

Angler experience and seasonal effects on the response of Lake Sturgeon
(*Acipenser fulvescens*) to catch and release angling

by

Eric Jacob Mullen

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Abstract

Lake sturgeon (*Acipenser fulvescens*), once widespread throughout North America, are now considered endangered in much of their natural range. However, there are areas where a presumably strong population has resulted in recreational fisheries, with catch and release (C&R) being common practice. Here, we evaluate physiological and behavioural impairment of lake sturgeon following C&R angling. Fish were captured by volunteer anglers and transferred to a holding pen in the Winnipeg River to recover and facilitate sampling post-angling. Measured physiological traits included blood osmolality, haematocrit, pH, glucose, cortisol, estradiol, and testosterone. Behavioural impairment was measured as a binary score using Reflex Action Mortality Predictors (RAMP) in addition to ventilation rate. The effect of season and angler experience on these measured variables were assessed. C&R angling resulted in an immediate physiological stress response and behavioural impairment in all lake sturgeon, regardless of treatment. Though generally we observed a recovery of changes in the acid-base balance, ion balance and reflex impairment by 24 h. Cortisol and glucose were elevated throughout the recovery period, possibly indicating interactive stressors of holding and repeat sampling. Sex steroid concentrations were highly variable, and indicated no clear response to C&R. Angling in the summer period caused increased ventilation rate and decreased osmolality at all timepoints, compared to spring angling. Novice anglers caused higher reflex impairment in sturgeon immediately post-capture compared to experienced anglers. This is the first study to examine the response of wild lake sturgeon to C&R. The data show an apparent resilience of lake sturgeon to C&R, and can be used to assess the validity of C&R as a management strategy in the conservation of lake sturgeon.

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Introduction

Recreational fisheries

Recreational fisheries exist across the globe, and can be broadly defined as the fishing for aquatic animals which are not required to sustain the physiological needs of the individual (Arlinghaus & Cooke, 2009). Rod and line angling has a large economic impact in North America, the increasing demand for the sustainable development of this recreational activity provides an opportunity for optimizing the science and management of our renewable fisheries resources and, by extension, the aquatic environment (Tufts et al., 2015). In Canada, recreational fisheries are closely monitored and managed through legal restrictions on catch season, harvest limits, gear selection, and local regulations created with the goal of sustainable fishery use.

Though some recreational anglers will harvest their catch for consumption, a considerable number of fish are immediately released. These fish may be viewed as unwanted bycatch and as such released due to their low food value, size, sex, or species (Cooke & Suski, 2005) or they may be voluntarily released due to their prized status as a trophy individual or iconic species (Landsman et al., 2011). In other cases, fisheries managers may choose to implement catch and release (C&R) programs as a management strategy to protect fish of a certain species, size, or life stage from harvest (Booth et al., 1995; Cooke & Schramm, 2007). According to the Department of Fisheries and Oceans Survey of recreational anglers in Canada (Department of Fisheries and Oceans, 2019) there were 184,475 active adult anglers in Manitoba in 2015. These anglers caught over 12 million fish and reportedly released up to 84 % of this catch. Given the large scale of fish release by recreational anglers, there is clearly a need to understand how the health of these non-retained fish is impacted by fishing activities.

C&R fisheries

The recent and widespread incorporation of C&R into North American fisheries management has provided a vital tool for managing and conserving recreational fisheries, and has offered a basis for sustainable use (Tufts et al., 2015). C&R fishing has the potential to reduce fish harvest without restricting recreational opportunities for anglers who are invested in the conservation of the fishery. The concept of C&R fishing is rooted in conservation, and operates under the assumption that released fish are not killed or otherwise impaired (Wydowski, 1977), however, angling events can have lethal or sublethal effects on fish through delayed stress and injury (Arlinghaus et al., 2007). There is a growing body of literature that exists to question the underlying assumptions of C&R fishing as a conservation tool. The results of these projects serve to demonstrate best practices for angling and species relevant information on the size, age, and season dependent response of individual fish to angling. The goal is that fisheries managers can then apply science-driven guidelines to the appropriate management of individual species (Cooke & Shramm, 2007). Assuming angling is not biased toward a more bold or shy phenotype, the information collected on individuals can be applied to the population level of certain fisheries to inform changes in management or current practice (Cooke & Shramm, 2007).

Effects of C&R on fish

In order to further our understanding on the effects of C&R on sportfish species, it is imperative to understand the nature of the challenges they experience during an angling event. The context and timing of these events provide helpful indices to the investigation of the condition of fish when returned to the water. Physical stressors such as capture, air exposure, handling, and rapid temperature changes may alter metabolic rate and food ingestion, and negatively impact the growth or condition of stressed fish (Wendelaar Bonga, 1997). In extreme

cases, physiological impairment related to C&R can cause mortality in many species. Consequently, C&R fisheries may feature restrictions on seasons and angling equipment to minimize stress, and ultimately, reduce mortality on recreationally significant species. Importantly, the physiological consequences of C&R angling are context and species dependant and the well-being of target species can be impacted by fish size, fight time, water temperature, and air exposure in addition to angler experience and gear selection (Cooke et al., 2013; Cooke & Suski, 2005). To the organism, an angling event may involve multiple acute stressors and the magnitude of those stressors may vary between species and individuals. Here we will review the physical stressors that can impact fish health, and explore how angler behaviour and abiotic factors may interact with these stressors.

Organism level response

No two C&R events are the same, variability occurs in the timing and severity of stressors. The following explanation of physical stressors should describe only what fish can experience under certain circumstances. To begin, tissue damage (hook puncture) occurs at the point of initial hooking, when a bait or lure has been ingested and the fish is subsequently hooked. Upon hooking, a fish will typically attempt to flee from the perceived threat. The forced and rigorous exercise that constitutes the ‘fight’ is then amplified by the resistance applied by the angler through the line and rod. Fish may suffer from exhaustion, decompression, predation, and rapid temperature changes during the fight (Arlinghaus et al., 2007). The handling of fish upon landing represents another stressor; fish may be netted or grabbed by the tail, gill, abdomen, or mouth and usually lifted out of the water by the angler. Air exposure then occurs as anglers observe, photograph, measure, or otherwise manipulate the fish above water. Fish retention by anglers may impact fish through scale/slime removal, hypoxia, temperature stress, and tissue

damage related to hook removal which may prolong post-release recovery and negatively affect the condition of released fish (Arlinghaus et al., 2007) Even after the fish has been released into the water, post-release stressors might include an increased susceptibility to predation (Arlinghaus et al., 2007), or complications related to the translocation of fish during an angling event outside of its optimal habitat (Cooke & Suski, 2005; McLean et al., 2019).

Angler behaviour & gear selection

When fish are angled, the duration and magnitude of the stress response is dependant on the severity and duration of the stress event (Schreck & Tort, 2016). Anglers may choose to control gear selection while C&R fishing to reduce the negative impacts of capture by choosing rods, reels, line, hooks, baits, terminal tackle, and other gear that are specialized to the target species (Arlinghaus et al., 2007). The decisions anglers make in terms of tactics and equipment while C&R fishing can have a real impact on the outcome of these events for fish. For example, a review by Cooke and Suski (2004) evaluated the effectiveness of circle hooks as a conservation tool in C&R fisheries. Circle hooks are designed specifically to reduce deep hooking in fish. Overall, the literature indicated a consistently lower mortality rate for fish caught on circle hooks over conventional J-style hooks. However, Cooke & Suski (2004) also found that hook removal times were greater in circle hooks than conventional J-hooks. The authors explained that hook type did not have a significant effect on physiological disturbance to the fish, but rather impacted the likelihood of direct mortality related to hooking injury.

The duration and seasonal effects of angling events are also an important consideration when quantifying the magnitude of a stress event on fish, particularly when the duration of an angling event (i.e. fight time) is often related to the suitability of angling gear to the size and species of fish that is being targeted (Arlinghaus et al., 2007). The duration of the fight time may

also relate to the length of fish being angled; for example, longer shortnose sturgeon, *Acipenser brevirostrum*, increased the duration of fight times compared to smaller individuals (Struthers et al., 2018). In addition, McLean et al. (2016) found that changes in blood parameters such as glucose, lactate, osmolality, and chloride increased with the length of treatment in simulated angling events on white sturgeon, *Acipenser transmontanus*.

Plasma cortisol and glucose increased with fight time in angled striped bass, *Morone saxatilis*, during summer angling but not in the fall, and increases in lactate and osmolality were positively correlated with fight times in both summer and fall angling events in striped bass (Tomasso et al., 1996). Meals & Miranda (1994) found that, in a largemouth bass, *Micropterus salmoides*, angling tournament scenario, large individuals had a significantly higher mortality rate (29 %) than smaller individuals (9 %). It should be noted that angling tournaments require fish to be handled more extensively than a typical angling scenario to meet the recording requirements of the competition.

Water temperature

The effect of temperature on physiological responses is perhaps the most pervasive environmental factor affecting fishes, in particular, the responses observed following exposure to a stressful event such as C&R angling (Gale et al., 2013). Generally, the potential for C&R related mortality and physiological impairment increases during the portion of the season when water temperature is at its peak (Cooke & Suski, 2005). At the cellular and organismal scale, high water temperatures may cause physiological changes to fish protein structure (Somero & Hoffman, 1996), enzyme activity, and metabolism (Fry, 1971; Hochachka & Somero, 1971) therefore amplifying the extent of physiological effects related to C&R.

In Atlantic salmon, *Salmo salar*, C&R at 22 °C resulted in a 40 % mortality rate (Wilkie et al., 1996) suggesting warmer temperatures negatively impacted recovery and resulted in significant delayed mortality. Anderson et al. (1998) also confirmed low survival rates and increased resting heart rates in Atlantic salmon captured and released at high water temperatures. Conversely, C&R on Atlantic salmon at 6 °C resulted in no mortalities yet still caused a rapid but transient increase in plasma lactate, K⁺, Cl⁻, and a decrease in pH but no changes in glucose levels. All measured parameters had been restored by 6 h post-angling (Booth et al., 1995). Similar effects of temperature have been observed in C&R studies on striped bass where temperatures of 26-32 °C resulted in a 11.5 % mortality whereas fish angled at 16-19 °C had a 5.7 % post-release mortality (Tomasso et al., 1996). Hypoxic conditions have also been used as a stressor in a variety of fishes to mimic the decreased oxygen availability a fish may experience during air exposure. Interestingly, Kieffer et al. (2011) reported a negative effect of lower temperature on the recovery of Atlantic sturgeon exposed to hypoxia, they hypothesize that at higher temperatures (15 °C) fish were able to clear plasma lactate at a faster rate than at lower temperatures (5 °C).

Interactive effects

The multiple stressors associated with C&R are likely to behave cumulatively (Barton, 2002) and accurate measurements and observations of angler behaviour and stressor timing will improve inferences that can be made on their physiological or behavioural effects. The interactive effects of water temperature and air exposure during C&R angling were reported by Gingerich et al. (2007) on bluegill, *Lepomis macrochirus*. These authors captured bluegill using standard angling techniques and exposed the fish to variations in temperature increase and air emersion duration. Unsurprisingly, they found that equilibrium loss and reduced ventilation

occurred most strongly to fish exposed to both the highest temperature and longest air exposure treatments post-angling. Further, immediate bluegill mortality was negligible at the lowest water temperatures, yet up to 80 % delayed mortality was observed in bluegill at the highest water temperature (27.4 °C) in the three highest air exposure groups.

Physiological response

Blood sampling can be used to provide information on the physiological responses of fish to C&R angling. Changes in blood variables will indicate the presence and veracity of a stress response, and demonstrate the recovery of these variables through time. Blood sampling and analysis holds appeal in the context of C&R research as it can be conducted non-lethally, allowing for observations of live fish for multiple sampling events. Typical physiological variables measured in the blood that are relevant to C&R studies and are key indicators of stress and/or physiological impairment include stress hormones (e.g. cortisol), ionic status (Sodium, Potassium, Chloride), metabolites (glucose or lactate), haematological characteristics (haematocrit/mean corpuscular haemoglobin content), and acid base status (Cooke et al., 2013).

Cortisol Response

Plasma cortisol concentrations are often used as a measure of the primary stress response in teleosts and chondrosteans. Through the activation of the hypothalamic pituitary inter-renal axis; exposure to an acute stressor will typically cause a transient increase in the circulating levels of cortisol that appears within minutes and then declines over the following hours. If the stressor persists and becomes chronic, there may be a general elevation in baseline levels of circulating cortisol for extended periods (Wendelaar-Bonga, 1997). While the acute cortisol response is considered an adaptive response driving energy release to fuel physiological function

following exposure to a stressor; prolonged exposure to elevated levels of cortisol are most likely maladaptive and may have negative effects on growth, reproduction, and immune function in fish that may lead to reduced fitness or delayed mortality (Wendelaar-Bonga, 1997). The physiological response to stressors and the extent of cortisol release varies substantially even among closely related fish species and the type of stressor used (Barton, 2002). It has, however, been well established that sturgeons exhibit a somewhat muted cortisol response in comparison to teleosts (Allen et al., 2009; Barton, 2002; Iwama et al., 2006; Pankhurst, 2011).

Osmoregulation

Osmotic disturbance is one of the most pronounced characteristics in the stress response of exhausted fish (Wendelaar Bonga, 1997; Wood, 1991). In a freshwater environment, the oxygen debt occurring in fish during the stress of exhaustive exercise alters gill blood flow and permeability, causing water to flow down its osmotic gradient into the fish and possible ionic loss resulting in decreased plasma osmolality (Schreck & Tort, 2016). The loss of electrolytes and the influx of water at the gills during intense exercise must be compensated by an increased expenditure of energy in fish adding further cost and possible time to the recovery period (Wood, 1991). The mineralocorticoid actions of cortisol promote active ion uptake upon disturbance of the hydromineral balance during stress (Wendelaar Bonga, 1997) and, as such, plasma osmolality may increase as an immediate response to a stressor (Baker et al., 2008; Donaldson et al., 2011; Tomasso et al., 1996). The gills of fish serve multiple functions as they control gas exchange, ion regulation, acid-base balance, and nitrogenous waste excretion; trade offs in gill function occur during periods of stress (Sardella and Brauner, 2016). For example, forced exercise cause a 70 % rise in sodium efflux in rainbow trout, *Oncorhynchus mykiss*, while

ventilatory and cardiovascular data indicated a 500 % increase in oxygen uptake (Wood & Randall, 1973).

Energy balance

During periods of acute stress, increased plasma catecholamines elicit the release of stored hepatic glycogen to the plasma as glucose (Wendelaar-Bonga, 1997). Elevated circulating levels of plasma glucose are often used as an indication of the secondary stress response, as cortisol is known to promote the release of glucose into circulation following exposure to a stressor (Barton, 2002). Glucose is mobilized to provide energy for the brain, gills, and muscles to compensate for the metabolic cost related to a stressor, that is the acute ‘fight or flight’ response (Iwama et al., 2006). Cortisol will promote glycogenolysis in the liver in the short-term following exposure to an acute stress, and gluconeogenesis over the longer term following an exposure to a chronic stress or repeated acute stressors (Vijayan & Moon, 1994; Vijayan et al., 1997). Both these mechanisms contribute to increases in circulating levels of glucose.

Reproduction

The release of cortisol as part of the stress response causes a reallocation of energy within an individual which may influence reproductive output (Wendelaar-Bonga, 1997). Under stressful conditions, spawning female fish may allocate energy into eggs or choose to abandon reproduction in a given year depending on the severity of a stressor and/or age of the individual. Further, exposure to stress may negatively impact gamete quality leading to reduced spawning success (Schreck et al., 2001). In one notable case, genetic analyses of Atlantic salmon caught and released by anglers indicated that larger salmon produced significantly less offspring than their non-caught counterparts, whereas the reproductive fitness of smaller salmon was not

impaired by the angling event (Richard et al., 2013). Air exposure was also reported to have a negative effect on Atlantic salmon fecundity. Salmon that were not exposed to air during an angling event produced twice the offspring as salmon removed from the water for <10 s and three times as many offspring as salmon exposed to air for >10 s (Richard et al., 2013).

The impacts of stress events on the circulation and levels of sex steroids such as testosterone and estradiol are variable, though there is a tendency for gonadal steroids to be depressed if a fish is exposed to a stressful event. In stellate sturgeon, *Acipenser stellatus*, the acute stressor of handling and stripping of eggs did not cause a significant deterioration in egg quality, however, exposure to chronic stress resulting in consistently high cortisol levels have been related to poor gamete quality in the same species (Bayunova et al., 2002). In lake sturgeon, *Acipenser fulvescens*, circulating levels of estradiol, testosterone, and cortisol in female and male spawning fish were typically highest immediately post-capture in 12 h gill net sets (Genz et al., 2014). However, exposure to the acute stress of hormonal induction of eggs and chronic stress of holding for two weeks in flow through aquaria had no significant effect on circulating levels of cortisol in female or male fish, or fertilization and hatching success of the progeny (Genz et al., 2014).

Vigorous exercise

The vigorous exercise associated with C&R angling is energy intensive and often requires recruitment of alternate fuel sources within the organism. Red muscle in fish is utilized to fuel locomotor activities such as aerobic swimming. Rapid and intense energy demands in fish related to predator-prey interactions, like burst and sprint movements, typically exceed the ability of aerobic respiration (Bennett, 1986). Thus, when exercise is strenuous and beyond the ability of the fish to respire aerobically (red muscle), then anaerobic respiration will be activated (white

muscle). Maximum muscle power is achieved as a result of anaerobic metabolism when the white muscle is utilized (Goolish, 1991). Exercise imposed by C&R fishing causes rapid energy expenditure in the white muscle, and the activation of anaerobic metabolic pathways (Cooke et al., 2013). During periods of exhaustive exercise lactate levels in fish blood usually increase significantly (Wood, 1991). This is a typical response seen during anaerobic activity in teleost's as the cellular production of lactic acid (Bennett, 1986) is dissociated into lactate and a hydrogen proton (Hochachka, 1991). This proton in addition to the protons sourced from ATP breakdown (Hochachka, 1991) can lead to a metabolic acidosis, causing a decrease in the pH of blood in exhausted teleost's (Wood, 1991). Therefore, it is reasonable to predict a marked decrease in blood pH in addition to a rise in blood lactate levels in sturgeon following strenuous exercise.

Oxygen demand

Forced activity requires additional oxygen uptake and use in organisms attempting to maintain internal homeostasis. The blood oxygen capacity represents the maximum amount of oxygen which can be carried by the blood, it is determined by the number of circulating erythrocytes (Nikinmaa, 2016). An increase in the haematocrit levels of fish during stress is a response to high oxygen demand (McLean et al., 2016) as in periods of vigorous exercise, increases in ventilation and an increased supply of haemoglobin to the erythrocytes are required to combat oxygen debt (Nikinmaa, 2016). After 5 min of forced exercise Japanese amberjack, *Seriola quinqueradiata*, displayed a 40 % increase in the blood haematocrit and haemoglobin concentration (Ken-Ichi et al., 1980). Kieffer et al. (2011) found an increase in the percent haematocrit of Atlantic sturgeon, *Acipenser oxyrinchus*, two hours after a hypoxic challenge. Temperature may also impact the variation in percent haematocrit in stressed fish. Under normal conditions, Atlantic sturgeon had significantly lower haematocrit levels at 5 °C than at 15 °C

(23.1 and 27.9 % respectively). This may be due to the increased dissolved oxygen content typical of colder water.

Behavioural effects

In addition to the use of physiological parameters to predict angling impacts, there are also a suite of behavioural responses which have been used to determine the extent of angling induced changes on fish. In some cases, observations collected on the behaviour of unstressed fish can be compared to observations on the recovery of stressed fish in lab settings (McLean et al., 2016), the results can potentially predict mortality based on the observed recovery and behaviour of individuals. Telemetry may also be used in some cases to track the movements of fish upon release in an angling scenario. Using radio telemetry, Thompson et al. (2008) assessed the behaviour of largemouth bass following an angling and air exposure event. They reported that longer air exposures tended to result in behavioral disruption as these fish remained at the release site longer than fish subjected to shorter air exposure.

Reflex impairment scoring

Physiological tools often produce unclear evidence for predicted mortality and, as such, there has been increasing interest in the use of reflex testing to assess whole animal responses and predict delayed mortality in C&R literature (Cooke et al., 2013). Wood et al. (1983) recorded a 40 % mortality rate over a 12 h period for rainbow trout exposed to severe exercise. The authors found that it was possible to reasonably predict mortality based on the inability of individuals to maintain orientation 1-2 h prior to morbidity and eventual mortality, and the same individuals exhibited rapid and shallow respiration during this time. Gingerich et al. (2007) found that the interactive effects of C&R stressors, in severe cases, would cause a loss of

equilibrium in bluegill that often led to delayed mortality. Reflex action mortality predictors (RAMP) are used to assess the vitality of fish based on the presence or absence of normal reflexes typically displayed in unstressed individuals of a given species. Normal reflexes may be observed and scored after stimulation by gravity, light, sound, or touch in both free swimming and restrained fish (Davis, 2010). Reflex impairment occurs rapidly and may continue for hours after the stress event, with eventual scores providing a quantitative link between the degree of physiological impairment of a fish and its fitness outcome (Davis, 2010).

In shortnose sturgeon, air exposure was positively correlated with reflex impairment scores after a simulated angling event (Struthers et al., 2018), and in white sturgeon, McLean et al. (2016) found that high reflex impairment scores predicted the delayed mortality of two individuals exposed to 10-15 min of vigorous exercise and partial air exposure. Furthermore, the same two individuals had higher than normal lactate levels and extreme haematocrit profiles (6 & 56 %). However, in general plasma lactate, osmolality, and Cl⁻ ion concentrations were positively correlated to reflex impairment scores for white sturgeon following a simulated angling event (McLean et al., 2016).

Lake sturgeon

Lake sturgeon are the largest fish species in Manitoba, they are specialized bottom feeders and benthic generalists which feed on small prey such as aquatic invertebrates, crayfish, fish, and carrion (Stewart & Watkinson, 2004). Sturgeon feed by slowly inspecting the substrate with their barbels, when prey is found the sturgeon will rapidly protrude its mouth and suck in the prey (Harkness and Dymond, 1961). Adult male lake sturgeon measure between 100-185 cm (11-30 kg) with females achieving lengths of 130-215 cm (25-100 kg) (Peterson et al., 2007). Lake sturgeon have an exceptional longevity and only reproduce periodically with spawning

events occurring in periods ranging from 4-7 years (Roussow, 1957). These fish do not typically reach sexual maturity until they are between 12 and 33 years old, with females taking longer to reach sexual maturity than males (Harkness & Dymond, 1961). Historically, lake sturgeon represent an important traditional food source to indigenous peoples in Manitoba. In the late 19th and early 20th centuries, commercial fisheries heavily pressured lake sturgeon populations in southern Manitoba (Stewart & Watkinson, 2004) causing population decrease and even extirpation in select Manitoba waterbodies.

Management

Lake sturgeon populations have become increasingly rare in the Manitoban Great Lakes as well as the Winnipeg, Red and Assiniboine river watersheds (Stewart & Watkinson, 2004). According to the COSEWIC report on the assessment and status of lake sturgeon (2017) the management unit containing the Saskatchewan-Nelson River populations of lake sturgeon, which includes the Winnipeg River, are listed as endangered. Dam construction and water management alongside fishing and harvest are listed as the main threats to lake sturgeon (COSEWIC, 2017). Due to habitat alterations and the relatively recent existence of a commercial fishery, the Lake Winnipeg drainage basin has hosted the most recent extirpation and population reductions observed for lake sturgeon within their range (Ferguson & Duckworth, 1997).

In 1995, a conservation closure was implemented on the Winnipeg River between the Ontario border and Pine Falls generating station to prohibit the harvest and development of a recreational fishery targeting lake sturgeon. The section of the Winnipeg River which is located between the Pointe du Bois generating station and the Slave Falls generating station stretches only ten kilometres. The population in this small reservoir has improved significantly during the past decades and is now expected to be nearing carrying capacity (COSEWIC, 2017). Despite the

conservation closure, an active recreational C&R fishery targeting lake sturgeon has existed and the intensity of this fishery has increased alongside the recovery of the adult sturgeon population in the Winnipeg River (D. Kroeker, personal communication, 2018).

Fishery

The Manitoba Master Angler program is a trophy recognition program where anglers can report catches for Manitoba sportfish if they are of a ‘trophy’ size, based on the minimum length requirements of the program for each species. For a lake sturgeon to meet these requirements it must have a total length >109 cm. The online master angler database is extensive, and the publicly available program contains thousands of reports for multiple species in various water bodies across the province. This database provides a useful interpretation of the lake sturgeon fishery trends on the Winnipeg River. Figure 1 illustrates the increase in trophy sturgeon catches being recorded in the Winnipeg River using data from the Manitoba Master Angler program. The number of reports increases strongly after the implementation of the conservation closure as anglers were either unaware or non-compliant with the restrictions of the closure to a recreational fishery and a muted regulatory and enforcement response. As a result, the 2018 Manitoba fishing guide stated “Winnipeg River between the Manitoba/Ontario border and the Pine Falls Generating Station – closed to lake sturgeon fishing all year”.

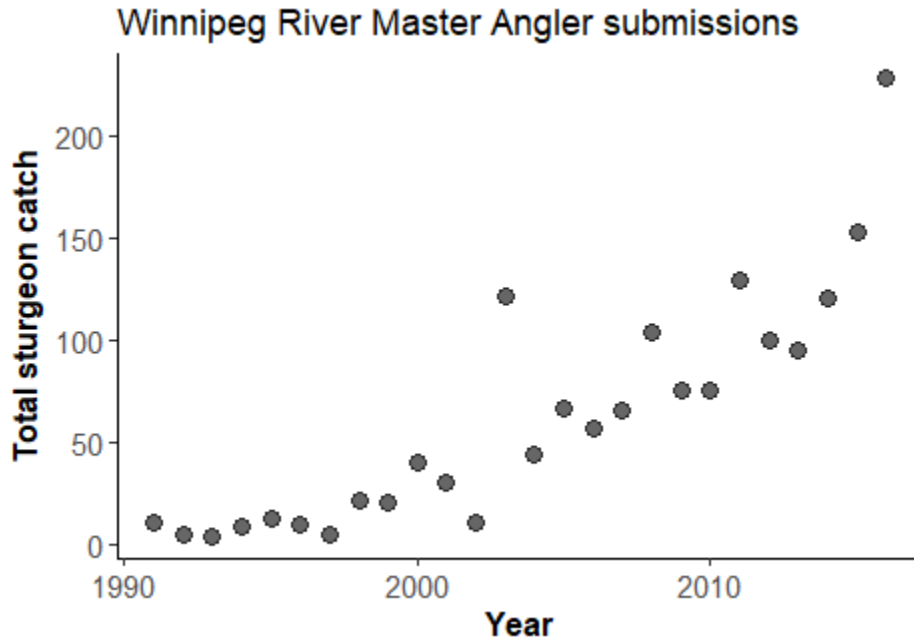


Figure 1: Reported catch of lake sturgeon per year on the Winnipeg River sourced from the public master angler program database. These records are limited to catches which meet the minimum length requirements of the program. Sturgeon must be 109 cm or larger to be reported. Data sourced from Travel Manitoba Master Angler database available online at https://anglers.travelmanitoba.com/master_angler_search.asp.

Typically, the open water fishing season in Manitoba begins in early May of each year. At this time, sturgeon are concentrating in areas of high flow below natural or artificial barriers in preparation for spawning (Peterson et al., 2007). Figure 2 illustrates the increase in master angler reports for lake sturgeon on the Winnipeg River beginning in May. The number of trophy sturgeon catches reported increases to a peak in June, remaining elevated in July and tapering as the summer continues into the fall.

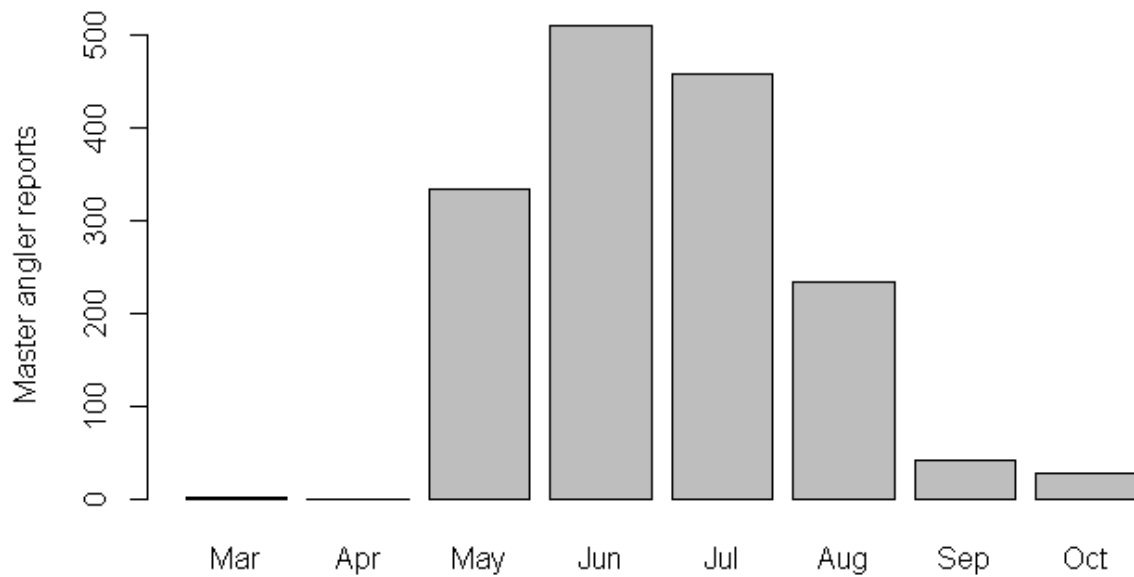


Figure 2: Reported total catch of lake sturgeon per month on the Winnipeg River sourced from the public master angler program database. Reports are described as the total number of master angler catches per month recorded from 1991 to 2016 in this dataset. These records are limited to catches which meet the minimum length requirements of the program. Sturgeon must be 109 cm or larger to be reported. Months not listed on the x axis had 0 records. Data sourced from Travel Manitoba Master Angler database available online at [.travelmanitoba.com/master_angler_search.asp](http://travelmanitoba.com/master_angler_search.asp).

Sturgeon C&R research

Members of the sturgeon family (*Acipenseridae*) share traits in their morphology, behaviour, and life history which make them vulnerable to the effects of human activities and, by

extension, fishing pressures (Boreman, 1997). Recent studies indicate white sturgeon are comparatively robust to the potential negative effects of C&R, yet behavioural and physiological disruption is exacerbated by increased temperature, fight time, and air exposure (McLean et al., 2019; McLean et al., 2016). Similarly, Struthers et al. (2018) reported that increased boat side holding times of shortnose sturgeon by C&R tournament anglers caused increased physiological disturbances indicative of stress. Baker et al. (2008) conducted a project to observe the stress response of lake sturgeon proceeding a gillnetting, handling, and PIT tagging program on the Winnipeg River. This project examined the effects of stressors on circulating stress signatures in the blood of wild lake sturgeon and found that measured levels of stress variables were lowest following a three day recovery period at temperatures below 17 °C.

In 2013, a pilot C&R study was conducted downstream of the Pointe du Bois generating station by provincial fisheries employees (Kansas and Geard, 2013). This project was conducted from May to July with 28 sturgeon being collected in water temperatures ranging from 9 to 19 °C. Fight times of these sturgeon ranged from 30 s to 33 min with the latter being a sturgeon that was foul hooked in the abdomen. This preliminary project aimed to determine whether sturgeon mortality occurred directly as a result of C&R angling. No mortalities were observed during the five day holding period and a continuation of this project that would extend observations of sturgeon post-release to include physiological variables indicative of stress, mortality, and sub-lethal effects was suggested by fisheries staff.

Objectives

Cooke & Suski (2005) warn against generalizations in management guidelines for C&R fisheries emphasizing that inter and intra-specific variation in the response to C&R has been seen in different life history stages, sizes, and even geographic distributions of fish. Anglers

sometimes adopt specialized techniques for releasing fish based on what species they target (Cooke & Schramm, 2007), and such guidelines are relevant in fisheries management to influence angler behaviour in situations where fish are being released. Mannheim et al. (2018) determined that long term strategic intervention to reduce handling time and air exposure improved C&R behaviour with recreational anglers.

In recent years, certain angling communities have advocated new techniques, gear and behaviours to improve the post-release survivability of desirable species (Cooke & Schramm, 2007; Landsman et al., 2011). The adoption of new behaviours by pro-active anglers is aimed to improve the overall performance of a fishery, and also to maintain a healthy size structure of a population by selectively releasing large, mature fish. The advancement of C&R science indicates that in some contexts, angler behaviour and self-regulation may have a profound impact on the fate of released fish (Landsman et al., 2011). For these reasons, the advancement of C&R as a sustainable use program in recreational fisheries is an ongoing and often collaborative effort between resource users, managers, and academics to provide information and recommendations on how to most responsibly interact with fish while angling. C&R research projects create a foundation for determining the extent of angling induced stress and how angler behaviour, or gear selection and timing may impact the fate of released fish. In this sense, adaptive regulations can be made to manage the predicted outcomes of C&R fisheries.

The objective of this investigation into the Winnipeg River lake sturgeon C&R fishery is to fill current knowledge gaps in this species response to angling, by replicating realistic angling interactions on wild caught lake sturgeon. To maintain the realistic variation of this fishery in this research, we will investigate the sturgeon's response to angling in various water temperatures, angler behaviour treatments, gear types, and by sampling sturgeon based on which

fish anglers are catching. This information can be used to suggest best handling practices for anglers who target lake sturgeon, and provide local fisheries regulators with insight into this prohibited fishery. This project will focus on understanding the physiological and behavioural impacts of C&R fishing on lake sturgeon in the short and medium term. Serial sampling will be conducted on wild caught lake sturgeon for physiological variables (Cortisol, Testosterone, Estradiol, Glucose, Osmolality, Haematocrit and pH) and behavioural indices (RAMP scores) to capture the extent of disruption and whether recovery can be observed within the holding period for sturgeon.

Hypothesis and predictions

Specifically, I hypothesize that lake sturgeon captured by rod and line will exhibit a change in physiology and behaviour that is directly related to the stress and exercise associated with the C&R event. A series of sampling events will be conducted on the Winnipeg River to assess potential seasonal effects of C&R on adult lake sturgeon. As approximately 70 % of the peer reviewed literature measuring the response of fishes to C&R indicate that higher water temperatures increase the risk of mortality and stress (Gale et al., 2013), I predict that angling events conducted in higher water temperatures (e.g. summer vs. spring sampling) will amplify the magnitude of the stress event in terms of behavioural and physiological responses.

The duration and severity of C&R related stressors is dictated strongly by gear selection and handling behaviours on behalf of the angler (Cooke & Suski, 2005), therefore, two angler behaviour treatments were designed in consideration of existing C&R guidelines for other species, sturgeon anglers and fisheries managers to replicate the gear selection and behaviour of an 'experienced' and a 'novice' angler. The experienced method has been designed to diminish angling related stressors to a reasonable minimum, whereas the novice method will challenge

these sturgeon with extended stressor durations and poor handling. For this experiment, I predict that sturgeon captured using the novice method will exhibit increased physiological and behavioural impairment when compared to an experienced angler treatment. It has been suggested that the response of sturgeon species to stressors may be muted in comparison to teleost's (Barton, 2002), thus I predict that lake sturgeon will demonstrate a robust response to C&R, but this response will be affected by season and angler experience.

Materials and methods

Lake sturgeon (n=60; Mean \pm S.E.M; Total length=954 \pm 228 mm; mass= 5154 \pm 467 g) were angled below the Pointe du Bois Generating Station on the Winnipeg River in Manitoba, Canada (50.297446, -95.547129). This project has benefitted immensely from a partnership with the Lac du Bonnet Wildlife Association, which provided volunteer support, boats, fishing equipment, and anglers for each field sampling effort. Anglers were briefed on the research project and given instructions towards the data they would be collecting, e.g. fight time, gear type, and angling experience (Table 1). The collection of information on angler behaviour in a realistic angling scenario demonstrated the air exposure, handling behaviour, and fight times typical of this catch and release fishery.

Lake sturgeon angling

Anglers were instructed to call out to the research vessel using two way radios when a sturgeon was hooked. The research vessel would then approach the boat and observe the anglers fighting the fish from a distance. When the sturgeon was caught and brought into the boat, the research vessel was attached to the side of the angling boat. At this point, anglers would typically de-hook, measure, and pose for pictures with the sturgeon.

It is important to note that, in 2018, anglers were instructed not to alter their angling behaviour for the project. Regular angling behaviour would demonstrate the variation in air exposure, angling equipment, and handling techniques which are typical of the catch and release fishery that has existed in this location for years. However, it is recognized as these individuals were specifically volunteering for this research project, there may be some inherent bias in how fish were treated during the angling event. During our angling events, between 3-5 volunteer

boats were angling simultaneously, each boat contained 2-4 anglers. All angling boats were visible to researchers who observed from a research vessel, prepared to collect fish for sampling. Volunteer anglers were provided with a data sheet for each fish their boat may collect, these sheets were divided into three short sections to capture information regarding the angler, the fish, and the equipment used, and completed by the anglers. Briefly, experience of the angler was requested; information regarding the rod, reel, line, hook, and bait was requested, then finally time at first hook up, time the fish was landed, hookset location, and time when fish was transferred to research vessel was recorded. These data sheets were collected with every fish sampled and the information was transcribed to a spreadsheet (Table 1).

Volunteers angled from anchored boats in the high flow tailwater of the generating station in depths between 6 to 7 m. Sturgeon caught by volunteers were handed over to research personnel in a separate vessel. Body mass and total length were recorded for each fish then a 2 ml blood sample was taken by caudal sinus puncture using a sterile 29 gauge needle connected to a sodium heparin coated 3 ml syringe (100 IU sodium heparin.ml⁻¹), and immediately placed on ice. Finally a binary RAMP score (see below) was assessed for each individual.

Table 1: Example of volunteer data sheet. Angler information, fish information and equipment information are recorded by anglers and handed to researcher with fish.

Angler information		
Name		
Experience fishing sturgeon at Pointe du Bois	Yes	No
Experience fishing sturgeon at other locations	Yes	No
Fish information		
Time at hook up:		
Time at boat:		
Time of landing if different than time at boat:		
Hookset location:		
Length:		
Photo taken?	Yes	No
Depth:		
Time of pass over to researcher:		
Equipment information		
Action of rod:		
Reel type:		
Line type/Strength:		
Hook size/Type		
Bait:		

Sample collection

The sampling procedure took approximately 2-3 min at which point the fish was transported to a holding pen on board the research vessel in a 378 L stock tank filled with fresh river water. A second blood sample and RAMP score was then taken approximately twenty minutes post-angling, before the fish was transferred to an in-river holding pen where the fish remained for subsequent sampling. If a fish did not already have an individual tag, it was given a uniquely coded external tag for identification at subsequent sampling timepoints. The tagging

procedure took less than 10 s, and during the sampling procedure each individual fish was inspected for signs of injury and evidence of spawning. All procedures outlined in this study were approved under Provincial collection permit #22-19 and protocol number F18-012 by the University of Manitoba Animal Care committee, pursuant to the guidelines in the Canadian Council for Animal Care. All fish were captured using barbless hooks.

Two nylon netted holding pens (3.66 x 3.66 x 2.12 m) were installed within approximately 200 m of the angling area in a depth of two meters of water and approximately three meters from shore but were only accessible by boat. The location of the holding pens had similar surface temperatures as the high flow area below the generating station, where the majority of angling occurred. These pens were installed side by side using six cedar poles (2-3.5 m) hammered into the sediment to form the structure which would hold the corners of the holding pens. Six downhaul poles were strapped to the main structure and used to drag the bottom corners of the holding pen downward so that contact with the river bed was made.

A maximum of four fish were held in each pen at any one time. At the 24 h, 48 h and 7 d sampling timepoints all the downhaul poles were disassembled, and foam blockers were rolled under the bottom of the holding pen and pushed so that the sturgeon were corralled to one side of the pen. Sturgeon were then haphazardly selected for sampling as described above with the exception that length and body mass was not taken. Individual fish were sampled and returned to the holding pen before selecting the next fish, total processing time for four sturgeon was 9-14 min. The order in which each individual was sampled was recorded to assess the potential impact of handling and sampling related stressors on the measured variable. Blood samples were transported to shore where a portion of whole blood was taken for haematocrit measurement. The remaining blood was centrifuged at 13,000 g for 3 min to separate red cells from plasma.

The plasma was decanted off and stored in a separate vial and both red cells and plasma were then stored at -20 °C prior to return to the laboratory, then transferred to a -80 °C freezer prior to further analysis.

Binary RAMP scores were assessed as previously described for white sturgeon where a 0 was unimpaired and a 1 was impaired (McLean et al., 2016). Briefly, mouth extensions in sturgeon have been previously associated with exhaustion, possibly caused by the loss of muscle tone due to physiological exhaustion after strenuous exercise causing the mouth to fully distend and cease to retract during opercular beats. This response was considered impaired if the fish fully extended the mouth within a period of 10 s while being held above water. Orientation reflex was considered impaired if the fish failed to right itself within three seconds after being turned over, and tail-grabbing was considered impaired if the fish failed to attempt an escape response when grabbed at the caudal peduncle. Finally, body flex was assessed and considered impaired if the fish did not wrestle from a loose grip within 10 s when being held above water around the central abdominal region. Respiration rate was measured by placing the fish in a small holding tank within the research boat and measuring the number of opercular beats that occurred during a 10 s period. Binary RAMP scores were assessed together to provide an overall RAMP score for each fish at each timepoint, respiration rate was assessed independently of the RAMP scores.

Seasonal and Angler effects

To assess the seasonal response of lake sturgeon to angling, sturgeon were collected in the spring and summer of 2018. In the spring, 24 fish (Mean total length: 927 +/- 33.3 mm) were captured from May 29th to June 6th. Water temperatures for the spring group ranged from 12.7-13.3 °C. In the summer, 12 fish (Mean total length: 893 +/- 37.0 mm) were captured from August 20th to 23rd. Water temperatures for the summer group were recorded between 20.9-21.2

°C. Volunteer anglers were asked to display normal angling behaviour during the 2018 angling events and anglers provided information regarding their level of experience angling sturgeon, gear selection, handling methods, and the durations of the angling event (i.e. Fight/Air exposure times). In 2018, all fish were held in the river pens for a maximum of 48 h post-capture. These fish were sampled for blood and RAMP immediately upon release when fish were transferred from the angling vessel to the research vessel (time 0), then 20 min, 24 h and 48 h post-angling.

In the spring of 2019, 24 lake sturgeon (n=12 for each treatment) were angled from May 21st to June 5th (Mean total length: 1011 +/- 40.5 mm) in water temperatures ranging from 8.1-12.3 °C. In this experiment, volunteer anglers were instructed to alter their behaviour and gear selection to the specifications of the following two angler behaviour treatments. In the novice angler group, volunteers used rods with a power rating of medium-heavy or lower, which were spooled with <9 kg test line. This tackle arrangement reflects the gear selection of an angler targeting other species (by-catch scenario) or a first-time sturgeon angler. It is possible that novice anglers are more likely to excessively air expose fish than anglers with experience handling and observing that species. To reflect this possibility, after being landed by hand, sturgeon were air exposed in the boat for five minutes during which anglers were allowed to photograph, measure, and otherwise manipulate the fish.

In the experienced angler treatment, anglers used a rod power of medium-heavy or larger, and line test rated for >9 kg, this gear selection would likely reduce fight time. In an effort to replicate the behaviours of specialized C&R muskellunge anglers (Landsman et al., 2011); large, rubber mesh bucket nets were used to confine and submerge the fish, rather than landing the fish for hook removal. Tools for dehooking were kept at hand in the case of deep hooking. With this method air exposure time was limited to a maximum of 30 s for photography and/or

measurement prior to the fish being handed over to the research vessel. All sturgeon were handled using wet cotton gloves to reduce abrasion and slime removal (Arlinghaus et al., 2007). Circulating levels of cortisol and glucose failed to return to baseline over the 48 h holding period in 2018. To facilitate examination of measured plasma parameters over the longer term in 2019, all fish were held in the river pens for a maximum of seven days post-capture. These fish were sampled for blood and RAMP immediately upon release (time 0), then at 20 min, 24 h, 48 h and 7 d post-angling. After the final sampling timepoint in both years, all lake sturgeon were released into the river at the point of capture.

Sample processing

All samples were randomly selected for blood chemistry analysis. Plasma samples for each fish, at each timepoint, were divided into three separate labelled tubes. During lab sampling, one of these tubes for each fish/timepoint combination would be randomly selected and mixed into a separate container, where they would be drawn in random order for sampling. The values associated with these samples were processed and linked to the fish and timepoint, thus ensuring random sampling across individuals, treatments, and timepoint. Plasma Osmolality was measured by placing 10 μ l of thawed plasma into a vapor pressure osmometer (Wescor Inc Vapro $\text{\textcircled{R}}$, model no. 5520). Extracellular and intracellular pH was measured using a benchtop pH meter (Fisher Scientific Accumet $\text{\textcircled{R}}$ model AE 150) on 20 μ l of freshly thawed plasma or lysed red blood cells. Red blood cells were measured for pH after being lysed by placing all individual vials in a container of liquid nitrogen. The frozen red blood cell samples were thawed immediately and placed back into the liquid nitrogen container. Thus, each red blood cell sample went through five freeze and thaw cycles before being measured for pH. Plasma glucose concentration was measured in duplicate using an enzymatic hexokinase assay following the

procedures outlined in Treberg et al. (2007) and adapted for a 96 well plate. Inter and intra-assay variation for glucose were 4.0 and 3.9 %, respectively.

Plasma cortisol, testosterone, and estradiol were measured in duplicate using enzyme linked immunosorbent assay kits (ELISA; Cayman Chemical Item no. 500360,582701, and 582251). The assays were performed following the instructions provided by the manufacturer. Sample extraction was required prior to performing the assay, 240 μ l of thawed plasma was mixed in a culture tube with diethyl ether (4 times sample volume), the mixture was vortexed for 10 s and the upper diethyl ether layer was removed and placed in a separate tube. This process was repeated for a total of five extractions, the diethyl ether layer was dried under a gentle stream of nitrogen and the water layer was discarded. Dried samples were reconstituted with 500 μ l ELISA assay buffer prior to plating, if necessary, more buffer was added to reach the desired dilution. Extraction efficiencies for cortisol, testosterone and estradiol were 78.7, 79.0, and 80.1 %, respectively. Dilution factors were determined by validating a series of dilutions against the standard curve of each assay. Inter and intra-assay variation was calculated for cortisol (18.1/0.93 %), testosterone (25.5/1.05 %), and estradiol (22.1/0.95 %).

Statistical analysis

Physiological variables were analyzed via linear mixed effect models (LMM) which measured the effect of treatment (Season/angler experience) and sampling timepoint on the response variable. Individuals were included as a random effect to account for the repeated sampling of sturgeon in the experiments. In cases where data deviated from the underlying assumptions of normality and equal variance (Shapiro test, Levene's test; $p < 0.05$) required for this parametric test, continuous data were natural logarithmically or rank transformed (Table 5/8). In some instances, additional fixed effects (Table 5/8) were added to the LMM in order to

strengthen the fit of models for certain response variables. In the case of a significant main effect of timepoint or treatment in these models, pairwise Tukey`s HSD post-hoc tests were used to determine significance between timepoints within treatments and vice versa.

RAMP scores were analyzed via general linear mixed models (GLMM) for discrete data with a poisson distribution. This model uses a penalized quasi likelihood estimation for model parameters (Bolker et al., 2009). Again, these models measured the effect of treatment and sampling timepoint on RAMP scores while including individual fish as a random effect. In the case of a significant main effect of timepoint or treatment, pairwise Tukey`s HSD post-hoc tests were used to determine significance between timepoints within treatments and vice versa. We tested the effect of sampling order on timepoints when sturgeon were removed from the holding pen for sampling during the recovery period. For this purpose, general linear models calculated the impact of sampling order on all response variables to determine if sampling methodology and pen manipulation had a significant effect on physiological and behavioural variables. General linear models were also used to measure the effect of sturgeon length and weight on the durations of fight and air exposure times. Analysis was conducted using R statistical software (Version 1.2.5019; R Core Team, 2019). In all cases, significance was accepted at $\alpha=0.05$.

Results

No mortalities occurred during the observation period of this study, none of the fish captured by volunteer anglers had visible signs of injury on their body and none showed signs of spawning behaviour (i.e. milt or egg release while handling) so we were not able to definitively assign sex to any of the fish used in the study. The order in which sturgeon were removed from the holding pen for sampling, in a few instances, related to increased cortisol (Spring 2018, Appendix A, $F_{1,45}=4.241$, $p=0.045$), decreased respiration rate (Spring 2018, Appendix A, $F_{1,45}=5.001$, $p=0.030$) and decreased testosterone (Spring 2018, Appendix A, $F_{1,46}=4.591$, $p=0.037$). For all other treatments and variables, no effect of sampling order on response variables were detected.

Sturgeon size and angling duration

Given that lake sturgeon total length and body mass had a strong positive relationship ($F_{1,55}=11.58$, $p<0.001$) and that anglers chose to measure sturgeon rather than collect weights, the following analysis of fight time and air exposure durations is based on length. The overall dataset ($n=60$) indicates, predictably, that fight times for lake sturgeon increased with total length (Appendix B; $F_{1,58}=7.21$, $p=0.009$). Total length was not a reasonable predictor of air exposure times (Appendix B; $F_{1,34}=0.261$, $p=0.613$) indicating that lake sturgeon anglers volunteering for this data collection maintained uniform handling, measuring, and photographing durations regardless of sturgeon size.

Angling techniques and handling

In 2018, volunteer anglers were instructed to behave normally during angling collections. In this way, we recorded an example of the ‘typical behaviour’ displayed by lake sturgeon

anglers in the field. 75 % of the volunteer anglers had fished sturgeon on the Winnipeg River previously, 11.1 % had angled sturgeon species elsewhere, and 13.9 % had no experience angling sturgeon at all. In this group, anglers preferred to use heavy (52.8 %) and medium heavy (36.1 %) action rod powers, though two anglers chose to use medium power and one angler opted for an extra heavy rod. Line test strength selected by volunteer anglers was, on average 13.8 kg (range 9-23 kg). 88.9 % of anglers landed lake sturgeon by grabbing the caudal peduncle and hauling the fish into the boat, the remaining 11.1 % used landing nets. Bait selection by our volunteer anglers consisted mainly of cut worms and baitfish, though one angler did prefer to use sliced deer heart as a lake sturgeon attractant. In our study, anglers posing with sturgeon for photographs typically supported the entire body with one hand below the abdomen, and the other supporting the tail.

Seasonal effects on behavioural responses

Sturgeon angled in the spring treatment endured fight times of 3.83 +/- 0.84 min and were air exposed for 4.00 +/- 0.62 min, on average. During the summer treatment, fight times and air exposure were 2.17 +/- 0.38 and 1.75 +/- 0.28 min, respectively. Fight times did not differ between season yet air exposure was significantly higher in the spring treatment (2 sample t test; $p=0.02$). C&R angling caused an immediate increase in reflex impairment for lake sturgeon in all treatments at both time 0 and 20 min post-angling ($p<0.01$; Figure 3). In both seasonal treatments, with the exception of two individuals at 24 h and one individual at 48 h in the summer treatment, RAMP scores at 24 and 48 h post-angling indicated that normal reflexes had been completely restored during the resting period suggesting a minimal effect of temperature on RAMP scores (Table 2).

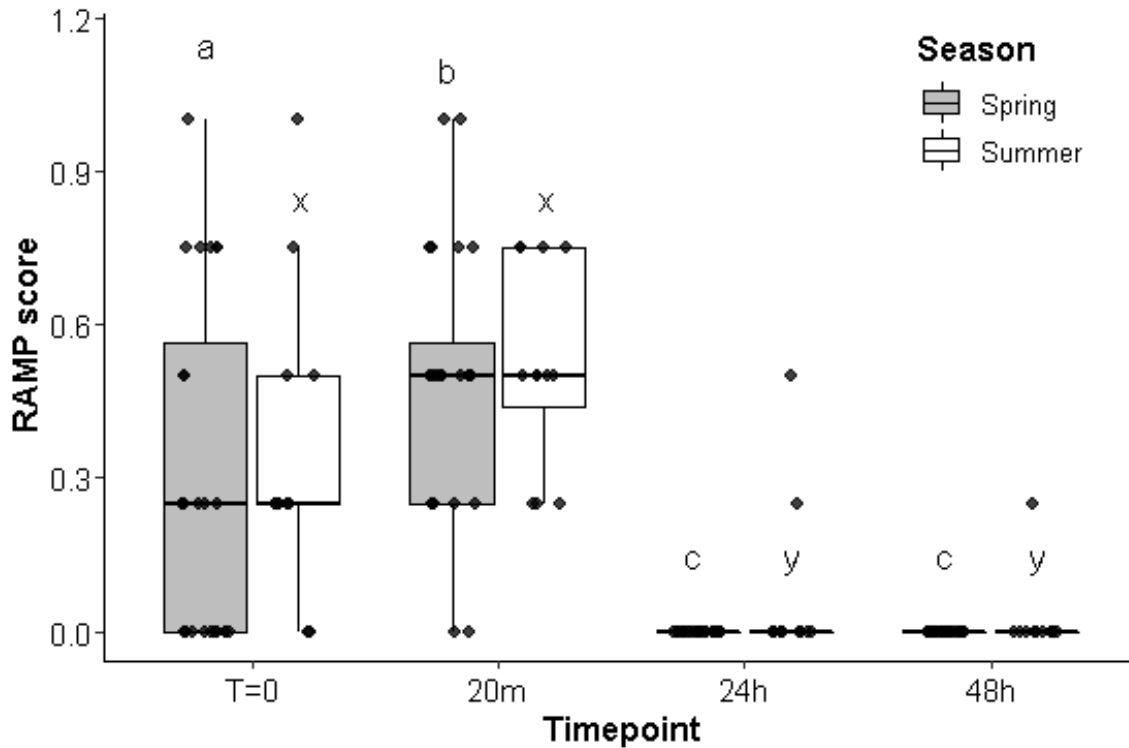


Figure 3: Seasonal comparison of RAMP scores for sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a season are noted by letter (abc for Spring, xyz for Summer).

Angler effects on behavioural responses

Fight times for novice anglers were higher, on average (5.20 +/- 0.92 min), than was observed in the experienced treatment (3.33 +/- 0.70 min) though the difference was not statistically significant. Sampling timepoint and treatment had significant effects on reflex impairment (Table 2) during the angler behaviour trials. Sturgeon experienced reflex impairment at time 0 in both the novice and experienced angler treatments (Figure 4). However, sturgeon released by novice anglers exhibited a greater increase in reflex impairment at time 0 compared

to those released by experienced anglers ($p < 0.001$; Figure 4). RAMP scores for sturgeon in the experienced treatment increased significantly at 20 min post-angling compared to time 0 values and there were no significant treatment differences by 20 min. Recovery of reflexes occurred within 24 h post-angling in both treatments, with the exception of a few individuals in either group, and at 48 h and 7 d post-angling there was no impaired RAMP scores in any individual.

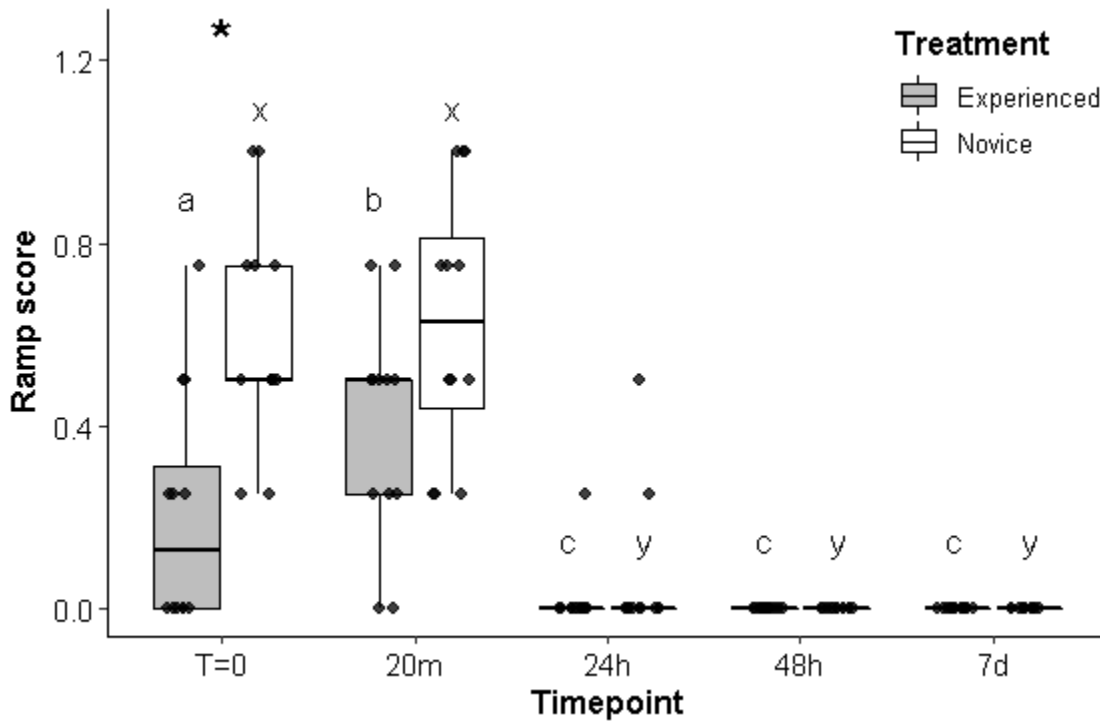


Figure 4: Angler behaviour comparison of RAMP scores for sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between angler experience treatments are noted with an * and significant differences between timepoints within a treatment are noted by letter (abc for experienced, xyz for novice).

Table 2: Summary of generalized linear mixed model results for reflex impairment data. The values, standard errors, degrees of freedom, T score and P value are reported for each fixed effect in the model.

	Parameter	Value	S.E.	Df	T	P
Seasonal 2018	Intercept	0.13	0.15	113	0.86	0.394
	20m	0.46	0.16	113	2.85	0.005
	24h	-29.4	188314.4	113	-0.00	1.000
	48h	-29.4	188314.4	113	-0.00	1.000
	Season	0.17	0.22	113	0.77	0.450
	20m: Summer	-0.08	0.27	113	-0.28	0.779
	24h: Summer	27.7	188314.4	113	0.00	1.000
	48h: Summer	26.6	188314.7	113	0.00	1.000
Angler behaviour 2019	Intercept	-0.34	0.2	87	-1.42	0.160
	20m	0.64	0.2	87	2.95	0.004
	24h	-2.30	0.6	87	-3.94	<0.001
	48h	-30.0	349316.8	87	-0.00	1.000
	7d	-30.0	349316.8	87	-0.00	1.000
	Treatment	1.19	0.3	22	3.83	<0.001
	20m: Novice	-0.61	0.3	87	-2.32	0.023
	24h: Novice	0.03	0.7	87	0.05	0.960
	48h: Novice	-1.15	505480.4	87	-0.00	1.000
	7d: Novice	-1.12	518117.6	87	-0.00	1.000

Seasonal effects on physiological responses

C&R angling triggered the cortisol stress response in lake sturgeon (Table 5/8); a steady rise in plasma cortisol was observed in the majority of sturgeon during the observation period. For sturgeon caught in the spring (Figure 5), plasma cortisol increased significantly from time 0 to the 24 h sampling period ($p < 0.001$; Figure 5), remaining elevated for at least 48 h after capture. In the summer, there was an increase in circulating levels of cortisol within 20 min post-angling ($p < 0.05$) with the highest levels of cortisol recorded 48 h post-capture ($p < 0.001$). The release of cortisol by sturgeon after angling and air exposure was not impacted by season.

When accounting for the effects of fight time, weight, and length on the plasma glucose response our model indicates that sampling timepoint had a significant effect on mobilized glucose in lake sturgeon following C&R, though there was no difference in glucose concentrations between the seasonal treatments (Table 5). Plasma glucose remained elevated throughout the recovery period and reached a peak 24 h after C&R relative to time 0 values ($p < 0.01$; Figure 6) without decreasing significantly by 48 h.

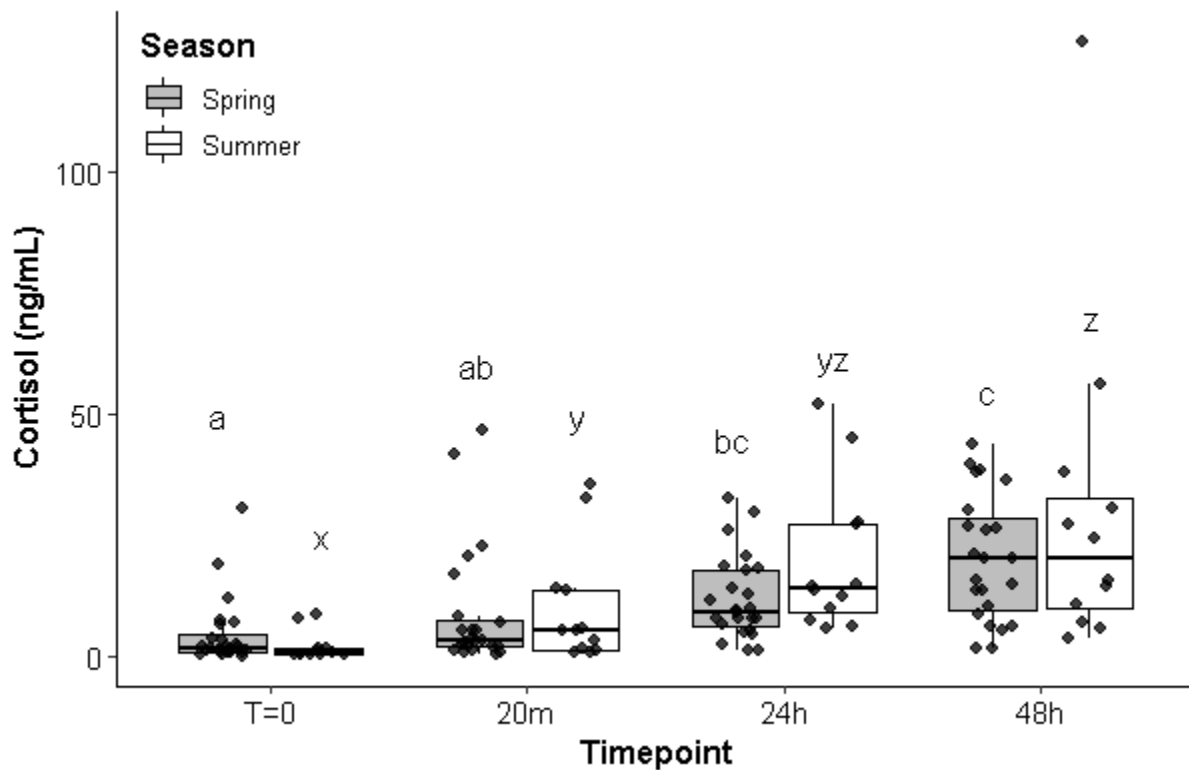


Figure 5: Seasonal comparison of plasma cortisol concentrations (ng/mL) of sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a season are noted by letter (abc for Spring, xyz for Summer).

In both the spring and summer, C&R caused a predictable marked decrease in extracellular pH at time 0, peaking at 20 min (Table 2). Extracellular pH in both spring and summer was significantly higher at 24 and 48 h post-angling compared to the 20 min timepoint. Interestingly, plasma pH at the 48 h timepoint was significantly lower in the summer sampled fish compared to the spring ($p < 0.001$; Table 2). Accounting for the effects of fight time and weight, timepoint had a significant effect on the disturbance of intracellular acid-base balance observed in both the spring and summer treatments (Table 5). In the spring treatment, pH of the red blood cells decreased at time 0 and 20 min ($p < 0.001$; Table 3) relative to the 24 and 48 h values. The same trend occurred between timepoints for sturgeon in the summer group. Sturgeon from the summer group experienced greater intracellular acidosis at time 0 ($p < 0.01$) and 20 min ($p < 0.05$) when compared to the spring group indicating some seasonal effect on acid base disturbance in the red blood cells.

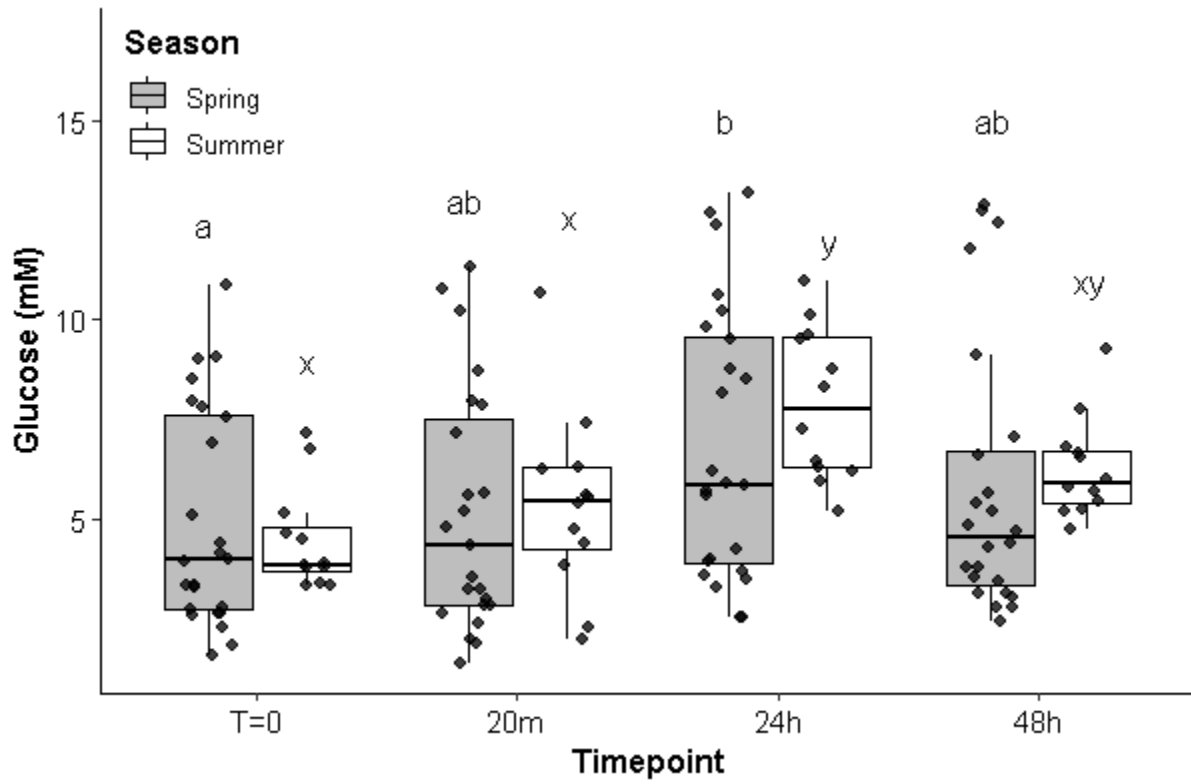


Figure 6: Seasonal comparison of plasma glucose concentrations (mM) of sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a season are noted by letter (abc for Spring, xyz for Summer).

Table 3: Seasonal comparison of physiological variables for sturgeon in a time series after capture and release. Data is presented as the mean +/- standard error. Statistical significance indicated by Tukey post-hoc tests, between spring and summer at one timepoint for individual variables are noted with an * in the summer cells. Significance between timepoints within a season are noted by letter (abc for Spring, xyz for summer).

		Time post-angling			
		T=0	20m	24h	48h
Extracellular pH	Spring	7.93 +/- 0.04 _{bc}	7.87 +/- 0.02 _b	8.03 +/- 0.04 _{bc}	8.16 +/- 0.03 _{ac}
	Summer	7.93 +/- 0.03 _{xy}	7.76 +/- 0.04 _x	7.98 +/- 0.04 _y	7.97 +/- 0.04 _y *
Intracellular pH	Spring	7.07 +/- 0.02 _a	7.00 +/- 0.01 _a	7.15 +/- 0.01 _b	7.18 +/- 0.02 _b
	Summer	6.98 +/- 0.02 _x *	6.92 +/- 0.02 _x *	7.14 +/- 0.02 _y	7.13 +/- 0.03 _y
Glucose (mM)	Spring	8.94 +/- 0.75 _a	10.75 +/- 1.33 _{ab}	15.79 +/- 1.10 _b	12.55 +/- 0.73 _{ab}
	Summer	9.88 +/- 1.12 _x	10.33 +/- 1.24 _x	13.71 +/- 1.38 _y	11.59 +/- 1.39 _{xy}
Osmolality (mmol/Kg)	Spring	278 +/- 1.14 _a	277 +/- 1.03 _a	270 +/- 1.68 _b	268 +/- 1.32 _b
	Summer	262 +/- 2.20 _x *	262 +/- 1.77 _x *	256 +/- 2.91 _x *	256 +/- 2.15 _x *
Respiration rate	Spring	9.12 +/- 0.64 _a	10.5 +/- 0.40 _a	10.7 +/- 0.37 _a	10.6 +/- 0.40 _a
	Summer	11.8 +/- 0.65 _x *	13.2 +/- 0.70 _x *	15.0 +/- 0.58 _x *	13.6 +/- 0.42 _x *
Haematocrit	Spring	22.6 +/- 1.34 _a	21.4 +/- 1.33 _a	23.2 +/- 1.20 _a	22.3 +/- 1.15 _a
	Summer	24.2 +/- 1.09 _x	24.2 +/- 1.15 _x	26.7 +/- 1.21 _x	27.1 +/- 0.93 _x *

After C&R angling, season and sampling timepoint both had significant effects on plasma osmolality (Table 5). Osmolality was elevated at time 0 and 20 min for sturgeon caught in the spring treatment, decreasing by 24 and 48 h (Figure 7). However, summer caught fish

exhibited no change in osmolality throughout the sampling period. Spring caught fish had significantly higher osmolality at each timepoint when compared to the summer. Furthermore, respiration rate remained unchanged across timepoints but was consistently higher in summer caught fish (Figure 8). Haematocrit profiles remained consistent over all sampling timepoints post C&R, however haematocrit was higher in summer caught fish after 48 h ($p=0.038$). Circulating levels of estradiol and testosterone were highly variable within and between individuals (Table 3). Interestingly, plasma testosterone (20 min) and estradiol (all timepoints) were higher in the summer caught fish when compared to the spring ($p<0.01$ for testosterone; $p<0.05$ for estradiol) but there was no effect of sampling time on either steroid in both treatments (Table 5).

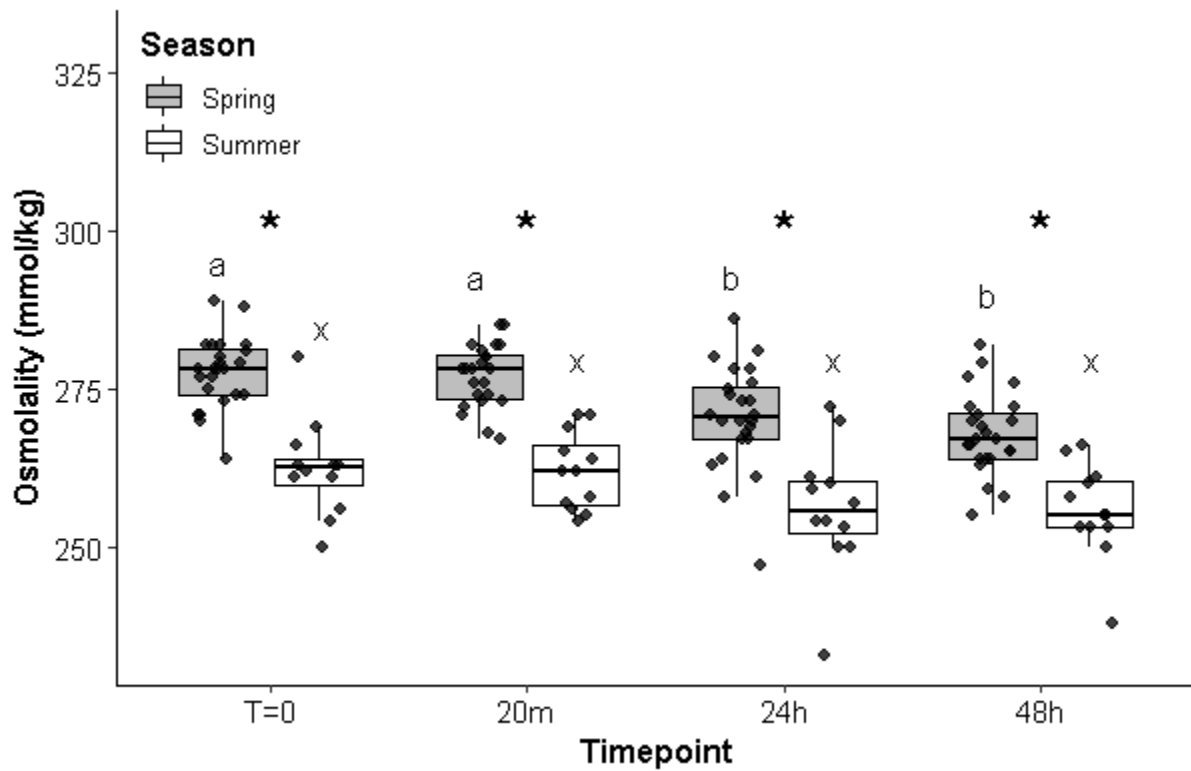


Figure 7: Seasonal comparison of plasma osmolality (mmol/kg) of sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between seasons is noted with an * and significant differences between timepoints within a season are noted by letter (abc for Spring, xyz for Summer).

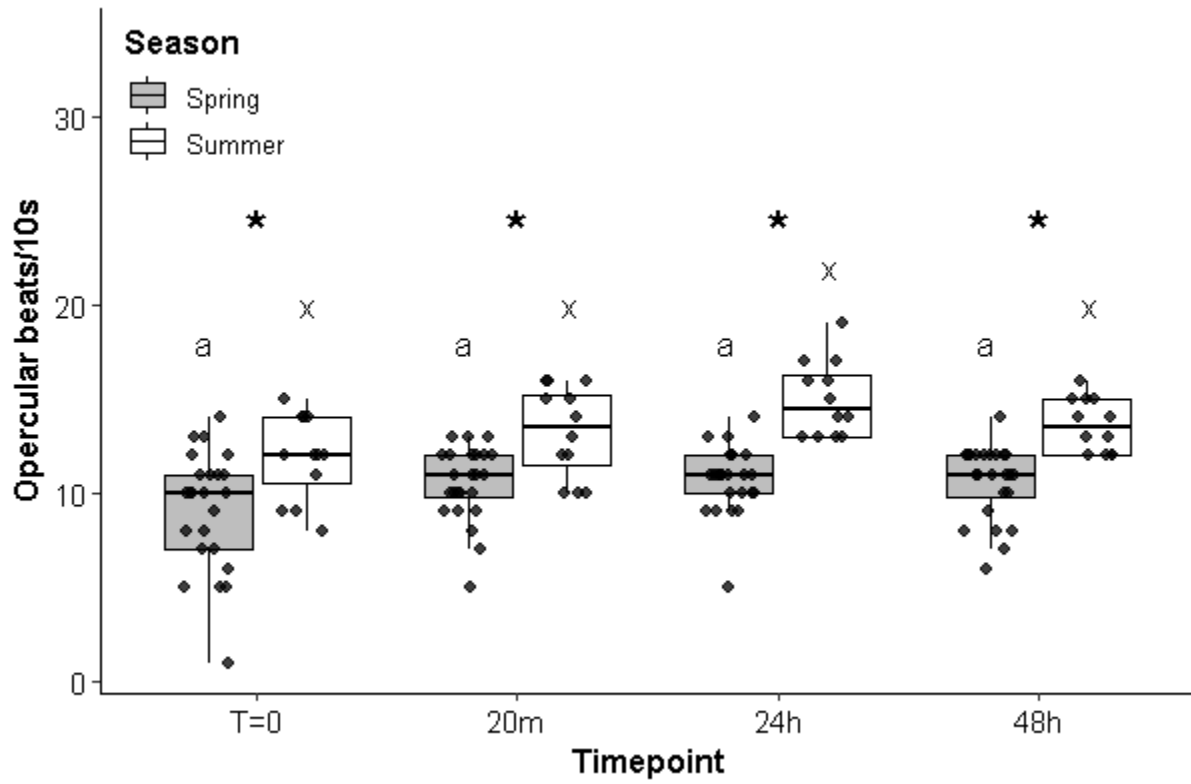


Figure 8: Seasonal comparison of respiration rate of sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between seasons is noted with an * and significant differences between timepoints within a season are noted by letter (abc for Spring, xyz for Summer).

Table 4: Seasonal comparison of plasma sex steroid concentrations for sturgeon in a time series after capture and release. Data is presented as the mean +/- standard error. Statistical significance indicated by Tukey post-hoc tests, between spring and summer at one timepoint for individual variables are noted with an * in the summer cells.

		Time post-angling			
		T=0	20m	24h	48h
Estradiol (ng/mL)	Spring	0.04 +/- 0.01	0.03 +/- 0.01	0.03 +/- 0.01	0.03 +/- 0.01
	Summer	0.06 +/- 0.01 *	0.05 +/- 0.01 *	0.06 +/- 0.01 *	0.04 +/- 0.01 *
Testosterone (ng/mL)	Spring	17.3 +/- 8.70	11.3 +/- 7.93	7.64 +/- 4.20	10.1 +/- 5.42
	Summer	29.9 +/- 14.1	38.4 +/- 12.1 *	14.1 +/- 5.99	18.5 +/- 10.8

Table 5: Summary of linear mixed effects model results for all physiological variables during seasonal treatments. The values, standard errors, degrees of freedom, T score and P value are reported for each fixed effect in the model. If data were transformed, the type of transformation is reported in the response column.

Response	Parameter	Value	S.E.	Df	T	P
Cortisol (Rank)	Intercept	41.56	6.83	120	6.08	<0.001
	20m	21.10	8.93	111	2.36	0.020
	24h	40.44	8.93	111	4.53	<0.001
	48h	60.52	8.93	111	6.78	<0.001
	Season	-15.56	11.12	120	-1.40	0.165
	20m: Summer	18.06	15.47	111	1.17	0.250
	24h: Summer	34.23	15.47	111	2.21	0.030
	48h: Summer	19.02	15.47	111	1.23	0.222
Extracellular pH (Rank)	Intercept	69.29	6.57	128	10.54	<0.001
	20m	-23.69	8.78	114	-2.70	0.008
	24h	17.00	8.78	114	1.94	0.055
	48h	50.90	8.78	114	5.80	<0.001
	Season	-13.40	10.90	123	-1.23	0.221
	20m: Summer	-9.44	15.20	114	-0.62	0.536
	24h: Summer	-0.08	15.20	114	-0.01	0.996
	48h: Summer	-36.44	15.20	114	-2.40	0.018
Intracellular pH (Rank)	Intercept	22.03	44.69	134	0.49	0.623
	20m	-20.63	7.96	134	-2.59	0.011
	24h	35.69	7.96	134	4.48	<0.001
	48h	42.94	7.96	134	5.39	<0.001
	Season	-39.65	9.85	134	-4.02	<0.001
	Fight time	-2.35	0.59	134	-4.00	<0.001
	Weight	6.55	5.48	134	1.20	0.234
	20m: Summer	7.25	13.79	134	0.53	0.600
	24h: Summer	27.40	13.79	134	1.99	0.049
	48h: Summer	12.35	13.79	134	0.90	0.372
Glucose (Log)	Intercept	12.39	4.66	97	2.66	0.009
	Weight	0.93	0.36	63	2.60	0.012
	Length	-2.75	1.09	85	-2.52	0.014
	Fight	0.02	0.01	59	2.22	0.030
	20m	0.06	0.11	107	0.57	0.574
	24h	0.43	0.11	106	3.99	<0.001
	48h	0.23	0.11	106	2.16	0.033
	Season	0.12	0.09	113	1.30	0.197
Osmolality	Intercept	277.58	1.38	127	201.05	<0.001
	20m	-0.56	1.82	122	-0.31	0.759
	24h	-7.17	1.80	121	-4.00	<0.001

	48h	-9.71	1.80	121	-5.40	<0.001
	Season	-15.40	2.24	129	-6.88	<0.001
	20m: Summer	0.23	3.13	121	0.07	0.942
	24h: Summer	0.92	3.11	120	0.30	0.769
	48h: Summer	2.96	3.11	120	0.95	0.344
Respiration rate (Rank)	Intercept	44.27	6.77	118	6.54	<0.001
	20m	14.44	8.85	108	1.63	0.106
	24h	16.06	8.85	108	1.82	0.072
	48h	16.23	8.85	108	1.83	0.069
	Season	38.55	11.02	108	3.50	<0.001
	20m: Summer	4.65	15.33	108	0.30	0.762
	24h: Summer	29.44	15.33	108	1.92	0.057
	48h: Summer	14.90	15.33	108	0.97	0.333
Haematocrit	Intercept	22.65	1.17	47	19.37	<0.001
	20m	-1.01	1.08	110	-0.93	0.354
	24h	0.55	1.05	110	0.52	0.604
	48h	-0.33	1.05	110	-0.31	0.754
	Season	1.12	1.34	113	0.84	0.404
	20m: Summer	0.93	1.84	110	0.51	0.614
	24h: Summer	1.90	1.83	110	1.04	0.300
	48h: Summer	3.15	1.83	110	1.73	0.087
Estradiol (Rank)	Intercept	65.83	7.81	58	8.43	<0.001
	20m	-6.83	7.81	112	-0.88	0.383
	24h	-10.67	7.81	112	-1.37	0.175
	48h	-12.52	7.81	112	-1.60	0.112
	Season	34.50	9.88	116	3.50	<0.001
	20m: Summer	10.79	13.52	112	0.80	0.427
	24h: Summer	13.71	13.52	112	1.01	0.313
	48h: Summer	6.90	13.52	112	0.51	0.611
Testosterone (Rank)	Intercept	69.33	8.17	70	8.49	<0.001
	20m	-4.46	8.81	112	-0.51	0.614
	24h	-12.00	8.81	112	-1.36	0.176
	48h	-12.29	8.81	112	-1.40	0.166
	Season	23.49	11.12	116	2.11	0.037
	20m: Summer	17.54	15.26	112	1.15	0.253
	24h: Summer	-0.50	15.26	112	-0.03	0.974
	48h: Summer	1.54	15.26	112	0.10	0.920

Angler effect on physiological responses

Sturgeon captured by experienced anglers showed an increase in circulating levels of cortisol from time 0 to 24 h ($p < 0.05$), continuing to increase by 48 h ($p < 0.01$) and remaining

elevated at 7 d post-angling compared to time 0 (Figure 9). In the novice group, cortisol increased above time 0 levels after 20 min, 24 h, and 7 d ($p < 0.05$). Again, circulating levels of cortisol were lowest immediately post-capture and were elevated for the remainder of the observation period (Figure 9). Angler behaviour treatments had no effect on plasma cortisol concentrations (Table 8).

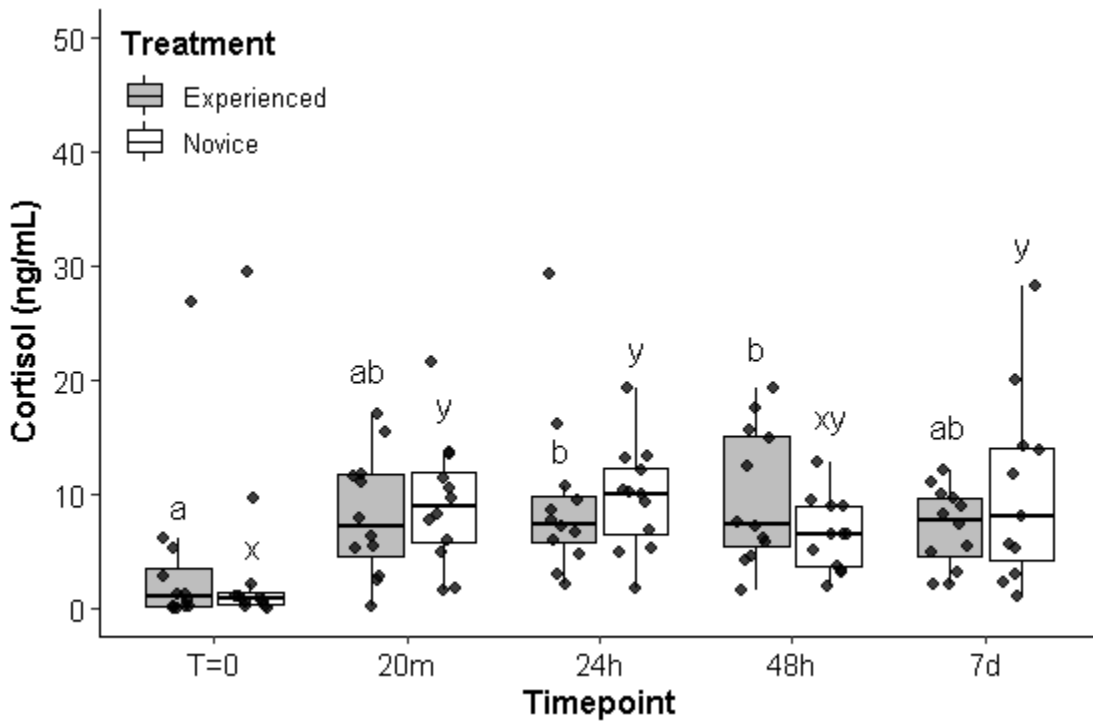


Figure 9: Angler behaviour comparison of plasma cortisol concentrations (ng/mL) of sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a treatment are noted by letter (abc for Experienced, xyz for Novice).

In both the novice and experienced angling treatments there was a significant intra- and extracellular acidosis at time 0 and 20 min relative to 24 and 48 h ($p < 0.05$; Figure 11/12) and 7 d post-angling. Angler experience had no effect on intra- or extra-cellular pH at any timepoint

(Table 8). In the novice treatment, plasma glucose increased significantly by 7 d post-angling when compared to time 0 samples ($p < 0.05$), otherwise, glucose was not impacted by handling treatments and remained stable across timepoints (Figure 10).

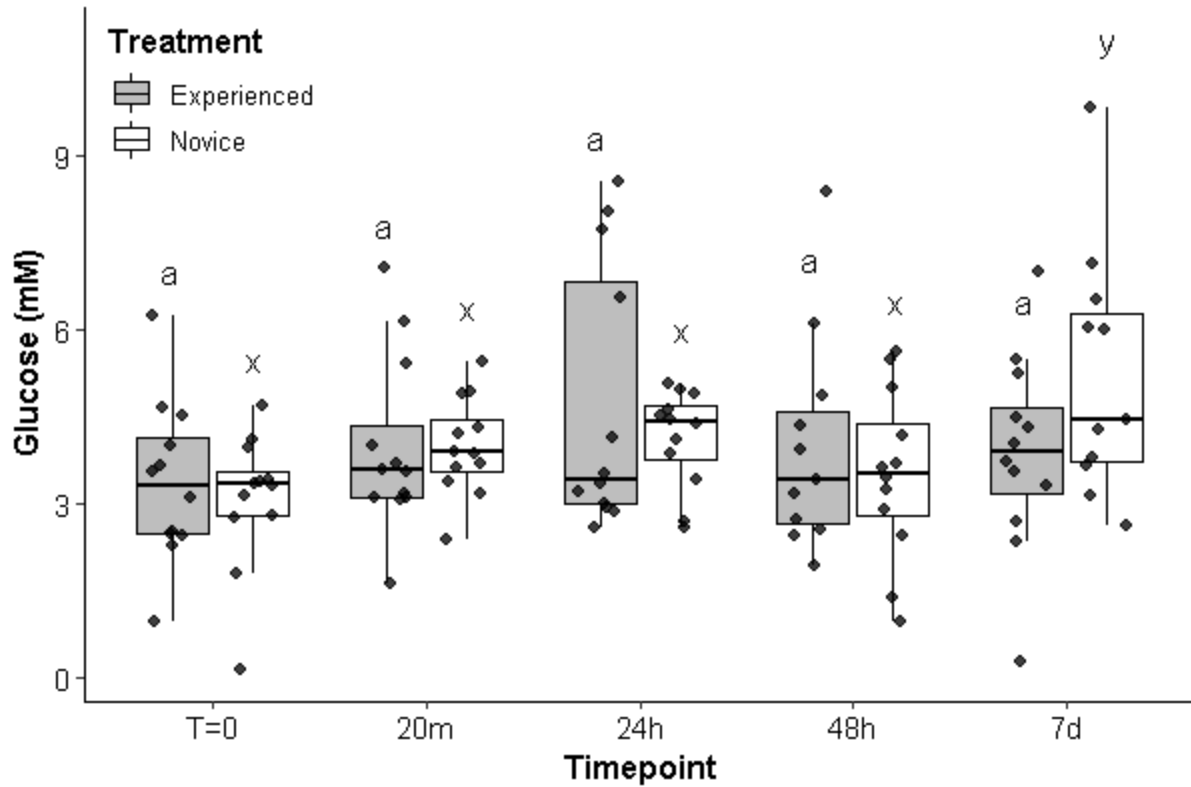


Figure 10: Angler behaviour comparison of plasma glucose concentrations (mM) of sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a treatment are noted by letter (abc for Experienced, xyz for Novice).

Table 6: Angler behaviour comparison of physiological variables for sturgeon in a time series after capture and release. Data is presented as the mean +/- standard error. Statistical significance, indicated by Tukey post-hoc tests, between the experienced angler group (Exp) and the novice angler group (Nov) at one timepoint for individual variables are noted with an * in the Novice cells. Significance between timepoints within a season are noted by letter (abc for Experienced, xyz for Novice).

		Time post-angling				
		T=0	20m	24h	48h	7d
Extracellular pH	Exp	7.89 +/- 0.07 _{ab}	7.75 +/- 0.05 _a	8.02 +/- 0.05 _b	8.04 +/- 0.05 _b	7.93 +/- 0.05 _{ab}
	Nov	7.98 +/- 0.08 _{xy}	7.77 +/- 0.05 _x	8.07 +/- 0.05 _y	8.04 +/- 0.07 _y	7.90 +/- 0.04 _{xy}
Intracellular pH	Exp	7.08 +/- 0.02 _a	7.05 +/- 0.03 _a	7.22 +/- 0.03 _b	7.19 +/- 0.02 _b	7.27 +/- 0.05 _b
	Nov	7.09 +/- 0.03 _{xy}	6.98 +/- 0.02 _x	7.15 +/- 0.02 _y	7.20 +/- 0.03 _z	7.19 +/- 0.04 _z
Glucose (mM)	Exp	3.35 +/- 0.40 _a	3.95 +/- 0.44 _a	4.70 +/- 0.66 _a	3.98 +/- 0.58 _a	3.86 +/- 0.49 _a
	Nov	3.05 +/- 0.34 _x	3.97 +/- 0.25 _{xy}	4.12 +/- 0.24 _{xy}	3.49 +/- 0.42 _{xy}	5.21 +/- 0.64 _y
Osmolality (mmol/Kg)	Exp	278 +/- 2.84 _a	292 +/- 1.93 _b	274 +/- 2.38 _{ac}	270 +/- 2.41 _{ac}	270 +/- 4.18 _c
	Nov	276 +/- 3.23 _{xy}	287 +/- 3.13 _x	270 +/- 2.25 _y	274 +/- 1.96 _y	269 +/- 4.06 _y
Respiration rate	Exp	9.75 +/- 0.45 _a	9.83 +/- 0.48 _a	10.9 +/- 0.48 _a	10.8 +/- 0.39 _a	10.8 +/- 0.51 _a
	Nov	8.17 +/- 0.51 _x	10.8 +/- 0.69 _y	10.3 +/- 0.50 _y	10.1 +/- 0.43 _y	10.1 +/- 0.51 _y
Haematocrit	Exp	31.0 +/- 1.73 _a	27.0 +/- 1.64 _a	28.7 +/- 1.39 _a	28.6 +/- 1.57 _a	29.8 +/- 1.58 _a
	Nov	25.0 +/- 1.70 _x	24.9 +/- 1.36 _x	24.2 +/- 1.01 _x	25.0 +/- 0.97 _x	25.8 +/- 1.54 _x

When accounting for the effects of sturgeon weight, sampling timepoint had a significant influence on plasma osmolality, though the effect of angler experience was not significant (Table 8). There was a transient increase in plasma osmolality in both the novice and experienced

treatments at 20 min post-angling followed by a return to time 0 values within 24 h remaining at that level until 7 d, though 7 d values in the experienced group were lower than time 0 ($p=0.01$; Table 6) Respiration rate was unaffected by angler experience but in the novice treatment, was significantly lower at time 0 compared to all other timepoints in that treatment ($p<0.05$; Table 6). Sampling timepoint and angler experience had significant effects on haematocrit profiles (Table 8), though post-hoc tests revealed no significant pairwise differences among the groups (Table 4). Furthermore, we found no effect of sampling time or angler experience on circulating levels of either testosterone or estradiol (Table 8).

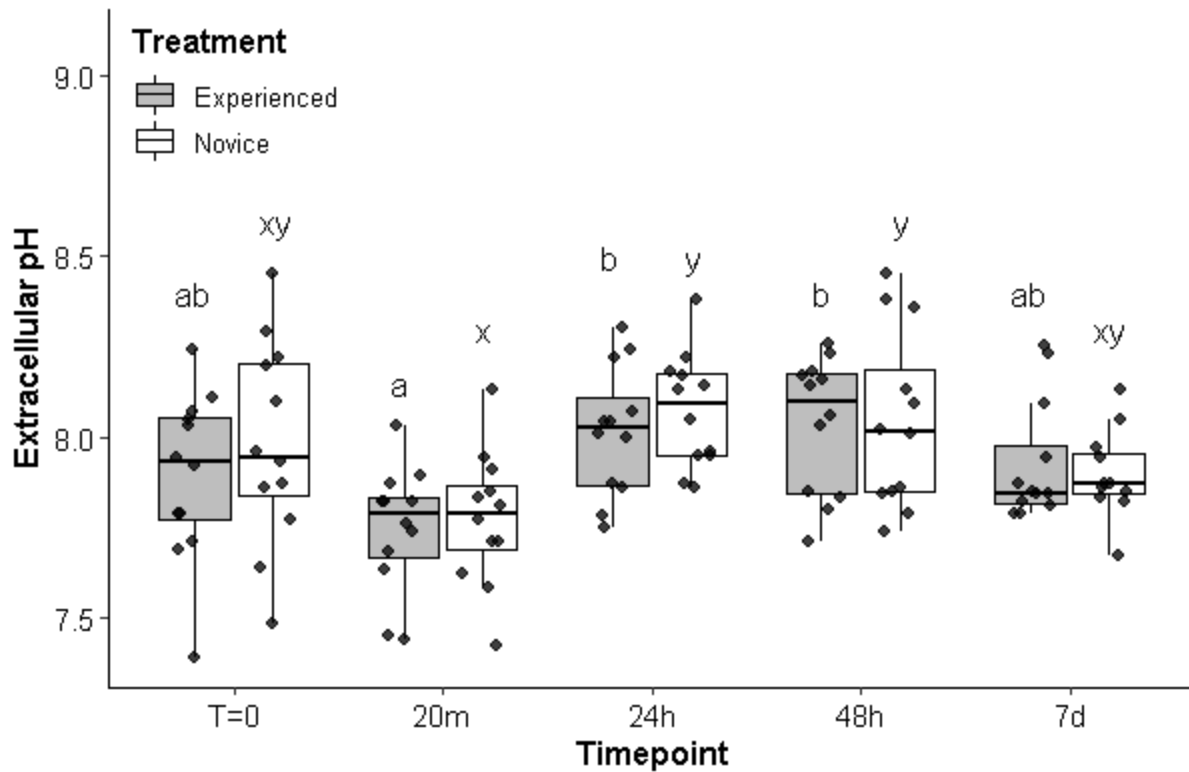


Figure 11: Angler behaviour comparison of plasma pHe response for sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a treatment are noted by letter (abc for Experienced, xyz for Novice).

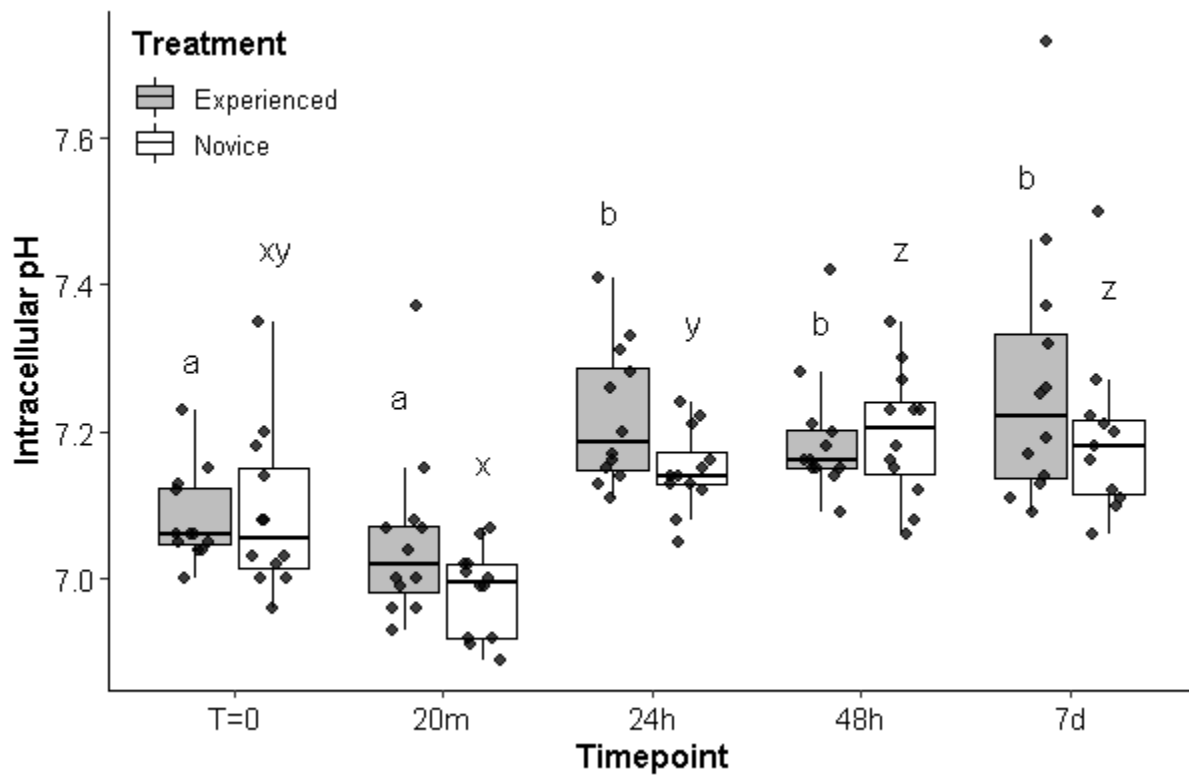


Figure 12: Angler behaviour comparison of red blood cell pH_i response for sturgeon in a time series after capture and release. Statistical significance, indicated by Tukey post-hoc tests, between timepoints within a treatment are noted by letter (abc for Experienced, xyz for Novice).

Table 7: Angler behaviour comparison of plasma sex steroid concentrations for sturgeon in a time series after capture and release. Data is presented as the mean +/- standard error, there are no statistically significant differences between these data.

		Time post-angling				
		T=0	20m	24h	48h	7d
Estradiol (ng/mL)	Exp	0.94 +/- 0.42	1.02 +/- 0.50	0.73 +/- 0.29	0.63 +/- 0.21	0.77 +/- 0.39
	Nov	0.94 +/- 0.44	0.82 +/- 0.40	0.76 +/- 0.37	0.49 +/- 0.20	0.97 +/- 0.48
Testosterone (ng/mL)	Exp	27.5 +/- 13.2	37.0 +/- 12.5	23.7 +/- 10.3	34.2 +/- 14.9	19.7 +/- 10.9
	Nov	14.0 +/- 9.81	10.9 +/- 8.66	4.62 +/- 3.19	1.45 +/- 0.42	20.2 +/- 12.4

Table 8: Summary of linear mixed effects model results for all physiological variables during angler behaviour treatments. The values, standard errors, degrees of freedom, T score and P value are reported for each fixed effect in the model. If data were transformed, the type of transformation is reported in the response column.

	Parameter	Value	S.E.	Df	T	P
Cortisol (Rank)	Intercept	26.92	8.97	107	3.00	<0.001
	20m	38.83	12.16	88	3.19	0.002
	24h	41.50	12.16	88	3.41	<0.001
	48h	46.08	12.16	88	3.79	<0.001
	7d	35.75	12.16	88	2.94	0.004
	Treatment	-0.17	12.69	107	-0.01	0.990
	20m: Novice	6.33	17.20	88	0.37	0.714
	24h: Novice	8.58	17.20	88	0.50	0.619
	48h: Novice	-15.33	17.20	88	-0.89	0.375
	7d: Novice	12.75	17.20	88	0.74	0.460
Extracellular pH	Intercept	7.89	0.06	99	137.39	<0.001
	20m	-0.15	0.07	87	-1.99	0.049
	24h	0.12	0.07	87	1.62	0.108

	48h	0.14	0.07	87	1.89	0.061
	7d	0.03	0.07	87	0.44	0.663
	Treatment	0.09	0.08	99	1.07	0.289
	20m: Novice	-0.06	0.11	87	-0.56	0.575
	24h: Novice	-0.03	0.11	87	-0.29	0.776
	48h: Novice	-0.07	0.11	87	-0.74	0.459
	7d: Novice	-0.11	0.11	87	-1.049	0.297
Intracellular pH (Rank)	Intercept	40.88	7.40	107	5.53	<0.001
	20m	-9.21	9.98	88	-0.92	0.359
	24h	43.83	9.98	88	4.39	<0.001
	48h	37.88	9.98	88	3.80	<0.001
	7d	45.33	9.98	88	4.54	<0.001
	Treatment	2.96	10.46	107	0.28	0.778
	20m: Novice	-20.29	14.11	88	-1.44	0.154
	24h: Novice	-22.42	14.11	88	-1.59	0.116
	48h: Novice	-1.46	14.11	88	-0.10	0.918
	7d: Novice	-10.04	14.11	88	-0.71	0.479
Glucose	Intercept	3.35	0.46	87	7.29	<0.001
	20m	0.60	0.57	85	1.06	0.293
	24h	1.30	0.57	86	2.28	0.025
	48h	0.74	0.58	86	1.27	0.208
	7d	0.51	0.57	85	0.90	0.373
	Treatment	-0.30	0.65	87	-0.46	0.650
	20m: Novice	0.32	0.80	85	0.40	0.693
	24h: Novice	-0.23	0.80	86	-0.29	0.773
	48h: Novice	-0.31	0.81	86	-0.38	0.707
	7d: Novice	1.65	0.81	86	2.03	0.045
Osmolality (Log)	Intercept	5.49	0.07	16	83.70	<0.001
	20m	0.05	0.01	67	3.85	<0.001
	24h	-0.02	0.01	67	-1.45	0.152
	48h	-0.03	0.01	67	-2.63	0.011
	7d	-0.04	0.01	67	-3.75	<0.001
	Treatment	-0.01	0.02	61	-0.53	0.601
	Weight	0.02	0.01	16	2.24	0.040
	20m: Novice	-0.01	0.02	67	-0.48	0.635
	24h: Novice	-0.01	0.02	67	-0.69	0.493
	48h: Novice	0.01	0.02	67	0.76	0.448
	7d: Novice	0.01	0.02	68	0.39	0.696
Respiration rate	Intercept	9.75	0.50	41	19.60	<0.001
	20m	0.08	0.41	87	0.20	0.841
	24h	1.16	0.41	87	2.82	0.006
	48h	1.00	0.41	87	2.41	0.017
	7d	1.08	0.41	87	2.61	0.010
	Treatment	-1.58	0.70	41	-2.25	0.030
	20m: Novice	2.50	0.59	87	4.27	<0.001
	24h: Novice	1.00	0.59	87	1.71	0.092

	48h: Novice	0.92	0.59	87	1.57	0.121
	7d: Novice	0.71	0.59	87	1.20	0.233
Haematocrit	Intercept	31.21	1.47	46	21.26	<0.001
	20m	-4.06	1.30	85	-3.11	0.003
	24h	-2.50	1.28	86	-1.94	0.056
	48h	-2.57	1.28	86	-2.00	0.048
	7d	-1.42	1.28	86	-1.11	0.271
	Treatment	-6.25	2.05	44	-3.04	0.004
	20m: Novice	4.04	1.81	85	2.24	0.028
	24h: Novice	1.75	1.79	85	0.98	0.332
	48h: Novice	2.56	1.79	85	1.43	0.160
	7d: Novice	2.23	1.82	85	1.23	0.223
Estradiol (Rank)	Intercept	67.50	10.11	42	6.68	<0.001
	20m	3.67	8.66	88	0.42	0.673
	24h	-1.50	8.66	88	-0.17	0.863
	48h	-10.33	8.66	88	-1.19	0.236
	7d	-14.17	8.66	88	-1.64	0.106
	Treatment	-8.00	14.29	42	-0.56	0.579
	20m: Novice	-4.08	12.25	88	-0.33	0.740
	24h: Novice	-1.08	12.25	88	-0.09	0.930
	48h: Novice	-7.33	12.25	88	-0.60	0.551
	7d: Novice	27.167	12.25	88	2.22	0.029
Testosterone (Rank)	Intercept	68.25	10.11	85	6.75	<0.001
	20m	5.50	12.21	88	0.45	0.653
	24h	-8.75	12.21	88	-0.72	0.475
	48h	-2.25	12.21	88	-0.18	0.854
	7d	1.50	12.21	88	0.12	0.902
	Treatment	-14.50	14.30	85	-1.01	0.313
	20m: Novice	2.33	17.27	88	0.14	0.893
	24h: Novice	-1.00	17.27	88	-0.06	0.954
	48h: Novice	0.75	17.27	88	0.04	0.965
	7d: Novice	0.92	17.27	88	0.05	0.958

Discussion

C&R angling initiated a physiological stress response and behavioural impairment in lake sturgeon; as indicated by the increased circulating levels of cortisol, increased energy mobilization (plasma glucose), the immediate disturbance of acid-base and osmotic balance in addition to the sudden increase in reflex impairment. These immediate physiological adjustments are typical of the adaptive response of a sturgeon species to external stressors (Baker et al., 2008; Bayunova et al., 2002; Genz et al., 2014; McLean et al., 2019; McLean et al., 2016). To date, there are no reports of the physiological and behavioural effects of C&R angling on lake sturgeon in the primary literature. Our data are unique given that we record the response of wild fish to volunteer anglers, thus effectively simulating the variation which may naturally occur in recreational fisheries.

We had predicted that cortisol values would decline to baseline within 24 h post-capture, however, in 2018, lake sturgeon experienced a steady increase in cortisol throughout the 48 h holding period. These results suggested a protracted cortisol response to the angling event, indicative of a chronic stressor (Wendelaar-Bonga., 1997). Thus, in 2019, we extended the holding period to 7 d, but despite the extended recovery period and lack of handling or manipulation between the 48 h and 7 d sampling timepoint, circulating levels of cortisol remained elevated above time 0 values. In 2018, sampling order had a significant effect on cortisol values, however, this was not evident in the 2019 dataset. Therefore the elevated levels of cortisol, in comparison to time zero values, when fish were maintained in the holding pens was most likely an interactive effect from stressors relating to repeated sampling protocols (removal from pen, handling, sampling) over the short-term, and confinement stress over the longer term.

In wild lake sturgeon, circulating levels of cortisol after gill netting (24 h set) and tagging increased immediately post-capture (\bar{x} =49.8 ng/ml), and a separate group of netted/tagged sturgeon had considerably lower cortisol values (\bar{x} =2.4 ng/m) after being maintained in riverside holding tanks for a three day resting period (Baker et al., 2008). In sockeye salmon, *Oncorhynchus nerka*, individuals held in net pens for 24 h after C&R showed decreased osmolality, a four-fold increase in plasma cortisol, and a two-fold increase in plasma glucose compared to salmon released immediately after angling and beach seining (Donaldson et al., 2011). Importantly, it is recognised that the repeated drawing of blood on wild fish can amplify the stress response over time, especially considering the additional handling requirements of blood collection. Hamlin et al. (2007) found that disturbances related to repeated blood sampling at time 0 and 1 h in Siberian sturgeon, *Acipenser baeri*, likely arrested the expected decrease in cortisol 4 h after a capture and confinement stressor, whereas sturgeon sampled only at 4 h demonstrated lower plasma cortisol indicative of a more rapid return to basal concentrations. Additionally, the physiological response to stressors and the extent of cortisol release varies substantially even among closely related fish species and the type of stressor used (Barton, 2002).

In this study, the range of plasma cortisol values in lake sturgeon at time 0 (\bar{x} =3.81 ng/ml; range: 0.07-30.7) were lower than those reported for wild white sturgeon sampled immediately after C&R (\bar{x} =15.6 ng/ml; range: 5.1-155.1; McLean et al., 2019) and those of wild lake sturgeon sampled directly after netting and tagging (\bar{x} =49.8 ng/ml; range: 3.5-142.9; Baker et al., 2008). The white sturgeon captured and sampled by McLean et al. (2019) were larger (FL; \bar{x} =1481+/-630mm) and endured longer fight times (\bar{x} =7.4+/-1.1 min) than our lake sturgeon. Similarly, the lake sturgeon sampled in Baker et al. (2008) were caught over the course of a 24 h

gill net set, these fish possibly struggled in the net for extended periods of time before facing tagging related confinement, air exposure and handling stressors. Taken together, it is likely that C&R of lake sturgeon recorded in this study was not as severe a stressor, in regard to cortisol levels, as those described in previous studies. Nevertheless, within our recovery period plasma cortisol had, in some instances, increased to comparatively high values (48 h, 2018; \bar{x} = 23.7 ng/ml; range: 1.82-127). These 48 h values are similar, though more variable, to the range observed in wild lake sturgeon held in tanks two days after netting and hormonal injection (range: 5-30 ng/ml; Genz et al., 2014).

The changes in plasma glucose concentrations in this study compared well with values reported for other sturgeon species after C&R. For example, we measured plasma glucose at time 0 after angling (\bar{x} =3.99 mM, range: 0.12-10.9), these data are higher on average to values reported on white sturgeon post-C&R (\bar{x} =3.2 mM; range: 1.9-8.4; McLean et al., 2016; McLean et al., 2019) but lower than those reported for lake sturgeon post-netting and tagging (\bar{x} =9.2 mM; range: 3-18.4; Baker et al., 2008). In spite of C&R stressors, our values taken after angling and air exposure compare to the resting plasma glucose concentrations of juvenile Atlantic sturgeon (\bar{x} =3.8 mM) and shortnose sturgeon (\bar{x} =3.7 mM; Baker et al., 2005) indicating that the immediate glucose response of lake sturgeon to C&R is limited, especially when compared to the values observed after extended stressors in other wild lake sturgeon (Baker et al., 2008). However, after 24 h holding, glucose values for some groups increased considerably (Spring 2018, \bar{x} =15.79 mM +/-1.10; Summer 2018, \bar{x} =13.71 mM +/-1.38) possibly indicating that the interactive effects of C&R, holding and repeat sampling can cause elevated glucose responses in wild lake sturgeon.

Anaerobic activity initiated by vigorous exercise typically results in metabolic acidosis in exhausted teleosts (Bennett, 1986; Hochachka, 1991; Wood, 1991). The stress associated with C&R angling caused a rapid decrease to both the extra and intra-cellular acid base balance of lake sturgeon (Table 2/4). However, recovery of the acid-base balance occurred within 24 h and was maintained for the remaining timepoints in both the seasonal and angler experience treatments. Restoration of acid base balance of lake sturgeon within 24 h was also recorded by Genz et al. (2014) after gill net capture and induced spawning treatments, despite the substantial decreases in extracellular pH exhibited by these fish (0.2-0.25 units). White sturgeon are capable of preferentially regulating intracellular pH in some vital tissues during challenging conditions, but when exposed separately to exhaustive exercise or 45 min of air exposure these fish exhibited extracellular acidosis (0.30 & 0.35 units, respectively) which, in turn, reduced red blood cell pH (Shartau et al., 2017). This degree of acidosis did not occur in our study where the pH of lake sturgeon plasma decreased by approximately 0.21 and 0.17 units from time 0 to 20 min in the novice angler and summer treatments, respectively. During these decreases in extracellular pH, red blood cell pH dropped, but to a lesser degree (Novice: 0.11 units; Summer: 0.08 units) suggesting some ability of lake sturgeon to buffer pH disturbance between extra and intracellular fluid compartments.

For sturgeon captured in the summer, plasma osmolality was consistently lower than the values observed in the spring. Meanwhile, respiration rates for these same sturgeon in the summer were higher than in the spring at every timepoint. The osmo-respiratory compromise describes the trade-off in gill function which dictates whether a fish under stress will maintain sufficient gas exchange or defend osmotic balance (reviewed in Sardella & Brauner, 2007). Vigorous exercise and air exposure during angling leads to an oxygen debt in fish, increased

respiration will benefit oxygen uptake but can also cause the efflux of ions across the gills (Wood & Randall, 1973), decreasing plasma osmolality (Schreck & Tort, 2016). The result of this osmotic imbalance will cause an increased expenditure of energy in fish, adding further cost and possibly time to the recovery period (Wood, 1991).

In our study, C&R angling at the highest environmental temperature of 21 °C may have exacerbated physiological changes and importantly oxygen debt to the lake sturgeon, causing them to accelerate respiration to regain homeostasis, resulting in a compromise in gill function and reduction in plasma osmolality (Sardella & Brauner, 2007) that was recovered within 24 h post-capture in all treatments. However, only respiration rate was measured in this study and the amplitude of opercular beats would be required to make an accurate assessment as to the volume of water being pushed through the gills, and thus oxygen exchange, during ventilation (Sundin et al., 1999). Ventilation rate, in some cases (Appendix A), was decreased as a result of sampling order, further pointing to some effect of sampling design on response variables. The Slave Falls Reservoir, where sturgeon were angled in this study, does not experience seasonal stratification and dissolved oxygen content is predictably lower in summer water temperatures (C. McDougall, personal communication, 2020). With less available oxygen, it is possible that sturgeon captured by anglers in the summer must ventilate more rapidly to re-pay the same oxygen debt as their counterparts caught in the spring, and suffer greater ion loss as a result.

Lake sturgeon haematocrit profiles remained largely unchanged after C&R. Baker et al. (2008) also found no difference in haematocrit profiles of lake sturgeon immediately post-netting/tagging, even when compared to three day resting values. Similarly, wild white sturgeon haematocrit profiles were unaffected by the length of fight time when angled on the Lower Fraser River (McLean et al., 2019). Notably, we did find that haematocrit was higher in summer

caught fish after 48 h during the seasonal treatment providing further evidence for an increase in oxygen debt post-angling in the summer group, given that respiration rate was increased at the same timepoint. Increases in the supply of erythrocytes represents a typical response to combat the oxygen debt caused by exercise and hypoxic conditions (Ken-Ichi et al., 1980; Kieffer et al., 2011; Nikinmaa, 2016). Still, our angler behaviour treatments revealed no effect whatsoever of C&R on haematocrit profiles or respiration rate, indicating that lake sturgeon angled within these experiments were able to resist the typical adaptive responses associated with increased oxygen demand, even when air exposed for five minutes.

Generally, the majority of C&R fishing pressure targeting lake sturgeon occurs in the spring and summer (Figure 2). However, recently there is a growing popularity for angling lake sturgeon during the winter, while ice fishing. The effect of C&R on lake sturgeon at the lowest environmental temperature is currently unknown. For this reason, in the winter of 2019 (February/ March) we attempted to capture sturgeon on the Winnipeg River, while replicating the tactics used by specialized sturgeon ice anglers on the Rainy and St. Croix Rivers in the United States. Despite our efforts, we were not able to capture lake sturgeon for sampling. Nonetheless, the timing of data collection in the spring and summer of 2018 is reflective of the vast majority of C&R sturgeon angling occurring in Manitoba, Canada.

Recreational fisheries often intentionally or accidentally target fish during periods of reproductive activity, causing a major conservation concern (Arlinghaus & Cooke, 2009), often because the target species may aggregate in small areas during the reproductive phase of their life history, but also due to the general negative effects of exposure to a stressful event on reproduction (Schreck et al., 2001). Arlinghaus et al. (2007) argued that because of the

importance of the reproductive period to population recruitment, that it is in the interest of managers to attempt to minimize any sublethal effects that may occur to fish during spawning.

C&R angling did not cause any discernable trend in the concentration of plasma testosterone and estradiol during the recovery period. Nevertheless, estradiol in the summer period was consistently higher at all timepoints and testosterone was greater at 20 min post-angling when compared to these same values in the spring. Sturgeon angled in the spring of 2018 were most likely caught at the post-spawn state, based on the timing of lake sturgeon spawn collections for other research programs in the Slave Falls Reservoir. During this time, estradiol and testosterone levels would likely have been depressed in both male and female fish (Allen et al., 2009b). Conversely, female lake sturgeon at the mid-late vitellogenic phase of oocyte development typically have increased levels of estradiol (Allen et al., 2009b), which may explain the elevated levels of estradiol observed in the fish captured in August 2018, a time of year when significant investment is being made in gonad development in preparation for the subsequent spawning season. Only mature and spawning males, however, show peak values of circulating testosterone (Allen et al., 2009b). Sex determination cannot be assigned visually on lake sturgeon and, without evidence of spawning, we were not able to assign sex to individuals within our dataset, making sound interpretation of C&R related trends in these highly variable steroids challenging.

The following year's (2019) data were collected over the entirety of the lake sturgeon spawning cycle, determined by the timing of spawn collection programs occurring in the Slave Falls Reservoir in 2019. However, no effect was observed on sex steroids despite the intense stressors defined by our novice treatment. It is recognised that elevated levels of cortisol usually have a negative impact on reproductive output in fish (Schreck et al., 2001) and in female lake

sturgeon captured during the spawning season, circulating levels of estradiol and cortisol were highest immediately post-gill netting, compared to circulating estradiol and cortisol values from the same fish maintained in holding tanks for two weeks post-capture (Genz et al., 2014). In the same study, there was no effect of capture stress on testosterone in either sex or estradiol in male fish (Genz et al., 2014). However, circulating levels of sex steroids and cortisol were not inversely related in Siberian sturgeon; capture and confinement had no effect on circulating estradiol, and plasma testosterone was significantly elevated during peak cortisol responses, suggesting cortisol may not impact reproductive output in the Siberian sturgeon (Hamlin et al., 2007). Regardless of the potential conflict in data interpretation from these two studies, based on previous evidence in teleosts (Schreck and Tort, 2016), caution should be exercised in regard to the timing of a C&R fishery for wild lake sturgeon and its relation to spawning.

We are aware that the pre-existing condition of individuals may have unknown effects on the response of lake sturgeon to C&R within our study. The concept of allostasis describes the ability of organisms to maintain internal physiological balance while coping with seasonal variation, social interactions, reproductive state, competition, weather events, and other common environmental challenges (McEwen & Wingfield, 2003). In the context of C&R, we recognize that anglers have no control on the nutritional, health, or pathological state of the fish they capture. It is therefore possible that the C&R of fish may exacerbate the existing allostatic load of certain individuals, leading to what has been described in the literature as allostatic overload (McEwen & Wingfield, 2003), and the subsequent increased risk of impairment and mortality. Animals are most resilient to this occurrence when the daily and seasonal variation in its activities are low (Romero et al., 2009). For this reason, there are additional uncertainties related to C&R angling of fish in a spawning state, as we are unaware of what the cumulative

physiological effects of these multiple conditions are on the survival of individuals in a compromised or sensitive state.

Our results did not reveal temperature dependant reflex impairment, at water temperatures between 13 °C in the spring and 21 °C in the summer. Mclean et al. (2016) found reflex impairment in white sturgeon to be temperature dependant, summer treated fish (\bar{x} =15.3 °C) having greater RAMP scores in each simulated C&R treatment compared to winter values (\bar{x} =6.6 °C). However, five minutes of air exposure, inappropriate gear selection, and rough handling (novice treatment) caused higher reflex impairment in sturgeon immediately after angling, compared to fish captured by anglers displaying presumptive best practices. This is consistent with the findings of Struthers et al. (2018) who determined reflex impairment was positively correlated with air exposure duration. Despite the multiple stressors and varying severity of temperature and angling treatments, reflex impairment for lake sturgeon recovered by 24 h and was unimpaired for the remainder of the sampling timepoints. Given that RAMP scores were designed to predict delayed mortality, and that our sturgeon regained reflex action during the recovery period (Davis, 2010; Mclean et al., 2016), delayed mortality as a result of C&R in lake sturgeon sampled in this study was likely to be negligible.

In spite of the context specific nature of this study`s design and implementation, the results of this research are broadly applicable to the management of lake sturgeon populations across their natural range. With consideration, to the variation which can occur in an animals response to stressors across individuals (e.g. allostatic load), populations, and the specific context in which they are challenged. This data would be most applicable in areas where the behaviours of anglers are reflected within the scope tested in our angler experience treatments, in terms of gear selection, stressor durations, and handling behaviours. Resource managers can investigate

the behaviours of anglers fishing for lake sturgeon in their region using surveys or observation. In addition, our data supports the resilience of lake sturgeon to seasonal variation, within the limited environmental conditions that we studied. In areas where water temperatures may exceed 21°C, additional caution should be advised to test for the effect of these conditions on lake sturgeon. Especially given the strong body of evidence supporting the notion that increases in environmental temperature can rapidly deteriorate the recovery of individuals after C&R (Gale et al., 2013).

Conclusion

The values of blood chemistry parameters measured in lake sturgeon after C&R represented a normal response to stressors of this nature in sturgeons, albeit, the prolonged stress response was likely an anomaly caused by the extended holding periods of wild animals. There is a robust body of literature indicating that sturgeon species are comparatively resilient to stressors such as air exposure and handling (Barton et al., 2000), hypoxia (Kieffer et al., 2011), and gill netting (Baker et al., 2008). C&R angling often represents a combination of stressors typically causing physiological and behavioural impairment in fish (Cooke & Suski, 2005), and these interactive stressors can have cumulative impacts (Barton, 2002; Gingerich et al., 2007). This study indicated that the response of lake sturgeon, in terms of acid-base balance, haematocrit, and reflex impairment is resilient to the effects of C&R angling in the short and medium term. The primary and secondary stress response (Plasma cortisol/glucose) were initiated upon C&R, though remained elevated during the recovery period, likely due to interactive stressors related to the study design. Though physiological and behavioural impairment did occur, water temperature did not intensify the disturbance of physiological variables over the study period up to 21 °C. Our data suggest that C&R anglers should optimize handling behaviours and gear selection when targeting this species, and to consider the potentially harmful effects of temperatures above 21 °C on the fate of lake sturgeon after release. Lake sturgeon have an exceptional longevity, late age at maturity, and only reproduce periodically (Harkness & Dymond, 1961; Roussow, 1957). These characteristics, in addition to the predictable congregation of spawning lake sturgeon under natural and artificial barriers, make this species vulnerable to angling during the reproductive period (Boreman, 1997). Further research on the reproductive implications of C&R on the lake sturgeon are advised, to calculate the effect of

C&R challenges on sturgeon fecundity and spawning success, as this information has implications for the long-term management of populations at risk.

These considerations are timely, as C&R fisheries develop for lake sturgeon in watersheds with healthy populations, and those recovering from historic decline. The economic, recreational, and cultural implications of a lake sturgeon C&R fishery in Manitoba are considerable, hence, why our understanding of this species response to C&R stressors is required, to inform the decisions of resource managers in regulating these fisheries. Lake sturgeon are hardy in their response to C&R, especially when compared to other more commonly targeted sportfish species which, under certain conditions, can suffer mortality and high levels of impairment after C&R (Anderson et al., 1998; Gingerich et al., 2007; Meals & Miranda, 1994; Thompson et al., 2008; Tomasso et al., 1996; Wilkie et al., 1996). C&R fisheries have the unique opportunity to create a sustainable relationship between recreational anglers and wildlife, and this relationship could afford better management and conservation measures for popular species in the long-term (Landsman et al., 2011; Tufts et al., 2015). Given the vulnerability of lake sturgeon to anthropogenic activities in other contexts (harvest, habitat loss, pollution), a well-managed C&R fishery could create an economic and cultural basis for meaningful protection. Specialized C&R anglers are inherently invested in the conservation of the species they target (Cooke & Suski, 2005; Landsman et al., 2011) and, as such, become important stakeholders in fisheries management. Lake sturgeon are unique among freshwater fishes, these prehistoric fish ignite the passion of many anglers who imagine interacting with a living, breathing fossil. So long as these anglers join the existing framework of stakeholders fighting for the future of this species, we might witness an uncompromising demand for future generations to experience healthy lake sturgeon populations.

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Appendix A

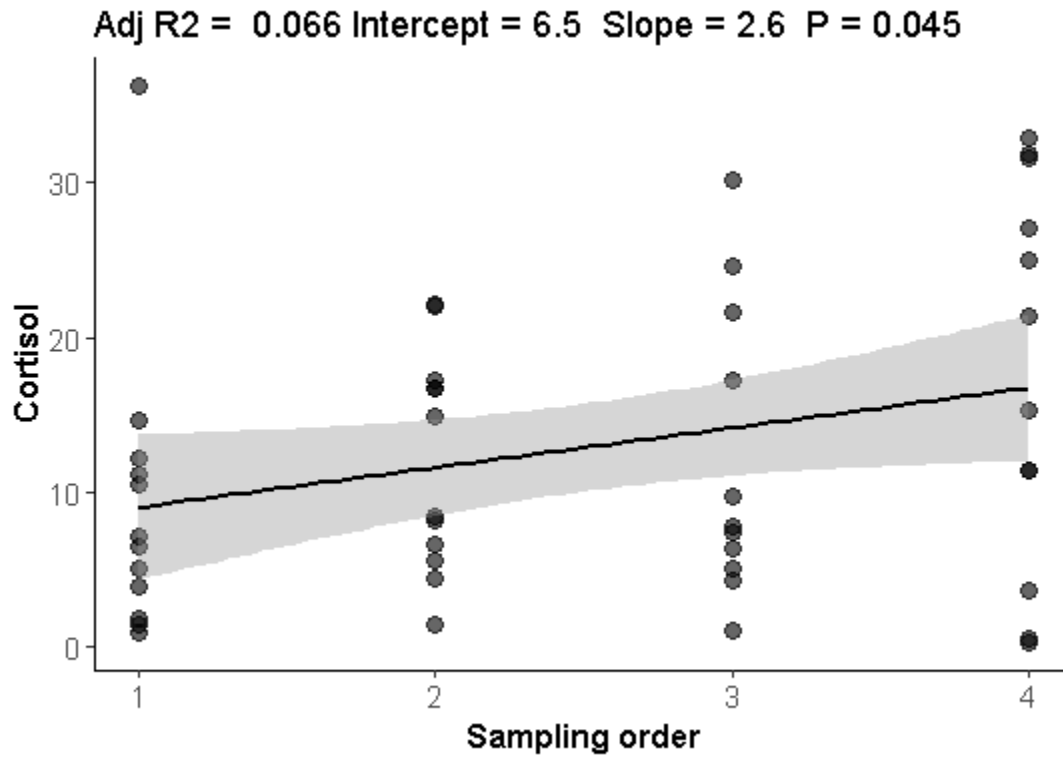


Figure 1: Linear regression model demonstrating the effect of sampling order on plasma cortisol concentrations in the spring of 2018. Statistical information is reported above the figure.

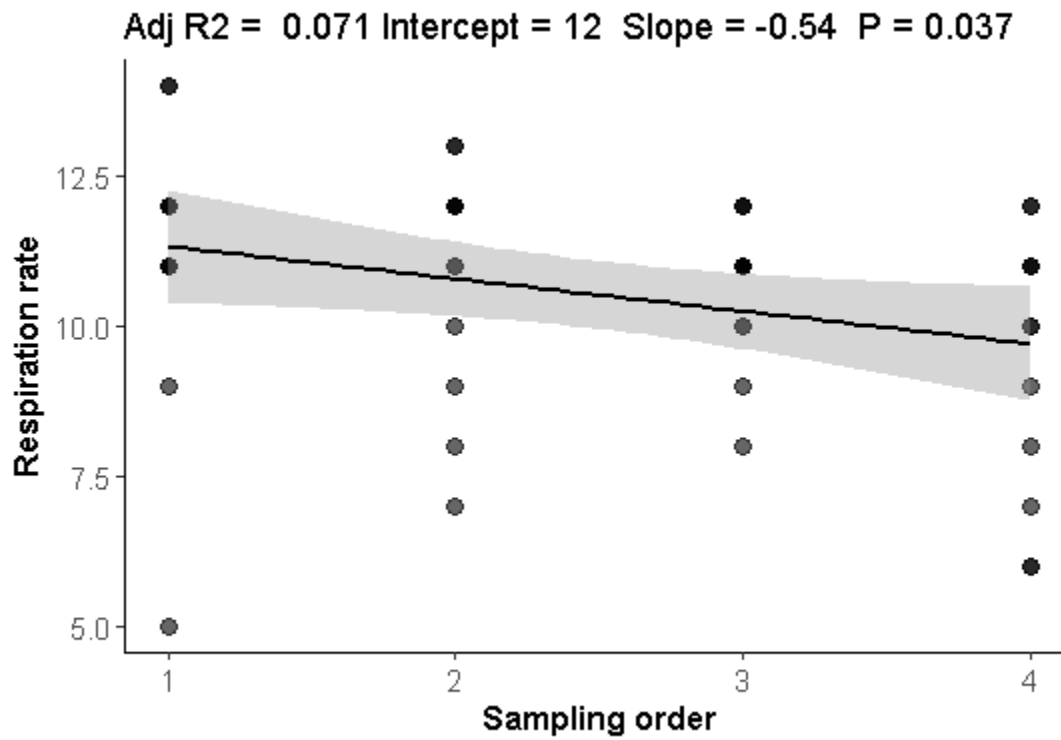


Figure 2: Linear regression model demonstrating the effect of sampling order on respiration rate in the spring of 2018. Statistical information is reported above the figure.

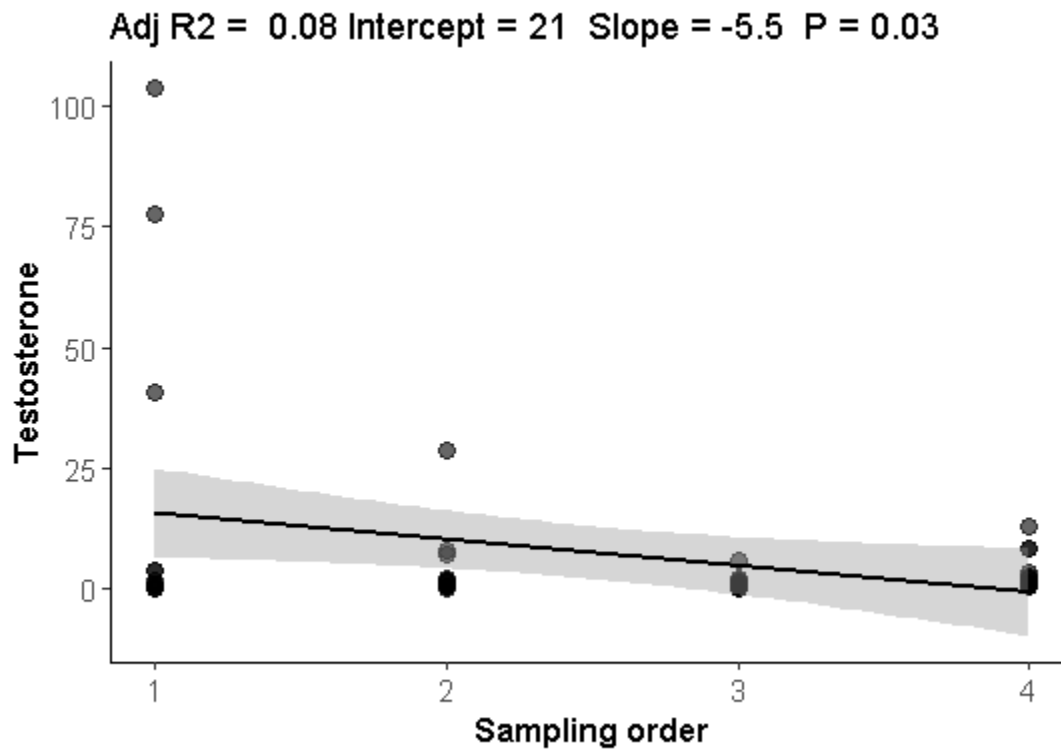


Figure 3: Linear regression model demonstrating the effect of sampling order on plasma testosterone concentrations in the spring of 2018. Statistical information is reported above the figure.

Appendix B

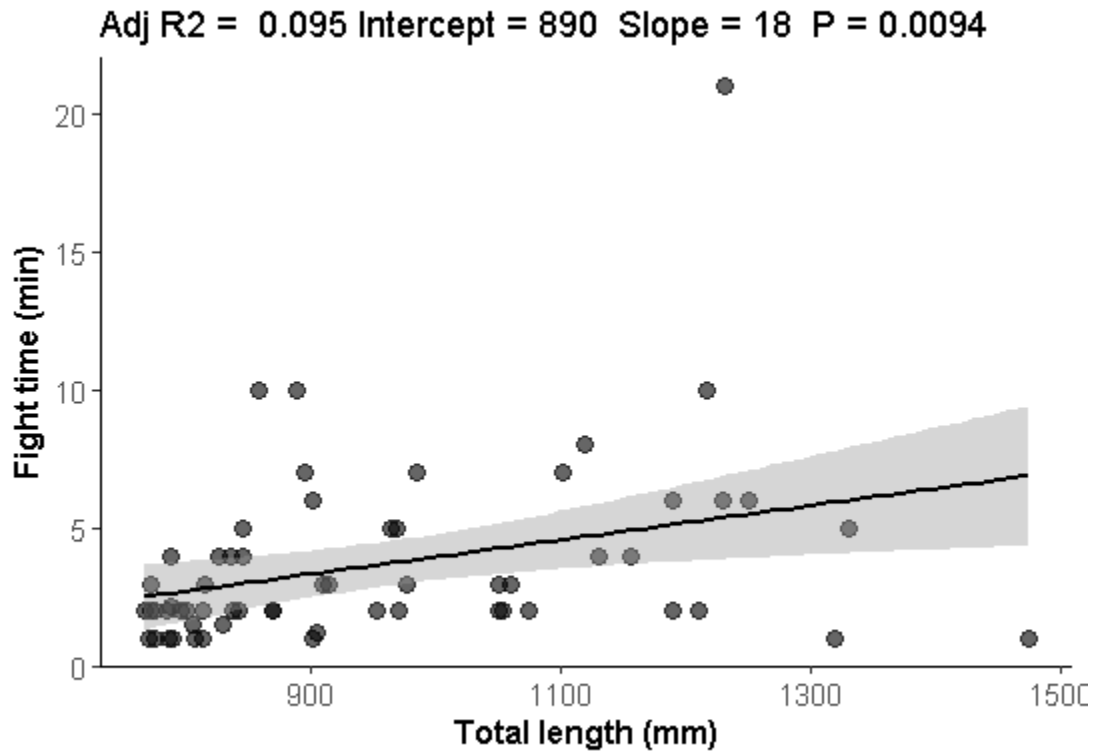


Figure 1: Linear regression model demonstrating the relationship between fight time (min) and total length (mm) for all lake sturgeon sampled in this study. Statistical information is reported above the figure.

