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Recovery from catch-and-release angling in Gulf of Mexico fishes

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Recovery from catch-and-release angling in Gulf of Mexico fishes

by

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Dedication

This work is dedicated to my family, friends, and mentors, for their overwhelming love and support throughout my life and especially here in Port Aransas, Texas. I especially want to thank my mother, Cheryl Ibarra, who first inspired me to go to The University of Texas at Austin and has since supported my dreams throughout my time here. I could not have done this without you. Thank you.

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Abstract

Recovery from catch-and-release angling in Gulf of Mexico fishes

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Catch-and-release angling (CAR), the process of releasing fish back to the wild post catch, has increasingly been regarded by various stakeholders as an effective measure to preserve wild fish populations. However, traditional fights on rod-and-reel between anglers and fish can cause sublethal physiological disturbances within a fish's blood and white muscle. Recently, studies are recognizing the importance of understanding the difference in intraspecific and interspecific species responses to these stressors, and many emphasize that larger fish have a larger internal disturbance and longer recovery time. With species specific studies to investigate the physiological responses to angling, stakeholders and policymakers can make species specific CAR regulations.

Atlantic tarpon (*Megalops atlanticus*), a sportfish in the Gulf of Mexico, are highly sought after but are physiologically unique as one of the only marine air breathing fishes. Physiologically, air breathing fishes have a modified air bladder, and which could therefore result in species specific guidelines in order to release

the fish safely. This research characterized Atlantic tarpon as facultative, or optional air breathers and saw no differences in recovery time with having access to air to breathe or not. Secondly, the study presented the recovery time for tarpon using common indices of stress, like lactate. Here, tarpon were stressed post-exercise but recovered within 4 h. This work suggests that although tarpon are a physiologically unique marine fish, their ability to air breathe likely does not play a role in recovery after exhaustive exercise. Thus, the study did not find a need for a different CAR protocol.

To study intraspecific variation of size on recovery from exhaustive exercise, this work used red drum (*Sciaenops ocellatus*), another economically important sportfish native to the Gulf of Mexico. This study characterized the recovery profile of red drum with the added variable of looking into the effect of size on magnitude of physiological disturbance and time to recovery. Red drum were found to have a larger disturbance shown in lactate build-up and increased plasma osmolalities with increasing size class, and thus had a longer recovery time compared to the smaller size class.

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Introduction

Locomotion in many animals is a critical component of organismal energetics, as it is important in foraging for food, finding mates for reproduction, and escaping predators which in turn determines an organism's ecological niche (Kieffer 2000). Understanding how and animal's morphology and biochemistry affect movement and exercise provides valuable insight into how animals succeed in nature (Weber et al. 2016). Fish have long been studied by physiologists to understand the mechanics and biochemistry of swimming and how different types of swimming behavior affect fish metabolism, acid-base balance and osmoregulation (Wood 1991). Fish have three different swimming behaviors: prolonged, sustained, and burst (Kieffer 2010). Sustained and prolonged swimming behaviors are typically characterized as aerobic swimming. These are slower swimming behaviors that utilize oxidative red muscle and can be associated with foraging or long-distance swimming (Brett, 1964; Beamish, 1978; Hammer, 1995; Plaut, 2001; Kieffer 2010). In contrast, burst swimming behaviors are used during predator-prey interactions and are categorized as anaerobic swimming powered by glycolytic white muscle (Beamish 1978; Kieffer 2000; Kieffer 2010).

Anaerobic swimming, or swimming without oxygen, relies on energy and substrate – including ATP, phosphocreatine (PCr) and glycogen – stored in the white muscle (Dobson and Hochachka, 1987). Glycogen is quickly broken down through glycolysis to produce small amounts of readily usable energy, ATP, which is used for

muscular contraction in burst swimming (Dobson and Hochachka, 1987). Glycolysis produces pyruvate that is converted into lactate by lactate dehydrogenase; this reaction recycles NAD⁺ that allows glycolysis to continue to produce ATP from glycogen (Dobson and Hochachka, 1987). PCr is another energy storing molecule, that when coupled with ADP and creatine phosphokinase generates ATP (Dobson and Hochachka, 1987). Extensive exhaustive exercise causes lactate to build-up in white muscle, and the concurrent ATP hydrolysis releases metabolic protons that induce a metabolic acidosis (Milligan and Wood, 1986, 1987; Wood 1991). Exhaustive exercise typically involves repeated burst swimming movements for an extended period of time (e.g. 3-5 minutes), and is often associated with a “leak” or “spill over” of excess lactate and metabolic protons from the tissue into the blood and plasma (Wood 1991; Kieffer 2000). During recovery, fish have to clear lactate from the muscle and plasma, replenish ATP and PCr stores, and compensate for the metabolic acidosis.

Energy stores, ATP and PCr, are replenished relatively quickly in the muscle during recovery, usually within 1 h (Wood 1991; Kieffer 2000). The process of removing lactate and regenerating glycogen in the muscle is slower, usually from 3 to 24 h (Wood 1991; Kieffer 2000). The bulk of the lactate is metabolized in the white muscle by oxidation and glyconeogenesis (Bilinski and Jonas, 1972; Wood 1991), and this process is aided by the lower intracellular pH of the muscle (Milligan and Wood 1986). The lower amount of lactate within the bloodstream is oxidized

and removed by the liver, heart, and red muscle (Mommensen et al. 1988; Milligan and Farrell, 1991; Bilinski and Jonas, 1972; Wood 1991). Lactate clearance and metabolic acid-base compensation are also an energetically costly processes, which is represented by excess post-exercise oxygen consumption (EPOC) observed during recovery (Wood 1991). Put simply, burst exercise consumes energy stores through anaerobic glycolytic pathways, while recovery is fueled by aerobic oxidative metabolism.

From an applied perspective, exercise physiology is an incredibly informative component to catch-and-release (CAR) angling protocols now favored by fisheries managers to limit population level impacts of recreational angling, by allowing smaller fish to be released back into the waters and grow to reproductive ages or keeping older and reproductive fish in the water. Traditional angling events are characterized by long fight times in which the fish is essentially fighting for its life. During these fights fish use burst swimming behavior for extended periods of time to try and escape from the line. These fights work the fish to exhaustion and therefore have the same physiological effect as forced exhaustive exercise (Cooke et al. 2013). Importantly, the patterns of exhaustion and recovery can vary widely between species, and within species, based on several biochemical and physiological traits. With this in mind, the overarching goal for this thesis was to characterize the recovery profiles of two recreationally and economically important sportfish species in the Gulf of Mexico, Atlantic tarpon (*Megalops atlanticus*) and red drum (*Sciaenops*

ocellatus). This work is divided into two chapters. The first chapter focuses on the effect of exhaustive exercise on Atlantic tarpon and the role of air-breathing on recovery from exhaustive exercise. The second chapter focuses on the exhaustive exercise and recovery profiles of red drum and explores the impact that individual size may have on these profiles.

Chapter 1: Recovery from exhaustive exercise in an air breathing fish, Atlantic tarpon (*Megalops atlanticus*)

INTRODUCTION

Recreational fisheries in the Gulf of Mexico (GoM) have been estimated to provide in excess of \$7 billion annually to the United States, and sportfish species like Atlantic tarpon (*Megalops atlanticus*) are thought to be major contributors (NOAA, 2016). These “Silver Kings” are highly sought after by anglers; tarpon’s massive size, acrobatics, and prolonged fight times have made them a prized saltwater catch (Ault and Luo, 2013). In the 1800s, tarpon were abundant throughout the Gulf of Mexico, especially around the Texas coastal bend where many flocked for tarpon fishing. However, in the 1950s anglers claimed tarpon populations abruptly declined, but the reasons were unknown (White and Brennan, 2010, Adams et al., 2014, Ault and Luo, 2013). Stakeholders deemed tarpon a catch-and-release (CAR) only species in Texas, in an effort to protect and rebuild their populations (Adams et al., 2014).

CAR angling is widely considered a useful conservation tool for fisheries managers to ensure that fish are returned to the wild and can repopulate the habitat. However, increasing evidence has shown that CAR angling is an intense stressor for fish – after fighting on the line, fish are exhausted (Cooke et al., 2013). Compounding factors such as type of hooks, lines, fight times, handling, and air exposure all increase the stress exerted on a fish (Cooke et al., 2013). Exhaustive exercise primarily relies on anaerobic metabolism, which results in fish utilizing energy stores like ATP and phosphocreatine while raising the concentration of waste products (e.g. lactate) in glycolytic skeletal (i.e.

white) muscle (Kieffer, 2000). When released back into the environment after prolonged stress, fish can be more susceptible to predation after release (Danylchuk et al., 2007).

While decades of research has helped refine the best practice protocols for CAR recreational angling, species specific considerations remain an integral to factor component to ensure the best chances of individual survival (Cooke and Suski 2005). For example, many shark species are highly sought after by anglers, but their unique physiological characteristics make many species particularly susceptible to CAR practices. This is particularly true of obligate RAM ventilating sharks such as the hammerhead (Shiffman and Hammerschlag, 2016). These animals are at a unique drowning risk following exhaustive exercise because they must maintain forward momentum to obtain oxygen and undertake lactate clearance. A similar line of thinking may also apply to air-breathing fishes, which may require access to the surface to obtain oxygen. Air-breathing fishes that require access to air during recovery from exhaustive exercise may drown if released into deep water and do not have the energy to resurface and air-breathe. While air-breathing has evolved multiple times in fishes, this unique mode of respiration is usually found in freshwater species (Johansen 1970; Graham, 1997). Air-breathing fish are known as bimodal breathers and there are two different classifications of air-breathing fishes: facultative and obligate (Graham, 1997). Facultative air-breathing fishes can effectively obtain oxygen via their gills, and thus do not need to air-breathe to survive (Graham, 1997). Conversely, obligate air-breathers cannot obtain sufficient oxygen through their gills, and therefore must have access to the surface to obtain oxygen. More simply, obligate air-breathers can drown without access

to air, which raises complications with respect to CAR procedures should released fish exhibit impaired swimming abilities.

Tarpon (*Megalops* spp.) are the only known genus of air-breathing marine fishes, and whether they are facultative or obligate air-breathers remains an open question. The available data for the Indo-Pacific tarpon (*Megalops cyprinoides*) suggests this species is a facultative air-breathers (Seymore et al., 2004, Graham, 1997). However, Atlantic tarpon's air-breathing behavior remains a source for debate due to differing results from studies over the years—some studies claim small juveniles are obligate air-breathers while others claim that sub-adults are facultative air-breathers (Seymore et al., 2008). As such, the purpose of this study was to explore the importance of air-breathing to recovery from exhaustive exercise in the Atlantic tarpon. More specifically, this study had three goals: 1) to use hypoxia exposure to determine if air-breathing behavior is facultative or obligate, 2) to determine if exhaustive exercise stimulates air-breathing during recovery, and 3) to assess the importance of air-breathing for lactate clearance and the recovery of muscle and plasma metabolites.

METHODS

Field sites and collections

All experiments were approved by the University of Texas at Austin's Institutional Animal Care and Use Committee (IACUC). Juvenile tarpon (*Megalops atlanticus*) (< 15 cm; N = 6) were caught using a 4' cast net in freshwater creeks in Port Aransas, Texas (27.819030 N, 97.073100 W) from October 2017 to January 2018 over three trips. Once

caught, fish were transported in aerated freshwater to the Fisheries and Mariculture Lab (FAML) at the University of Texas Marine Science Institute (Port Aransas, TX). Sub-adult tarpon (> 30 cm; N = 25) were caught using a seine net at a brackish water channel in Aransas Pass, TX (27.891130 N, 97.151890 W) from February 2018 to August 2019 over eight collection trips. Fish were placed in a 500 L transportation tank filled with freshwater, transported to FAML, and placed in husbandry tanks.

Housing and husbandry

Juveniles were housed in 40 L aquaria with recirculating and aerated freshwater (dechlorinated Port Aransas tap water) maintained at 25 °C. Ammonia was controlled using biofiltration, and a 50% water change was performed once a week. Fish were withheld from experimentation until they acclimated to the holding facilities, which was assessed by normal routine behavior and feeding approximately 1-month post housing. Fish were initially fed to satiation with freshly cut and peeled shrimp, and subsequently transferred to dry food pellets (Aquamax, Purina). Sub-adults were housed in 1000 L tanks containing aerated freshwater and filtration and maintained at 26-27 °C. Sub-adults were fed fresh shrimp until satiation every other day. Sub-adults were housed for at least 48 h before experimentation. In the event that temperature acclimation was needed (i.e. the environment was colder than the holding facility), the temperature was changed 1 °C per day.

Air-breathing experiments

Juveniles ($N = 6$; mass = 339.7 ± 74.97 g) were used to assess whether air-breathing was obligate or facultative in two experimental series. Series 1 measured the frequency of air-breathing over 15-minute intervals of declining oxygen saturation (% O₂ saturation: 100, 80, 60, 40, 20, and 10). A recirculating system with a central header tank supplied water to two 5 L observation chambers. An automated oxygen control system consisting of an electrode and gas solenoid (Loligo Systems) bubbled nitrogen gas into the header tank to displace oxygen as needed. Each observation chamber held a single juvenile tarpon, and fish were acclimated to the chamber for 12 h at normoxia prior to experimentation. A GoPro Hero 4 recorded video of each 15-minute interval. Videos were manually analyzed for air-breathing frequency, which was defined by surfacing, gulping air, and a subsequent bubble release from the operculum. Breaths were standardized to breath/minute. Series 2 used the same juvenile fish ($N = 6$) but investigated a longer exposure interval) and under normoxia (100% O₂) and hypoxia (20% O₂), as well as investigated the impacts of exhaustive exercise on air-breathing. Tarpon were first filmed for 1 hour under normoxia or hypoxia to achieve a pre-exhaustive exercise control. Fish were then removed from the observation tank and chased to exhaustion using a standard chase protocol (Turner et al. 1983; Wood 1991; Roche et al. 2013; Ackerly and Esbaugh et al. 2020). Fish were chased by a pinch tail method in a 10 L tank for 3 minutes or until exhaustion. Exhaustion was defined as when the fish lost equilibrium for at least 3 seconds. They were then returned to the observation chamber and filmed for 1 hour in either normoxia or hypoxia. Note that no

air exposure was included in the chase protocol since Atlantic tarpon can breathe air. Also note that the same fish were used for all experiments, which was necessitated by the limited supply of animals. All fish were provided at least 7 days between experimentation, during which they were successfully fed (Milligan 1996).

Exhaustive exercise and recovery experiments

Sub-adult tarpon were utilized to explore the importance of air-breathing on recovery profiles following exhaustive exercise. Fish were chased using a standard protocol that involved chasing a single fish in a tank by hand or net for 5 minutes, or until exhaustion (Turner et al. 1983; Wood 1991; Roche et al. 2013; Ackerly and Esbaugh et al. 2020). Tarpon were then transferred to the recovery chamber and allowed to recover for 0 h, 1 h, and 4 h (N = 5; N = 10; N = 5, respectively). The 1 h and 4 h time points had two types of recovery chambers: one with access to air and one without access to air. No-exercise control fish were in placed in recovery chambers for 36 h (N = 5) to provide an unstressed baseline for comparison. As above, no air exposure period was included in the chase protocol. Tarpon were anesthetized by adding buffered MS-222 (250 mg/L MS-222; 500 mg/L NaHCO₃) to the recovery chamber through a hole in the lid, euthanized by spinal transect, and sampled for blood and white muscle. Blood was collected using a 22-gauge heparinized needle by caudal puncture, and white muscle was dissected by scalpel. Blood samples were kept on ice until analyses, and white muscle was flash frozen in liquid N₂.

Physiological and biochemical assays

Hematocrit (Hct) was determined using heparinized capillary tubes filled with 5 μ L of whole blood and centrifuged for 1 minute (StatSpin MP). Remaining blood was centrifuged for 2 minutes at 10,000 g to separate RBCs from plasma. Plasma was flash frozen and stored at -80°C until further use. RBCs were washed three times with cold isotonic saline and assessed for intracellular pH (pH_i) and hemoglobin (Hb) concentration. The pH_i was measured by first lysing the cells via freeze thaw, after which the sample was placed in a thermostated bath (26°C) and measured using a micro pH electrode (Accumet, Fisher Scientific). Mean corpuscal hemoglobin concentration (MCHC) was determined using a standard Drabkin's colorimetric assay as performed using a plate spectrophotometer (Drabkin and Austin 1935). Plasma osmolality on thawed samples was determined using a vapor pressure osmometer (Wescor; VAPRO 5520). Plasma pH_e was determined as described above for pH_i . Plasma lactate, tissue lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP) concentrations were measured following the enzymatic methods of Lowry and Passonneau (1972). The muscle was processed according to the procedure described in Booth et al. (1995).

Statistical analyses

Behavior analysis of air-breathing under varying levels of oxygen saturation was completed using a one-way analysis of variance (ANOVA), while behavioral analysis of air-breathing under hypoxia and/or following exercise was completed using a two-way ANOVA. For all tests a Shapiro-Wilk test was used to verify normality and a Brown-

Forsythe test was used to assess equal variance, and if assumptions were not met data was assessed via a Kruskal-Wallis One Way Analysis of Variance on Ranks. Physiological and biochemical analyses investigating recovery period on tarpon with access to air were analyzed using an ANOVA. While, a two-way ANOVA was used to assess the differences in recovery between tarpon with and without air access. All statistical tests were performed using Sigma Plot version 13, and the fiducial level of significance is $P \leq 0.05$.

RESULTS

Air-breathing experiments

Series 1 examined air-breathing using a 15 min stepwise hypoxia protocol, which revealed no significant difference in breathing rate (Fig. 1a). However, a trend toward increased breathing at and below 40% O₂ saturation was observed. Therefore, a second trial (Series 2) exposed fish to 20% O₂ saturation for 1 h, which revealed Atlantic tarpon significantly increased breathing rate under hypoxia as compared to normoxia (Fig. 1b). Series 2 also demonstrated that there was no significant increase in breathing rate post-exercise in normoxia, but again a significantly increased breathing rate was observed when recovered in hypoxia (Fig. 1b). There was a trend towards increased post-exercise breathing rate under hypoxia as compared to normoxia; however, the two-way interaction term was not significant ($P = 0.209$).

Blood biochemical analyses

There was no significant effect of exercise on hematocrit (Hct) (Fig. 2A) and plasma osmolality (Fig. 2B) at any recovery time relative to the no-exercise control. Immediately post-exercise, plasma pH_e and red blood cell pH_i significantly decreased and remained low at 1 h post-exercise but returned to pre-exercise levels by 4 h (Fig. 3). Plasma lactate was significantly higher at 1 h post-exercise and remained elevated more than control levels at 4 h (Fig. 4). There was no significant difference in recovery of any endpoint when fish had no access to air. There was also no significant interaction between air access and recovery time.

Muscle biochemical analyses

Muscle ATP and PCr concentrations significantly decreased immediately post-exercise but had partially recovered by 1 h post-exercise and had reached pre-exercise levels by 4 h post-exercise (Fig. 5). Muscle lactate significantly increased immediately post-exercise, peaked at 1 h, and returned to control levels by 4 h (Fig. 6a, c). Similarly, muscle pH_i significantly decreased immediately post-exercise, remained low at 1 h, and returned to pre-exercise levels by 4 h (Fig 6b, d). There was no significant difference in recovery when fish had no access to air. There was also no significant interaction between air access and recovery time.

DISCUSSION

The first goal of this study was to assess whether the air-breathing behavior of Atlantic tarpon is obligate or facultative. While the results of Series 1 did not demonstrate

a significant increase in air-breathing rate, there did appear to be a trend toward increased air-breathing O₂ saturation decreased, with a marked increase beginning at 40% O₂ saturation (Fig. 1a). Series 2 used a longer exposure and observation interval and demonstrated that Atlantic tarpon significantly increased their air-breathing rate under hypoxia compared to normoxia (Fig. 1b). This suggests that Atlantic tarpon are facultative air-breathers. By definition, facultative air-breathing fish exhibit differences in air-breathing frequency in normoxia and hypoxia; they can physiologically meet oxygen requirements from their gills in normoxia but need to air-breathe to obtain oxygen under hypoxic conditions (Graham 1997). It is presumed that if Atlantic tarpon were obligate air-breathers then there would not have been an increase in air-breathing rate under hypoxia, rather air-breathing rates would have been the same under both treatments (Graham 1997). These findings are in agreement with our hypothesis and are congruent with knowledge of Pacific tarpon, which are also facultative air-breathers (Seymore et al. 2004).

While Atlantic tarpon are clearly a facultative air-breather with respect to hypoxia, this response is not stimulated during recovery from exhaustive exercise. When Atlantic tarpon were chased to exhaustion and allowed to recover for 1 h in either normoxia or hypoxia, only those fish recovering in hypoxia increased their air-breathing rate (Fig. 1b). Even further, there was no significant difference in air-breathing rate for non-exercised hypoxia exposed and post-exercise hypoxia exposed fish (Fig. 1b). This suggests that exhaustive exercise does not stimulate air-breathing in Atlantic tarpon, which was counter to our original hypothesis. Thus, tarpon do not need access to air post CAR

events. Although hypoxia driven air-breathing is well-known (Johansen 1970; Graham 1997), there have also been multiple studies that suggest exercise can stimulate air-breathing during or after exercise (Becker 1983; Johansen et al. 1970; Burleson et al. 1998; Farmer and Jackson 1998). Specifically, spotted gar (*Lepisosteus oculatus*), a facultative air-breathing fish, increased air-breathing rate as a direct result of exhaustive exercise in addition to hypoxic conditions (Burleson et al. 1998).

The biochemical recovery profiles of Atlantic tarpon following exhaustive exercise generally support the above observations that air-breathing is not stimulated by exercise. This is evident from the fact that there were no effects of restricting access to air on any biochemical trait after either 1 h or 4 h of recovery. While we must acknowledge the low samples size at 4 h, it is important to note that the lack of interaction between treatment and recovery time results in a pooled sample size of N=5 for the two treatments across the experiment, and the p values for the main effects across all biochemical endpoints were strongly not-significant (e.g. P = 0.141 - 0.827).

In general, exhaustive exercise is linked to anaerobic swimming, which is associated with a sharp decline in energy stores in the white muscle, including ATP, PCr, and glycogen (Wood 1991; Kieffer 2000). Anaerobic swimming also leads to lactate and metabolic proton build-up in the muscle, which “spills over” into the plasma (Wood 1991; Kieffer 2000) (Fig. 4). These biochemical impacts of exercise must be corrected for fish to return to normal activity. The exercise and recovery profiles of Atlantic tarpon generally conformed to a common pattern that has been described for other teleosts, including rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo*

salar)(Ferguson et al. 1993; Milligan and Wood 1986; Turner et al 1983; Wakefield et al. 2004). This included a sharp decline in PCr and ATP at 0 h (i.e. immediately post-exercise), with partial recovery by 1 h post-exercise and full recovery within 4 h (Fig. 5). A similar pattern was observed for pH_e and both red blood cell and muscle pH_i , which is expected owing to the impact of ATP hydrolysis on exercise induced metabolic acidosis (Robergs et al. 2004; Robergs 2019). Exhaustive exercise also caused the expected increase in muscle lactate at 0 h post-exercise, which also peaked at 1 h and returned to pre-exercise concentrations by 4 h of recovery (Fig. 6a). The plasma lactate also significantly increased at 0 h and peaked at 1 h post-exercise, but did not return to pre-exercise levels by 4 h (Fig. 4). Plasma lactate is thought to be metabolized through gluconeogenesis or oxidation in the liver, red muscle and heart (Weber et al. 2016). The slower recovery rate of plasma lactate as compared to muscle lactate following exhaustive exercise is relatively common in fishes (e.g. Omlin and Weber 2013).

Fisheries managers need to understand the biology of different target species to ensure successful management (Cooke and Suski 2005). Tarpon are rare and are a target for elite anglers, and, to protect them, many areas have instituted mandated catch-and-release restrictions (FFWCC 2013; Adams et al. 2014). There has been suggestions lately that catch-and-release angling can lead to mortality for tarpon (FFWCC 2013), which could be thought of as linked to their unique biology. This study found Atlantic tarpon to be facultative air-breathers, and that the air-breathing behavior is stimulated by hypoxia but not exhaustive exercise. Furthermore, air-breathing does not aid Atlantic tarpon recovery from exhaustive exercise, but rather Atlantic tarpon recovery mirrors that

of well-studied fish species, like rainbow trout (*Oncorhynchus mykiss*) and largemouth bass (*Micropterus salmoides*) (Ferguson et al. 1993; Milligan and Wood 1986; Turner et al 1983; Gingerich and Suski 2012). This work provides important validation for the premise that specialized CAR protocols are likely not needed to address the unique air-breathing physiology of Atlantic tarpon. This work instead suggests that typical CAR best practices should suffice to facilitate survival post-capture. The most effective CAR protocols for limiting stress in water-breathing fish involve minimized fight time on the line, fish quickly removed from the hook, and minimized amounts of air exposure (Cooke et al. 2013).

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FIGURES

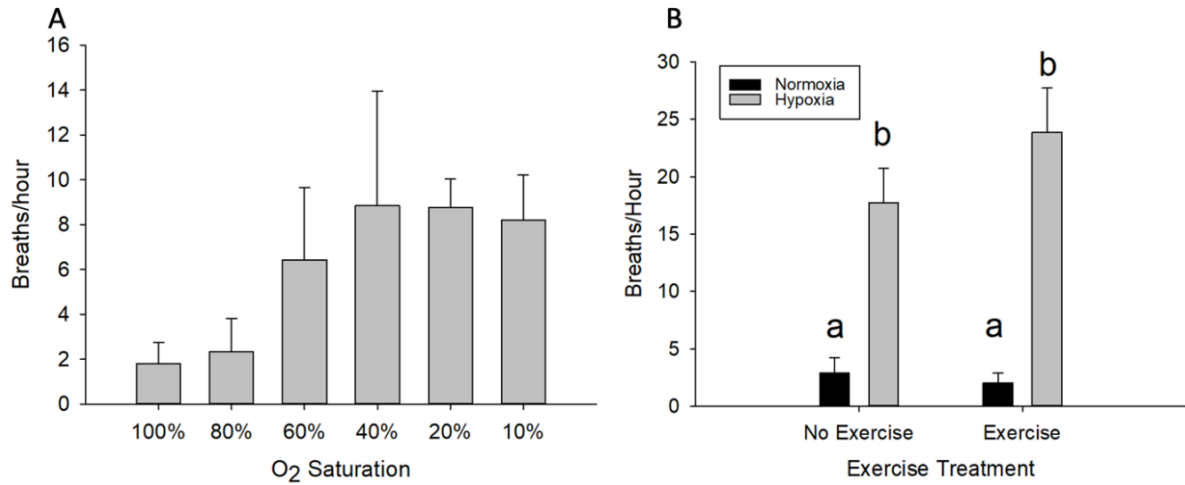


Figure 1: (A) Series 1: stepwise decreasing O₂ saturation to examine breaths per hour in Atlantic tarpon (*Megalops atlanticus*), was determined by a One-Way ANOVA ($P > 0.05$; $N = 6$). (B) Series 2: examined air-breathing rate in breaths per hour in normoxia (100% O₂) and hypoxia (20% O₂), as well as tested air-breathing rates in response to exhaustive exercise. Results were tested in a Two-Way ANOVA, significant difference is noted by a difference in letters. Interactions was detected for O₂ saturation but not for exercise ($P \leq 0.05$; $N = 6$).

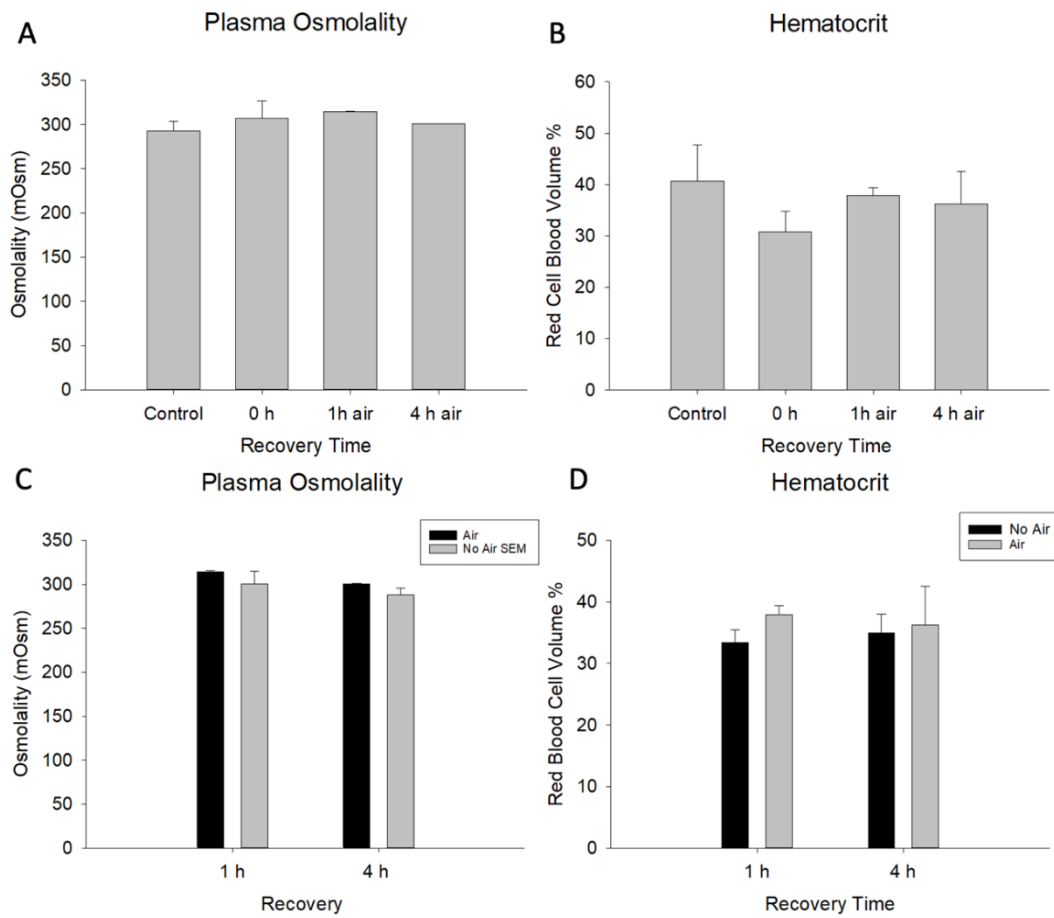


Figure 2: Plasma osmolality, all with access to air (A) hematocrit, all with access to air (B) plasma osmolality, differing in access to air (C), hematocrit, differing in access to air (D) ($P \leq 0.05$; $N = 2-5$) following exhaustive exercise in Atlantic tarpon (*Megalops atlanticus*). A and B were analyzed with a One-Way ANOVA ($P > 0.05$; $N = 2-5$). C and D were analyzed with a Two-Way ANOVA ($P > 0.05$; $N = 2-5$).

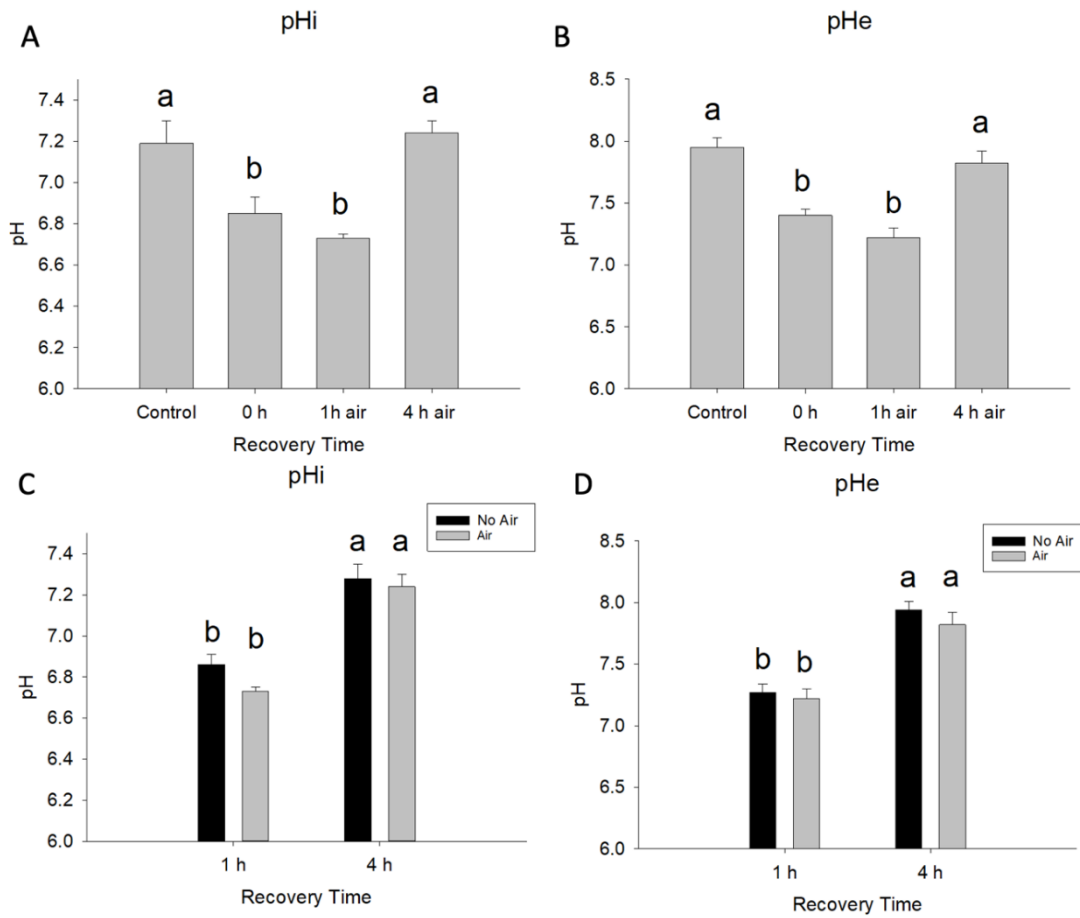


Figure 3: Measured pHe with air access (A) measured pH_i with air access (B), pHe, differing in access to air (C), pH_i differing in access to air (D) following exhaustive exercise in Atlantic tarpon (*Megalops atlanticus*). A and B were analyzed with a One-Way ANOVA ($P \leq 0.05$; $N = 2-5$). C and D were analyzed with a Two-Way ANOVA ($P > 0.05$; $N = 2-5$).

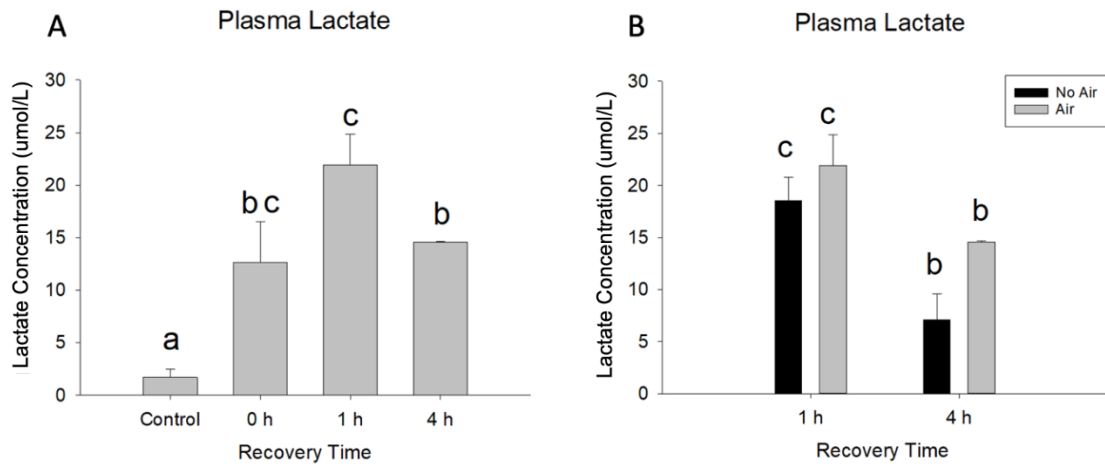


Figure 4: Plasma lactate with air access (A) plasma lactate, differing in access to air (B), following exhaustive exercise in Atlantic tarpon (*Megalops atlanticus*). A was analyzed with a One-Way ANOVA ($P \leq 0.05$; $N = 2-5$). B was analyzed with a Two-Way ANOVA ($P > 0.05$; $N = 2-5$).

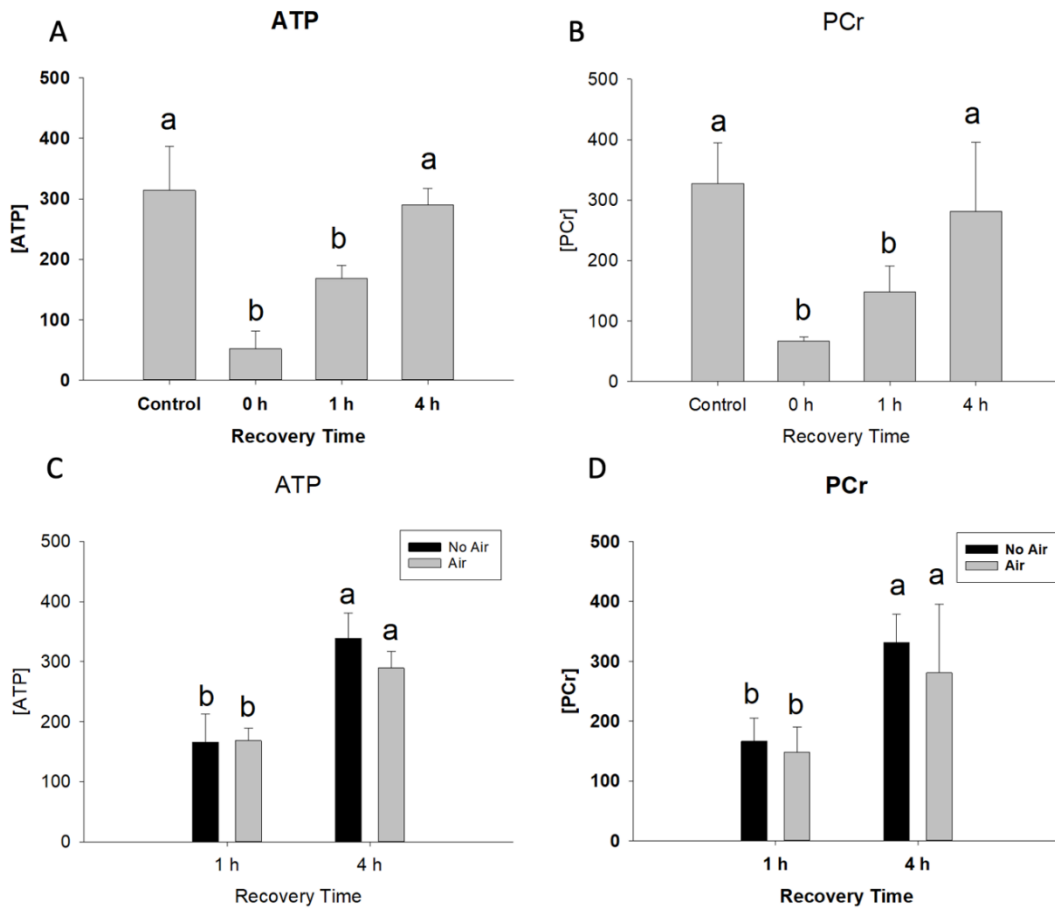


Figure 5: ATP with air access (A) PCr with air access (B), ATP, differing in access to air (C), PCr differing in access to air (D) following exhaustive exercise in Atlantic tarpon (*Megalops atlanticus*). A and B were analyzed with a One-Way ANOVA ($P \leq 0.05$; $N = 2-5$). C and D were analyzed with a Two-Way ANOVA ($P > 0.05$; $N = 2-5$).

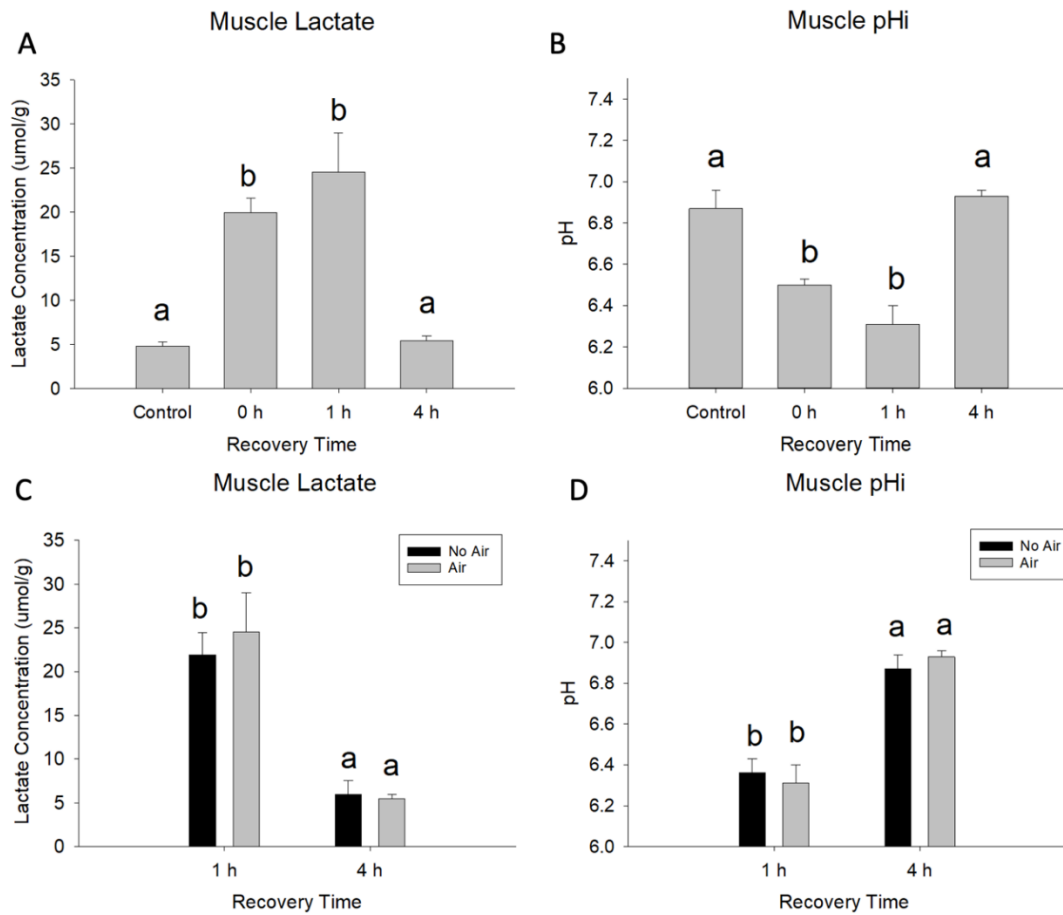


Figure 6: Muscle lactate with air access (A) muscle pHi with air access (B), muscle lactate, differing in access to air (C), muscle pHi, differing in access to air (D) following exhaustive exercise in Atlantic tarpon (*Megalops atlanticus*). A and B were analyzed with a One-Way ANOVA ($P \leq 0.05$; $N = 2-5$). C and D were analyzed with a Two-Way ANOVA ($P > 0.05$; $N = 2-5$).

Chapter 2: The effects of size and exhaustive exercise on recovery in red drum (*Sciaenops ocellatus*)

INTRODUCTION

Recreational fisheries in the Gulf of Mexico (GoM) are a \$7 billion industry (FEU NOAA 2016), but angling has been implicated in fish mortalities and declining sportfish populations (Cooke and Cowx 2004). Many conservation strategies have been implemented to limit the effect of recreational angling on fish populations, and a central component of these strategies is the adoption of catch and release (CAR) protocols. As a management tool CAR is assumed to cause minimal physiological disturbance (Wydoski 1977; Cooke et al. 2013), and by returning the fish to the environment, CAR works to limit angling-based population impacts (Cooke and Suski 2005). However, it is important to recognize that individual species may have different tolerances to CAR, and thus species-specific validation of CAR recovery profiles provides valuable information about the effectiveness of these protocols (Cooke and Suski 2005).

Many factors can contribute to the stress and recovery profiles of CAR. These include fight times (i.e. exercise duration), the weight of fishing line, the type of hook, handling, and air exposure (Cooke et al. 2013; Arlinghaus et al. 2007). From a physiological perspective CAR is simply an intense exercise stress (Cooke and Suski 2005; Cooke et al. 2013). As such, two factors that can contribute to survival are the magnitude of the initial stress, and the speed of biochemical recovery. In fact, recovery from exhaustive exercise has been increasingly studied in different species

to understand how fish respond to, and recover from, angling stress (Wydoski 1977; Cooke et al 2002; Cooke and Suski 2005). In general, exhaustive exercise via angling utilizes burst swimming behavior, which relies on glycolytic white muscle (Kieffer 2000). These anaerobically powered swimming bursts can only be sustained for a short period of time, and quickly deplete glycogen, phosphocreatine (PCr) and ATP stores within the muscle (Kieffer 2000). Furthermore, anaerobic metabolism results in a build-up of toxic lactate in the muscle and plasma, which often occurs in conjunction with a metabolic acidosis caused by ATP hydrolysis (Kieffer 2000; Cooke et al. 2002). The rate of lactate clearance, acid-base compensation, and the regeneration of energy stores can vary substantially between species and governs how quickly a fish can return to an active lifestyle (Kieffer 2000). Importantly, post-release predation, presumably due to a fish's state of exhaustion, is increasingly being recognized as a major source of species susceptibility following CAR (Cooke et al. 2013).

Another factor that has received relatively less attention is body mass. Larger fish have been shown to have a reduced cost of swimming (Schmidt-Nielsen 1972), a lower mass-specific metabolic rate (e.g. Pan et al. 2018), and an increased amount of anaerobic fuel (Kieffer et al. 1996), which may infer greater recovery potential. Yet larger salmonids have been shown to exhibit increased PCr consumption, elevated lactate production, and a greater exercise-induced acid-base disturbance (Wakefield et al. 2004; Kieffer et al. 1996; Goolish 1989). Similarly, larger

largemouth bass exhibited an increased exercise-induced stress response and longer recovery times, as demonstrated by higher lactate and glucose concentrations in the plasma and muscle, as well as a slower clearance rate of these metabolites (Gingerich and Suski 2012). As such, we cannot generalize intraspecies responses to exhaustive exercise when alluding to different size classes (Gingerich and Suski 2012).

On this background, this study sought to examine the biochemical consequences and recovery profiles of exhaustive exercise in the marine sportfish, the red drum (*Sciaenops ocellatus*). Furthermore, we sought to test the hypothesis that larger red drum would exhibit a greater magnitude of disturbance and longer times to return to control levels following exercise as compared to smaller fish. Red drum are an economically important sportfish throughout the GoM and southeastern United States, where they are a crucial part of recreational fisheries (Rooker et al. 2010, Carson et al. 2014). Furthermore, red drum are a tightly regulated fishery that consists of both daily bag limits and slot sizes, yet surprisingly little is known about their physiological responses to exercise.

METHODS

Experimental Fish

All experiments were approved by the University of Texas at Austin's Institutional Animal Care and Use Committee (IACUC). Large juvenile red drum (*Sciaenops ocellatus*) (N = 24; 50.8—73.7 cm; 3.0 ± 0.4 kg; mean ± S.E.M.) were

collected by hook and handline at the shipping channel in Port Aransas, TX (27°50'23" N, 97°04'11" W) from June 2019 to April 2020. This size fish was targeted based on current slot size regulations for recreational red drum angling in Texas (TPWD). Once caught, fish were placed in a 500-L tank, and transported to the Fisheries and Mariculture Laboratories (FAML) at the University of Texas Marine Science Institute (Port Aransas, TX). Fish were subsequently held in 40,000 L tanks with recirculating and aerated seawater held at 22 °C and 35 ppt salinity. A 50% water change was performed once per week. Ammonia, salinity, and temperature were monitored daily and pH was monitored weekly. Fish were withheld from experimentation until acclimated to the facility, determined by successful feeding and routine behavior. Fish were fed to satiation with frozen shrimp every other day.

Small juvenile red drum (N = 48; 20.3—30.5 cm TL; 159.1 ± 26.1 g; mean \pm S.E.M.) were obtained from Ekstrom Aquaculture, LLC in Palacios, TX and transported to the University of Texas at Austin Marine Science Institute in Port Aransas, TX. Fish were held in a recirculating system with filtered, ozone treated seawater (35 ppt) originating from the Corpus Christi ship channel. Water quality was tested daily for ammonia, salinity, and temperature, and pH was monitored weekly. A 25% water change was performed weekly. All fish were held in a single 40,000 L tank maintained at 22 °C with constant aeration. Fish were fed commercial pellets (Aquamax, Purina) daily to satiation, but were starved for at least 48 h prior to experimentation.

Exhaustive exercise and recovery protocol

Both large and small juvenile red drum were chased using a standard chase protocol, which involved chasing the fish in a tank by hand or net for 5 minutes, or until exhaustion, followed by one minute or air exposure (Turner et al. 1983; Wood, C.M. 1991; Roche et al. 2013; Ackerly and Esbaugh et al. 2020). Small juvenile red drum were then sampled immediately or transferred to a size appropriate recovery chamber and allowed to recover for 0.5 h, 1 h, 3 h, and 6 h (N = 8 each). Large juvenile red drum were sampled immediately or transferred to a size appropriate recovery chamber and allowed to recover for 1 h, and 3 h (N = 6 each). Both large and small red drum size classes included a no-exercise control group, which involved placing fish in the recovery chamber for 36 h (N = 6; N = 8 respectively). Fish were anesthetized in MS-222 bath (250 mg/L; 500 mg/L NaHCO₃), euthanized by spinal transect, and sampled for blood and white muscle. Blood was collected using a 16-gauge heparinized needle for large juvenile red drum and 22-gauge heparinized needle for small juvenile red drum. Blood samples were kept on ice until analyses, and white muscle was flash frozen in liquid N and stored at -80 °C until use.

Physiological and biochemical analyses

Hematocrit (Hct) was determined using heparinized capillary tubes filled with 5 uL of whole blood and centrifuged for 1 min (StatSpin MP). Remaining blood was centrifuged for 2 min at 10,000 × *g* (gravity) to separate RBCs from plasma. Plasma was flash frozen and stored at -80 °C until further use. RBCs were washed three times with cold isotonic saline and assessed for intracellular pH (pHi) and hemoglobin

(Hb) concentration. The pH_i was measured by first lysing the cells via freeze thaw, after which the sample was placed in a thermostated bath (26 °C) and measured using a micro pH electrode (Accumet, Fisher Scientific). Mean corpuscal hemoglobin concentration (MCHC) was determined using a standard Drabkin's colorimetric assay as performed using a plate spectrophotometer (Drabkin and Austin 1935). Plasma osmolality on thawed samples was determined using a vapor pressure osmometer (Wescor; VAPRO 5520). Plasma pH_e was determined as described above for pH_i . Plasma lactate, tissue lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP) concentrations were measured following the enzymatic methods of Lowry and Passonneau (1972). The muscle was processed according to the procedure described in Booth et al. (1995).

Statistical analyses

All physiological and biochemical analyses were analyzed using a one-way analysis of variance (ANOVA). A Shapiro-Wilk test was used to verify normality and a Brown-Forsythe test was used to assess equal variance. A Grubb's test was ran for outliers and if any were identified they were removed from the dataset. All statistical tests were performed using Sigma Plot version 13, and the fiducial level of significance is $P \leq 0.05$.

RESULTS

Blood biochemical analyses

Plasma osmolality in small juveniles was significantly elevated by exhaustive exercise at 0h, 0.5 h, and 1 h and returned to control levels by 3 h (Fig, 1a). Large juveniles had significantly higher plasma osmolality at 0 h, 1 h and 3 h and did not see

their return to control levels (Fig. 1c). Small juveniles Hct was significantly elevated at 0.5 h and 1h, and returned to control levels by 3 h post exercise (Fig. 1b), while large juveniles Hct was significantly elevated at 0 h and 1 h post exercise and also returned to control levels by 3 h. (Fig. 1d) MCHC showed no significant differences from the control at any time point for both small and large juveniles. Small juveniles showed no significant change in pHi or pHe, but a slight acidification and recovery trend was indicated for both (Fig 2a, b). Large juveniles showed significantly decreased pHi and pHe at 1 h, which returned to control by 3 h (Fig. 2c, d). Plasma lactate was significantly elevated in small juveniles at 0.5 h, 1 h, and 3 h, and returned to control baselines by 6 h of recovery (Fig. 3b). Large juveniles demonstrated significantly elevated plasma lactate at all post-exercise time points. Both size classes saw increases in lactate at 0 h that were more than 4 times greater than controls (Fig. 3c). Across all variables, the peak exercise-induced response occurred within the first hour of recovery as opposed to immediately after exercise. For all affected variables, the magnitude of the peak response was greater for large juveniles than small juveniles.

Muscle biochemical analyses

There were no significant changes in the muscle ATP concentration for either small or large juveniles, however, a downward trend in ATP content post-exercise was notable (Table 1). Similarly, there was no significant change in muscle PCr concentration for either size class (Table 1). Muscle lactate peaked at 0 h and returned to control baselines by 1h and 3 h for small and large juveniles, respectively (Fig. 3a, c). Small

juveniles had significantly decreased muscle pH_i at 0 h, 0.5 h, and 1 h post-exercise, returned to control levels at 3 h, and significantly increased at 6 h (Fig. 4a). Large juveniles muscle pH_i was significantly decreased at 0 h, 1 h, and 3 h (Fig. 4b). The magnitude of the peak lactate and pH_i responses was larger for large juveniles than small juveniles.

DISCUSSION

Both size classes following exhaustive exercise experienced physiological disturbances, mostly consistent with other well-studied fish species' responses to exhaustive exercise (Kieffer 2000; Gingerich and Suski 2012; Ferguson et al. 1993; Kieffer et al. 1994). As expected muscle lactate for both size classes increased significantly post-exercise at 0 h; large juvenile red drum returned to pre-exercise levels by 1 h (Fig.3a), while large juvenile red drum did not return to control levels until 3 h (Fig.3b). Large juvenile drum took longer to clear lactate, but the initial concentration of lactate post-exercise was not higher than small juvenile red drum. Consistent with this increase in muscle lactate the pH_i of the muscle for both small and large juvenile red drum significantly decreased at 0 h (Fig. 4a, b). Large juveniles continued to have lower a muscle pH_i through the 3 h timepoint (Fig. 4b), which means that although large juvenile red drum are able to clear muscle lactate within 3 h they are not able to compensate for the subsequent metabolic acidosis in the muscle by this time (Wood et al. 1982). Small juveniles returned to control levels by 3 h post-exercise, but then showed a significant increase at 6 h (Fig. 4a),

suggesting that they have overcompensated for this metabolic acidosis (Tang et al. 1988). Surprisingly, energy stores in the white muscle, ATP and PCr, did not show a significant decrease post-exercise (Table 1), although we did observe the typical trend of decreasing ATP and PCr stores immediately after exercise at 0 h (Table 1). These results are unusual compared to the general literature of exhaustive exercise responses in fish (Wood 1991; Kieffer 2000; Kieffer 2010) and are likely indicative of some activity during the control time points for both of the small and large juveniles.

Plasma lactate immediately increased post-exercise at 0 h, peaked at 1 h, and remained significantly higher through 3 h for both size classes (Fig. 3b, d); small juvenile red drum did not show recovery of plasma lactate until 6 h. Many active fish species, like salmonids or red drum, retain lactate within the plasma and white muscle for hours post-exercise (Omlin and Weber 2013; Weber et al. 2016), and most species clear this lactate by 24 h (Weber et al. 2016; Wood and Perry 1985). In congruence with this lactate build-up, a decrease was exhibited in the pH_e and pH_i of the blood in the large juveniles at 1 h, which recovered by 3 h (Fig. 2c, d). Small juveniles showed similar trends in both parameters; however, these results were not significant and generally reduced in magnitude as compared to the large juvenile size class (Fig. 2a, b). These decreases in plasma and blood pH are to be expected; lactate and metabolic protons are released from the hydrolysis of ATP (Robergs et al. 2004; Robergs 2019). Similarly, we observed significantly increased plasma osmolality and hematocrit immediately post-exercise at 0 h and a measured peak at 1 h for both small and large juveniles (Fig. 1a, c). This increase

is in large due to the high muscle lactate concentration (Milligan and Wood, 1986), while red blood cells swell in response to the metabolic acidosis (Milligan and Wood, 1987; Milligan et al. 1989; Wood et al. 1990). Large juvenile's plasma osmolality remains elevated through 3 h compared to 1 h for small juveniles. Thus, red drum provide further evidence that larger fish experience more significant disturbances that takes longer to recover from following exhaustive exercise compared to smaller fish.

As mentioned above, lactate is a byproduct of the breakdown of glycogen and is responsible for extending the potential for glycolytic metabolism by recycling NAD⁺. While both size classes of red drum exhibited a peak in muscle lactate at 0 h and demonstrated recovery within 3 h – with smaller fish recovering earlier – it is also interesting to consider how these patterns can inform on the mechanisms of lactate clearance. It is typical for the high levels of lactate produced in the white muscle to “leak” into the plasma (Wood 1991; Kieffer 2000), which results in two pools of lactate that must be utilized. In contrast to the available evidence in fish, mammals actively move lactate from white muscle to the blood and subsequently to the liver, heart, and red muscle where it is either recycled to glycogen/glucose or oxidized; a process known as the Cori Cycle (Weber et al. 2016). Many fish species, notably salmonids, appear to undertake glyconeogenesis directly in the white muscle and therefore do not utilize the Cori Cycle (Milligan and McDonald 1987; Milligan and Girard 1993; Wood 1991). It is tempting to ascribe the relative patterns of lactate clearance in the muscle and plasma of red drum to a greater reliance on the Cori Cycle, as muscle lactate peaks immediately after exercise and declines while plasma lactate peaks at 0.5 – 1 h post-exercise.

Furthermore, the relative concentration of lactate in the plasma is quite high relative to the muscle (i.e. 35 and 40% peak concentrations for small and large juveniles, respectively) when compared to patterns observed in other fish species (Black et al. 1962; Turner et al. 1983; Dobson and Hochachka, 1987; Mommsen and Hochachka, 1988). As such, plasma lactate remains a small pool relative to the muscle and is likely cleared via oxidative processes in the heart, red muscle and red blood cells, as described for other fish species (Mommsen et al. 1988; Milligan and Farrell, 1991; Bilinski and Jonas, 1972).

In a variety of parameters, larger red drum experienced a longer recovery time compared to the smaller red drum. Notably, the plasma osmolality remained significantly higher than the control at 3 h in large juveniles compared to the small juveniles, which returned to normal by 3 h (Fig. 1). In addition, both muscle lactate and muscle pH_i disturbances lasted longer in the large juveniles, and both blood pH_i and pH_e had larger shifts in large juveniles compared to the small juveniles. This pattern holds true with other studies that have demonstrated larger fish of the same species experienced longer and larger physiological disturbances (Gingerich and Suski 2012; Kieffer 2010). Larger largemouth bass (*Micropterus salmoides*) compared to smaller largemouth bass exhibited four times more muscle lactate even at 4 h, and a 25% greater concentration of plasma lactate compared to the smaller size class (Gingerich and Suski 2012). Lactate was shown to increase with increasing size, and muscle pH_i decreased with increasing size in rainbow trout (Ferguson et al. 1993). While these studies still account for only a small number of sportfish species subject to CAR, it appears that size-dependent stress responses may be a widely applicable phenomenon in teleosts.

The results of this study demonstrate that red drum conform to typical patterns of exhaustive exercise and recovery, which can inform on best CAR practices. Importantly, this work illuminated the differences in CAR risk factors between small and large juvenile red drum. In practical terms, red drum are typically managed both through slot size limitations (e.g. 20-28 in TL in Texas) and daily catch limits, which means that CAR protocols are incredibly important in managing the ecological impact of recreational angling. Importantly, many anglers pursue trophy “bull” reds that exceed slot size limitations and are thus subject to CAR. These species are also a crucial component of the spawning stock biomass, and as such represent a very important segment of the population of fisheries management. Our work suggests that these fishes may be the most at risk to the ancillary effects of CAR that result from slow recovery, such as predation. Through management practices, like slot size, smaller fish are able to quickly recover as demonstrated in this study and are given the chance to mature to reproductive age. Vecchio and Wenner 2007 examined how red drum behave after CAR events through tagging and looked at the immediate physiological response to being caught on the line for different times and types of gear used. As red drum fight on the line longer, the larger their immediate physiological disturbances, like lactate (Gallman et al. 1999). Understanding the foundational response to exhaustive exercise as well as responses to different angling practices enhance our overall knowledge and understanding of how red drum respond to angling pressures in the wild.

ACKNOWLEDGEMENTS

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TABLES AND FIGURES

		Control	0 h	0.5 h	1 h	3 h	6 h
ATP	<i>Sub-adult</i>	69.9 ± 22.0	13.0 ± 2.2	25.7 ± 5.25	14.3 ± 3.3	15.0 ± 3.4	40.9 ± 12.4
	<i>Adult</i>	171.1 ± 62.9	23.3 ± 1.3		79.5 ± 19.7	71.3 ± 34.3	
PCr	<i>Sub-adult</i>	16.2 ± 2.9	11.6 ± 2.4	20.7 ± 6.9	59.2 ± 22.7	15.8 ± 3.2	45.7 ± 12.4
	<i>Adult</i>	85.1 ± 32.8	25.6 ± 1.4		29.0 ± 1.9	119.1 ± 63.4	

Table 1: [ATP] and [PCr] in the white muscle at all time points for small and large juvenile red drum (*Sciaenops ocellatus*). ($P > 0.05$; small juveniles N = 6 - 8; large juveniles N = 5 - 6).

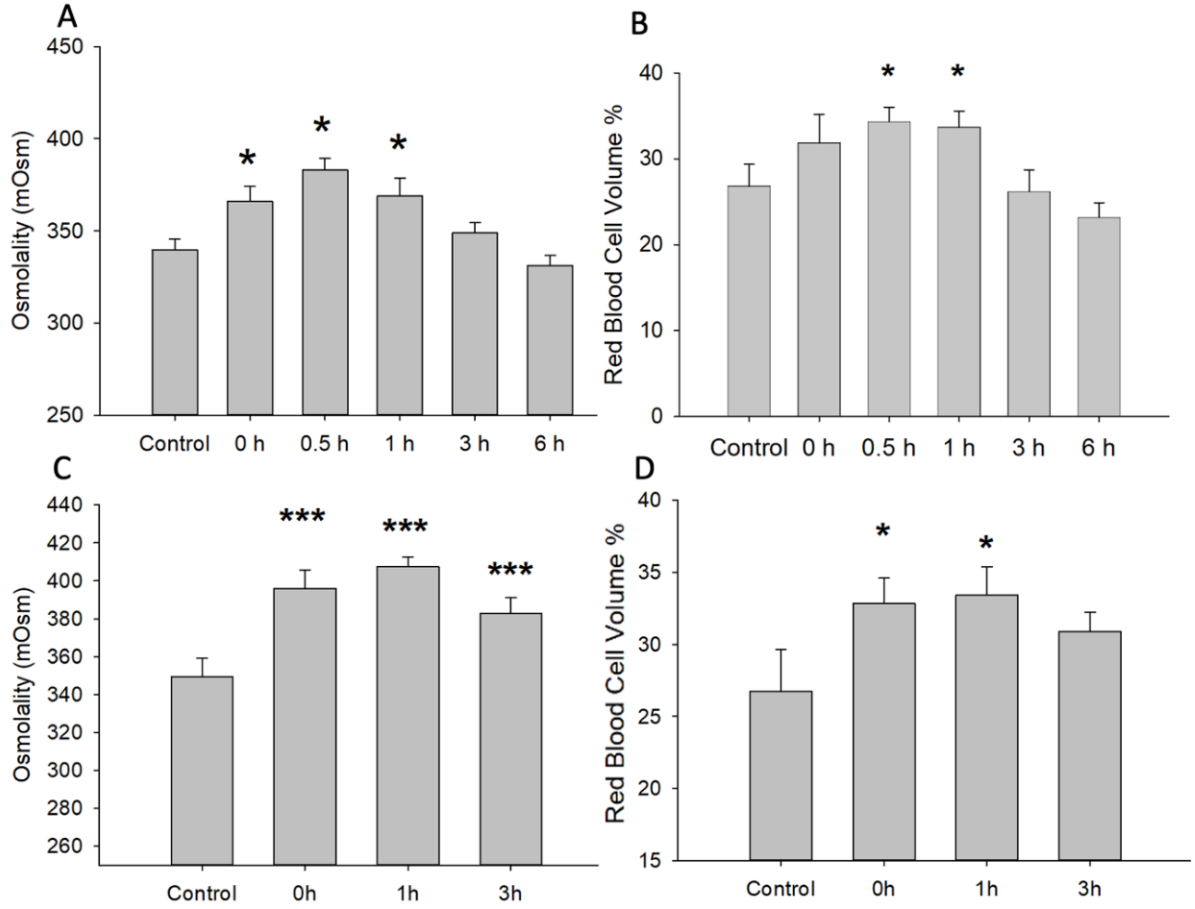


Figure 1: Small juvenile plasma osmolality (A), small juvenile hematocrit (B) ($P \leq 0.05$; $N = 8$), large juvenile plasma osmolality (C) and large juvenile hematocrit (D) ($P \leq 0.05$; $N = 6$ each) following exhaustive exercise in red drum (*Sciaenops ocellatus*). Significant differences from the 36 h no-exercise control time point as determined by ANOVA are denoted by an asterisk (* = $P \leq 0.05$; ** = $P \leq 0.001$; *** = $P \leq 0.001$).

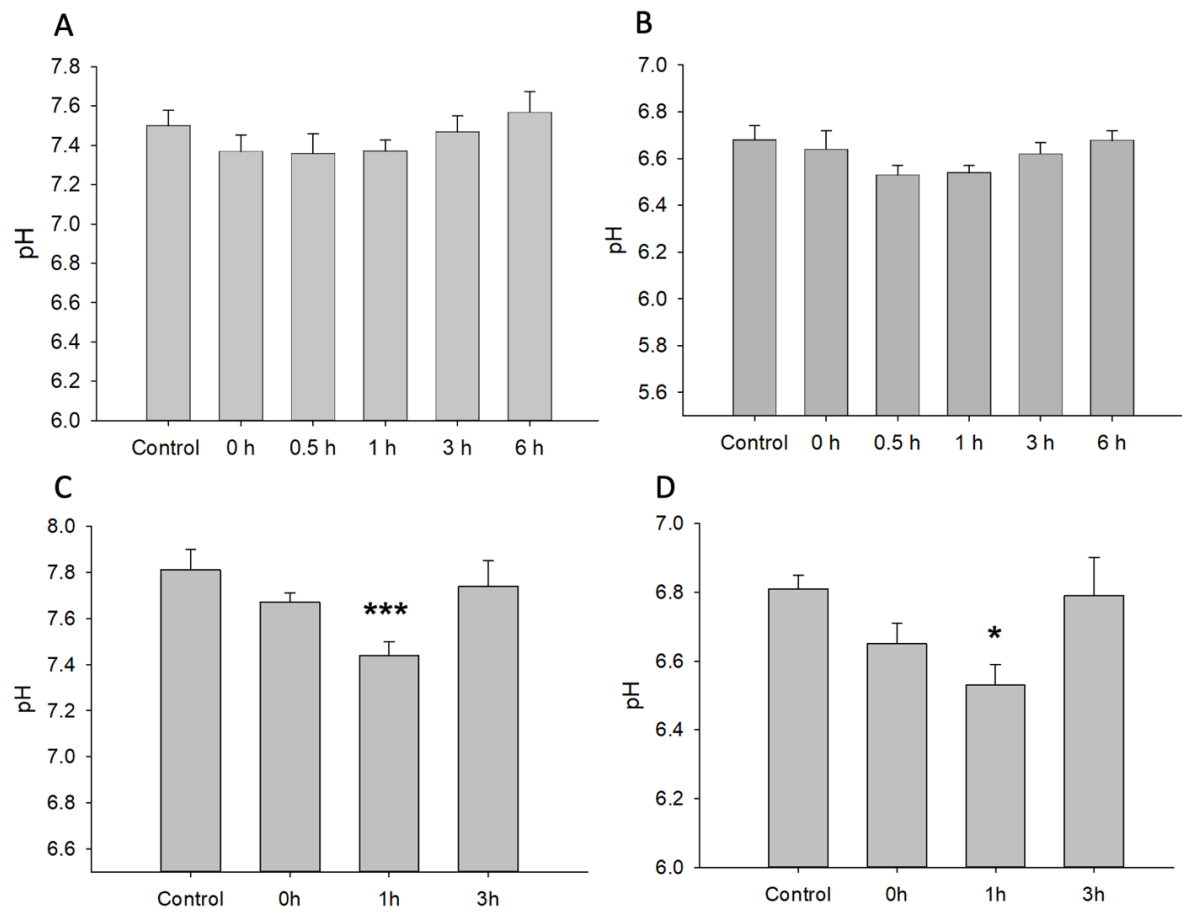


Figure 2: Small juvenile pHe (A) small juvenile pHi (B) ($P \leq 0.05$; $N = 8$), large juvenile pHe (C), large juvenile pHi (D) ($P \leq 0.05$; $N = 6$) following exhaustive exercise in red drum (*Sciaenops ocellatus*). Significant differences from the 36 h no-exercise control time point as determined by ANOVA are denoted by an asterisk (* = $P \leq 0.05$; ** = $P \leq 0.001$; *** = $P \leq 0.001$).

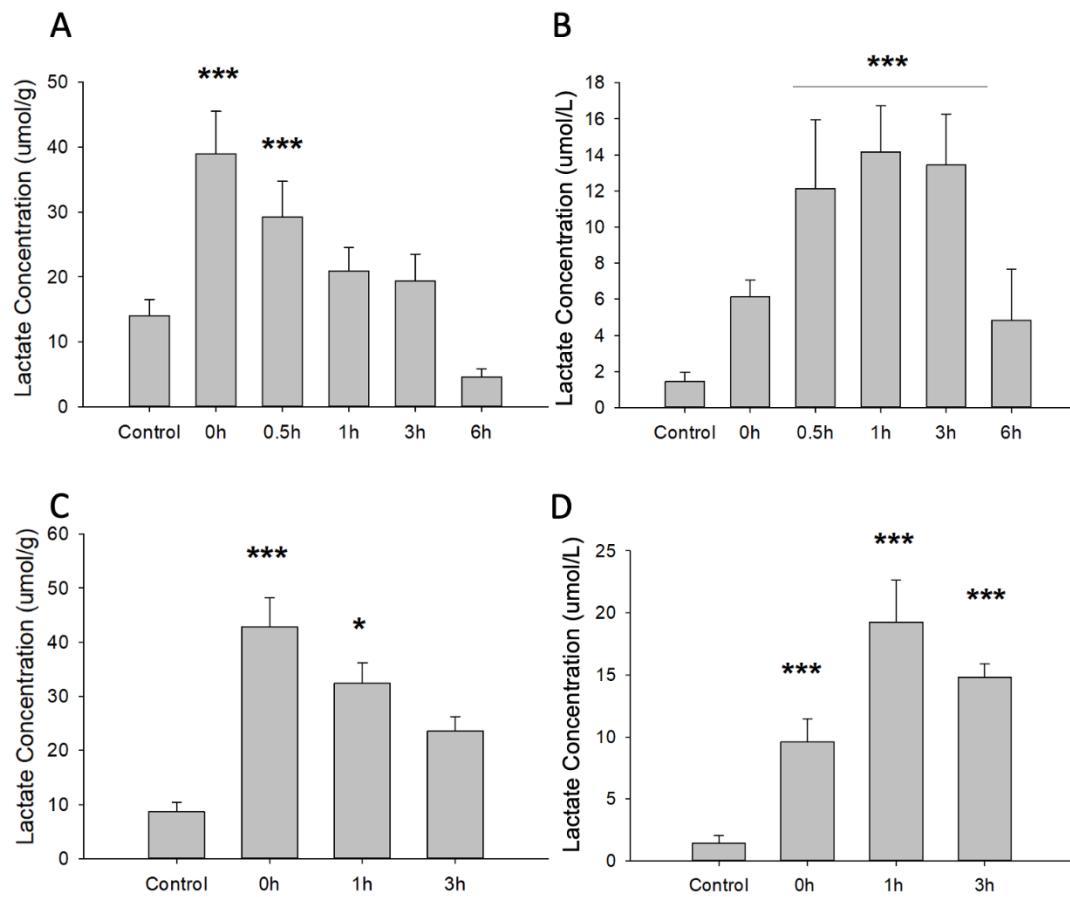


Figure 3: Small juvenile muscle lactate (A) small juvenile plasma lactate (B) ($P \leq 0.05$; $N = 8$), large juvenile muscle lactate (C), large juvenile plasma lactate (D) ($P \leq 0.05$; $N = 6$) following exhaustive exercise in red drum (*Sciaenops ocellatus*). Significant differences from the 36 h no-exercise control time point as determined by ANOVA are denoted by an asterisk (* = $P \leq 0.05$; ** = $P \leq 0.001$; *** = $P \leq 0.001$).

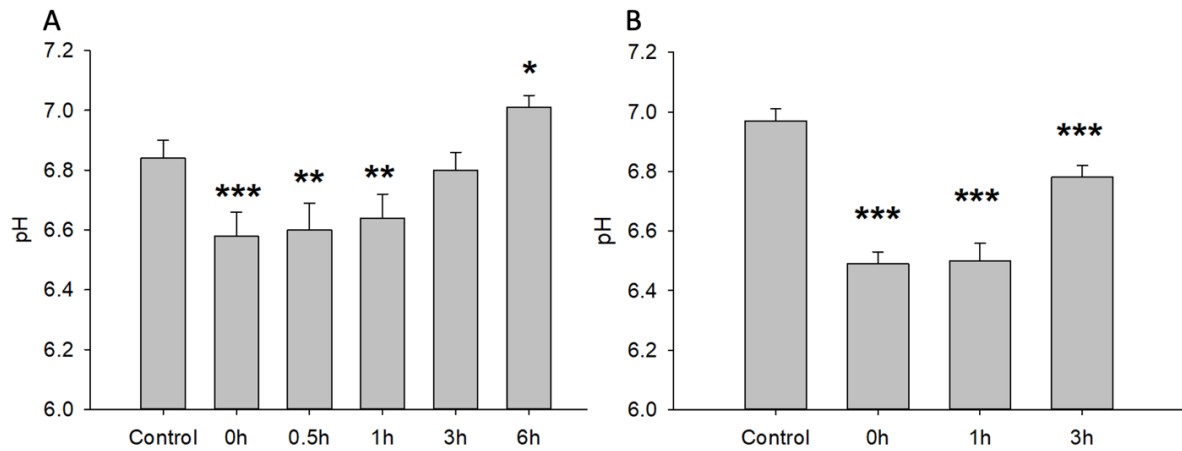


Figure 4: Small juvenile muscle pHi (A) ($P \leq 0.05$; $N = 8$) and large juvenile muscle pHi (B) ($P \leq 0.05$; $N = 6$) following exhaustive exercise in red drum (*Sciaenops ocellatus*). Significant differences from the 36 h no-exercise control time point as determined by ANOVA are denoted by an asterisk (* = $P \leq 0.05$; ** = $P \leq 0.001$; **** = $P \leq 0.001$).

Conclusions

PERSPECTIVES

Recreational fisheries and catch-and release (CAR) angling contributes to the Gulf of Mexico economy on the order of \$7 billion dollars annually, and this amount is increasing each year (FEU NOAA 2016). CAR angling is meant to help preserve fish populations, so that the ecosystem stays healthy and anglers will have access to popular species for generations to come. To this end, CAR is thought to be an effective conservation management tool (Wydoski 1977; Cooke et al. 2013). However, in recent years it has become apparent that effective management comes from species-specific regulations based on their physiological needs (Cooke et al. 2013).

This work found Atlantic tarpon (*Megalops atlanticus*) to be facultative air-breathers, and that the air-breathing behavior is stimulated by hypoxia but not exhaustive exercise. Furthermore, air-breathing did not aid Atlantic tarpon recovery from exhaustive exercise, but rather Atlantic tarpon recovery mirrors that of other well-studied fish species, like rainbow trout (*Oncorhynchus mykiss*) and largemouth bass (*Micropterus salmoides*) (Ferguson et al. 1993; Milligan and Wood 1986; Turner et al 1983; Gingerich and Suski 2012). Based on the observed physiological responses, this study did not find a need to have a specialized CAR protocols that address the air-breathing physiology of Atlantic tarpon. The second chapter of this thesis focused on the recovery from exhaustive exercise in another popular Texas sportfish species, the red drum (*Sciaenops ocellatus*), and whether recovery would differ between size classes. This work demonstrated that the general response of red drum was similar to that of other sportfish

(Ferguson et al. 1993; Milligan and Wood 1986; Turner et al 1983), and that larger red drum experienced stress of higher magnitude with slower recovery times. This is of particular importance in the context of the CAR fishing that focuses on “bull” red drum, which includes those reproductively active fish that exceed the angling slot size. These larger fish represent the most product component of the spawning stock biomass, which is a crucial segment of the population with respect to conservation management. This work demonstrates that it is important that anglers targeting these “oversized reds” follow best practices for CAR, which includes limiting fight time by using heavy gauge fishing line, reducing handling, and limiting air exposure.

FUTURE DIRECTIONS

In chapter 1, the 4-hour recovery time points for tarpon suffered from small sample size. This 4-hour time point would allow us to better describe the recovery processes of Atlantic tarpon and allow for more predictive power. A further step could be taken to add another treatment at 4 h recovery that incorporated 1-minute of air exposure before recovery. This is a common addition to CAR-style exercise recovery studies and seeks to determine if air exposure has an additional effect on the physiological disturbance. Since Atlantic tarpon are air-breathing fish, we hypothesize that a 1-minute air exposure would not have an additive effect on stress indices, however, this should be tested. To further confirm the results of my thesis, it would be beneficial to add plasma cortisol, a hormone released after stressful events, to the recovery profile. A spike in cortisol can play a direct role in the re-synthesis of glycogen in the muscle, among numerous other physiological

implications (Milligan 2003). These steps would allow us to better understand the impacts of CAR techniques on this facultative air-breathing marine fish. It would also be informative to directly assess blood collected from Atlantic tarpon and red drum caught on rod-and-reel in the field. Laboratory experiments are unlikely to perfectly replicate the conditions that exist in the natural environment, and this is especially true for an exhaustive exercise protocol versus a true CAR event. Quantifying the true physiological stress following CAR would allow us to properly contextualize the results of this study.

LIMITATIONS

In 2018 Atlantic tarpon were categorized as a vulnerable species on the ICUN Red List, meaning the Atlantic tarpon removed from the wild should be kept to a minimum. Due to this and the challenging nature of fish collections for sub-adult Atlantic tarpon, the sample size for the treatments were kept low ($N = 5$). Unfortunately, there were additional limitations placed on Atlantic tarpon collections by COVID-19, which stopped collection trips two months earlier than originally planned. These limitations significantly affected the completion of the 4 h recovery time point in Chapter 1, where two of the recovery time points, 4 h with air access and 4 h without air access, had an $N = 2$ and $N = 3$, respectively. While I believe the findings of the study would not be different had the sample size been increased to 5, this remains a limitation of the current work.

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Vita

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