2012), which is useful for designing best practices for capturing and releasing fish (Cooke and Suski 2005; Wikelski and Cooke 2006; Madliger et al. 2016) and changing social norms in recreational fisheries (Danylchuk et al. 2018).

Best practices ideally minimize harm in handled fish and physiological indicators are useful metrics to assess the severity of the disruption to homeostasis caused by an angling event (Ferguson and Tufts 1992; Pankhurst and Dedual 1994; Wilkie et al. 1996). When homeostasis is disrupted, catecholamines and corticosteroids are released into the circulatory system and several 'downstream' changes occur, such as the release of glucose and alterations to the ionic balance within blood (Wendelaar Bonga 1997). Examples of physiological disturbances exhibited in fish during or after an angling event include increased plasma cortisol, blood glucose, plasma lactate, tissue lactate and reduction in blood pH (Wood 1991; Wendelaar Bonga 1997; Arlinghaus et al. 2007; McLean et al. 2016; Lawrence et al. 2018). Additionally, monitoring reflexes (i.e., innate and automatic responses to stimuli) can be used to determine fish vitality following C&R events (Davis 2010; McLean et al. 2016). Reflex impairment is easily measured in a field setting and is useful in predicting post-release mortality because lacking certain reflexes indicates a compromised physiological state (Raby et al. 2012; McLean et al. 2016). Overall, if physiological consequences and reflex impairment are quantified, they can be used as indicators of immediate fish health following a C&R angling event and are therefore useful metrics when developing best practices.

Though much has been learned about the physiological responses of angling in fish, applying physiological research to fishes broadly to create best practices is a challenge because data from previous studies is difficult to generalize for several reasons (Gingerich and Suski 2012). Firstly, a major influence on physiological disturbances in fish is temperature, as fish angled in warmer temperatures display greater physiological disturbances (Gustaveson et al. 1991; Thompson et al. 2002) and higher mortality rates (Wilkie et al. 1996, 1997; Wilde 1998; Wilde et al. 2000; Dempson et al. 2002; Thorstad et al. 2003; Arlinghaus et al. 2007; Gingerich et al. 2007) so environmental context is important when assessing fish responses to C&R angling. Secondly, angler behaviour can also influence the degree to which fish experience physiological effects of C&R because both length of time spent fighting against the line and time exposed to air have implications on post-release vitality of fishes (Cooke and Suski 2005; Meka and McCormick 2005; Schreer et al. 2005; Suski et al. 2007; Arlinghaus et al. 2009; Cooke et al. 2017; Louison et al. 2017a). Therefore, it is important to experimentally vary time spent reeling in fish and time that the fish is exposed to air to gain a complete understanding of the physiological effects of C&R angling. Thirdly, morphological characteristics such as mouth shape, teeth type and pattern are also different across species (Venkatesh 2003). These differences may pose challenges for anglers handling them, and studies on one species may not apply to another due to these differences in characteristics. A species that is unique in several ways and is targeted by anglers is freshwater drum (*Aplodinotus grunniens*). To date, no study has characterized the individual-level consequences of C&R angling on freshwater drum in any context.

Freshwater drum are vulnerable to C&R angling. Drum grow large and are treated as a 'trophy fish' species throughout their range (e.g., Texas Parks and Wildlife Department 2020; Travel Manitoba 2020). Additionally, freshwater drum are released as bycatch while anglers are attempting to catch other species that can be targeted with similar gear and tackle used for capturing freshwater drum (e.g., channel catfish [*Ictalarus punctatus*] and walleye [*Sander vitreus*]). Simply applying other physiological findings to the species to understand the potential consequences of C&R angling is difficult, as freshwater drum have a large, uniquely round body shape, large scales, teeth in many rows including well-developed pharyngeal teeth, and they are the only freshwater species within the family Sciaenidae (Scott and Crossman 1973). The morphological characteristics pose challenges for hook retrieval and removal from nets, potentially leading to longer air exposure and injury from prolonged handling time. Additionally, severe hooking injuries such as swallowing the baited hook are more likely to occur as the pharyngeal teeth are located in the back of the mouth next to the esophageal tissue (Beckwith Jr. and Rand 2005). Lastly, because freshwater drum are a long-lived species (maximum published age of 71 years; Pereira et al. 1995; Jacquemin et al. 2015), determining the potential for physiological and reflex responses and C&R-induced mortality is important for fisheries management (Cooke and Suski 2005; Davie and Kopf 2006), as populations of long-lived fish species can be more severely impacted by fisheries interactions (Schroeder and Love 2002; Cooke and Cowx 2006). Generally, there is a knowledge gap related to the consequences of C&R angling on freshwater drum, and it must be filled to support the development of best practices for this species.

The objective of the study was to quantify physiological and reflex responses to variable C&R angling practices in freshwater drum across seasons. I hypothesized that longer C&R angling events (i.e., longer fight time and air exposure) during warmer time periods would result in biological consequences for freshwater drum, which has been shown in a study on their saltwater conspecific, red drum (*Sciaenops ocellatus*; Gallman et al. 1999). I predicted that fish that undergo longer C&R angling events will have higher rates of injury and reflex impairment, higher levels of blood glucose, plasma cortisol, plasma lactate, and lower levels of blood pH, and that greater changes in these parameters will occur during the warmer sampling period.

Methods

Study Site

Sampling occurred near Selkirk, Manitoba, Canada (50.127881°, -96.879764°) on Treaty 1 Territory along the Red River, also known as Miscousipi in Cree (meaning “Red Water River”). Angling occurred during two seasons: spring (May 26–June 1, 2019) and summer (July

10–July 23, 2019). In the spring, mean (± SD) water temperature was 15.9 ± 1.2 °C and mean (± SD) dissolved oxygen (DO) was 10.14 ± 0.29 mg/L. In the summer, mean (± SD) water temperature was 24.8 ± 0.8 °C and mean (± SD) DO was 8.30 ± 1.02 mg/L. Water temperature (Kruskal-Wallis test; $F = 104.7$; d.f.= 1, 36; $P < 0.001$) and dissolved oxygen (Kruskal-Wallis test; $F = 88.51$; d.f.= 1, 36; $P < 0.001$) were significantly different between seasons.

The Red River is considered productive and supports a variety of fish species including 51 native species and six introduced species (North/South Consultants Inc. 2010). Recreational anglers on the Red River largely target trophy channel catfish (Siddons et al. 2016), though other fish species are often captured while anglers are attempting to catch channel catfish, thus the fishery is also C&R for many species, including freshwater drum, through bycatch. Freshwater drum are also targeted as trophy fish (Travel Manitoba 2020) and in the Manitoba recreational fishery there is a limited harvest of 10 individuals with a maximum size limit of 60 cm. Thus, anglers intending on harvesting will likely release some smaller individuals while angling, and when trophy freshwater drum are caught they must be released. Locally, freshwater drum are ranked as Secure (S1) and are not a conservation concern in Manitoba (NatureServe 2020). To my knowledge abundance data is not collected in Manitoba because the population is deemed secure, and no major threats are currently documented for this species in Manitoba, which is the northern periphery of a wide North American distribution that extends south into Guatemala.

Field Sampling

Freshwater drum were angled from shore using rod and reel, pickerel rigs (size 2/0 barbless hooks, 20 lb test line), and earthworms as bait. Pickerel rigs are lures that tie onto the end of the main line and include two spreader arms that extend outwards perpendicular to the rig.

There is a J hook that hangs off each spreader arm and the rig has a weight attached to the bottom so that upon casting, the rig sits upright in the water column and does not drift. Once a fish was determined to be hooked, the fight time and air exposure times were varied to account for differences in angler skill level (fight time range: 5 s to 2 min; air exposure range: 20 s to 3 min). These ranges were assigned randomly prior to the fish being captured to avoid body size bias, but to ensure an even spread of times a haphazard approach was taken if one of the times was more prevalent in the data than another time. Upon capture, an injury assessment was performed to evaluate amount of bleeding and hooking location. Amount of bleeding was classified as either none, slight, or flowing (Bettoli et al. 2000), and hooking location was classified as critically hooked in the throat, or non-critically hooked in the mouth or jaws (Bartholomew and Bohnsack 2005). Critical hooking indicates difficulty in removing the hook as well as a greater chance of severe injury, whereas non-critical hooking indicates ease of removing the hook and less chance of severe injury. After the hook was removed fish were placed in aerated recovery bins (volume: 113.5 L; dimensions: 82.4 x 37.6 x 35.8 cm) containing fresh river water. Within 3 min of capture, a blood biopsy was taken using a 21-gauge needle and heparinized vacutainer, which drew blood through caudal puncture (Lawrence et al. 2020). The initial blood sample was taken within 3 min upon capture because stress biomarkers in the blood at this time are representative of the physiology of the organism prior to the angling event (i.e., a 'baseline' for the second blood sample; Lawrence et al. 2018). Blood pH (Professional Portable Cheese pH Meter; Hanna Instruments, Inc., Woonsocket, RI, USA) and blood glucose (OneTouch UltraMini Glucose Meter) levels were taken in the field (Wells and Pankhurst 1999), and the blood sample was then centrifuged for 2 min at 2000 RPM to separate plasma from red blood cells (Louison et al. 2017b). Plasma was then immediately flash frozen in liquid nitrogen before being moved to a -80 °C freezer in the laboratory until further analysis. Thirty minutes after the angling event, a second blood sample was taken in a similar fashion as described above, as stress biomarkers in most teleost fishes are expected to peak around 30 min (Lawrence et al. 2018), however, at the time of sampling, I did not know if this was the case for freshwater drum. Drawing blood was not always successful, which explains differences in sample size for some analyses.

Following a 15 min recovery period after the second blood sample, a reflex action mortality predictor (RAMP) test was given to assess impairment. Reflexes are innate and automatic responses to stimuli that can lead to more complex behaviours. Five reflexes were scored: 'body flex' (if the fish attempted to escape while being held out of water by the midsection); 'tail grab' (if the fish attempted to burst swim after being pinched on the caudal peduncle); 'orientation' (if the fish attempted to right themselves after being placed upside down submerged); 'ventilation' (if the fish attempted to ventilate through movement of the operculum); and, 'vestibular-ocular response' (if the fish maintained eye contact with the handler after being rotated on its side) (Davis 2007; McLean et al. 2016; Louison et al. 2017b). The reflex was scored '1' when deemed impaired and scored '0' when deemed unimpaired, therefore a score of five indicates the fish was fully impaired. Any fish exhibiting full impairment was euthanized using cerebral percussion and severing of the cervical spine. Due to the length of time that passed between the landing and sampling of RAMP, the measured RAMP score is confounded by the 45 min holding period. However, I believe it is still a useful metric to assess possible long-term effects of the independent variables. Finally, the fish was measured for length and weight and was then released back into the river. All fish were handled and processed following protocols that were approved by the University of Winnipeg University Animal Care Committee (UACC), protocol #10491.

Laboratory Analyses

Both plasma cortisol and lactate were measured in blood plasma. A cortisol enzyme-linked immunosorbent assay (ELISA) test kit (Neogen Toxicology, Lexington, KY, USA) was used to assess cortisol concentration. ELISA kits were previously validated for use with fish (Sink et al. 2008). Plasma samples were diluted with extraction buffer at a 1:50 ratio. Plasma lactate concentrations were obtained via an enzymatic assay using a 96-well plate following the methods outlined in Lowry and Passonneau (1972) with a dilution factor of 3.75 (Suski et al. 2003). For the assay, the enzyme lactate dehydrogenase increases the speed that pyruvate and NADH are converted to lactate and NAD^+, respectively (Valvona et al. 2016). The reaction produces a color change that can be read using a spectrophotometer at 340 nm.

Statistical Analyses

Two-way ANOVAs were used to assess if plasma cortisol, plasma lactate, blood glucose or blood pH indicated that the stress response was activated as a result of the angling treatment by comparing 0 min (baseline) to 30 min values, as well as assessing if any seasonal variation was present in these values between the spring and the summer. To determine if fight duration, length of air exposure, reflex impairment, hooking location, or seasonality were predictors of the physiological variables at 30 min, linear models were generated for plasma cortisol, plasma lactate, blood glucose and blood pH. Second-order Akaike information criterion (AICc) were used to compare models and determine the most parsimonious linear model for each physiological variable using the package MuMIn in R (Bartoń 2019). Because of co-variation with total length, season was excluded from the glucose models to avoid bias, as only smaller fish were caught in the summer. This co-variation was ruled out for all other models. A Poisson linear regression was used to assess differences in RAMP scores (i.e., reflex impairment) between seasons, as it accounts for ranked data. The level of significance was assessed at $\alpha \leq 0.05$ for all statistical tests and all statistical analyses were performed using R version 3.6.1 (R Core Team 2019).

Results

Forty-eight freshwater drum were angled and sampled in total between both seasons (28 in the spring, 20 in the summer). The injury assessment revealed that 33 freshwater drum were non-critically hooked in the mouth and two non-critically hooked fish experienced slight bleeding. Fifteen fish were critically hooked in the throat and two experienced bleeding (one slight, one flowing). Additionally, five mortalities were observed following a critical hooking in the throat. Reflex impairment did not differ between seasons (Poisson linear regression; $\chi^2 = 0.163$; d.f. = 1; $P = 0.686$) and three individuals were considered fully impaired (RAMP score of five). The most common reflex to be impaired was 'orientation' which was impaired fourteen times, followed by 'tail grab' which was impaired in ten fish. The 'body flex' reflex was missing in eight fish, 'vestibular-ocular response' was missing in five fish, and 'ventilation' was the least impaired reflex, which was only found to be missing in four individuals.

Physiological parameters increased from 0 min to 30 min and were found to be significantly different for both spring and summer for all variables except blood pH during the summer (Table 2.1; Figure 2.1). Plasma cortisol increased 80-fold from 0 min to 30 min in the spring, and, increased 60-fold in the summer (Figure 2.1A). Blood glucose increased by 164 % in the spring when 0 min was compared to 30 min, and by 334 % in the summer (Figure 2.1B). Similarly, plasma lactate increased by 189 % in the spring and 221 % in the summer (Figure 2.1C). Finally, comparing blood pH taken at 0 min and 30 min, there was a 2-fold decrease in the spring but no change in the summer (Figure 2.1D).

Table 2.1. Results for two-way ANOVA for effects of blood sample time (baseline values or 30 minutes following an angling event) or season (spring or summer) on four different physiological variables (plasma cortisol, blood glucose, plasma lactate and blood pH) for freshwater drum (*Aplodinotus grunniens*) following an angling event.

Variable	Main Effects	d.f.	SS	*F* value	*P* value
Plasma Cortisol	Blood Sample Time	1	327462	122.692	**< 0.001**
	Season	1	45104	16.899	**< 0.001**
	Blood Sample Time X Season	1	6735	2.523	0.117
Blood Glucose	Blood Sample Time	1	124.90	66.94	**< 0.001**
	Season	1	27.66	14.82	**< 0.001**
	Blood Sample Time X Season	1	39.49	21.16	**< 0.001**
Plasma Lactate	Blood Sample Time	1	335.7	127.251	**< 0.001**
	Season	1	0.1	0.034	0.855
	Blood Sample Time X Season	1	3.8	1.429	0.236
Blood pH	Blood Sample Time	1	0.384	22.747	**< 0.001**
	Season	1	0.519	30.751	**< 0.001**
	Blood Sample Time X Season	1	0.111	6.573	**0.013**

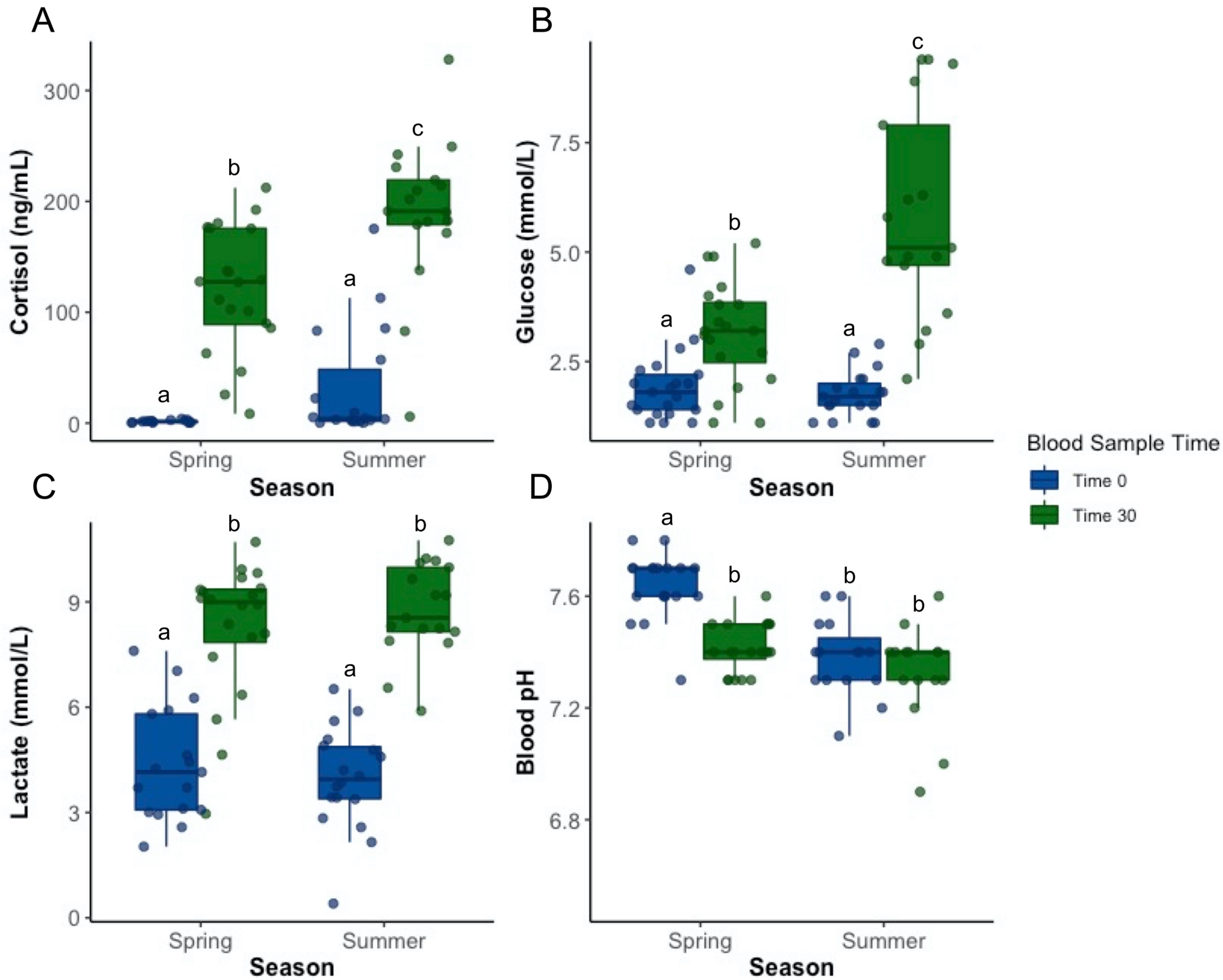

Figure 2.1. Levels of (A) plasma cortisol, (B) blood glucose, (C) plasma lactate and (D) blood pH in freshwater drum (*Aplodinotus grunniens*) following an angling event sampled in both the spring and summer seasons. Letters represent significant differences between blood sampling times indicating a stress response occurred, as well as seasonal differences in plasma cortisol, blood glucose and blood pH 30 minutes following an angling event.

The most parsimonious models to predict plasma cortisol, blood glucose, plasma lactate and blood pH were found via AICc (Table 2.2). RAMP score and season were significant predictors of plasma cortisol (Table 2.2 and 2.3). In the spring, individuals that were more impaired had lower plasma cortisol (Figure 2.2; linear regression; t = -2.641; d.f. = 1, 18; P = 0.017). Individuals in the summer had higher plasma cortisol at 30 min following an angling event than those at 30 min in the spring (Table 2.3; Figure 2.1). Four different models significantly predicted blood (Table 2.2 and 2.3) and these models were the only models to include total length as a co-variate (Figure 2.3; linear regression; t = -5.261; d.f. = 1, 35; P < 0.001). In addition to total length, air exposure was also selected as a predictor in the most parsimonious model and it was found to have a negative relationship with blood glucose (Table 2.3; Figure 2.4C; linear regression; t = -2.519; d.f. = 2, 34; P = 0.017). The most parsimonious model for plasma lactate included air exposure time as the only predictor. Increasing air exposure time significantly decreased plasma lactate concentration (Table 2.3; Figure 2.4B; linear regression; t = -2.976; d.f. = 35; P = 0.005). A second model including air exposure and fight duration was also identified for plasma lactate, but fight duration was not a significant predictor of plasma lactate (Table 2.3) nor any of the other physiological variables (Figure 2.5). Plasma lactate also did not vary seasonally (Table 2.3; Figure 2.1C). Lastly, season was found to be a significant predictor of blood pH overall as pH was lower in the summer than it was in the spring (Table 2.3; Figure 2.1D).

Table 2.2. Second-order Akaike information criterion (AICc) comparing linear models for each physiological variable (package MuMIn in R). The dependent variable is the 30 min value for each physiological variable following an angling event (plasma cortisol, blood glucose, plasma lactate and blood pH). Weight represents the relative likelihood of the model. The selected model for statistical analyses for each physiological variable is bolded. Season was not included in blood glucose models, as no small fish were caught during the spring and these were the only models which included total length as an independent variable.

Dependent Variable	Model	d.f.	AICc	Weight
Plasma Cortisol	**RAMP Score + Season**	**4**	**414**	**0.97**
	RAMP Score	3	422	0.02
	Fight Duration + Air Exposure + Hook Location + Season	6	422	0.01
Blood Glucose	**Total Length + Air Exposure**	**4**	**146**	**0.44**
	Total Length + RAMP Score + Air Exposure	5	147	0.21
	Total Length + RAMP Score	4	148	0.17
	Total Length + Air Exposure + Fight Duration	5	148	0.13
	Total Length + RAMP Score + Air Exposure + Fight Duration	6	150	0.05
Plasma Lactate	**Air Exposure**	**3**	**141**	**0.59**
	Fight Duration + Air Exposure	4	143	0.27
	Fight Duration + Air Exposure + Hook Location	5	146	0.07
	RAMP Score + Fight Duration + Air Exposure	5	146	0.07
Blood pH	**Season**	**3**	**-37**	**0.46**
	Air Exposure	3	-35	0.22
	Air Exposure + Season	4	-35	0.19
	RAMP Score + Season	4	-34	0.13

Table 2.3. Summary statistics for the most parsimonious linear models for each physiological variable. Significance was assessed at $P \leq 0.05$ and significant results are bolded. Models that were not statistically significant were not included in this table. The dependent variable is the 30 min value for each physiological variable following an angling event (plasma cortisol, blood glucose, plasma lactate and blood pH).

Variable	Factor	Value	SEM	d.f.	*t*-value	*P*-value
Plasma Cortisol	(Intercept)	134.196	14.887	2, 34	9.015	< 0.001
	RAMP Score	-13.246	6.214	2, 34	-2.131	**0.040**
	Season	66.836	19.767	2, 34	3.381	**0.002**
Blood Glucose	(Intercept)	13.015	1.471	2, 34	8.849	< 0.001
	Total Length	-0.019	0.004	2, 34	-4.933	**< 0.001**
	Air Exposure	-0.018	0.007	2, 34	-2.519	**0.017**
	(Intercept)	13.197	1.479	3, 33	8.924	< 0.001
	Total Length	-0.020	0.004	3, 33	-5.049	**< 0.001**
	RAMP Score	-0.199	0.190	3, 33	-1.047	0.303
	Air Exposure	-0.014	0.008	3, 33	-1.703	0.098
	(Intercept)	13.018	1.516	2, 34	8.588	< 0.001
	Total Length	-0.022	0.004	2, 34	-5.630	**< 0.001**
	RAMP Score	-0.354	0.171	2, 34	-2.078	**0.045**
	(Intercept)	12.831	1.541	3, 33	8.327	< 0.001
	Total Length	-0.019	0.004	3, 33	-4.845	**< 0.001**
	Air Exposure	-0.019	0.008	3, 33	-2.494	**0.018**
	Fight Duration	0.005	0.011	3, 33	0.459	0.649
Plasma Lactate	(Intercept)	10.006	0.571	1, 35	17.528	< 0.001
	Air Exposure	-0.020	0.007	1, 35	-2.976	**0.005**
	(Intercept)	9.714	0.651	2, 34	14.914	< 0.001
	Fight Duration	0.009	0.010	2, 34	0.937	0.355
	Air Exposure	-0.022	0.007	2, 34	-3.112	**0.004**
Blood pH	(Intercept)	7.415	0.030	1, 33	248.108	< 0.001
	Season	-0.095	0.046	1, 33	-2.081	**0.045**

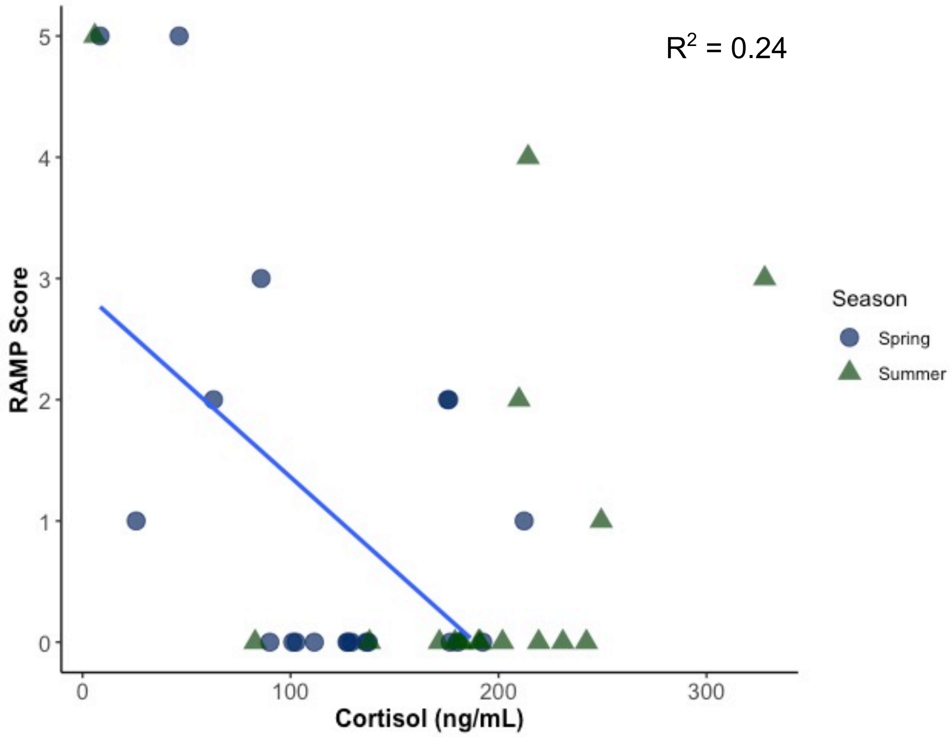

Figure 2.2. Relationship between the concentration of plasma cortisol (ng/mL) and RAMP scores in freshwater drum (*Aplodinotus grunniens*) following an angling event in the spring.

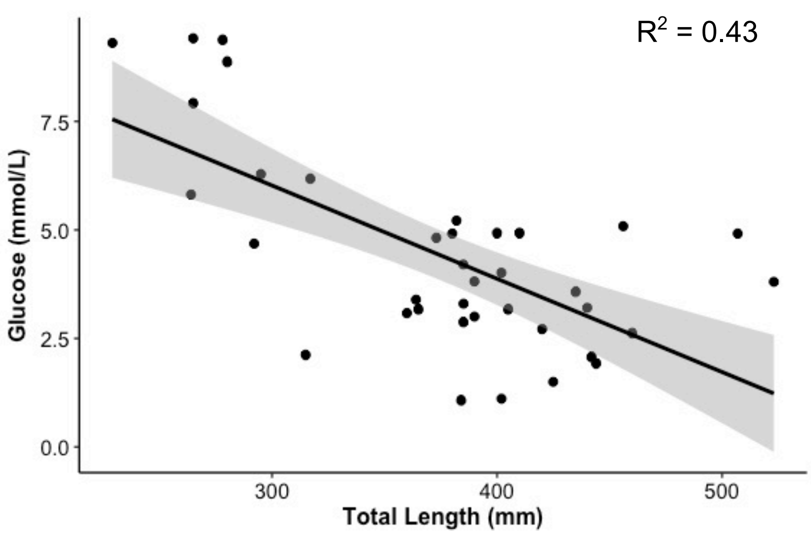

Figure 2.3. Relationship between glucose (mmol/L) 30 minutes following an angling event and total length (mm) of freshwater drum (*Aplodinotus grunniens*).

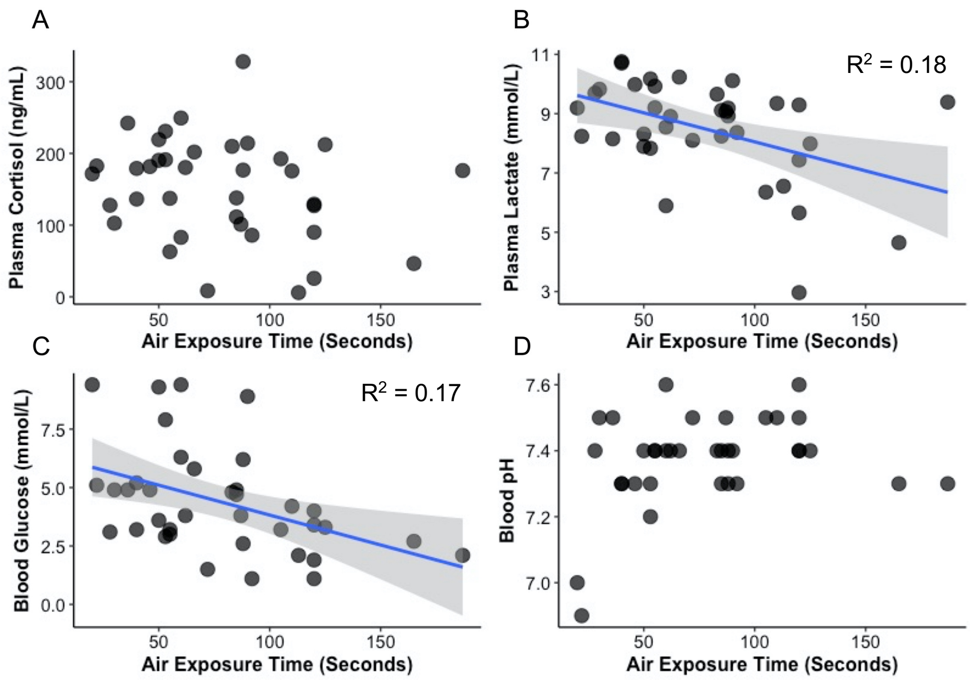

Figure 2.4. Relationship between air exposure time (seconds) and the values of (A) plasma cortisol (ng/ml), (B) plasma lactate (mmol/L), (C) blood glucose (mmol/L) and (D) blood pH in freshwater drum (*Aplodinotus grunniens*) 30 minutes following an angling event.

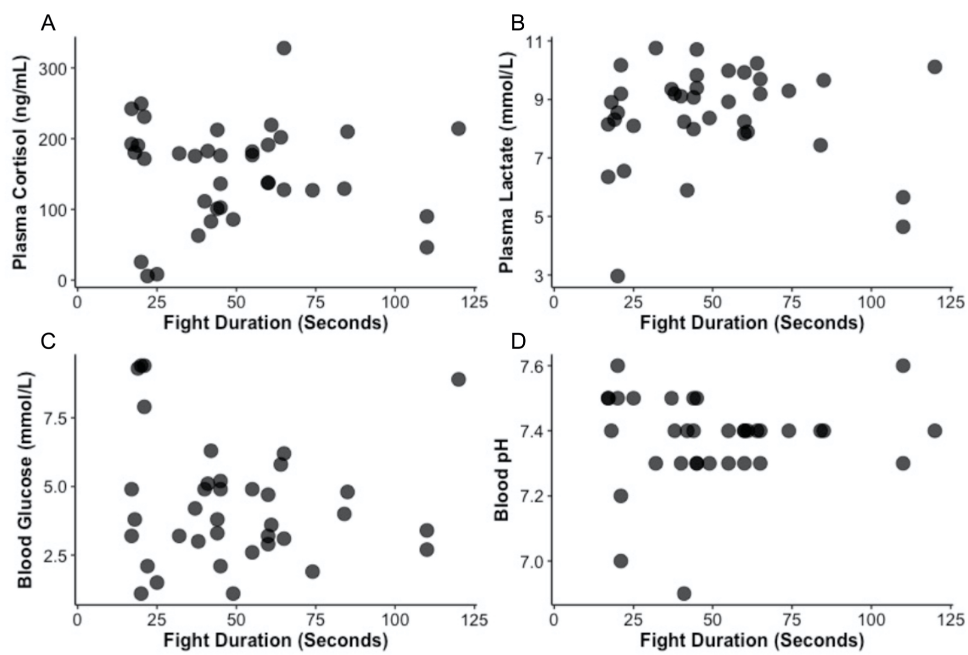

Figure 2.5. Relationship between fight duration (seconds) and the values of (A) plasma cortisol (ng/ml), (B) plasma lactate (mmol/L), (C) blood glucose (mmol/L) and (D) blood pH in freshwater drum (*Aplodinotus grunniens*) 30 minutes following an angling event.

Discussion

Physiological responses and RAMP

Freshwater drum in my study experienced elevated plasma cortisol, plasma glucose and plasma lactate following an angling event, which is consistent with what is known for other teleost fishes (e.g., Suski et al. 2003; Thompson et al. 2008; Pankhurst 2011; Cooke et al. 2013; Lawrence et al. 2018). When comparing differences in physiological parameters between the 0 min and 30 min sampling times, significant changes for most physiological variables were observed, indicating a disruption in homeostasis (Wendelaar Bonga 1997). These changes following angling events are expected (e.g., Gallman et al. 1999; Suski et al. 2003), because the physiological changes increase the amount of energy available for fish to cope with stressors and avoid death (Schreck and Tort 2016). Blood glucose increases at a slower rate than corticosteroids because of hepatic glycogenolysis, where blood glucose is released to provide immediate energy for muscles (Wendelaar Bonga 1997; Schreck and Tort 2016). Additionally, lactate levels indicated an exhaustion of aerobic energy stores and a hypoxic cellular environment (Wardle 1978; Wood et al. 1983). Often as a result of lactic acid production, blood pH decreases (Wardle 1978). As to why blood pH did not decline following angling in the summer, it could be due to a decline in blood pH occurring at an accelerated rate in warmer water temperatures (Wilkie et al. 1996) and I was not able to sample the blood fast enough to capture the true baseline blood pH. Furthermore, the sample size for each season was small and is a limitation of the seasonal comparison of the physiological responses, however, it was clear that an angling event induced a consistent physiological response in freshwater drum.

Physiological metrics are useful in understanding what is occurring in the body, but often fail to predict post-release mortality when they are the only metric being examined (Wood et al.

1983; Davis 2002; Brownscombe et al. 2017). In my study not only did the physiology of freshwater drum change, but I also observed a change in reflexes that may help understand the potential for post-release mortality. Overall, reflexes appeared to be consistent between seasons and only three individuals received a RAMP score of five meaning they were deemed fully impaired and were euthanized. The most commonly impaired reflex was 'orientation'. Twenty-nine percent of fish were unable to maintain their orientation within the water column following the angling event, which indicates a complete lack of coordinated movement, suggesting a shutdown of the neuromuscular system (Raby et al. 2012). Lacking the ability to maintain an upright position leaves fish susceptible to post-release mortality, as lacking the orientation reflex is a predictor of delayed mortality in other species (Brownscombe et al. 2017) such as bluegill (*Lepomis macrochirus*; Gingerich et al. 2007) and coho salmon (*Oncorhynchus kisutch*; Raby et al. 2012). Although freshwater drum do not have many natural aquatic predators in the Red River watershed, they may be especially vulnerable to avian predation (e.g., by American white pelicans [*Pelecanus erythrorhynchos*]), particularly if the fish floats along the surface of the water following release. Once the orientation reflex is lost, mortality by avian predation is a potential outcome as they are unable to submerge themselves out of sight (Ross and Hokenson 1997; Jarvis and Lowe 2008; Nguyen et al. 2009; Raby et al. 2014; Brownscombe et al. 2017). Given this vulnerability to predation, it is possible that fish lacking the orientation reflex in my study did not survive upon release. However, given the length of time between angling and the RAMP assessment, I cannot be confident that the loss of reflexes was entirely due to angling.

RAMP score had a negative relationship with plasma cortisol in the spring season which contradicted my prediction that higher physiological stress metrics would be found in more impaired fish. RAMP score has been found to have a negative relationship with plasma cortisol in ice-angled bluegill sampled 30 min following capture (Louison et al. 2017b). Louison et al. (2017b) speculated that the sampled fish had not yet responded physiologically when RAMP scores were taken due to lower enzymatic activity in colder water temperatures. Because my study was completed during warmer periods and I observed physiological responses, it is difficult to compare the two findings, thus I believe further study is warranted to understand why RAMP scores would be negatively related to plasma cortisol.

Seasonal and angler effects

Seasonal differences were observed in some physiological variables, but not all. Generally, the stress response is increased in teleosts during warmer water temperatures following angling events (Gustaveson et al. 1991; Wilkie et al. 1996; Thompson et al. 2002, 2008; Meka and McCormick 2005; Arlinghaus et al. 2007; Landsman et al. 2011; McLean et al. 2016). Plasma cortisol and blood glucose sampled 30 minutes following angling were found to be 63 % and 54 % higher in the summer than in the spring, respectively. This elevation of stress parameters in warmer water may be caused by lower levels of available dissolved oxygen in the water as temperatures increase (as suggested by Cooke and Suski 2005), or by higher metabolic rate experienced in warmer water temperatures (Johnston and Dunn 1987). Furthermore, the rate of physiological processes such as the synthesis and release of cortisol following the angling event is increased in warmer water (i.e., Q_{10} effects), therefore temperature-mediated effects on plasma cortisol and glucose were expected (Schreck and Tort 2016).

Plasma lactate was the only variable that did not differ seasonally. Other studies have failed to find a temperature effect on lactate levels in the range of temperatures present in my study. For example, largemouth bass exhaustively exercised and air exposed had similar lactate levels at 14 and 20 ºC, and significantly higher levels at 32 ºC (Suski et al. 2006). Other studies have found higher temperatures to induce higher levels of lactate, however, these studies were completed on cold water species (e.g., Meka and McCormick 2005; Kieffer et al. 2011; McLean et al. 2016; Morrison et al. 2020). Therefore, the lack of seasonal effect on plasma lactate is likely due to freshwater drum being a warm water species and my study being conducted over a relatively narrow temperature range. Sampling blood at a variety of time periods sufficient to observe the rise and fall of lactate could provide clarity on the lack of a seasonal lactate response.

To understand how angling techniques specifically impact freshwater drum, I compared physiological responses to fight duration and length of air exposure. Length of time exposed to air was found to be a significant negative predictor of both blood glucose and plasma lactate. Although contrary to my prediction of expecting to observe higher physiological stress values with longer air exposure, other studies have also failed to find positive correlation between air exposure and elevated physiological variables, such as a study on northern pike (*Esox Lucius* L.) where air exposure between 0 and 300 seconds did not correlate positively with any stress physiology metrics (Arlinghaus et al. 2009). Fight duration was not related to any of the stress physiology metrics, thus it is possible that my fight times were short enough that they did not physically exhaust the fish. Similarly, fight duration had no effect on physiological variables in permit (*Trachinotus falcatus*) with fight duration ranging from 1 to 12 min (Holder et al. 2020), which is longer than the fight duration used in my study (5 seconds to 2 min). Overall, both length of time exposed to air and fight duration did not appear to have much of an impact on freshwater drum, as positive relationships with physiological variables were not found for either. Because both fight duration and length of time exposed to air were representative of angling events in this environment, the lack of a positive relationship with longer fight and air exposure times is promising for the conservation goal of catch-and-release angling for freshwater drum.

Hooking location

Delayed hooking mortality as a result of angling is influenced by the location of the hook wound (Arlinghaus et al. 2007). Thirty-one percent of fish (15 individuals) were critically hooked in the throat and 33 % of those critically hooked fish (5 individuals) died prior to release. The critically hooked fish tended to 'swallow' the hook or were hooked deeply in the esophagus tissue. Other studies have demonstrated that fish hooked in esophageal tissue are less likely to survive angling events (Muoneke and Childress 1994; Bartholomew and Bohnsack 2005; Brownscombe et al. 2017). For example, lake trout (*Salvelinus namaycush*) that were hooked internally in sensitive organs experienced a delayed mortality rate of 71.4 %, whereas when hooked in the jaw only experienced a delayed mortality rate of 6.9 % (Loftus et al. 1988). Therefore, it is possible that released freshwater drum in the study eventually died as a result of the angling event, though further study is needed.

It is possible that the type of tackle used in my study is the cause of the frequency of critical-hooking and there may be options to reduce this in the future. The tackle I used mimicked what is commonly used by anglers in Manitoba to catch freshwater drum (i.e., baited barbless J hooks), thus critical hooking may be a common occurrence in the fishery. Studies have shown that circle hooks minimize the frequency at which fish swallow the hook in species such as white seabass (*Atractoscion nobilis*; Aalbers et al. 2004), red drum (Aguilar et al. 2002), and rock bass (*Ambloplites rupestris*; Cooke et al. 2003). Red drum are a close relative of freshwater drum, and Aguilar (2003) found that 52 % of red drum caught using J hooks were deeply hooked, whereas only 4.2 % of red drum caught using circle hooks were deeply hooked. Furthermore, a mortality rate of 15.9 % was observed for red drum deeply hooked with J hooks, compared to a 0 % mortality rate for red drum deeply hooked with circle hooks (Aguilar 2003). Freshwater drum seem to be more sensitive to deep hooking than red drum, as 33 % of deeply hooked freshwater drum died in my study, compared to the 15.9 % mortality rate in red drum (Aguilar 2003). It is also possible that more freshwater drum died upon release, as we did not track delayed mortality. Pickerel rigs with circle hooks may lead to fewer instances of freshwater drum swallowing the hooks, and work to implement a change in practice in Manitoba may benefit the fishery.

Best practices

General best practices for the handling of freshwater drum that an angler intends to release should always be practiced to avoid unintended mortality. Firstly, fish should be kept submerged underwater as much as possible to minimize air exposure and fight times should be minimized to avoid physical exhaustion (Cooke and Suski 2005; Brownscombe et al. 2017). Secondly, it is especially important to focus on these strategies while angling during warmer time periods because greater physiological disturbances were observed in freshwater drum during warmer water temperatures in the summer. Thirdly, techniques and equipment that minimize deep hooking should be sought by anglers. It was clear from my findings that freshwater drum are sensitive to deep hooking, and post-release mortality might be a common occurrence, although further study is required. Lastly, freshwater drum may be an important model for studying best practices in teleost fishes because they have the largest latitudinal range of any North American freshwater fish and this unique species characteristic may help to understand the convergence between climate change and recreational angling impacts on freshwater fishes.

Acknowledgements

This research was carried out on the traditional territories of the Anishinaabeg, Cree, Dakota, Dene, Métis, and Oji-Cree Nations (Treaty 1 Territory). Funding support for this research was provided through an NSERC Alexander Graham Bell master's studentship award held by J. T. Card, an NSERC discovery grant held by C. T. Hasler, and a University of Winnipeg start-up grant held by C. T. Hasler. I extend a heartfelt thank you to both Anne-Laure Card and Dan Card for hosting us while I carried out my research. Additionally, I extend a huge thank you to everyone that assisted with catching fish and sampling them in the field including Theresa Mackey (whom also assisted with laboratory analysis), Jenna Fleet, Dan Card, James Maclean, Christian Ridao, Dr. Jen Jeffrey, Aidan Novalkowski, Dr. Mike Lawrence, Marianne Geisler, McKenzie Hauger, Alex Schoen, Matt Jensby, Emily McIntosh, Caleb Wong and Kelsie Oliphant. Two anonymous reviewers provided helpful revisions and comments on a previous version of the manuscript. A scientific collection permit was obtained and followed regarding fish collection by the Manitoba Sustainable Development Fisheries Science and Fish Culture Section Fisheries Branch, permit #14-19.

Chapter 3: Examination of potential histological, thermal, and reflex effects of ice and air exposure on yellow perch (*Perca flavescens*) following ice-angling

Abstract

Ice-angling is a popular activity practiced across northern regions during the winter season. Despite this popularity and due to inherent issues with sampling fish in the wintertime, few studies have quantified the sublethal impacts of ice-angling and ice and air exposure on fish species that will be released back into the water. To address this knowledge gap, yellow perch (*Perca flavescens*) were exposed to ice and air for 3 min following an ice-angling event. The surface temperature of various tissues was quantified throughout this exposure, reflex impairment using RAMP (reflex action mortality predictor) scores was assessed following the exposure, and tissues were excised for histological analyses. Sixty percent of all fish captured showed impaired 'body flex' reflex. Fifty six percent of fish exposed to the treatment were impaired for the 'orientation' reflex compared to only 14 % of control fish lacking this reflex. Heat loss occurred at the midbody throughout the 3-min exposure, and gills, eye, and caudal fin all remained below 0 °C throughout the exposure and for the control treatment. Histologically, aneurysms within the secondary lamellae of the gills were found in 68.75 % of all fish (both control and treatment, no significant difference between groups) that were caught via ice-angling, which may result from exposure to sub-zero temperatures that may have compromised pillar cells. To my knowledge, this is the first observation of aneurysms occurring within the secondary lamellae of the gills of a teleost fish following an angling event. I recommend that anglers limit air exposure when ice-angling in sub-zero temperatures by keeping fish submerged

in water or releasing any fish immediately that they do not intend on harvesting, to limit negative biological consequences of cold air exposure coupled with the stress of an ice-angling event, which has been found in other studies.

Introduction

Recreational angling is the practice of catching fish using a rod and reel for non-consumptive or non-economic reasons (Cooke and Cowx 2004). In northern regions where ice freezes thick enough during winter, recreational angling takes the form of ice-angling, which involves capturing fish through a hole drilled in the ice (Margenau et al. 2003; Deroba et al. 2007; Logan et al. 2019). Ice-angling contributes billions of dollars to local economies. For example, the walleye (*Sander vitreus*) recreational ice fishery in Lake Winnipeg, Manitoba has been estimated to contribute hundreds of millions of dollars to the regional economy (Manitoba Wildlife Federation 2018; Lawrence et al. in review). Despite the popularity of ice-angling, the consequences of it on fish have seldom been studied (Arlinghaus et al. 2007; Lawrence et al. in review).

The process of ice-angling requires that hooked fish be retrieved and then handled out of water. Retrieval times vary from a few seconds to several minutes, depending on the type of gear used and the size of the fish captured (Althoff et al. 2020), and landed fish are often held in the air or on ice for a few seconds to several minutes to remove hooks and to take measurements and photos (Lawrence et al. in review). While fish are frequently harvested, many fish are released back through the hole in the ice for a variety of reasons, including conservation ethics and morals, bycatch, culling and harvest regulations (Cooke and Suski 2005; Arlinghaus et al. 2007). The assumption of releasing fish after capture is that they continue to survive and reproduce (Cooke and Schramm 2007; Arlinghaus et al. 2007; Brownscombe et al. 2017).

Only a handful of ice-angling studies have been completed, primarily focusing on hooking location and delayed mortality (Dextrase and Ball 1991; Persons and Hirsch 1994; DuBois et al. 1994; Twardek et al. 2018; Althoff et al. 2020; Somers et al. 2021). The results indicate that deeply hooked fish may be susceptible to higher rates of delayed mortality following ice-angling, and these studies focus on species commonly targeted by ice-anglers such as lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*) and walleye (Dextrase and Ball 1991; Persons and Hirsch 1994; DuBois et al. 1994; Twardek et al. 2018; Althoff et al. 2020). Fewer studies have focused on physiological or behavioural consequences of ice-angling on fish (Louison et al. 2017b, 2017a; Winter et al. 2018; Logan et al. 2019; Bieber et al. 2019). These physiological assessments have shown an attenuated stress response following ice-angling in a variety of species when compared to studies performed in warmer water temperatures, which may result from colder water temperatures decelerating enzyme activity (Louison et al. 2017b, 2017a; Logan et al. 2019). Levels of plasma lactate and cortisol in yellow perch (*Perca flavescens*) following an ice-angling event have been previously quantified to map a recovery profile, as they were sampled at different time points following the ice-angling event (0 min, 30 min, 2 h and 4 h; Louison et al. 2017b). Louison et al. (2017b) found that lower temperatures appear to dampen the magnitude of the physiological response, because lower levels of plasma cortisol and lactate were observed when compared to those experienced by yellow perch in warmer water temperatures (Eissa and Wang 2013; Louison et al. 2017b). Not only were these values lower than expected, but the response was delayed, as significantly higher levels of plasma cortisol at 2 and 4 h post-capture were observed, even though it is expected that these values would peak approximately 30 min post-capture if the study was performed in warmer water temperatures (Louison et al. 2017b; Lawrence et al. 2018). This same trend was observed in northern pike following an ice-angling event (Louison et al. 2017a). It is hypothesized that both shorter fight times associated with ice-angling events and reduced enzymatic activity in colder temperatures play a role in this delayed physiological response in colder water temperatures (Louison et al. 2017b, 2017a; Logan et al. 2019). While the stress response shown in ice-angled fish appears dampened, sub-zero air exposure following a simulated winter angling event impairs swimming ability in bluegill (*Lepomis macrochirus*), which could diminish their ability to evade predators or carry out life sustaining activities such as feeding (Bieber et al. 2019). Ultimately, without understanding all the effects of ice-angling on released fish, mortality and recruitment estimates may be underestimated or overestimated, respectively, and this would have significant consequences for fisheries management (Cooke et al. 2002; Davis 2002; Cooke and Schramm 2007; Arlinghaus et al. 2007; Brownscombe et al. 2017).

Yellow perch are commonly targeted by recreational ice-anglers. Catch rates are usually high for yellow perch, and thus anglers may catch their entire harvest limit and continue to fish throughout the day to maximize the size of the fish they intend on retaining, termed 'culling' (e.g., Logan et al. 2019). During this time, anglers often leave captured fish on the ice, which deprives the fish of oxygen and may induce freezing of vital tissues such as the gills. In addition to anglers releasing previously caught smaller fish for larger ones, large fish may also be released, as some trophy fish record-keeping programs require that the fish be released to meet submission rules (Travel Manitoba 2020). These events often lead to prolonged air exposure and handling as programs also require photographs and measurements for submissions (Travel Manitoba 2020). The physiological stress response following ice-angling was previously quantified in yellow perch revealing a delayed stress response (Louison et al. 2017b), but this study did not address surface tissue freezing which could impact recovery of released fish during winter. Furthermore, the need to histologically assess damage to gills from sub-zero temperatures was highlighted by Logan et al. (2019), as damage within the gills may have long-term consequences for the fish following release. Research that simulates real-world angler behaviour is needed to improve our understanding of how fish are impacted following release during cold temperatures in northern climates.

As catch-and-release angling is continually occurring in recreational fisheries (Cooke and O'Connor 2010), fisheries managers must understand the effect that ice-angling has on released fish. Without this understanding, improper management of populations could result from a lack of knowledge surrounding both sublethal and lethal effects of ice-angling on fish that will be released back into the population. I aim to build on the findings that there are sublethal effects of ice-angling on freshwater fish (e.g., Louison et al. 2017b) by further assessing reflex impairment, quantifying tissue freezing by recording the surface temperature of important tissues exposed to sub-zero air temperatures and assessing tissue damage using histological methods. The objective of my study was to quantify the severity of winter catch-and-release angling on yellow perch using an exploratory approach by looking at reflex impairment, temperature loss, and injury to the gills or fins through histological assessment. My hypothesis is that winter catch-and-release angling has physical consequences for yellow perch, and I predict that winter catch-and-release angling will impair reflexes, induce heat loss, and damage tissues through freezing. The potential for tissue freezing in the gills in ice-angled fish has only been assessed once before (Bieber et al. 2019) and has never been assessed for fins. It is important that both aspects are investigated in fish following ice-angling events as sublethal effects have the potential to impact whole organismal performance in both the short and long-term (Arlinghaus et al. 2007; Cooke et al. 2013a).

Methods

Study Locations

Fieldwork was completed at two locations: Fox Lake, WI, USA (43.573953°, -88.929323°) on January 8 and 9, 2019 and Gull Lake, MB, Canada (50.414498°, -96.518582°) on March 9, 2019. Air temperature ranged from -8.0 °C to 1.0 °C at Fox Lake, WI and -4.1 °C to -2.6 °C at Gull Lake, MB. Water temperature was approximately 4 °C at both sampling locations though was not continuously monitored. Fox Lake, WI had an oxygen saturation of 132.5 %, and dissolved oxygen at Gull Lake, MB was not monitored as there is an aeration system installed on the lake during the winter months to prevent hypoxic conditions. Sport fish species present in Fox Lake, WI include yellow perch, muskellunge (*Esox masquinongy*), largemouth bass (*Micropterus salmoides*), northern pike, walleye, bluegill, black crappie (*Pomoxis nigromaculatus*) and white crappie (*Pomoxis annularis*; Hey and Associates, Inc. and University of Wisconsin-Milwaukee 2008), whereas sportfish species present in Gull Lake, MB only include yellow perch and northern pike (Angler's Atlas 2021). Both lakes are regularly targeted by recreational ice-anglers.

Sampling Methodology: Fox Lake, WI, USA

Yellow perch were angled through holes in the ice using standard ice-fishing rods spooled with 1.8 kg monofilament fishing line. A size 7 barbless J-hook with a coloured weighted head was tied onto the fishing line and was dropped down the hole into the water after baiting with a white grub while performing a motion called "jigging," whereby the angler bobs the fishing rod up and down. Upon capture, the hook was immediately removed, and the fish was brought to a central location (no more than 20 m from any hole) for group assignment and processing.

Fish were randomly assigned to one of two groups: control or treatment. Control fish (n = 6) were measured for total length, weighed, and assessed for reflex impairment (Raby et al. 2012). I used a reflex action mortality predictor (RAMP) assessment to score each fish on four metrics: “body flex” (fish attempts escape while being held out of water by the midsection), “tail grab” (fish attempts to burst swim away after being pinched on the caudal peduncle), “orientation” (fish attempts to right themselves after being placed upside down submerged), and “vestibular-ocular response” (fish keeps its eye on the handler after being rotated on its side; Davis 2007; McLean et al. 2016; Louison et al. 2017b). The reflex was scored “0” when deemed unimpaired and scored “1” when deemed impaired (McLean et al. 2016). The RAMP assessment was completed in a shallow container which allowed fish to be submerged, and fresh lake water was added each time an assessment was completed. The treatment group (n = 8) underwent the same RAMP test, but it was conducted following a 3-min ice and air exposure where the right side of the fish was laid on the ice. Surface temperature assessments were not completed at the Fox Lake location and therefore the focal point for data collection from Fox Lake was exclusively reflex impairment. All fish were handled and processed following protocols approved by the University of Winnipeg University Animal Care Committee (UACC), protocol #10491, and the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois Urbana-Champaign, protocol #18215.

Sampling Methodology: Gull Lake, MB, Canada

The angling methodology and the ice and air exposure treatment at Gull Lake was the same as Fox Lake, but the post-capture methodology differed. The focal points for data collection from Gull Lake were reflex impairment, temperature readings of important tissues throughout the treatment, and tissue collection for histological analysis. Upon capture, the treatment group (n = 8) were measured for total length, weighed, and were then placed on the ice for a 3-min ice and air exposure (ice temperature: -3 ± 0.73 °C). Surface temperature was recorded using a non-contact infrared thermometer (FLIR TG165 Imaging IR Thermometer; FLIR Systems, Inc. Wilsonville, OR, USA) for both sides of the fish at various landmarks including: gills, eyes, midbody beneath the dorsal fin, and caudal fin. Temperature readings were taken each minute of the air-exposed side throughout the treatment, and at the end of the ice and air exposure the fish was flipped over, and temperature readings of the same tissues were taken of the other side of the fish that had been exposed to the ice (ice-side readings were only measured at the end of the treatment period). To measure temperature of the gills, the operculum was opened to allow for the thermometer to be pointed directly at the gill filaments. Following the temperature readings, the previously described reflex impairment test was performed, and fish were then euthanized. Tissue samples including the first gill arch and pectoral fins from both sides of the fish, and a fin clip of the caudal fin were dissected and placed in fixative (modified Karnovksy's fixative: 1 % paraformaldehyde + 2.5 % glutaraldehyde in 0.1 M sodium phosphate, pH 7.2 buffer) for histological analysis. The control group (n = 8) was sampled in the same manner, except these fish were not subjected to the ice and air exposure and instead temperature readings were just measured for the tissues from both sides of the fish.

Histological Analysis

Fixed tissue samples were stored at room temperature. Samples were dehydrated by rinsing with 70 %, 80 %, 95 % and 100 % ethanol at -20°C. The last rinse was allowed to warm up to room temperature, and one final 100 % ethanol rinse was then performed for 30 min. Dehydrated tissues were embedded using a Leica Historesin Embedding Kit (7022-18 500 Leica Historesin Embedding Kit, Heidelberg, Germany). I then placed the samples in infiltration solution (50 mL Leica Historesin + 0.5 g dibenzolperoxide + 1.3 mL polyethylene glycol) and changed the solution twice over a 24 h period while refrigerated. Tissues were trimmed, placed, and oriented into molds using embedding solution (15 mL infiltration solution + 1 mL hardener). Chucks were placed on top of each mold for use in the microtome (Sorvall JB-4 Microtome, Ivan Sorvall, Inc., Norwalk, Connecticut) and were left for 24 h prior to sectioning. Samples were sectioned along the sagittal plane at 1.5 µm thickness and were placed onto slides and stained with 1 % toluidine blue + 1 % borax. Slides were examined using a microscope (CX41 Upright Microscope, Olympus Corporation, Tokyo, Japan) and imaged using the software Infinity Analyze 2-1 (Lumenera Corporation, Ottawa, Canada).

Imaged gills were measured for interlamellar cell mass (ILCM; Figure 3.1) and lamellar length (Figure 3.1). For lamellar length, ten lengths were randomly measured, as previously described by Ong et al. (2007) and Blair et al. (2016). Pectoral fins (both the right and left side) and caudal fins were assessed by measuring the size of mucous cells in the epidermis of the fins (Figure 3.2). The area of ten randomly selected mucous cells were measured using the software ImageJ (Schneider et al. 2012). Any abnormalities observed in the imaged tissues were also noted. The experimental groups were unknown to me when conducting histological analysis to avoid bias and were only revealed upon completion of the imaging and subsequent analysis.

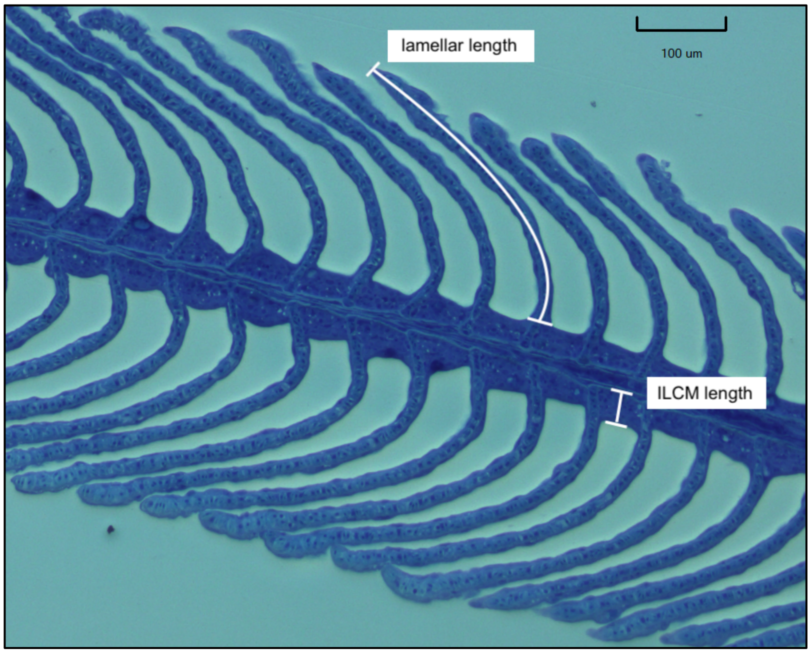

Figure 3.1. Reference image of a primary lamellae with secondary lamellae branching off of it, with the two measurements labeled that were taken for the gills: lamellar length and interlamellar cell mass (ILCM) length. The image is from the left side (air exposed) gill of a yellow perch (*Perca flavescens*) that experienced the treatment (3-min ice and air exposure following an ice-angling event). Tissues were stained with 1 % toluidine blue + 1 % borax stain and sectioned along the sagittal plane at 1.5 μm thickness. This image was taken at 100X magnification and the scale bar in the image represents 100 μm.

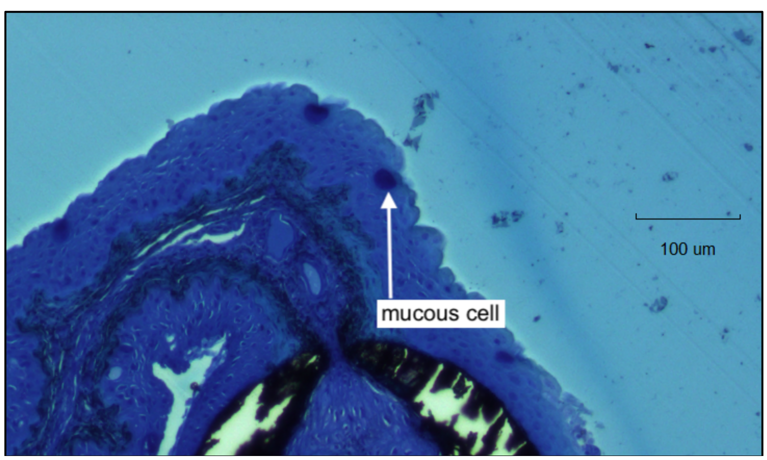

Figure 3.2. Reference image highlighting the mucous cells that were quantified within the epidermis of a caudal fin sample. The image is from the caudal fin of a yellow perch (*Perca flavescens*) that experienced the treatment (3-min ice and air exposure following an ice-angling event). Tissues were stained with 1 % toluidine blue + 1 % borax stain and represent a cross section of 1.5 μm thickness. This image was taken at 100X magnification and the scale bar in the image represents 100 μm.

Statistical Analysis

Surface temperatures of the midbody, eye and gill that were exposed to the air throughout the treatment were tested using one-way repeated measures ANOVAs to determine if these temperatures differed throughout the air and ice exposure (left side of fish only). The time point at which the temperature was taken was the independent variable and surface temperature was the dependent variable. When significant effects were detected, I used multiple pairwise paired t-tests to determine significance between the time points that the surface temperatures were taken of the air-exposed tissues. The results of the one-way repeated measures ANOVA for the midbody were corrected using the Greenhouse-Geisser correction as these data did not meet the assumption of sphericity. Surface temperatures of the caudal fin failed the Shapiro-Wilk test for normality, so instead a non-parametric Friedman test was performed. These temperatures were not compared to controls, as the 0-min time point acts as a baseline comparison within the 3-min treatment. The 3-min air-exposed surface temperature reading (left side of the fish) was compared to the 3-min ice-exposed surface temperature reading (right side of the fish) using paired t-tests for each tissue, because the surface temperature of the ice-exposed side was only measured at the end of the treatment. Differences in RAMP scores (i.e., reflex impairment) between control and treatment individuals were assessed using a Poisson linear regression to account for ranked data, where RAMP score was the dependent variable and treatment group was the independent variable. Two-way ANOVAs were performed to assess differences in ILCM, lamellar length and ILCM:length (ILCM divided by lamellar length) with control vs. treatment as one factor and left vs. right gill as the second factor. A binomial logistic regression was used to assess treatment effects on the presence of aneurysms in the gill, where the presence of aneurysms was the dependent variable, and both treatment group and gill (left or right) were the independent variables. Student's t-tests were used to assess differences in mucous cell area between control and treatment individuals for the right pectoral fin, left pectoral fin, and the caudal fin. Significance was assessed at $\alpha \leq 0.05$ for all statistical tests and all statistical analyses were performed using R version 3.6.1 (R Core Team 2019).

Results

RAMP Scores

Thirty yellow perch were angled and sampled for reflex impairment between both locations (16 at Gull Lake, MB and 14 at Fox Lake, WI). Reflex impairment did not differ between the control and treatment individuals at Gull Lake (Poisson linear regression; χ^2 = 0.455; P = 0.500) or Fox Lake (Poisson linear regression; χ^2 = 0.117; P = 0.733). One treatment individual at Gull Lake was considered fully impaired (RAMP score of four). From both sampling locations, eighteen fish were missing the reflex 'body flex,' making it the most common reflex to be impaired regardless of treatment (Table 3.1). The 'tail grab' and 'orientation' reflexes were both missing in eleven fish, and the 'vestibular-ocular response' was only missing in two fish making it the least impaired reflex. Including both control and treatment individuals, 76.7 % of individuals were missing one or more reflexes upon assessment.

Table 3.1. The number of yellow perch (*Perca flavescens*) from both study locations post-ice-angling event and from both the control and treatment (3 min ice and air exposure) groups that experienced reflex loss of the four reflexes assessed: body flex, orientation, vestibular-ocular response and tail grab.

Impaired Reflex	Gull Lake Control (n=8)	Gull Lake Treatment (n=8)	Fox Lake Control (n=6)	Fox Lake Treatment (n=8)
Body Flex	6	4	4	4
Orientation	1	4	1	5
Vestibular-Ocular Response	0	2	0	0
Tail Grab	3	4	2	2

Surface Temperature Readings

The surface temperature of the air-exposed midbody musculature decreased over the exposure period (Figure 3.3A; one-way repeated measures ANOVA; F = 24.907; d.f. = 1.51, 10.54; P < 0.001), starting at an average of 1.34 °C and decreasing an average of 1.57 °C resulting in a mean temperature of -0.23 °C at the end of the treatment for the air-exposed midbody (Figure 3.3A). The temperature of the eye, gill, or caudal fin did not differ across the exposure for the air-exposed side of the fish (eye: Figure 3.3B; one-way repeated measures ANOVA; F = 0.537; d.f. = 3, 21; P = 0.662, gill: Figure 3.3C; one-way repeated measures ANOVA; F = 2.448; d.f. = 3, 21; P = 0.092, caudal fin: Figure 3.3D; non-parametric Friedman test; χ^2 = 4.70; d.f. = 3; P = 0.195). However, all three of these tissues remained below 0 °C throughout the entire treatment (Figure 3.3). Midbody (Figure 3.4A; paired t-test; t = 4.02; d.f. = 4; P = 0.005) and eye (Figure 3.4B; paired t-test; t = 2.55; d.f. = 7; P = 0.038) surface temperatures differed between the air-exposed side and the ice-exposed side at the end of the treatment period, with the ice-exposed side being colder in both cases. The temperature decrease resulted in average surface temperatures of -1.11 °C for the midbody and -2.43 °C for the eye for the ice-exposed side at the end of the treatment. The temperature of the gill and the caudal fin did not decrease between the air-exposed side and the ice-exposed side at the 3-min time point (gill: Figure 3.4C; paired t-test; $t = 0.561$; d.f. = 7; $P = 0.592$, caudal fin: Figure 3.4D; paired t-test; $t = -1.03$; d.f. = 7; $P = 0.337$), however, surface temperature readings of all four tissues were recorded below 0 °C for the ice-exposed side at the end of the treatment (Figure 3.4).

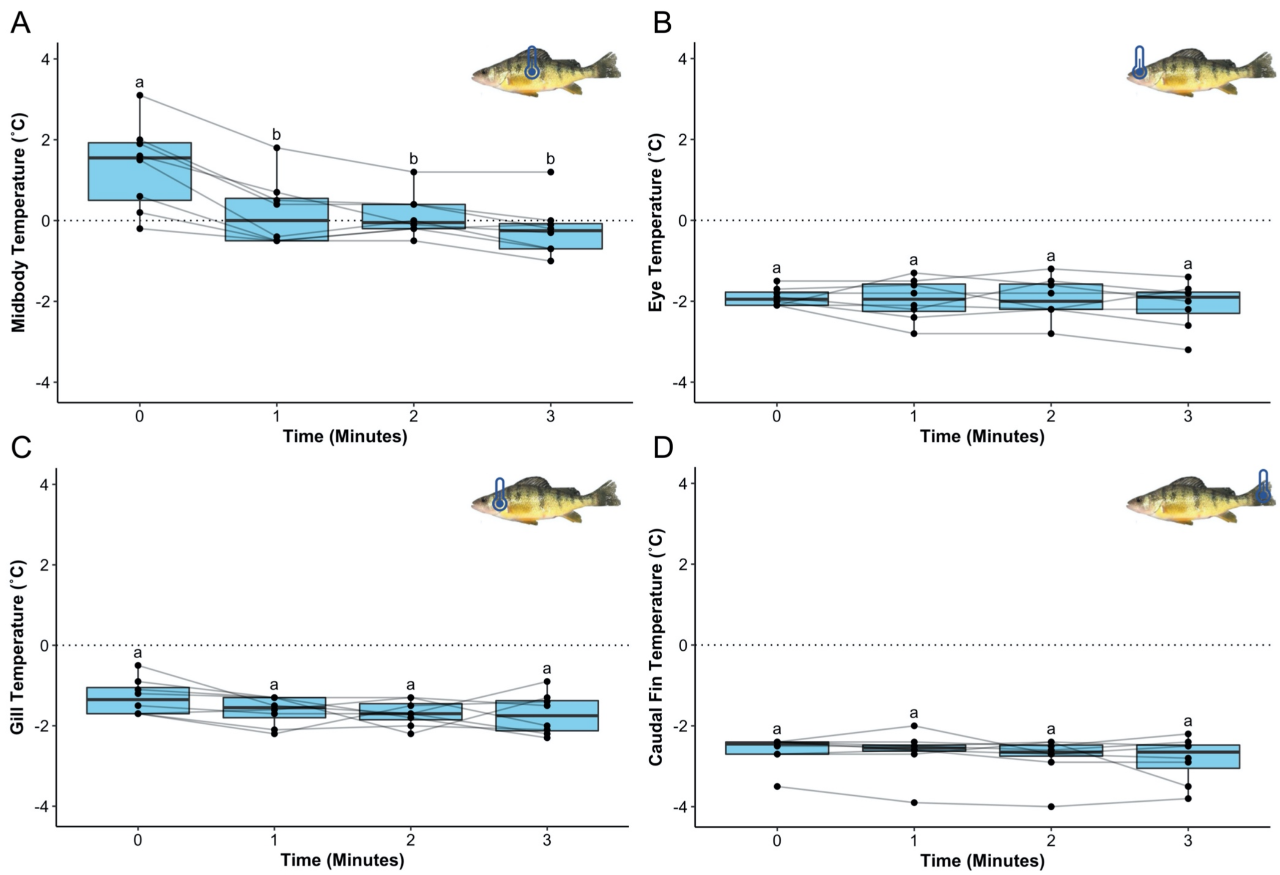

Figure 3.3. Surface temperature of the (A) midbody, (B) eye, (C) gill and (D) caudal fin of yellow perch (*Perca flavescens*) assessed at each minute throughout the 3-min ice and air exposure following an ice-angling event (Gull Lake location only), with significance demonstrated by dissimilar letters using one-way repeated measures ANOVA tests. Statistical significance was assessed at $\alpha \leq 0.05$.

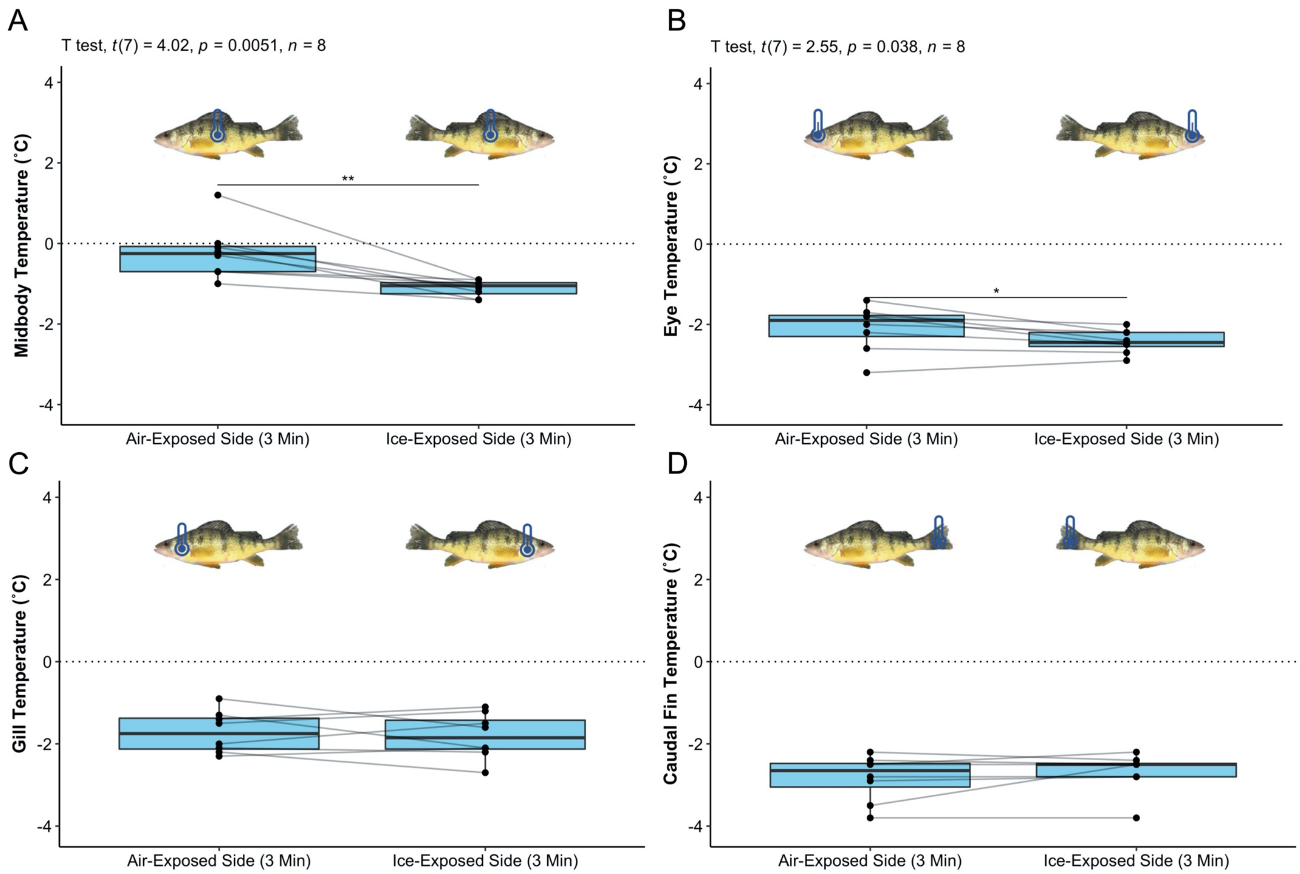

Figure 3.4. Surface temperature of the (A) midbody, (B) eye, (C) gill and (D) caudal fin in yellow perch (*Perca flavescens*) for both the air-exposed side (left) and ice-exposed side (right) at the 3-min time point of the treatment (3-min ice and air exposure following an ice-angling event), from the Gull Lake location only. Paired t-tests were used to assess differences and statistical significance is demonstrated by asterisks, where * represents $\alpha \leq 0.05$ and ** represents $\alpha \leq 0.01$.

Histology

There was no effect of treatment on any metrics related to the gills. Specifically, ILCM (Figure 3.5A; two-way ANOVA; F = 1.151; d.f. = 1, 29; P = 0.292), lamellar length (Figure 3.5B; two-way ANOVA; F = 1.679; d.f. = 1, 29; P = 0.205) or ILCM:lamellar length (Figure 3.5C; two-way ANOVA; F = 0.053; d.f. = 1, 29; P = 0.819) showed no visible differences when comparing control and treatment samples. Additionally, there was no treatment effect on gills when samples from air exposed gills were compared to ice exposed gills (ILCM: Figure 3.5A; two-way ANOVA; F = 1.789; d.f. = 1, 29; P = 0.192, lamellar length: Figure 3.5B; two-way ANOVA; F = 0.412; d.f. = 1, 29; P = 0.526, or ILCM:lamellar length: Figure 3.5C; two-way ANOVA; F = 3.292; d.f. = 1, 29; P = 0.080).

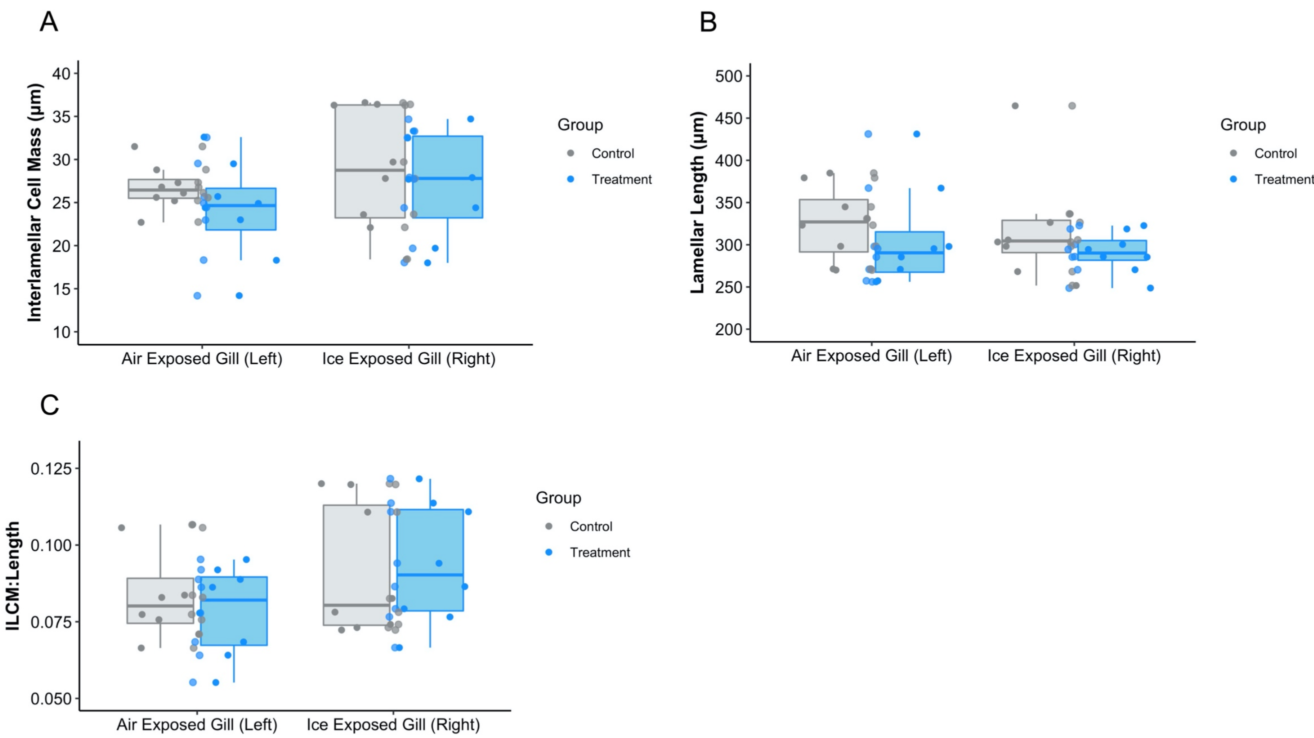

Figure 3.5. Measurements of the (A) interlamellar cell mass, (B) lamellar length, and (C) the ILCM:length for both the left and right gills of yellow perch (*Perca flavescens*) from both control and treatment (ice and air exposure for 3-min following an ice-angling event) groups. No statistically significant differences were found amongst any groups which was assessed using two-way ANOVAs.

Reference images of the left (Figure 3.6A) and right gill (Figure 3.6B) of control fish are provided, as well as references images of the air exposed left gill (Figure 3.6C) and the ice exposed right gill (Figure 3.6D) of the treatment fish. During the scan for general abnormalities, aneurysms of the secondary lamellae were observed in the gills of both treatment and control individuals with no significant difference between treatment group (Figure 3.7; binomial logistic regression; $z = -0.734$; $P = 0.463$) or gill (Figure 3.7; binomial logistic regression; $z = -1.418$; $P = 0.156$). A reference image of the aneurysms within the secondary lamellae in both control and treatment fish is provided (Figure 3.8).

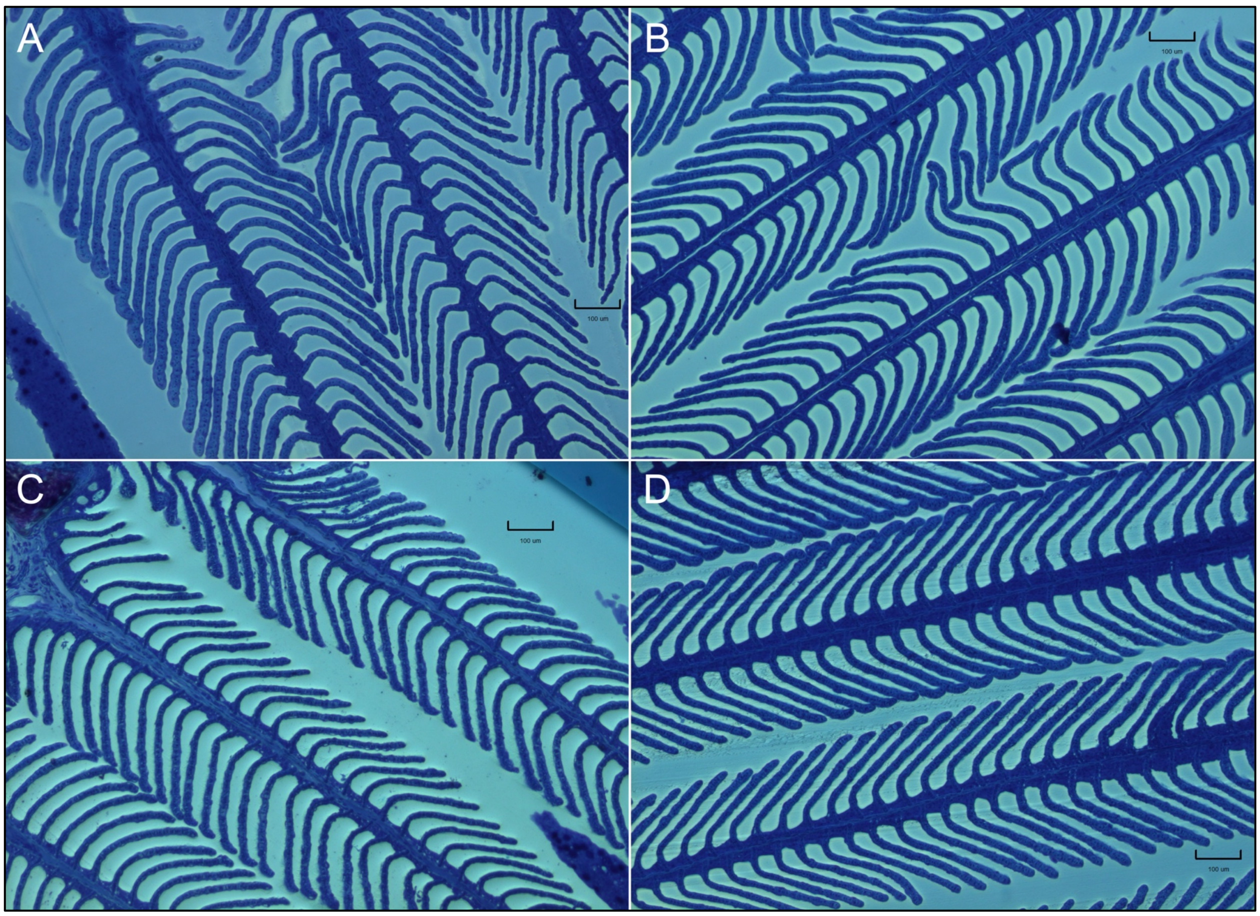

Figure 3.6. Reference images of gill sections from yellow perch (*Perca flavescens*) from both control and treatment (3-min ice and air exposure following an ice-angling event) groups. The images represent examples of (A) the left gill from a control fish, (B) the right gill from a control fish, (C) the left gill from a treatment fish (air exposed side) and (D) the right gill from a treatment fish (ice exposed side). Tissues were stained with 1 % toluidine blue + 1 % borax stain and sectioned along the sagittal plane at 1.5 μm thickness. These images were taken at 100X magnification and the scale bars in the image represent 100 μm.

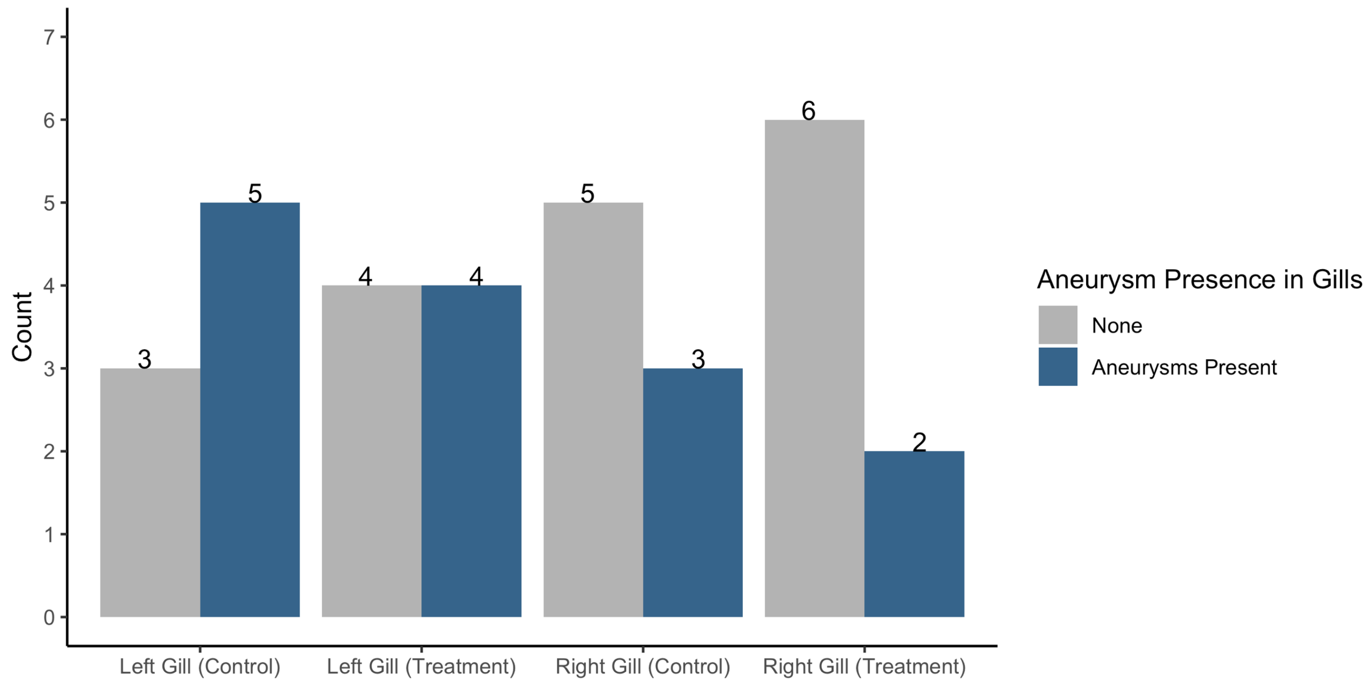

Figure 3.7. Presence and absence of aneurysms in the secondary lamellae of yellow perch (*Perca flavescens*) following an ice-angling event, where the treatment was laying the fish on the ice while simultaneously being exposed to air for 3 min. No statistically significant differences were found using a binomial logistic regression, where presence of aneurysms was the dependent variable and both treatment and gill (left or right) were the independent variables.

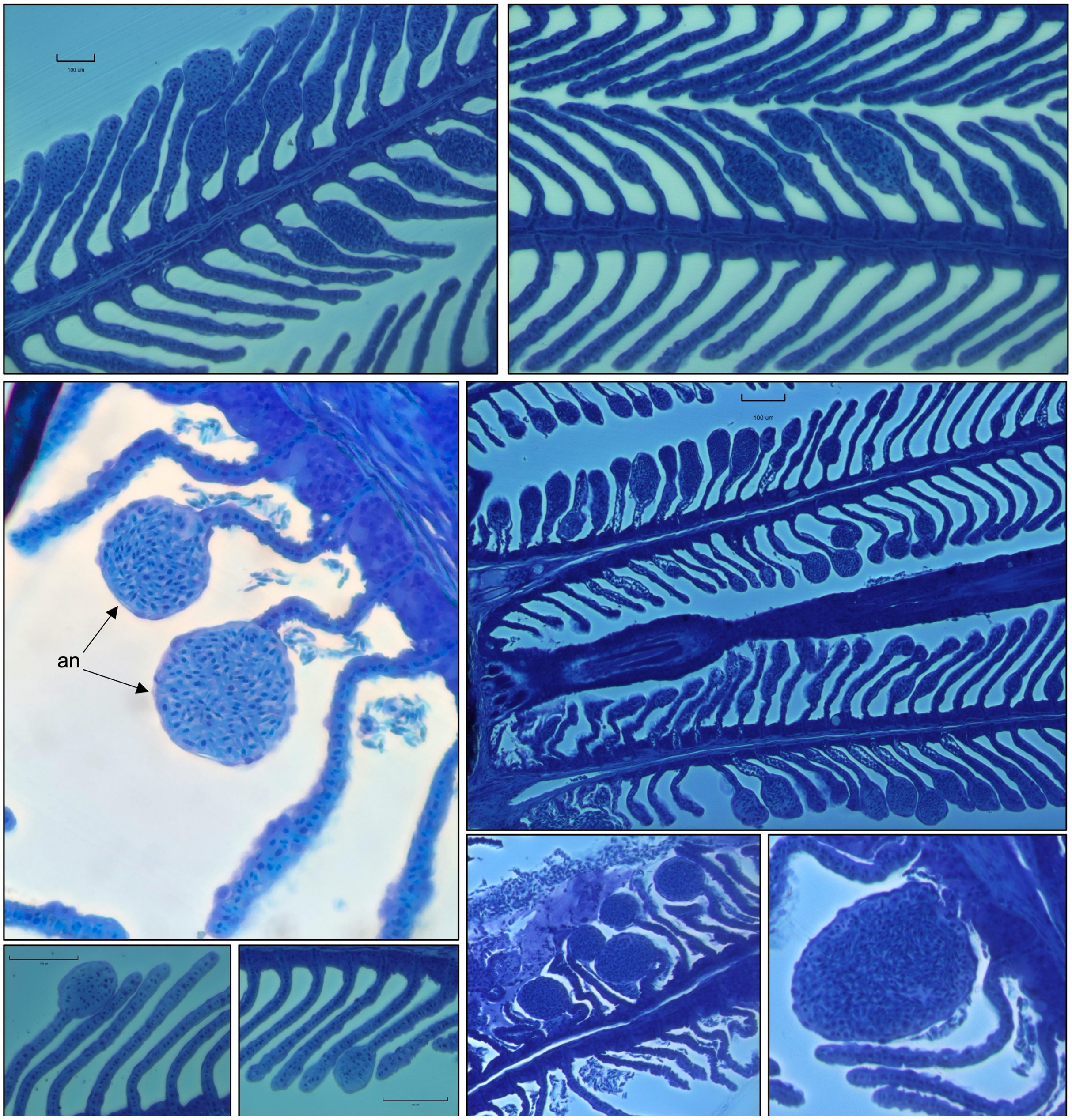

Figure 3.8. Example images of aneurysms (an) occurring in the secondary lamellae of the gills in yellow perch (*Perca flavescens*) following an ice-angling event. Images are taken from both control and treatment (3-min ice and air exposure following an ice-angling event) fish as aneurysms were found to occur in both groups. Tissues were stained with 1 % toluidine blue + 1 % borax stain and sectioned along the sagittal plane at 1.5 µm thickness. These images were taken at a variety of magnifications and the scale bars in the image, where present, represent 100 µm.

There was no effect of treatment on the area of mucous cells within the caudal fin (Figure 3.9A; Student's t-test; t = 0.367; d.f. = 12; P = 0.720), the air exposed left pectoral fin (Figure 3.9B; Student's t-test; t = 0.275; d.f. = 10; P = 0.790), or the ice exposed right pectoral fin (Figure 3.9C; Student's t-test; t = -0.684; d.f. = 9; P = 0.511). Reference images of the left pectoral fin from a control fish (Figure 3.10A), the right pectoral fin from a control fish (Figure 3.10B), the left pectoral fin from a treatment fish (ice exposed side; Figure 3.10C), the right pectoral fin from a treatment fish (air exposed side; Figure 3.10D), the caudal fin from a control fish (Figure 3.10E) and the caudal fin from a treatment fish (Figure 3.10F) are provided.

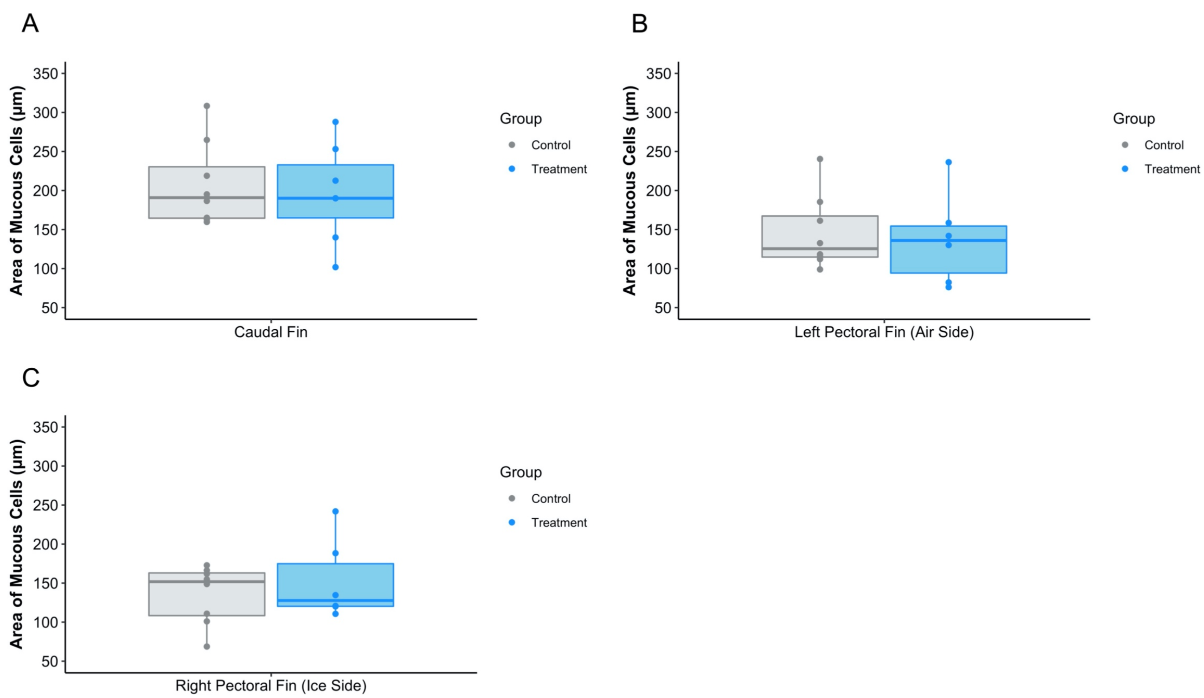

Figure 3.9. Area of mucous cells measured in the epithelium of the (A) caudal fin, (B) left pectoral fin (air exposed side), and (C) the right pectoral fin (ice exposed side) comparing between yellow perch (*Perca flavescens*) in both the control and treatment (3-min ice and air exposure following an ice-angling event) groups. No statistically significant differences were found when comparing with Student's t-tests.

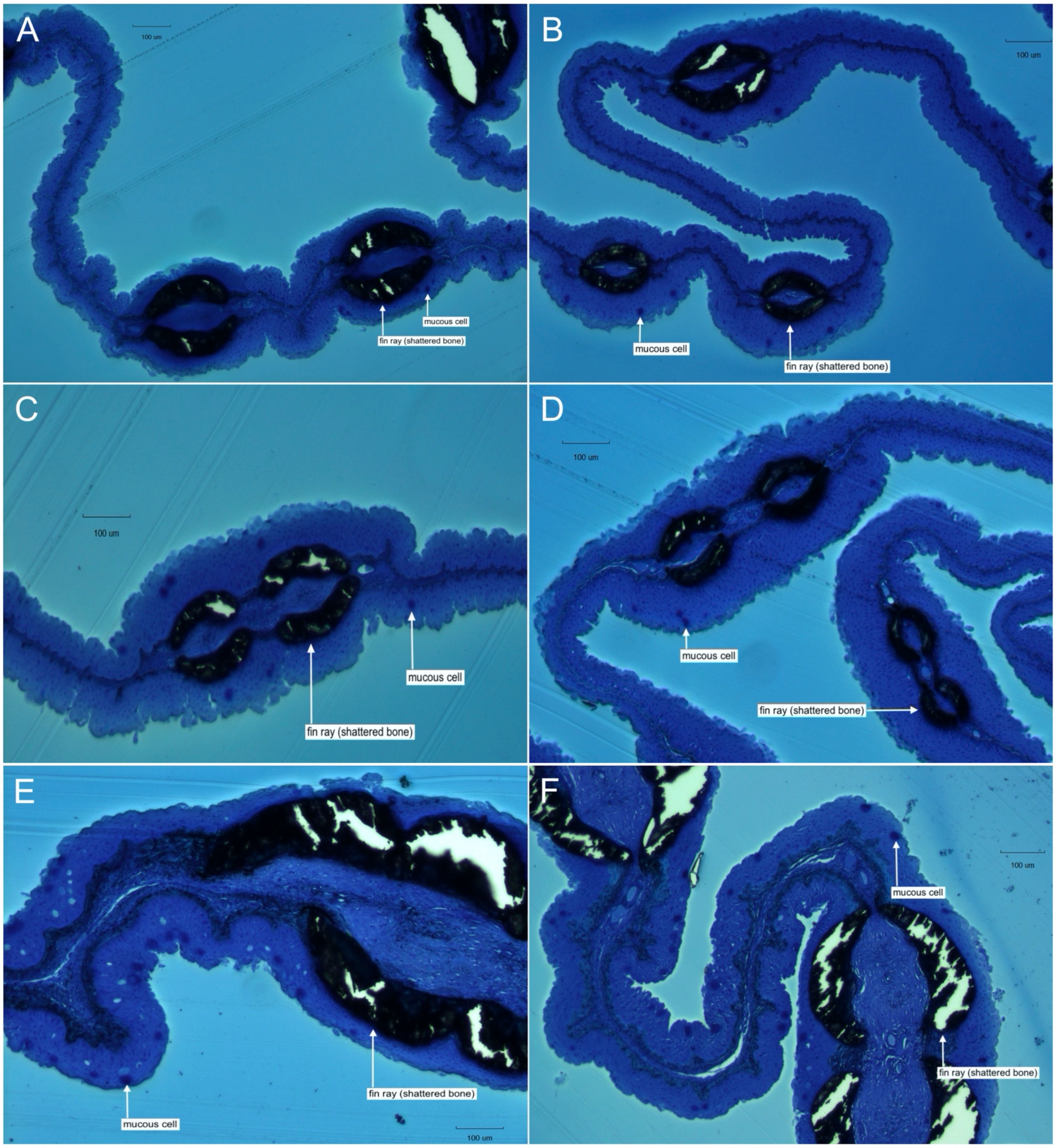

Figure 3.10. Reference images of fin cross sections from yellow perch (*Perca flavescens*) from both control and treatment (3-min ice and air exposure following an ice-angling event) groups, highlighting the mucous cells that were quantified within the epidermis of fins. The images represent examples of (A) the left pectoral fin from a control fish, (B) the right pectoral fin from a control fish, (C) the left pectoral fin from a treatment fish (air exposed side), (D) the right pectoral fin from a treatment fish (ice exposed side), (E) the caudal fin from a control fish and (F) the caudal fin from a treatment fish. Tissues were stained with 1 % toluidine blue + 1 % borax stain and represent a cross section of 1.5 μm thickness. These images were taken at 100X magnification and the scale bars in the image represent 100 μm.

Discussion

The results of my study support my hypothesis that ice-angling has physical consequences for yellow perch, and my predictions that ice-angling would impair reflexes, induce heat loss, and damage tissues through freezing. The high occurrence of impairment of the body flex reflex following an ice-angling event is consistent with other ice-angling studies on yellow perch and walleye (Louison et al. 2017b; Logan et al. 2019), supporting the notion that released fish may experience reflex impairment following ice-angling events. Midbody surface temperature in yellow perch decreased to below 0 °C after being laid on the ice for 3 min, and the surface temperature of the gills, eye and caudal fin were also all below 0 °C once the fish was removed from the lake. This freezing may damage gill tissue as aneurysms in the secondary lamellae were observed, which may result from exposure to sub-zero air temperatures, although further study is needed to confirm.

Though RAMP scores did not differ statistically between treatments, in total, 60 % of yellow perch subjected to ice-angling (ignoring the treatment applied) were missing the body flex reflex, so it is likely that the ice-angling event itself impairs reflexes in captured fish. Lack of body flex reflex following an ice-angling event was found in 35 % of yellow perch in a similar study, and further, body flex was the only reflex impaired in yellow perch of the four reflexes assessed in their study (Louison et al. 2017b). Similarly, 46 % of walleye, another species within the family Percidae, lacked the body flex reflex following an ice or air exposure after an ice-angling event (Logan et al. 2019). However, the walleye studied by Logan et al. (2019) were held for 24 hrs following the ice-angling event and ice or air exposure, and all but one fish were considered to be fully recovered prior to their release. Despite this finding, wild fish are not typically held for a period of 24 hrs following an ice-angling event, so delayed mortality because of reflex impairment may still occur. Any reflex impairment that may result from catch-and-release could leave fish susceptible to predation, particularly in game fish species that are not apex predators within their system (Gingerich et al. 2007), such as yellow perch in the case of this present study. Further, 56 % of yellow perch that experienced the ice and air exposure in my study were found to lack the orientation reflex, which could also render the fish vulnerable to predation once released if the fish does not recover in a timely manner. Although my study did not assess reflex impairment over a temporal recovery gradient, both Louison et al. (2017b) and Logan et al. (2019) found evidence that the highest point of reflex impairment occurred shortly after capture which was then followed by recovery. This finding indicates that it is possible that if the fish in my study had been held in recovery containers following the ice-angling event, they may have experienced recovery as well. Lastly, no differences were found between control and treatment individuals for reflex impairment within my study, which is consistent with other findings as reflex impairment in walleye following an ice-angling event was not linked to air and snow exposure or duration of exposure (Logan et al. 2019).

Yellow perch experienced heat loss during the 3 min ice and air exposure treatment. Average midbody surface temperature for both sides of the fish decreased below 0 °C at the end of the treatment. This finding reveals that fish were losing heat in the core part of their body during this period spent out of water and on the ice, which is inferred because a parallel relationship exists between internal and surface body temperature (Garrick 2008; Romanovsky 2014) as described by Wosnick et al. (2018). However, core body temperature was not measured in my study, thus it is not clear how exposing the fish to ice and air following an ice-angling event impacts internal body temperature in yellow perch. Wosnick et al. (2018) deployed a similar methodology using infrared thermography to observe body surface temperature warming in blacktip sharks (*Carcharhinus limbatus*) during air exposure following capture. Temperature changes in the body surface of blacktip sharks were significantly impacted by time spent out of water while exposed to air (Wosnick et al. 2018). Although my study is looking at an opposite effect and season, our findings are similar because the midbody surface temperature in yellow perch decreased significantly following ice and air exposure, thus the time the fish spent out of water in this case also significantly impacted surface body temperature. Further, the surface temperatures of the eye, gill and caudal fin were recorded below 0 °C at the beginning of the treatment and throughout, which reveals that these tissues likely reach sub-zero temperatures once the fish is removed from the water during ice-angling events. The right eye (exposed to ice) was significantly colder than the left eye (exposed to air) at the end of the treatment. If the freezing of the eye causes any damage to the cornea, both opacity and stromal swelling are possible outcomes (Smelser 1962; Edelhauser et al. 1968; Ubels and Edelhauser 1987) which could potentially reduce vision upon release. I did not assess damage to the eye following ice and air exposure, but it would be useful to investigate this in future studies.

Cold shock occurs when a fish that is acclimatized to a certain temperature range is then exposed to a rapid decrease in temperature that they are not acclimated to, causing a fast decrease in body temperature which can ultimately alter the physiology and behaviour of the organism (Donaldson et al. 2008). In extreme cases, cold shock can result in death, depending on the rate of temperature decrease, the magnitude of the temperature change or the duration of the exposure to the cold temperature (Donaldson et al. 2008; Wosnick et al. 2018). Yellow perch are poikilotherms, meaning their body temperature is fully dependent on the temperature of their surrounding environment (Peoples 2015). Cold shock is often studied through looking at rapid decreases in water temperature (e.g., Szekeres et al. 2014), or by removing a fish from cold water, exposing them to warmer air temperatures, and then releasing them back into cold water (Gingerich et al. 2007; Donaldson et al. 2008; Wosnick et al. 2018). Although rarely studied, rapid temperature decrease when a fish is moved from cold water and exposed to sub-zero air temperatures could also inflict cold shock (Louison et al. 2017a). When coupled with the already negative effects of air exposure, this could compound into more negative biological consequences for the fish. It is likely that the fish in my study experienced some level of cold shock while exposed to ice and air, as surface temperatures below freezing were present and reflex impairment was observed with high occurrence. Additionally, the exposure time in my study was relatively short compared to what may occur while anglers are out ice-fishing, so it is possible that a longer exposure period would result in even colder surface body temperatures and higher rates of reflex impairment, although further study is needed to confirm. While fish were euthanized in my study, both temperature readings and RAMP scores provide a non-invasive approach to studying thermal dynamics (Wosnick et al. 2018) and reflex impairment (Brownscombe et al. 2017) in teleost fish, because fish may be released after these assessments are completed.

Assessing the impact of cold air exposure on ice-angled fish is complicated as important tissues are being exposed to harsh temperatures, which impacts the core body temperature of the fish. The result of reduced body temperature on the assessment of ice-angling on fish means that commonly measured physiological processes such as the stress response are delayed and or attenuated during cooler water temperatures (Van Ham et al. 2003; Meka and McCormick 2005; Brownscombe et al. 2015; Louison et al. 2017b, 2017a; Card and Hasler 2021), so effects can be difficult to discern using physiological techniques (Logan et al. 2019). Therefore, to assess if sub-zero air temperatures cause damage to important tissues in ice-angled yellow perch, histological techniques may be useful (Bieber et al. 2019). Within all tissues assessed in my study, no differences were found between the control and treatment individuals. These results are consistent with previous work that also did not find any differences among treatment groups within the gills following a simulated winter angling event, although different metrics were measured in their study such as primary lamellae length, secondary lamellae count, and secondary lamellae area (Bieber et al. 2019). Generally, the evidence suggests that exposure to air and ice for 3 min has no discernable impact on gill morphology.

Whether ice-angling and air and ice exposure influences gill function remains unclear. The primary function of the secondary lamellae in the gills of teleost fish is to facilitate ion and gas transfer into the bloodstream (e.g., oxygen uptake and carbon dioxide release; Ferguson and Tufts 1992), and their delicate structure provides a large surface area for gas and ion transfer to occur (Wilson and Laurent 2002). If the gill is damaged or altered as a result of an environmental change, respiratory function may be impaired (Jiraungkoorskul et al. 2002). The aneurysms observed in my study are an example of a change within the delicate structure of the secondary lamellae, and these aneurysms or "ballooning" have been observed in other studies. Most commonly, aneurysms are associated with exposure to adverse metals, organic compounds, toxicants or sediments in the water (van den Heuvel et al. 2000; Bhagwant and Elahee 2002; Stentiford et al. 2003; Hassaninezhad et al. 2014; Mabika and Barson 2014). As there were no differences between the control and treatment fish regarding the frequency of aneurysms, and because aneurysms were found in 68.75 % of fish in the present study, I hypothesize that the occurrence of aneurysms in the secondary lamellae may be related to the ice-angling event itself and subsequent below freezing air exposure, as all individuals in my study experienced this same ice-angling event and temperature reduction. This potential cold air exposure effect happens almost immediately once the fish is removed from the lake, as the fish assessed in my study did not differ between treatment and controls, and the temperature of the gills was below 0 °C at the beginning of the treatment for every fish assessed.

The occurrence of aneurysms in the secondary lamellae have been linked to the death of pillar cells that connect the two epithelial sheets of the secondary lamellae to one another, as these pillar cells maintain the structural integrity of the lamellae (van den Heuvel et al. 2000; Hassaninezhad et al. 2014; Strzyżewska-Worotyńska et al. 2017). If the functionality of pillar cells is lost, the structural integrity of the secondary lamellae will be compromised as these pillar cells regulate blood flow, thus the lamellae will swell and fill with erythrocytes resulting in aneurysm (van den Heuvel et al. 2000; Hassaninezhad et al. 2014; Strzyżewska-Worotyńska et al. 2017). Aneurysm in the secondary lamellae is considered a severe type of lesion meaning that recovery is possible, but difficult, compared to other changes that may occur in the secondary lamellae such as changes in the epithelial tissue (Hassaninezhad et al. 2014; Mabika and Barson 2014; Strzyżewska-Worotyńska et al. 2017). Because the surface temperature of the gills was recorded as below 0 °C for all fish in my study following an ice-angling event, it is likely that these tissues are freezing once the fish is removed from the lake and exposed to sub-zero air temperatures. It is possible that this freezing ruptures pillar cells through crystallization because pillar cells are composed of collagen columns that exist within the infoldings of the cell membrane (Bettex-Galland and Hughes 1973), and freezing has been found to destabilize collagen fibrils because of the expansion of intrafibrillar space through ice formation (Ozcelikkale and Han 2016). To my knowledge, this study represents the first to have described aneurysms in the secondary lamellae of teleost fish following an ice-angling event. As my study was executed in a field setting with less ability for control than a laboratory setting, and because

I did not undertake a water quality assessment to rule out any environmental contamination that could also result in ruptured pillar cells, further study is necessary to investigate the definitive cause of aneurysms. Future studies could also replicate these conditions and confirm that pillar cells are being damaged by taking a cross-section of the lamellae pillar cell and using transmission electron microscopy to assess structural integrity and pillar cell morphology. Lastly, a similar angling study should be executed in the summer season at this same lake to compare what the gills look like following an angling event and subsequent air exposure during warmer air temperatures, and a water quality analysis should also be completed to rule out any potential environmental effects.

The exploratory approach undertaken in my study poses many questions for future study as the impacts of ice-angling on freshwater fishes in northern climates is severely understudied. My results indicate that a high percentage of yellow perch are lacking reflexes following ice-angling, important tissues are freezing in environments with air temperatures below 0 °C following ice-angling events, and that aneurysms are occurring within the delicate secondary lamellae of the gills which may result from this tissue freezing, potentially impairing respiratory function. As I cannot be fully confident that sub-zero temperatures are causing aneurysms in the secondary lamellae of the gills, and because my results show that the temperature of the gills are below 0 °C once the fish is removed from the lake in sub-zero air temperatures, I recommend that anglers practice a precautionary approach and limit the amount of air exposure when ice-angling. This particularly applies when the air temperature is below freezing to minimize any potential damage that may be occurring in the gills. I recommend that anglers release any fish that they do not intend to keep immediately following capture while ice-angling, as this will limit both the amount of air exposure that the fish is subjected to and the length of time that the fish is exposed to sub-zero air temperatures, hopefully limiting any damage to vital tissues such as the gills. Future research could explore other techniques to investigate the sublethal effects of ice-angling on the tissues assessed in the present study, such as investigating mRNA abundances related to genes associated with cell damage. Lastly, I recommend that future studies undertake post-release monitoring following ice-angling events where fish are exposed to sub-zero air temperatures to assess the potential for delayed mortality resulting from the combination of air exposure, freezing tissues and the stress of an ice-angling event (Wosnick et al. 2018).

Acknowledgements

This research was carried out on the traditional territories of the Anishinabek and Métis Nations, as well as the traditional territories of the Očhéthi Šakówiŋ, Waazija (Ho-Chunk/Winnebago) and Myaamia Nations. Funding support for this research was provided through an NSERC Alexander Graham Bell master's scholarship award held by J. T. Card, an NSERC discovery grant held by C. T. Hasler, and a University of Winnipeg start-up grant held by C. T. Hasler. We extend a huge thank you to Theresa Mackey, Andrew Althoff, Logan Cutler and Derek Kroeker for assisting with catching fish and sampling them in the field. Additionally, we would like to thank Dr. Alyssa Weinrauch for consulting on histological methods, Jenna Fleet for helping investigate the cause of the gill aneurysms, as well as Dr. Erwin Huebner for training on histological techniques and allowing us the use of his lab space and equipment.

Chapter 4: General Discussion

The practice of catch-and-release (C&R) angling on a global scale is considerable, as best estimates predict that over 28 billion caught-fish are released after capture by recreational anglers annually (Cooke and Cowx 2004; Danylchuk et al. 2018). Effects of C&R angling have been widely documented, however, significant gaps in the literature exist for understudied species and practices, such as ice-angling (Cooke and Suski 2005; Arlinghaus et al. 2007). C&R research conveys that anglers have a negative impact on the fish that they catch when proper handling techniques are not practiced (Cooke et al. 2013a; Brownscombe et al. 2017). To combat this, C&R research focuses on the development of best practices regarding how to best handle caught fish when anglers intend on releasing them. However, these suggestions should be species-specific (Cooke and Suski 2005) because bias exists against certain species that are not considered valued sportfish by all anglers (Rypel et al. 2021). If research does not cover all species and all types of angling these recommendations will not be all encompassing, and anglers may believe that general best practices do not apply across all species based on personal bias against species that are considered 'rough fish' (Rypel et al. 2021). For example, anglers may fail to use general best handling practices with 'rough fish' species if they place less value on them, but if best practices are developed for these species, it may help positively influence angler behaviour when handling all caught fish. Further, lacking knowledge on certain species is a problem regarding the sustainability of fisheries, as 'good' policy cannot be developed without a scientific basis to support it. My aim for this thesis was to assist in filling these knowledge gaps so that best practices may be developed for a species that has never been studied in this capacity (freshwater drum) and to support the growing literature base on C&R ice-angling.

In chapter 2 my hypothesis was partially supported, as I hypothesized that longer C&R angling events (i.e., longer fight duration and air exposure) during warmer time periods would result in biological consequences for freshwater drum. Contrary to my prediction, I did not find a positive relationship between fight duration or air exposure and any of the stress physiology metrics. However, physiological disturbances were greater during warmer water temperatures in the summer when compared to the spring. The lack of a positive relationship with fight duration and air exposure is encouraging for the use of C&R angling on freshwater drum as a conservation measure because I used fight duration and air exposure times that mimicked real-world angling circumstances. Despite this finding, the occurrence of deep hooking in freshwater drum when angling using J hooks was high, and a study on their saltwater conspecific (red drum [*Sciaenops ocellatus*]) indicates that the use of circle hooks may reduce the likelihood of deep hooking (Aguilar 2003), but further study is needed to confirm. The results of this chapter indicate that it is important to minimize angling stress particularly during warmer water temperatures, so limiting air exposure and fight duration are still recommended.

Future studies on freshwater drum should focus on quantifying the occurrence of deep hooking when circle hooks are used, compared to commonly used J hooks. Additionally, these studies should assess delayed mortality following C&R angling events, particularly because the orientation reflex was impaired so frequently, and the frequency of delayed mortality in freshwater drum following angling events remains unknown. Studies could also be broadened to include other species that are commonly caught from the Red River to determine if the use of circle hooks could limit the instance of deep hooking in these species as well. Encouraging specific tackle use may not be effective when only focused on one species that is not targeted by all anglers. Lastly, recovery profiles should be documented, as stress physiology sampling was completed 30 min following the angling event based on other species (Lawrence et al. 2018), and this may not be the peak of these stress physiology metrics in freshwater drum following an environmental stressor.

In chapter 3 my hypothesis was fully supported, as I hypothesized that winter C&R angling has physical consequences for yellow perch and predicted that winter C&R angling would induce heat loss, impair reflexes, and damage tissues through freezing. My results revealed that reflex impairment is high in yellow perch following ice-angling events, tissues are reaching sub-zero temperatures immediately once the fish is removed from the hole in the ice, and aneurysms within the secondary lamellae of the gills are occurring at a high frequency following ice-angling events. The results of this chapter indicate that it is important for ice-anglers to minimize air exposure to the greatest extent possible while ice-angling in sub-zero temperatures to limit any damage to the gills through freezing.

Future studies should prioritize further investigation of the occurrence of aneurysms in the secondary lamellae of the gills in teleost fish species following ice-angling. It is not known how these aneurysms may impact fish upon release, although we can predict that they may impede gas exchange as they are categorized as a severe lesion meaning they damage the regular function of the tissue (Hassaninezhad et al. 2014). This finding warrants further study as it is the first documentation of the occurrence of aneurysms in the gill of a teleost fish species following ice-angling events, and additional investigation should explore if this is occurring in other species during exposure to sub-zero temperatures. Furthermore, ice-angling research should continue to assess the effects of ice-angling on a diverse group of fish species that are ice-angled in northern climates.

The common theme between these two chapters is that angling events can negatively impact these two fish species, and anglers can modify their approach to mitigate these impacts when they plan on releasing the fish that they capture. However, communicating best practices to anglers can be challenging, and regulations surrounding the proper handling of fish are not possible as they would be remarkably difficult to enforce. Additionally, top-down methods such as traditional regulatory approaches (i.e., government-imposed) that attempt to influence voluntary changes in angler behaviour have been considered ineffective (Cooke et al. 2013b), as they often promote best practices alongside regulations that dilute the importance of the material (Danylchuk et al. 2018). Communicating with anglers on policy regarding best angling practices may be an avenue of sharing science-based recommendations but influencing behavioural changes in anglers can be difficult (Danylchuk et al. 2018). Keep Fish Wet (https://www.keepfishwet.org) is a non-government organization with the sole focus of promoting best C&R angling practices to recreational anglers to promote conservation in recreational fisheries. This bottom-up approach has proven effective at changing angler behaviours because it encourages anglers to adopt a conservation stewardship philosophy and to share that with others (e.g., sharing via social media or other platforms), and its success may be attributed to its non-partisan identity (Danylchuk et al. 2018). Further, voluntary changes in angler behaviour increase compliance and reduce the need for monitoring (Cooke et al. 2013b). Keep Fish Wet communicates science-based best practices in an accessible format, as they explain recommendations from C&R research in easily understandable language (Danylchuk et al. 2018). For example, the first principle listed on their website is to minimize air exposure, which aligns with what I am promoting as best practice for both freshwater drum during warmer temperatures and yellow perch during ice-angling. Although 'keeping fish wet' sounds like a sensible practice to adopt, anglers often hold fish out of the water to admire their catch, obtain measurements, or take photographs. A disconnect can exist between what scientists recommend and the angling practices that are adopted by anglers, so grassroots movements such as Keep Fish Wet help combat this disconnect to ensure the sustainability of recreational fisheries (Danylchuk et al. 2018).

Despite the general suggestions of best practices for C&R angling such as limiting air exposure and reducing handling time, species-specific guidelines should be developed (Cooke and Suski 2005; Danylchuk et al. 2018). This is because most of the angling base that has currently adopted the Keep Fish Wet principles are fly fishers targeting cold-water species, which only comprises a small proportion of total angling effort (Danylchuk et al. 2018). Although a precautionary approach implies that it would be a good idea to limit the air exposure of caught fish, my chapter 2 results provide specific science-based advice to minimize the stress of angling events on freshwater drum during warmer water temperatures, and to explore other tackle options that may reduce the occurrence of deep hooking. Keep Fish Wet currently highlights cold-water species that predominantly fly fishers target (Danylchuk et al. 2018), whereas the results of my study on freshwater drum provide an opportunity to increase their outreach to target a diverse range of fish species, waterbodies, and types of anglers. If these recommendations are not species-specific, anglers may not understand that these best practices should be widely adopted for use on all fish species caught (e.g., including fish species that they are not targeting or species that they consider to be insignificant or are sometimes referred to as ‘trash fish’). Additionally, Keep Fish Wet has translated the results of winter ice-angling studies to promote general best practices for ice-angling (Danylchuk 2021). My chapter 3 results surrounding gill damage could easily be added to this type of communication to support their speculation that exposure to freezing temperatures could damage gill tissue (Danylchuk 2021). In conclusion, the best practices supported by the results of both chapters will be most effectively communicated to anglers using bottom-up approaches that encourage a shift in angler behaviour to adopt a conservation stewardship mentality.

References

Aalbers, S. A., G. M. Stutzer, and M. A. Drawbridge. 2004. The effects of catch-and-release angling on the growth and survival of juvenile white seabass captured on offset circle and J-type hooks. North American Journal of Fisheries Management 24(3):793–800. DOI: 10.1577/M03-034.1.

Aguilar, R. 2003. Short-term hooking mortality and movement of adult red drum (*Sciaenops ocellatus*) in the Neuse River, North Carolina. Master of Science, North Carolina State University, North Carolina. Available: https://repository.lib.ncsu.edu/handle/1840.16/2547. (October 2020).

Aguilar, R., P. S. Rand, and G. H. Beckwith Jr. 2002. Quantifying the catch-and-release mortality rate of adult red drum in the Neuse River Estuary. North Carolina Fisheries Resource Grant Program 01-FEG-07. Raleigh, North Carolina.

Almodovar, A., and G. G. Nicola. 2004. Angling impact on conservation of Spanish stream-dwelling brown trout *Salmo trutta*. Fisheries Management and Ecology 11(3–4):173–182. DOI: 10.1111/j.1365-2400.2004.00402.x.

Althoff, A. L., C. T. Hasler, and M. J. Louison. 2020. Impact of retrieval time and hook type on hooking depth in ice-angled northern pike caught on tip-ups. Fisheries Research [online serial] 225:105502. DOI: 10.1016/j.fishres.2020.105502.

Anderson, R. M., and R. B. Nehring. 1984. Effects of a catch-and-release regulation on a wild trout population in Colorado and its acceptance by anglers. North American Journal of Fisheries Management 4(3):257–265. DOI: 10.1577/1548-8659(1984)4<257:EOACRO>2.0.CO;2.

Angler’s Atlas. 2021. Available: https://www.anglersatlas.com/place/159535/gull-lake/fish/northern-pike. (January 2021).

Arlinghaus, R., S. J. Cooke, J. Lyman, D. Policansky, A. Schwab, C. Suski, S. G. Sutton, and E. B. Thorstad. 2007. Understanding the complexity of catch-and-release in recreational fishing: an integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. Reviews in Fisheries Science 15(1–2):75–167. DOI: 10.1080/10641260601149432.

Arlinghaus, R., T. Klefoth, S. J. Cooke, A. Gingerich, and C. Suski. 2009. Physiological and behavioural consequences of catch-and-release angling on northern pike (*Esox lucius* L.). Fisheries Research 97(3):223–233. DOI: 10.1016/j.fishres.2009.02.005.

Barthel, B. L., S. J. Cooke, C. D. Suski, and D. P. Philipp. 2003. Effects of landing net mesh type on injury and mortality in a freshwater recreational fishery. Fisheries Research 63(2):275–282. DOI: 10.1016/S0165-7836(03)00059-6.

Bartholomew, A., and J. A. Bohnsack. 2005. A review of catch-and-release angling mortality with implications for no-take reserves. Reviews in Fish Biology and Fisheries 15(1–2):129–154. DOI: 10.1007/s11160-005-2175-1.

Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42(3):517–525. DOI: 10.1093/icb/42.3.517.

Bartoń, K. 2019. MuMIn: multi-model inference. R package version 1.43.15. Available: https://CRAN.R-project.org/package=MuMIn. (February 2020).

Beckwith Jr., G. H., and P. S. Rand. 2005. Large circle hooks and short leaders with fixed weights reduce incidence of deep hooking in angled adult red drum. Fisheries Research 71(1):115–120. DOI: 10.1016/j.fishres.2004.08.023.

Bettex-Galland, M., and G. M. Hughes. 1973. Contractile filamentous material in the pillar cells of fish gills. Journal of Cell Science 13(2):359–370. DOI: 10.1242/jcs.13.2.359.

Bettoli, P. W., C. S. Vandergoot, and P. T. Horner. 2000. Hooking mortality of saugers in the Tennessee River. North American Journal of Fisheries Management 20(3):833–837. DOI: 10.1577/1548-8675(2000)020<0833:HMOSIT>2.3.CO;2.

Bhagwant, S., and K. B. Elahee. 2002. Pathologic gill lesions in two edible lagoon fish species, *Mulloidichthys flavolineatus* and *Mugil cephalus*, from the Bay of Poudre d'Or, Mauritius. Western Indian Ocean Journal of Marine Science 1(1):35–42.

Bieber, J. F., M. J. Louison, J. A. Stein, and C. D. Suski. 2019. Impact of ice-angling and handling on swimming performance in bluegill and largemouth bass. North American Journal of Fisheries Management 39(6):1301–1310. DOI: 10.1002/nafm.10366.

Blair, S. D., D. Matheson, Y. He, and G. G. Goss. 2016. Reduced salinity tolerance in the Arctic grayling (*Thymallus arcticus*) is associated with rapid development of a gill interlamellar cell mass: implications of high-saline spills on native freshwater salmonids. Conservation Physiology [online serial] 4(1):cow010. DOI: 10.1093/conphys/cow010.

Bodensteiner, L. R., and W. M. Lewis. 1992. Role of temperature, dissolved oxygen, and backwaters in the winter survival of freshwater drum (*Aplodinotus grunniens*) in the Mississippi River. Canadian Journal of Fisheries and Aquatic Sciences 49(1):173–184. DOI: 10.1139/f92-021.

Brownscombe, J. W., S. D. Bower, W. Bowden, L. Nowell, J. D. Midwood, N. Johnson, and S. J. Cooke. 2014. Canadian recreational fisheries: 35 years of social, biological, and economic dynamics from a national survey. Fisheries 39(6):251–260. DOI: 10.1080/03632415.2014.915811.

Brownscombe, J. W., A. J. Danylchuk, J. M. Chapman, L. F. G. Gutowsky, and S. J. Cooke. 2017. Best practices for catch-and-release recreational fisheries–angling tools and tactics. Fisheries Research 186:693–705. DOI: 10.1016/j.fishres.2016.04.018.

Brownscombe, J. W., L. P. Griffin, T. Gagne, C. R. Haak, S. J. Cooke, and A. J. Danylchuk. 2015. Physiological stress and reflex impairment of recreationally angled bonefish in Puerto Rico. Environmental Biology of Fishes 98(11):2287–2295. DOI: 10.1007/s10641-015-0444-y.

Card, J. T., and C. T. Hasler. 2021. Physiological effects of catch-and-release angling on freshwater drum (*Aplodinotus grunniens*). Fisheries Research [online serial] 237:105881. DOI: 10.1016/j.fishres.2021.105881.

Coleman, F. C., W. F. Figueira, J. S. Ueland, and L. B. Crowder. 2004. The impact of United States recreational fisheries on marine fish populations. Science 305(5692):1958–1960. DOI: 10.1126/science.1100397.

Cooke, S. J., B. L. Barthel, and C. D. Suski. 2003. Effects of hook type on injury and capture efficiency of rock bass, *Ambloplites rupestris*, angled in south-eastern Ontario. Fisheries Management and Ecology 10(4):269–271. DOI: 10.1046/j.1365-2400.2003.00329.x.

Cooke, S. J., and I. G. Cowx. 2004. The role of recreational fishing in global fish crises. BioScience 54(9):857–859. DOI: 10.1641/0006-3568(2004)054[0857:TRORFI]2.0.CO;2.

Cooke, S. J., and I. G. Cowx. 2006. Contrasting recreational and commercial fishing: searching for common issues to promote unified conservation of fisheries resources and aquatic environments. Biological Conservation 128(1):93–108. DOI: 10.1016/j.biocon.2005.09.019.

Cooke, S. J., M. R. Donaldson, C. M. O’Connor, G. D. Raby, R. Arlinghaus, A. J. Danylchuk, K. C. Hanson, S. G. Hinch, T. D. Clark, D. A. Patterson, and C. D. Suski. 2013a. The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. Fisheries Management and Ecology 20(2–3):268–287. DOI: 10.1111/j.1365-2400.2012.00867.x.

Cooke, S. J., and C. M. O’Connor. 2010. Making conservation physiology relevant to policy makers and conservation practitioners. Conservation Letters 3(3):159–166. DOI: 10.1111/j.1755-263X.2010.00109.x.

Cooke, S. J., L. Y. Palensky, and A. J. Danylchuk. 2017. Inserting the angler into catch-and-release angling science and practice. Fisheries Research 186:599–600. DOI: 10.1016/j.fishres.2016.10.015.

Cooke, S. J., D. P. Philipp, K. M. Dunmall, and J. F. Schreer. 2001. The influence of terminal tackle on injury, handling time, and cardiac disturbance of rock bass. North American Journal of Fisheries Management 21(2):333–342. DOI: 10.1577/1548-8675(2001)021<0333:TIOTTO>2.0.CO;2.

Cooke, S. J., and H. L. Schramm. 2007. Catch-and-release science and its application to conservation and management of recreational fisheries. Fisheries Management and Ecology 14(2):73–79. DOI: 10.1111/j.1365-2400.2007.00527.x.

Cooke, S. J., J. F. Schreer, K. M. Dunmall, and D. P. Philipp. 2002. Strategies for quantifying sublethal effects of marine catch-and-release angling: insights from novel freshwater applications. American Fisheries Society Symposium 30:121–134.

Cooke, S. J., and L. U. Sneddon. 2007. Animal welfare perspectives on recreational angling. Applied Animal Behaviour Science 104(3–4):176–198. DOI: 10.1016/j.applanim.2006.09.002.

Cooke, S. J., and C. D. Suski. 2005. Do we need species-specific guidelines for catch-and-release recreational angling to effectively conserve diverse fishery resources? Biodiversity and Conservation 14(5):1195–1209. DOI: 10.1007/s10531-004-7845-0.

Cooke, S. J., C. D. Suski, R. Arlinghaus, and A. J. Danylchuk. 2013b. Voluntary institutions and behaviours as alternatives to formal regulations in recreational fisheries management. Fish and Fisheries 14(4):439–457. DOI: 10.1111/j.1467-2979.2012.00477.x.

Cowx, I. G. 2002. Recreational fishing. Pages 367–390 *in* P. J. B. Hart and J. D. Reynolds, editors. Handbook of Fish Biology and Fisheries. Volume 2: fisheries. Blackwell Publishing, Oxford, UK.

Daiber, F. C. 1952. The food and feeding relationships of the freshwater drum, *Aplodinotus Grunniens* Rafinesque in Western Lake Erie. The Ohio Journal of Science 52(1):35–46.

Dalla Via, J., G. van den Thillart, O. Cattani, and P. Cortesi. 1997. Environmental versus functional hypoxia/anoxia in sole *Solea solea*: the lactate paradox revisited. Marine Ecology Progress Series 154:79–90. DOI: 10.3354/meps154079.

Danylchuk, A. J., S. C. Danylchuk, A. Kosiarski, S. J. Cooke, and B. Huskey. 2018. Keepemwet Fishing—An emerging social brand for disseminating best practices for catch-and-release in recreational fisheries. Fisheries Research 205:52–56. DOI: 10.1016/j.fishres.2018.04.005.

Danylchuk, S. C. 2021. The forgotten end of the temperature spectrum: winter fishing. Available: https://www.keepfishwet.org/keepemwet-news-1/2021/1/19/winter-fishing. (May 2021).

Davie, P. S., and R. K. Kopf. 2006. Physiology, behaviour and welfare of fish during recreational fishing and after release. New Zealand Veterinary Journal 54(4):161–172. DOI: 10.1080/00480169.2006.36690.

Davis, M. W. 2002. Key principles for understanding fish bycatch discard mortality. Canadian Journal of Fisheries and Aquatic Sciences 59(11):1834–1843. DOI: 10.1139/f02-139.

Davis, M. W. 2007. Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. ICES (International Council for the Exploration of the Sea) Journal of Marine Science 64(8):1535–1542. DOI: 10.1093/icesjms/fsm087.

Davis, M. W. 2010. Fish stress and mortality can be predicted using reflex impairment. Fish and Fisheries 11(1):1–11. DOI: 10.1111/j.1467-2979.2009.00331.x.

Dempson, J. B., G. Furey, and M. Bloom. 2002. Effects of catch and release angling on Atlantic salmon, *Salmo salar* L., of the Conne River, Newfoundland. Fisheries Management and Ecology 9(3):139–147. DOI: 10.1046/j.1365-2400.2002.00288.x.

Deroba, J. J., M. J. Hansen, N. A. Nate, and J. M. Hennessy. 2007. Temporal profiles of walleye angling effort, harvest rate, and harvest in northern Wisconsin lakes. North American Journal of Fisheries Management 27:717–727. DOI: 10.1577/M06-125.1.

Dextrase, A. J., and H. E. Ball. 1991. Hooking mortality of lake trout angled through the ice. North American Journal of Fisheries Management 11(3):477–479. DOI: 10.1577/1548-8675(1991)011<0477:HMOLTA>2.3.CO;2.

Domeier, M. L., H. Dewar, and N. Nasby-Lucas. 2003. Mortality rate of striped marlin (*Tetrapturus audax*) caught with recreational tackle. Marine and Freshwater Research 54(4):435–445. DOI: 10.1071/MF01270.

Donaldson, M. R., S. J. Cooke, D. A. Patterson, and J. S. Macdonald. 2008. Cold shock and fish. Journal of Fish Biology 73(7):1491–1530. DOI: 10.1111/j.1095-8649.2008.02061.x.

DuBois, R. B., T. L. Margenau, R. S. Stewart, P. K. Cunningham, and P. W. Rasmussen. 1994. Hooking mortality of northern pike angled through ice. North American Journal of Fisheries Management 14(4):769–775. DOI: 10.1577/1548-8675(1994)014<0769:HMONPA>2.3.CO;2.

Dunmall, K. M., S. J. Cooke, J. F. Schreer, and R. S. McKinley. 2001. The effect of scented lures on the hooking injury and mortality of smallmouth bass caught by novice and experienced anglers. North American Journal of Fisheries Management 21(1):242–248. DOI: 10.1577/1548-8675(2001)021<0242:TEOSLO>2.0.CO;2.

Edelhauser, H. F., J. R. Hoffert, and P. O. Fromm. 1968. A comparative study of sodium permeability in lake trout and rabbit corneas. American Journal of Physiology-Legacy Content 214(2):389–394. DOI: 10.1152/ajplegacy.1968.214.2.389.

Eissa, N., and H. P. Wang. 2013. Physiological stress response of yellow perch subjected to repeated handlings and salt treatments at different temperatures. North American Journal of Aquaculture 75(3):449–454. DOI: 10.1080/15222055.2013.799622.

European Inland Fisheries Advisory Commission (EIFAC). 2008. EIFAC code of practice for recreational fisheries. EIFAC Occasional Paper No. 42, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Ferguson, R. A., and B. L. Tufts. 1992. Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*): implications for “catch and release” fisheries. Canadian Journal of Fisheries and Aquatic Sciences 49(6):1157–1162. DOI: 10.1139/f92-129.

Fisheries and Oceans Canada. 2019. Survey of Recreational Fishing in Canada, 2015. Available: https://www.dfo-mpo.gc.ca/stats/rec/can/2015/doc/2015-rec-fish-eng.pdf. (November 2019).

Food and Agriculture Organization of the United Nations (FAO). 2011. Expert consultation to develop the FAO technical guidelines for responsible fisheries: recreational fisheries, Berlin, Germany, 5-6 August 2011. FAO Fisheries and Aquaculture Report No. 979, Ankara, Turkey.

French III, J. R. P., and M. T. Bur. 1996. The effect of zebra mussel consumption on growth of freshwater drum in Lake Erie. Journal of Freshwater Ecology 11(3):283–289. DOI: 10.1080/02705060.1996.9664450.

French III, J. R. P., and J. G. Love. 1995. Size limitation on zebra mussels consumed by freshwater drum may preclude the effectiveness of drum as a biological controller. Journal of Freshwater Ecology 10(4):379–383. DOI: 10.1080/02705060.1995.9663460.

Gallman, E. A., J. J. Isely, J. R. Tomasso, and T. I. J. Smith. 1999. Short-term physiological responses of wild and hatchery-produced red drum during angling. North American

Journal of Fisheries Management 19(3):833–836. DOI: 10.1577/1548-8675(1999)019<0833:STPROW>2.0.CO;2.

Garrick, D. 2008. Body surface temperature and length in relation to the thermal biology of lizards. Bioscience Horizons 1(2):136–142. DOI: 10.1093/biohorizons/hzn014.

Gingerich, A. J., S. J. Cooke, K. C. Hanson, M. R. Donaldson, C. T. Hasler, C. D. Suski, and R. Arlinghaus. 2007. Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. Fisheries Research 86(2–3):169–178. DOI: 10.1016/j.fishres.2007.06.002.

Gingerich, A. J., and C. D. Suski. 2012. The effect of body size on post-exercise physiology in largemouth bass. Fish Physiology and Biochemistry 38(2):329–340. DOI: 10.1007/s10695-011-9510-3.

Gorissen, M., and G. Flik. 2016. The endocrinology of the stress response in fish: an adaptation-physiological view. Pages 75–111 *in* C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner, editors. Volume 35: fish physiology. Academic Press, Cambridge, Massachusetts. DOI: 10.1016/B978-0-12-802728-8.00003-5.

Grover, A. M., M. S. Mohr, and M. L. Palmer-Zwahlen. 2002. Hook-and-release mortality of Chinook salmon from drift mooching with circle hooks: management implications for California’s ocean sport fishery. Pages 39–56 *in* J. A. Lucy and A. L. Studholme, editors. Catch and release in marine recreational fisheries. American Fisheries Society, Symposium 30, Bethesda, Maryland.

Gustaveson, A. W., R. S. Wydoski, and G. A. Wedemeyer. 1991. Physiological response of largemouth bass to angling stress. Transactions of the American Fisheries Society 120(5):629–636. DOI: 10.1577/1548-8659(1991)120<0629:PROLBT>2.3.CO;2.

Hasler, C. T., A. H. Colotelo, T. Rapp, E. Jamieson, K. Bellehumeur, R. Arlinghaus, and S. J. Cooke. 2011. Opinions of fisheries researchers, managers, and anglers towards recreational fishing issues: an exploratory analysis for North America. American Fisheries Society Symposium 75:51-74.

Hassaninezhad, L., A. Safahieh, N. Salamat, A. Savari, and N. E. Majd. 2014. Assessment of gill pathological responses in the tropical fish yellowfin seabream of Persian Gulf under mercury exposure. Toxicology Reports 1:621–628. DOI: 10.1016/j.toxrep.2014.07.016.

van den Heuvel, M. R., M. Power, J. Richards, M. MacKinnon, and D. G. Dixon. 2000. Disease and gill lesions in yellow perch (*Perca flavescens*) exposed to oil sands mining-associated waters. Ecotoxicology and Environmental Safety 46(3):334–341. DOI: 10.1006/eesa.1999.1912.

Hey and Associates, Inc., and University of Wisconsin-Milwaukee. 2008. Fox Lake Management Strategy. Available: https://dnr.wi.gov/lakes/grants/largereports/LPT-244-04.pdf. (November 2018).

Hilborn, R., T. A. Branch, B. Ernst, A. Magnusson, C. V. Minte-Vera, M. D. Scheuerell, and J. L. Valero. 2003. State of the world's fisheries. Annual Review of Environment and Resources 28(1):359-399. DOI: 10.1146/annurev.energy.28.050302.105509.

Holder, P. E., L. P. Griffin, A. J. Adams, A. J. Danylchuk, S. J. Cooke, and J. W. Brownscombe. 2020. Stress, predators, and survival: exploring permit (*Trachinotus falcatus*) catch-and-release fishing mortality in the Florida Keys. Journal of Experimental Marine Biology and Ecology [online serial] 524:151289. DOI: 10.1016/j.jembe.2019.151289.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, J. A. Estes, T. P. Hughes, S. Kidwell, C. B. Lange,

H. S. Lenihan, J. M. Pandolfi, C. H. Peterson, R. S. Steneck, M. J. Tegner, and R. J. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293(5530):629–637. DOI: 10.1126/science.1059199.

Jacquemin, S. J., J. C. Doll, M. Pyron, M. Allen, and D. A. S. Owen. 2015. Effects of flow regime on growth rate in freshwater drum, *Aplodinotus grunniens*. Environmental Biology of Fishes 98(4):993–1003. DOI: 10.1007/s10641-014-0332-x.

Jacquemin, S. J., M. Pyron, M. Allen, and L. Etchison. 2014. Wabash River freshwater drum *Aplodinotus grunniens* diet: effects of body size, sex, and river gradient. Journal of Fish and Wildlife Management 5(1):133–140. DOI: 10.3996/032013-JFWM-027R.

Jarvis, E. T., and C. G. Lowe. 2008. The effects of barotrauma on the catch-and-release survival of southern California nearshore and shelf rockfish (Scorpaenidae, *Sebastes* spp.). Canadian Journal of Fisheries and Aquatic Science 65(7):1286–1296. DOI: 10.1139/F08-071.

Jiraungkoorskul, W., E. S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grams, and P. Pokethitiyook. 2002. Histopathological effects of roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*). ScienceAsia 28(3):121–127. DOI: 10.2306/scienceasia1513-1874.2002.28.121.

Johnston, I. A., and J. Dunn. 1987. Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. Symposia of the Society for Experimental Biology 41:67–93.

Kieffer, J. D., D. W. Baker, A. M. Wood, and C. N. Papadopoulos. 2011. The effects of temperature on the physiological response to low oxygen in Atlantic sturgeon. Fish Physiology and Biochemistry 37(4):809–819. DOI: 10.1007/s10695-011-9479-y.

Kieffer, J. D., M. F. Kubacki, F. J. S. Phelan, D. P. Philipp, and B. L. Tufts. 1995. Effects of catch-and-release angling on nesting male smallmouth bass. Transactions of the American Fisheries Society 124(1):70–76. DOI: 10.1577/1548-8659(1995)124<0070:EOCARA>2.3.CO;2.

Landsman, S. J., H. J. Wachelka, C. D. Suski, and S. J. Cooke. 2011. Evaluation of the physiology, behaviour, and survival of adult muskellunge (*Esox masquinongy*) captured and released by specialized anglers. Fisheries Research 110(2):377–386. DOI: 10.1016/j.fishres.2011.05.005.

Lavery, M. 2015. Winter: the forgotten study season. Available: https://thefisheriesblog.com/2015/02/09/winter-fieldwork/. (December 2018).

Lawrence, M. J., E. J. Eliason, J. W. Brownscombe, K. M. Gilmour, J. W. Mandelman, and S. J. Cooke. 2017. An experimental evaluation of the role of the stress axis in mediating predator-prey interactions in wild marine fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 207:21–29. DOI: 10.1016/j.cbpa.2017.02.001.

Lawrence, M. J., S. Jain-Schlaepfer, A. J. Zolderdo, D. A. Algera, K. M. Gilmour, A. J. Gallagher, and S. J. Cooke. 2018. Are 3 minutes good enough for obtaining baseline physiological samples from teleost fish? Canadian Journal of Zoology 96(7):774–786. DOI: 10.1139/cjz-2017-0093.

Lawrence, M. J., G. D. Raby, A. K. Teffer, K. M. Jeffries, A. J. Danylchuk, E. J. Eliason, C. T. Hasler, T. D. Clark, and S. J. Cooke. 2020. Best practices for non-lethal blood sampling of fish via the caudal vasculature. Journal of Fish Biology 97(1):4-15. DOI: 10.1111/jfb.14339.

Lawrence, M. J., K. M. Jeffries, S. J. Cooke, E. C. Enders, C. T. Hasler, C. M. Somers, C. D. Suski, and M. J. Louison. *In Review*. Catch and release ice fishing: status, issues, and research needs. Transactions of the American Fisheries Society 00:000-000.

LeBlanc, D. M., C. M. Wood, D. S. Fudge, and P. A. Wright. 2010. A fish out of water: gill and skin remodeling promotes osmo- and ionoregulation in the mangrove killifish *Kryptolebias marmoratus*. Physiological and Biochemical Zoology 83(6):932–949. DOI: 10.1086/656307.

Loftus, A. J., W. W. Taylor, and M. Keller. 1988. An evaluation of lake trout (*Salvelinus namaycush*) hooking mortality in the upper Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 45(8):1473–1479. DOI: 10.1139/f88-172.

Logan, J. M., M. J. Lawrence, G. E. Morgan, W. M. Twardek, R. J. Lennox, and S. J. Cooke. 2019. Consequences of winter air exposure on walleye (*Sander vitreus*) physiology and impairment following a simulated ice-angling event. Fisheries Research 215:106–113. DOI: 10.1016/j.fishres.2019.03.014.

Louison, M. J., C. T. Hasler, M. M. Fenske, C. D. Suski, and J. A. Stein. 2017a. Physiological effects of ice-angling capture and handling on northern pike, *Esox lucius*. Fisheries Management and Ecology 24(1):10–18. DOI: 10.1111/fme.12196.

Louison, M. J., C. T. Hasler, G. D. Raby, C. D. Suski, and J. A. Stein. 2017b. Chill out: physiological responses to winter ice-angling in two temperate freshwater fishes. Conservation Physiology [online serial] 5(1):cox027. DOI: 10.1093/conphys/cox027.

Lowry, O. H., and J. V. Passonneau. 1972. A flexible system of enzymatic analysis. Academic Press, Cambridge, Massachusetts.

Mabika, N., and M. Barson. 2014. A survey of gill histopathology of thirteen common fish species in the Sanyati Basin, Lake Kariba, Zimbabwe. Zoologica Poloniae 59(1–4):25–34. DOI: 10.2478/zoop-2014-0002.

Madliger, C. L., S. J. Cooke, E. J. Crespi, J. L. Funk, K. R. Hultine, K. E. Hunt, J. R. Rohr, B. J. Sinclair, C. D. Suski, C. K. R. Willis, and O. P. Love. 2016. Success stories and emerging themes in conservation physiology. Conservation Physiology [online serial] 4(1):cov057. DOI: 10.1093/conphys/cov057.

Manitoba Wildlife Federation. 2018. Economics of the recreational fishery on Lake Winnipeg. Available: https://mwf.mb.ca/archives/674. (May 2021).

Margenau, T. L., S. J. Gilbert, and G. R. Hatzenbeler. 2003. Angler catch and harvest of northern pike in northern Wisconsin lakes. North American Journal of Fisheries Management 23:307–312. DOI: 10.1577/1548-8675(2003)023<0307:ACAHON>2.0.CO;2.

McEwen, B. S., and J. C. Wingfield. 2010. What is in a name? Integrating homeostasis, allostasis and stress. Hormones and Behavior 57(2):105–111. DOI: 10.1016/j.yhbeh.2009.09.011.

McLean, M. F., K. C. Hanson, S. J. Cooke, S. G. Hinch, D. A. Patterson, T. L. Nettles, M. K. Litvak, and G. T. Crossin. 2016. Physiological stress response, reflex impairment and delayed mortality of white sturgeon *Acipenser transmontanus* exposed to simulated fisheries stressors. Conservation Physiology [online serial] 4(1):cow031. DOI: 10.1093/conphys/cow031.

Meka, J. M., and S. D. McCormick. 2005. Physiological response of wild rainbow trout to angling: impact of angling duration, fish size, body condition, and temperature. Fisheries Research 72(2–3):311–322. DOI: 10.1016/j.fishres.2004.10.006.

Millard, M. J., S. A. Welsh, J. W. Fletcher, J. Mohler, A. Kahnle, and K. Hattala. 2003. Mortality associated with catch and release of striped bass in the Hudson River. Fisheries Management and Ecology 10(5):295–300. DOI: 10.1046/j.1365-2400.2003.00363.x.

Morrison, S. M., T. E. Mackey, T. Durhack, J. D. Jeffrey, L. M. Wiens, N. J. Mochnacz, C. T. Hasler, E. C. Enders, J. R. Treberg, and K. M. Jeffries. 2020. Sub-lethal temperature thresholds indicate acclimation and physiological limits in brook trout *Salvelinus fontinalis*. Journal of Fish Biology 97(2):583-587. DOI: 10.1111/jfb.14411.

Muoneke, M. I., and W. M. Childress. 1994. Hooking mortality: a review for recreational fisheries. Reviews in Fisheries Science 2(2):123–156. DOI: 10.1080/10641269409388555.

NatureServe. 2020. NatureServe Explorer. NatureServe, Arlington, Virginia. Available: https://explorer.natureserve.org/. (October 2020).

Nguyen, V., M. A. Gravel, A. Mapleston, K. C. Hanson, and S. J. Cooke. 2009. The post-release behaviour and fate of tournament-caught smallmouth bass after ‘fizzing’ to alleviate distended swim bladders. Fisheries Research 96(2–3):313–318. DOI: 10.1016/j.fishres.2008.12.003.

North, R. 2002. Factors affecting the performance of stillwater coarse fisheries in England and Wales. Pages 284–298 *in* I. G. Cowx, editor. Management and Ecology of Lake and Reservoir Fisheries. Fishing News Books, Oxford, UK.

North/South Consultants Inc. 2010. Bipole III transmission project: existing aquatic environment. Available: https://www.hydro.mb.ca/projects/bipoleIII/pdfs/eis/AquaticsTechnicalReport/BPIII_Aquatics_Technical_Report_November_2011.pdf. (November 2018).

Ong, K. J., E. D. Stevens, and P. A. Wright. 2007. Gill morphology of the mangrove killifish (*Kryptolebias marmoratus*) is plastic and changes in response to terrestrial air exposure. Journal of Experimental Biology 210(7):1109–1115. DOI: 10.1242/jeb.002238.

Ozcelikkale, A., and B. Han. 2016. Thermal destabilization of collagen matrix hierarchical structure by freeze/thaw. PLoS (Public Library of Science) One [online serial] 11(1):e0146660. DOI: 10.1371/journal.pone.0146660.

Pankhurst, N. W. 2011. The endocrinology of stress in fish: an environmental perspective. General and Comparative Endocrinology 170(2):265–275. DOI: 10.1016/j.ygcen.2010.07.017.

Pankhurst, N. W., and M. Dedual. 1994. Effects of capture and recovery on plasma levels of cortisol, lactate and gonadal steroids in a natural population of rainbow trout. Journal of Fish Biology 45:1013–1025. DOI: 10.1111/j.1095-8649.1994.tb01069.x.

Pauly, D., J. Alder, E. Bennett, V. Christensen, P. Tyedmers, and R. Watson. 2003. The future for fisheries. Science 302(5649):1359–1361. DOI: 10.1126/science.1088667.

Payer, R. D., R. B. Pierce, and D. L. Pereira. 1989. Hooking mortality of walleyes caught on live and artificial baits. North American Journal of Fisheries Management 9(2):188–192. DOI: 10.1577/1548-8675(1989)009<0188:HMOWCO>2.3.CO;2.

Pelzman, R. J. 1978. Hooking mortality of juvenile largemouth bass, *Micropterus salmoides*. California Fish and Game 64(3):185–188.

Peoples, B. 2015. Why are fish cold blooded? Available: https://thefisheriesblog.com/2015/07/19/why-are-fish-cold-blooded/. (May 2021).

Pereira, D. L., C. Bingham, G. R. Spangler, D. J. Conner, and P. K. Cunningham. 1995. Construction of a 110-year biochronology from sagittae of freshwater drum (*Aplodinotus* *grunniens*). Pages 177–196 *in* D. H. Secor, J. M. Dean, and S. E. Campanda, editors. New Developments in Fish Otolith Research. University of South Carolina Press, Columbia, South Carolina.

Persons, S. E., and S. A. Hirsch. 1994. Hooking mortality of lake trout angled through ice by jigging and set-lining. North American Journal of Fisheries Management 14(3):664–668. DOI: 10.1577/1548-8675(1994)014<0664:HMOLTA>2.3.CO;2.

Pickering, A. D., and T. G. Pottinger. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7(1–4):253–258. DOI: 10.1007/BF00004714.

Policansky, D. 2002. Catch-and-release recreational fishing: a historical perspective. Pages 74–94 *in* T. J. Pitcher and C. Hollingworth, editors. Recreational fisheries: ecological, economic and social evaluation. Blackwell Science, Oxford, UK.

Post, J. R. 2013. Resilient recreational fisheries or prone to collapse? A decade of research on the science and management of recreational fisheries. Fisheries Management and Ecology 20(2–3):99–110. DOI: 10.1111/fme.12008.

Post, J. R., L. Persson, E. A. Parkinson, and T. van Kooten. 2008. Angler numerical response across landscapes and the collapse of freshwater fisheries. Ecological Applications 18(4):1038–1049. DOI: 10.1890/07-0465.1.

Post, J. R., M. Sullivan, S. Cox, N. P. Lester, C. J. Walters, E. A. Parkinson, A. J. Paul, L. Jackson, and B. J. Shuter. 2002. Canada's recreational fisheries: the invisible collapse? Fisheries 27(1):6–17. DOI: 10.1577/1548-8446(2002)027<0006:CRF>2.0.CO;2.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raat, A. J. P., J. G. P. Klein Breteler, and S. A. W. Jansen. 1997. Effects on growth and survival of retention of rod-caught cyprinids in large keepnets. Fisheries Management and Ecology 4(5):355–368. DOI: 10.1046/j.1365-2400.1997.00059.x.

Raby, G. D., M. R. Donaldson, S. G. Hinch, D. A. Patterson, A. G. Lotto, D. Robichaud, K. K. English, W. G. Willmore, A. P. Farrell, M. W. Davis, and S. J. Cooke. 2012. Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears. Journal of Applied Ecology 49(1):90–98. DOI: 10.1111/j.1365-2664.2011.02073.x.

Raby, G. D., J. R. Packer, A. J. Danylchuk, and S. J. Cooke. 2014. The understudied and underappreciated role of predation in the mortality of fish released from fishing gears. Fish and Fisheries 15(3):489–505. DOI: 10.1111/faf.12033.

Romanovsky, A. A. 2014. Skin temperature: its role in thermoregulation. Acta Physiologica 210(3):498–507. DOI: 10.1111/apha.12231.

Romero, L. M., M. J. Dickens, and N. E. Cyr. 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. Hormones and Behavior 55(3):375–389. DOI: 10.1016/j.yhbeh.2008.12.009.

Ross, M. R., and S. R. Hokenson. 1997. Short-term mortality of discarded finfish bycatch in the Gulf of Maine fishery for northern shrimp *Pandalus borealis*. North American Journal of Fisheries Management 17(4):902–909. DOI: 10.1577/1548-8675(1997)017<0902:STMODF>2.3.CO;2.

Rotllant, J., and L. Tort. 1997. Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. Journal of Fish Biology 51(1):21–28. DOI: 10.1111/j.1095-8649.1997.tb02510.x.

Rypel, A. L., P. Saffarinia, C. C. Vaughn, L. Nesper, K. O'Reilly, C. A. Parisek, M. L. Miller, P. B. Moyle, N. A. Fangue, M. Bell-Tilcock, D. Ayers, and S. R. David. 2021. Goodbye to "Rough Fish": Paradigm Shift in the Conservation of Native Fishes. Fisheries. Accepted Author Manuscript. DOI: 10.1002/fsh.10660.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7):671–675. DOI: 10.1038/nmeth.2089.

Schreck, C. B., and L. Tort. 2016. The concept of stress in fish. Pages 1–34 *in* C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner, editors. Volume 35: fish physiology. Academic Press, Cambridge, Massachusetts. DOI: 10.1016/B978-0-12-802728-8.00001-1.

Schreer, J. F., D. M. Resch, M. L. Gately, and S. J. Cooke. 2005. Swimming performance of brook trout after simulated catch-and-release angling: looking for air exposure thresholds. North American Journal of Fisheries Management 25(4):1513–1517. DOI: 10.1577/M05-050.1.

Schroeder, D. M., and M. S. Love. 2002. Recreational fishing and marine fish populations in California. California Cooperative Oceanic Fisheries Investigations Report 43:182–190.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Bulletin Fisheries Research Board of Canada No. 184.

Siddons, S. F., M. A. Pegg, N. P. Hogberg, and G. M. Klein. 2016. Age, growth, and mortality of a trophy channel catfish population in Manitoba, Canada. North American Journal of Fisheries Management 36(6):1368–1374. DOI: 10.1080/02755947.2016.1224783.

Sink, T. D., R. T. Lochmann, and K. A. Fecteau. 2008. Validation, use, and disadvantages of enzyme-linked immunosorbent assay kits for detection of cortisol in channel catfish, largemouth bass, red pacu, and golden shiners. Fish Physiology and Biochemistry 34(1):95–101. DOI: 10.1007/s10695-007-9150-9.

Smelser, G. K. 1962. Corneal hydration: comparative physiology of fish and mammals. Investigative Ophthalmology and Visual Science 1(1):11–32.

Somers, C. M., U. Goncin, S. Hamilton, M. Chupik, and R. Fisher. 2021. Chasing northern pike *Esox lucius* under ice: long-distance movements following catch-and-release ice angling. North American Journal of Fisheries Management. Accepted Author Manuscript. DOI: 10.1002/nafm.10638.

Stentiford, G. D., M. Longshaw, B. P. Lyons, G. Jones, M. Green, and S. W. Feist. 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Research 55(2):137–159. DOI: 10.1016/S0141-1136(02)00212-X.

Strzyżewska-Worotyńska, E., J. Szarek, I. Babińska, and D. Gulda. 2017. Gills as morphological biomarkers in extensive and intensive rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) production technologies. Environmental Monitoring and Assessesment 189(12):611. DOI: 10.1007/s10661-017-6278-7.

Stunz, G. W., and D. A. McKee. 2006. Catch-and-release mortality of spotted seatrout in Texas. North American Journal of Fisheries Management 26(4):843–848. DOI: 10.1577/M05-181.1.

Suski, C. D., S. J. Cooke, and B. L. Tufts. 2007. Failure of low-velocity swimming to enhance recovery from exhaustive exercise in largemouth bass (*Micropterus salmoides*). Physiological and Biochemical Zoology 80(1):78–87. DOI: 10.1086/509058.

Suski, C. D., S. S. Killen, J. D. Kieffer, and B. L. Tufts. 2006. The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. Journal of Fish Biology 68(1):120–136. DOI: 10.1111/j.0022-1112.2006.00882.x.

Suski, C. D., S. S. Killen, M. B. Morrissey, S. G. Lund, and B. L. Tufts. 2003. Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. North American Journal of Fisheries Management 23(3):760–769. DOI: 10.1577/M02-042.

Szekeres, P., J. W. Brownscombe, F. Cull, A. J. Danylchuk, A. D. Shultz, C. D. Suski, K. J. Murchie, and S. J. Cooke. 2014. Physiological and behavioural consequences of cold shock on bonefish (*Albula vulpes*) in The Bahamas. Journal of Experimental Marine Biology and Ecology 459:1–7. DOI: 10.1016/j.jembe.2014.05.003.

Taylor, M. J., and K. R. White. 1992. A meta-analysis of hooking mortality of nonanadromous trout. North American Journal of Fisheries Management 12(4):760–767. DOI: 10.1577/1548-8675(1992)012<0760:AMAOHM>2.3.CO;2.

Texas Parks and Wildlife Department. 2020. Fish records and awards. Available: https://tpwd.texas.gov/fishboat/fish/programs/fishrecords/. (October 2020).

Thompson, J. A., S. G. Hughes, E. B. May, and R. M. Harrell. 2002. Effects of catch-and-release on physiological responses and acute mortality of striped bass. American Fisheries Society Symposium 30:139–143.

Thompson, L. A., S. J. Cooke, M. R. Donaldson, K. C. Hanson, A. Gingerich, T. Klefoth, and R. Arlinghaus. 2008. Physiology, behavior, and survival of angled and air-exposed largemouth bass. North American Journal of Fisheries Management 28(4):1059–1068. DOI: 10.1577/M07-079.1.

Thorstad, E. B., T. F. Naesje, P. Fiske, and B. Finstad. 2003. Effects of hook and release on Atlantic salmon in the River Alta, northern Norway. Fisheries Research 60(2–3):293–307. DOI: 10.1016/S0165-7836(02)00176-5.

Tomasso, A. O., J. J. Isely, and J. R. Tomasso Jr. 1996. Physiological responses and mortality of striped bass angled in freshwater. Transactions of the American Fisheries Society 125(2):321–325. DOI: 10.1577/1548-8659(1996)125<0321:NPRAMO>2.3.CO;2.

Travel Manitoba. 2020. Master angler awards. Available: https://anglers.travelmanitoba.com. (October 2020).

Twardek, W. M., R. J. Lennox, M. J. Lawrence, J. M. Logan, P. Szekeres, S. J. Cooke, K. Tremblay, G. E. Morgan, and A. J. Danylchuk. 2018. The postrelease survival of walleyes following ice-angling on Lake Nipissing, Ontario. North American Journal of Fisheries Management 38(1):159–169. DOI: 10.1002/nafm.10009.

Ubels, J. L., and H. F. Edelhauser. 1987. Effects of corneal epithelial abrasion on corneal transparency, aqueous humor composition, and lens of fish. The Progressive Fish-Culturist 49(3):219–224. DOI: 10.1577/1548-8640(1987)49<219:EOCEAO>2.0.CO;2.

Valvona, C. J., H. L. Fillmore, P. B. Nunn, and G. J. Pilkington. 2016. The regulation and function of lactate dehydrogenase A: therapeutic potential in brain tumor. Brain Pathology 26(1):3–17. doi:10.1111/bpa.12299.

Van Ham, E. H., R. D. Van Anholt, G. Kruitwagen, A. K. Imsland, A. Foss, B. O. Sveinsbø, R. FitzGerald, A. C. Parpoura, S. O. Stefansson, and S. E. Wendelaar Bonga. 2003. Environment affects stress in exercised turbot. Comparative Biochemistry and

Physiology Part A: Molecular & Integrative Physiology 136(3):525–538. DOI: 10.1016/S1095-6433(03)00083-7.

Venkatesh, B. 2003. Evolution and diversity of fish genomes. Current Opinion in Genetics & Development 13(6):588–592. DOI: 10.1016/j.gde.2003.09.001.

Wang, Y., G. J. Heigenhauser, and C. M. Wood. 1994. Integrated responses to exhaustive exercise and recovery in rainbow trout white muscle: acid-base, phosphogen, carbohydrate, lipid, ammonia, fluid volume and electrolyte metabolism. Journal of Experimental Biology 195(1):227–258. DOI: 10.1242/jeb.195.1.227.

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. DOI: 10.1242/jeb.77.1.141.

Watson, R., P. Tyedmers, A. Kitchingman, and D. Pauly. 2003. What's left: the emerging shape of the global fisheries crisis. Conservation in Practice 4(3):20–21.

Wells, R. M. G., and N. W. Pankhurst. 1999. Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. Journal of the World Aquaculture Society 30(2):276–284. DOI: 10.1111/j.1749-7345.1999.tb00876.x.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiological Reviews 77(3):591–625. DOI: 10.1152/physrev.1997.77.3.591.

Wikelski, M., and S. J. Cooke. 2006. Conservation physiology. Trends in Ecology and Evolution 21(1):38–46. DOI: 10.1016/j.tree.2005.10.018.

Wilde, G. R. 1998. Tournament-associated mortality in black bass. Fisheries Management 23(10):12–22. DOI: 10.1577/1548-8446(1998)023<0012:TMIBB>2.0.CO;2.

Wilde, G. R., M. I. Muoneke, P. W. Bettoli, K. L. Nelson, and B. T. Hysmith. 2000. Bait and temperature effects on striped bass hooking mortality in freshwater. North American Journal of Fisheries Management 20(3):810–815. DOI: 10.1577/1548-8675(2000)020<0810:BATEOS>2.3.CO;2.

Wilkie, M. P., M. A. Brobbel, K. G. Davidson, L. Forsyth, and B. L. Tufts. 1997. Influences of temperature upon the postexercise physiology of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 54(3):503–511. DOI: 10.1139/f96-305.

Wilkie, M. P., K. Davidson, M. A. Brobbel, J. D. Kieffer, R. K. Booth, A. T. Bielak, and B. L. Tufts. 1996. Physiology and survival of wild Atlantic salmon following angling in warm summer waters. Transactions of the American Fisheries Society 125(4):572–580. DOI: 10.1577/1548-8659(1996)125<0572:PASOWA>2.3.CO;2.

Wilson, J. M., and P. Laurent. 2002. Fish gill morphology: inside out. Journal of Experimental Zoology 293(3):192–213. DOI: 10.1002/jez.10124.

Winter, H. N., M. J. Louison, J. A. Stein, and C. D. Suski. 2018. Metabolic response of bluegill to exercise at low water temperature: implications for angling conservation. Environmental Biology of Fishes 101(12):1657–1667. DOI: 10.1007/s10641-018-0814-3.

Wong, C. H. S., E. C. Enders, and C. T. Hasler. 2021. Limited evidence of zebra mussel (*Dreissena polymorpha*) consumption by freshwater drum (*Aplodinotus grunniens*) in Lake Winnipeg. Journal of Great Lakes Research 47(3):592-602. DOI: 10.1016/j.jglr.2020.08.020.

Wood, C. M. 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. Journal of Experimental Biology 160(1):285–308. DOI: 10.1242/jeb.160.1.285.

Wood, C. M., J. D. Turner, and M. S. Graham. 1983. Why do fish die after severe exercise? Journal of Fish Biology 22(2):189–201. DOI: 10.1111/j.1095-8649.1983.tb04739.x.

Wood, J. 2015. Fishing to the beat of a different drum. Available: http://www.hookedmagazine.ca/fishing-to-the-beat-of-a-different-drum/. (December 2018).

Wosnick, N., C. A. Navas, Y. V. Niella, E. L. A. Monteiro-Filho, C. A. Freire, and N. Hammerschlag. 2018. Thermal imaging reveals changes in body surface temperatures of blacktip sharks (*Carcharhinus limbatus*) during air exposure. Physiological and Biochemical Zoology 91(5):1005–1012. DOI: 10.1086/699484.

Appendix

Table A.1. Summary statistics for linear models assessing seasonal differences using water temperature instead of the categorical season covariate used in Card and Hasler (2021). The dependent variable is the 30 min value for each physiological variable following an angling event in freshwater drum (plasma cortisol, blood glucose, plasma lactate and blood pH). Significance was assessed at $P \leq 0.05$ and significant results are bolded. In conclusion, using water temperature as a covariate still shows the same seasonal variation in plasma cortisol, blood glucose and blood pH as using the categorical season covariate used in Card and Hasler (2021).

Variable	Factor	Value	SEM	d.f.	*t*-value	*P*-value
Plasma Cortisol	(Intercept)	-107.627	31.026	2, 67	-3.469	< 0.001
	Water Temperature	6.163	1.463	2, 67	4.213	**< 0.001**
	Sampling Time	136.494	12.543	2, 67	10.882	**< 0.001**
Blood Glucose	(Intercept)	-1.303	0.856	2, 72	-1.523	0.132
	Water Temperature	0.157	0.041	2, 72	3.823	**< 0.001**
	Sampling Time	2.574	0.354	2, 72	7.270	**< 0.001**
Plasma Lactate	(Intercept)	3.918	0.974	2, 67	4.024	< 0.001
	Water Temperature	0.014	0.046	2, 67	0.295	0.769
	Sampling Time	4.271	0.394	2, 67	10.850	**< 0.001**
Blood pH	(Intercept)	7.956	0.073	2, 64	108.251	< 0.001
	Water Temperature	-0.023	0.004	2, 64	-6.285	**< 0.001**
	Sampling Time	-0.142	0.031	2, 64	-4.639	**< 0.001**