Comparative Biochemistry and Physiology, Part B 266 (2023) 110844

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

The effects of size on exhaustive exercise and recovery in a marine sportfish, the red drum (*Sciaenops ocellatus*)

Leighann Martin, Benjamin Negrete Jr, Andrew J. Esbaugh *

Marine Science Institute, The University of Texas at Austin, Port Aransas, TX 78373, USA

ARTICLE INFO

Edited by: Chris Moyes

Keywords:
Lactate clearance
Angling
Catch and release
Anaerobic energy expenditure
Recreational fisheries

ABSTRACT

Recreational angling is an economically important activity in many communities around the world. One conservation strategy adopted to offset the population-level consequences of recreational angling is "catch-and-release" (CAR), which is the act of returning fish to the environment following an angling event. While an expansive literature has helped to generalize CAR best practices, species-specific validation of recovery profiles remains a crucial component of species-specific angling guidance. This study sought to define the injury and recovery profiles in the plasma and white muscle following exhaustive exercise in two size classes of a common Gulf of Mexico sportfish, the red drum (*Sciaenops ocellatus*). The two sizes included a "small" (20–30 cm) and "slot" size (51–74 cm), the latter of which is a common angling target. Both size classes showed a characteristic injury profile that consisted of significantly elevated muscle and plasma lactate, plasma osmolality and haematocrit, as well as decreased muscle ATP and phosphocreatine, and lowered plasma and muscle pH. In small fish, muscle metabolites returned to control values by 1 h post-exercise and plasma metabolites returned to control between 3 and 6 h post-exercise. In contrast, slot sized fish had recovery periods of $\geq$3 h for all metabolites. The maximum injury effect size was also greater in the slot size class. These data suggest that while red drum conform to typical patterns of post-exercise recovery, larger trophy-sized fish may be more at risk to the ancillary effects of exhaustive exercise owing to greater exercise injury and slower recovery rates.

1. Introduction

Recreational angling is a tremendously popular activity and provides an important economic contribution to many communities. For example, recreational fisheries in the Gulf of Mexico (GoM) are a multi-billion dollar per year industry (National Marine Fisheries Service, 2018). Nonetheless, angling activities have been implicated in fish mortalities and declining sportfish populations (Cooke and Cowx, 2004). As such, policy makers are tasked with balancing the economic contributions of recreational angling with conservation strategies that will ensure the long-term health of sportfish populations. Many conservation strategies have been implemented to limit the effect of recreational angling on fish populations including seasonal closures, catch-limits, and "slot" size regulations that limit the size of fish that can be kept to a narrow range. Intrinsic to many of these strategies is the concept of catch-and-release (CAR) angling, which is the act of returning fish to the environment following an angling event. When performed in accordance with "best practices" CAR will cause minimal physiological disturbance to a fish and thus communities can reap the economic benefits of recreational angling while limiting angling-based population impacts (Arlinghaus et al., 2007; Cooke and Cowx, 2004; Cooke et al., 2013; Cooke and Suski, 2005). While a plethora of studies have helped to refine the general best-practices for CAR (e.g. Arlinghaus et al., 2009; Cooke et al., 2013; Danylchuk et al., 2014; Danylchuk et al., 2007; O'Toole et al., 2010) it is important to recognize that individual species may have different tolerances to CAR, and thus species-specific validation of CAR recovery profiles are a crucial component of species-specific angling guidance (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 (reviewed by Arlinghaus et al., 2007; Cooke et al., 2013). Yet, from a physiological perspective, a central factor in the efficacy of CAR protocols is the intensity of the exercise-induced metabolic 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 (e.g. Arlinghaus et al., 2009; Booth et al., 1995; Danylchuk et al., 2014; Donaldson et al., 2014;

* Corresponding author.
E-mail address: a.esbaugh@austin.utexas.edu (A.J. Esbaugh).

https://doi.org/10.1016/j.cbpb.2023.110844
Received 29 November 2022; Received in revised form 10 February 2023; Accepted 20 February 2023
Available online 23 February 2023

Gingerich et al., 2007; Kieffer et al., 1994; Landsman et al., 2011; Suski et al., 2007; White et al., 2008). In general, exhaustive exercise utilizes burst swimming behavior, which relies on glycolytic white muscle (Kieffer, 2000; Kieffer, 2010; Milligan, 1996). 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; Kieffer, 2010; Milligan, 1996). Furthermore, anaerobic metabolism results in a build-up of lactate in muscle and plasma, which often occurs in conjunction with a metabolic acidosis (e.g. Esbaugh et al., 2009; Kieffer et al., 1994; Kieffer, 2000; Milligan, 1996; Milligan and Wood, 1986a, 1986b; Turner et al., 1983; Wood, 1991). 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. Importantly, post-exercise predation, presumably due to a fish's state of exhaustion, is increasingly being recognized as a major source of species susceptibility following CAR (Arlinghaus et al., 2007; Cooke et al., 2013; Danylchuk et al., 2007).

Another factor that can affect exercise "injury" and recovery, which has received relatively little 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., 2016), and an increased amount of anaerobic fuel (Kieffer et al., 1996), which collectively may infer greater recovery potential. In fact, large freshwater drum exhibited smaller exercise-induced elevations in plasma glucose as compared to smaller fish (Card and Hasler, 2021). Yet, larger salmonids have shown increased PCr consumption, elevated lactate production, and a greater exercise-induced acid-base disturbance (Goolish, 1991; Kieffer et al., 1996; Wakefield et al., 2004). 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. In fact, individual responses to exhaustive exercise are increasingly being highlighted with respect to fisheries conservation (e.g. Killen et al., 2016; Ward et al., 2016).

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*). The red drum is a popular sportfish in the GoM and southeastern United States (National Marine Fisheries Service, 2018) that was nearly fished to extinction prior to the implementation of conservation strategies throughout the United States in the 1980s. Current conservation efforts in the United States are subject to both federal and state level oversight which include limits on commercial fishing, daily bag limits, and slot-size regulations. Despite the conservation interest in red drum there has yet to be a species-specific study detailing the metabolic time course of exhaustive exercise and recovery. To this end, we first performed a thorough exercise and recovery time course using typical aquaculture reared laboratory sized red drum which detailed an initial window of recovery for blood and white muscle biochemical metabolites. A second series of experiments used a truncated recovery time course to explore the potential allometric responses in exhaustive exercise and recovery in red drum while simultaneously providing applicable data on the wild caught slot-sized red drum targeted by anglers.

2. Methods

2.1. Animal husbandry

All experiments were approved by the University of Texas at Austin's Institutional Animal Care and Use Committee (IACUC). Lab-sized "small" juvenile red drum ($N = 48$; 20.3–30.5 cm TL; 159.1 ± 26.1 g; mean ± 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 weekly for pH, and daily for ammonia, salinity, and temperature. 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, and fish were acclimated to the facility for at least 2-weeks prior to experimentation. Fish were fed commercial pellets (Aquamax, Purina) daily to satiation, but were starved for at least 48 h prior to experimentation.

"Slot-sized" red drum ($N = 24$; 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 except for a 48 h fasting period prior to experimentation.

2.2. Exhaustive exercise and recovery protocol

Both slot-sized 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 min, or until exhaustion. This was followed by one minute of air exposure, which was performed by holding the exhausted fish in a net out of water. Note that this exhaustion protocol has been used extensively in red drum for the successful determination of maximum metabolic rate and elevated post-exercise oxygen consumption (Ackerly and Esbaugh, 2020, 2021; Ern et al., 2016; Khursigara et al., 2018, 2021; Negrete Jr. et al., 2022). 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). Slot-sized 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 slot-sized 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 a buffered MS-222 bath (250 mg/L; 500 mg/L $NaHCO_3$), euthanized by spinal transection, 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.

2.3. Physiological and biochemical analyses

Haematocrit (Hct) was determined using heparinized capillary tubes filled with 5 uL of whole blood and centrifuged for 1 min (StatSpin MP). The remaining blood was centrifuged for 2 min at 10,000 × *g* (gravity) to separate red blood cells (RBC) from plasma. Half of the plasma was flash frozen and stored at −80 °C until further use, while the other half was used for the determination of plasma osmolality and pH (see below). Plasma osmolality was determined using a vapor pressure osmometer (Wescor; VAPRO 5520). Plasma pH (pH_e) was measured using a micro pH electrode (Accumet, Fisher Scientific) placed in a thermostated bath (26 °C). Muscle pH_i was measured using the homogenate method of Portner et al. (1990). Briefly, muscle was ground to powder on liquid N_2 after which 100 mg was added to approximately 5 volumes of 160 mM KF, 6 mM Na_2NTA solution in a 600 μL microfuge tube with no remaining headroom. The slurry was briefly vortexed and centrifuged at

Table 1
Statistical output detailing the effects of size class on exhaustive exercise stress indicators in fish at rest and at the time point representing their peak exercise "injury". All indicators were first assessed using a 2-way ANOVA (size class x exercise status). In cases where the interaction between size class and exercise status was not significant, a series of Bonferroni corrected *t*-tests were used to specifically test the effect of size class on resting and exercised stress indicators. Values denote *p*-values for the effects of size within resting and exercised individuals, as well as the interaction term. Significant differences are denoted in bold.

	Interaction	Rest	Exercise	Statistical Test
Plasma lactate	0.503	0.436 [1]		2-way ANOVA
Muscle lactate	NA	**0.018**	0.087	Bonferroni corrected t-test ($\alpha = 0.025$)
Muscle pH_i	0.016	**0.042**	0.139	2-way ANOVA
Muscle ATP	NA	**<0.001**	0.041	Bonferroni corrected t-test ($\alpha = 0.025$)
Muscle PCr	NA	**0.013**	0.345	Bonferroni corrected rank sum test ($\alpha = 0.025$)
Plasma pH	0.072	**0.002** [1]		2-way ANOVA
Haematocrit	0.787	0.756 [1]		2-way ANOVA
Osmolality	0.042	0.330	**<0.001**	2-way ANOVA

[1] When no significant interaction is observed only the main size effect p-value is denoted. Note that in these instances the Bonferroni corrected t-test was also not significant for the effects of size, both at rest and exercise time points.

13,000 *g* for 15 s, after which the supernatant pH was measured as described above.

Plasma lactate, tissue lactate, phosphocreatine (PCr), and adenosine triphosphate (ATP) concentrations were all measured on frozen samples following the enzymatic methods of Lowry and Passonneau (1972) and Booth et al. (1995) using a plate spectrophotometer. Briefly, tissue samples were ground to powder using a mortar and pestle over liquid N_2, followed by deproteination with $HClO_4$, after which metabolite supernatants were isolated via centrifugation and neutralized (2 M KOH, 0.4 M KCl, 0.3 M Imidazole) to pH 7–8. Muscle lactate was determined by the oxidation of NADH to NAD^+ in the presence of hydrazine (2 mM). Muscle ATP was measured from the breakdown of glucose-6-phosphate in the presence of the enzymes hexokinase and glucose-6-phosphate dehydrogenase. PCr concentration was measured as the breakdown of PCr in the presence of creatine kinase. Plasma was deproteinated using $HClO_4$, mixed, centrifuged, and the supernatant neutralized (2.5 M K_2CO_3) and plasma lactate concentration ([lactate]) was determined using a spectrophotometric method of detecting the reduction of NADH to NAD^+.

2.4. Statistical analyses

All physiological and biochemical analyses were analyzed using a one-way analysis of variance (ANOVA) followed by Holm-Sidak post-hoc test to identify pairwise statistical differences. A Shapiro-Wilk test was used to verify normality and a Brown-Forsythe test was used to assess equal variance. In the event of non-normal data distribution a natural log transformation was performed. In the instances that this transformation did not normalize the data, a Kruskal-Wallis one-way ANOVA on rank was performed. A Grubb's test was performed to identify outliers, which were removed from the dataset. To compare the

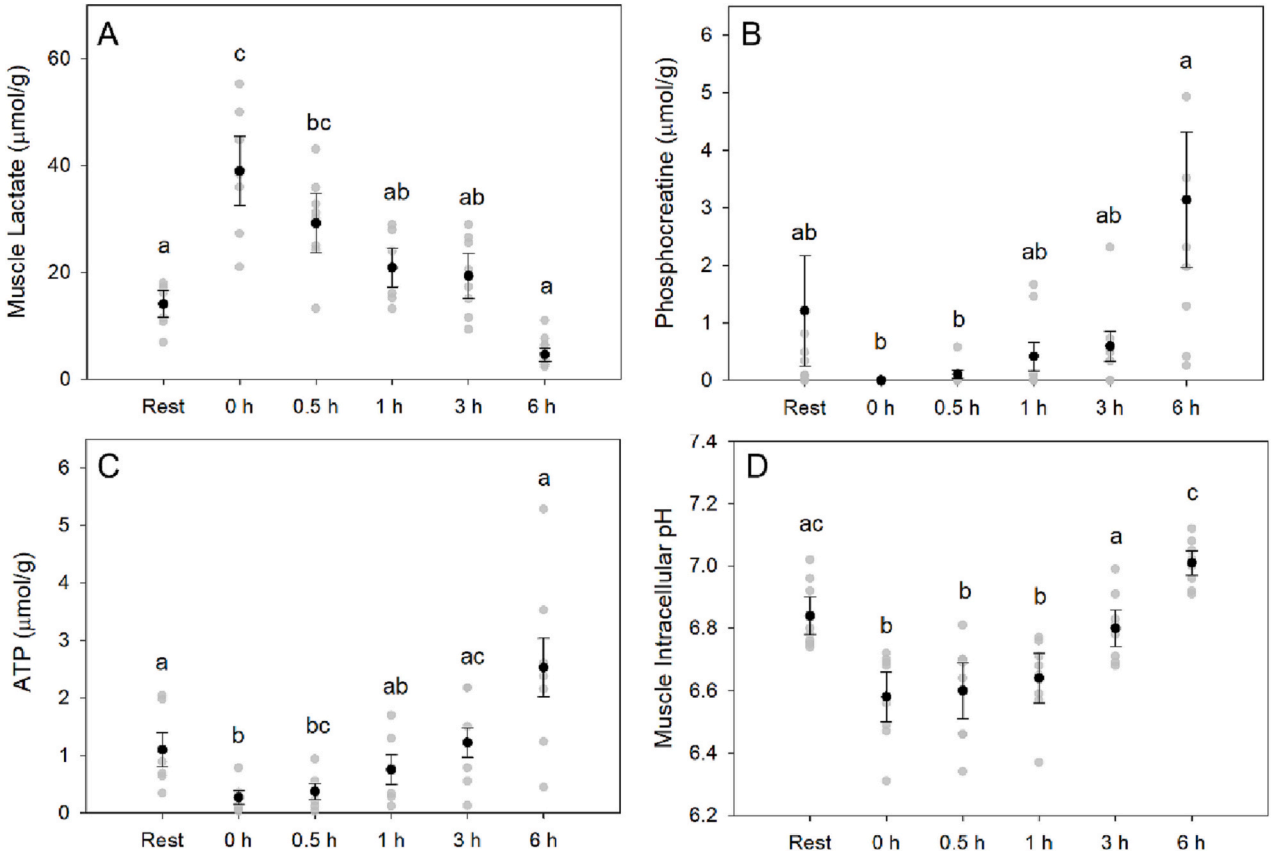

Fig. 1. The effects of exhaustive exercise and time course of recovery on white muscle in small size class red drum ($N = 48$; 20.3–30.5 cm total length; 159.1 ± 26.1 g body mass). The mean and SEM are denoted by the black dots and error bars and individual raw data points are represented by the gray dots. Rest denotes the non-exercised control fish and the time points represent the duration of post-exercise recovery. The specific exercise stress indicators in white muscle are as follows: A) muscle lactate ($N = 7$–8 per time point), B) phosphocreatine ($N = 8$), C) ATP ($N = 6$–8) and D) intracellular pH ($N = 8$). Significant differences are noted by the different letters ($P \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

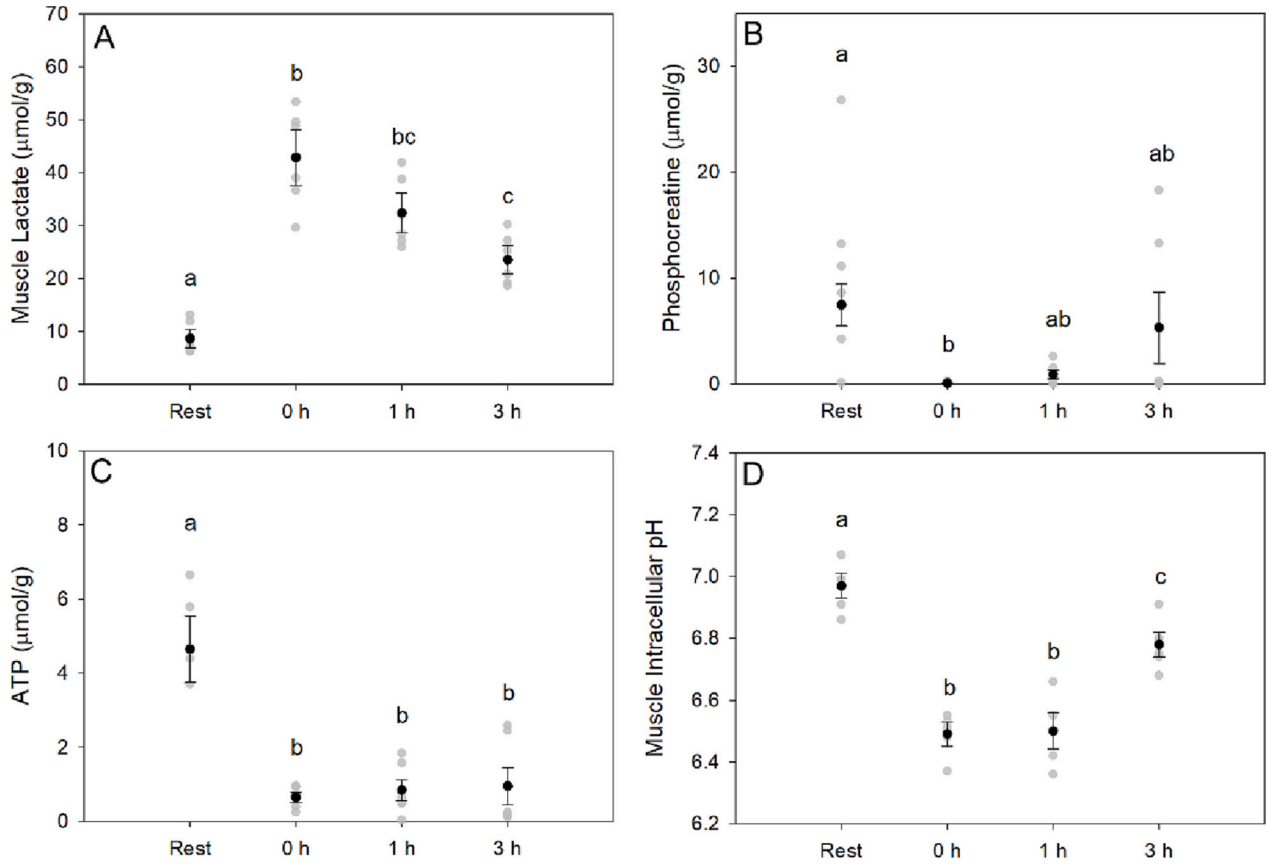

Fig. 2. The effects of exhaustive exercise and time course of recovery on white muscle in large slot size class red drum ($N = 24$; 50.8–73.7 cm total length; 3.0 ± 0.4 kg body mass). The mean and SEM are denoted by the black dots and error bars and individual raw data points are represented by the gray dots. Rest denotes the non-exercised control fish and the time points represent the duration of post-exercise recovery. The specific exercise stress indicators in white muscle are as follows: A) muscle lactate ($N = 6$ per time point), B) phosphocreatine ($N = 6$), C) ATP ($N = 5$–6) and D) intracellular pH ($N = 6$). Significant differences are noted by the different letters ($P \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effects of exercise "injury" between small and slot-sized fishes, we calculated Cohen's d effect sizes for all metabolites between the resting treatment and the time point representing the peak response. The Cohen's d effect sizes for small and slot-sized fish were then compared using a paired *t*-test, whereby the pair was defined as effect size in a single metabolite. A second series of tests were also performed to assess whether changes in effect size were driven by size-based differences in resting or exercised time points (Table 1). In this case, a 2-way ANOVA was first employed, but in cases where a non-significant interaction between size and exercise status precluded the desired comparison (i.e. size x exercise status) a Bonferroni corrected *t*-tests ($\alpha = 0.025$) were performed to compare between small and slot-sized individuals at rest and at the point of peak exercise stress (i.e. two t-tests). All statistical tests were performed using Sigma Plot version 13, and the fiducial level of significance is $P \leq 0.05$. Cohen's d effect sizes were calculated manually as the difference in means divided by the pooled standard deviation.

3. Results

3.1. White muscle metabolites and pH

Three of the four measured muscle metabolites were significantly different from resting values immediately post-exercise in the small sized red drum (Fig. 1), with only PCr showing no significant decrease as a consequence of exercise. The immediate post-exercise timepoint also represented the peak response of all metabolites in the white muscle. Muscle lactate (Fig. 1A), PCr (Fig. 1B) and ATP (Fig. 1C) all recovered to resting values by 1 h post-exercise, while muscle pH_i remained significantly below resting values until the 3 h time point (Fig. 1D). A similar profile was observed in the slot-sized red drum with the immediate post-exercise interval showing the peak deviation from resting values in muscle lactate, PCr, ATP and pH_i (Fig. 2A, B, C, D, respectively). Only muscle PCr showed full recovery during the 3 h recovery window. Muscle lactate and pH_i showed a significant reduction in the magnitude of the exercise response by 3 h; however, neither metric fully recovered to resting values. Muscle ATP did not show significant evidence of recovery throughout the 3 h time window.

3.2. Blood exercise stress indicators

The time course of exhaustive exercise and recovery for blood metabolites of small and slot-sized red drum can be found in Figs. 3 and 4, respectively. In small red drum the earliest stress response was noted in plasma osmolality (Fig. 3A), which was significantly elevated above controls immediately post-exercise, peaked at 0.5 h post-exercise and returned to control levels by 3 h post-exercise. Both haematocrit (Fig. 3B) and plasma lactate (Fig. 3C) exhibited a delayed response with elevation not detected until 0.5 h post-exercise. Haematocrit also peaked at 0.5 h post-exercise and returned to control by 3 h, while plasma lactate peaked at 1 h post-exercise and did not return to control levels until 6 h post-exercise. Interestingly, plasma pH showed significant variation within the data set based on ANOVA analysis (Fig. 3D; $P = 0.025$) yet post-hoc analysis was unable to detect pairwise differences.

In slot-sized red drum the time course of detectable metabolic responses in the blood was generally faster than observed for smaller red

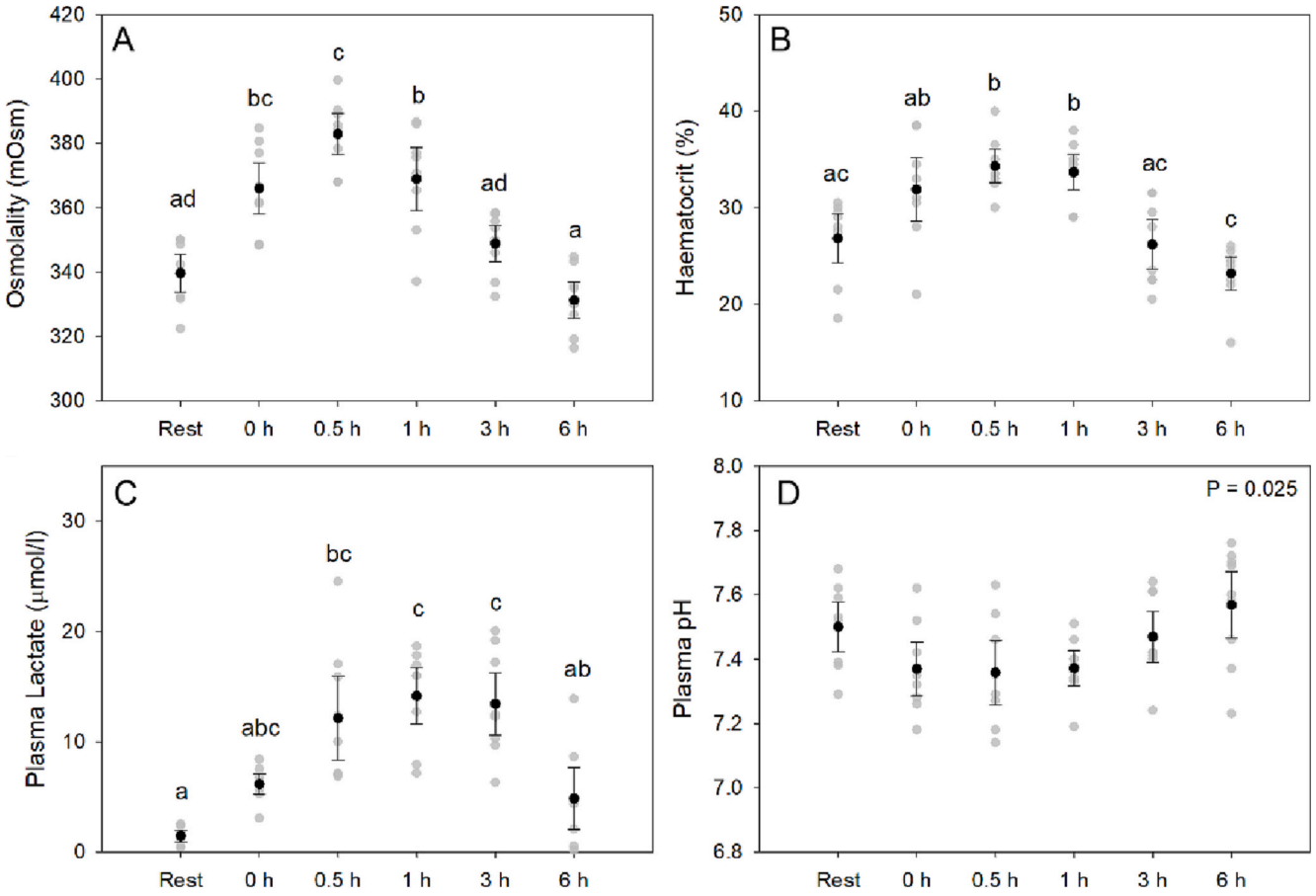

Fig. 3. The effects of exhaustive exercise and time course of recovery on blood stress indices in small size class red drum ($N = 48$; 20.3–30.5 cm total length; 159.1 ± 26.1 g body mass). The mean and SEM are denoted by the black dots and error bars and individual raw data points are represented by the gray dots. Rest denotes the non-exercised control fish and the time points represent the duration of post-exercise recovery. The specific exercise stress indicators in the blood are as follows: A) plasma osmolality ($N = 8$ per time point), B) haematocrit ($N = 8$), C) plasma lactate ($N = 8$) and D) plasma pH ($N = 8$). Significant differences are noted by the different letters ($P \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

drum. Specifically, osmolality, haematocrit and plasma lactate (Fig. 4A, B, and C, respectively) were all significantly different from resting controls immediately post exercise. All three measures peaked at 1 h post-exercise, but only haematocrit returned to control values by 3 h post-exercise. Plasma pH was significantly lower than resting values at 1 h post-exercise and had recovered by 3 h post-exercise (Fig. 4D).

3.3. Comparison of exhaustive exercise indicators between size classes

Two approaches were used to assess whether the magnitude of the exercise-induced disturbances in exhaustive exercise indicators were consistently different between size classes. We first used a Cohen's d effect size approach to quantitatively compare the magnitude of exercise injury in small and slot-sized red drum. The peak effect size for each metabolite (i.e. the time point with the largest mean difference relative to the resting mean) for each size class is shown in Fig. 5. The larger slot-sized red drum had consistently greater effect sizes than those found in smaller fish (paired *t*-test; $P = 0.024$; $N = 8$). We then used a 2-way ANOVA approach using only the resting values and the time point that represented the peak response for each stress indicator to assess whether the overall change in effect size was due to deviation in resting values or peak stress values between size classes (Table 1). Neither plasma lactate nor haematocrit showed a significant difference on the basis of size, which corresponds to the small differences in effect size between small and slot-sized individuals (Fig. 5). Significant differences in muscle lactate, ATP and PCr were observed only within the resting time points, while significant differences in osmolality were only observed within the peak stress time point. For muscle pH_i there was no significant interaction between time point and size class, however, a significant main effect of size was observed. This demonstrates that the differences in observed effect size were the product of size effects on both resting and peak stress time points.

4. Discussion

Here we sought to provide species-specific information related to exhaustive exercise and recovery in the blood and white muscle of red drum to help inform on the efficacy of catch-and-release for this valuable sportfish. Our results indicate that red drum conform to the typical patterns of exhaustive exercise and recovery. However, there was a notable difference between the smaller lab sized fish and the slot-size typically targeted by anglers, both with respect to the magnitude of exercise induced disturbance and the time for recovery.

The effects of exhaustive exercise on the glycolytic white muscle of fishes is well documented (see reviews Cooke et al., 2013; Kieffer, 2000; Milligan, 1996) and involves the consumption of energy stores (e.g. ATP and PCr) and energetic substrates (e.g. glycogen) while producing metabolic end-products such as lactate and H^+. The general responses of red drum conformed to expectation whereby ATP and PCr were almost entirely consumed immediately post-exercise, which coincided with a significant increase in muscle lactate and significant decline in pH_i. The peak response observed in all four stress indicators was similar between the two size classes of red drum; however, several indicators in the larger slot-sized red drum were slower to recover. The smaller red drum

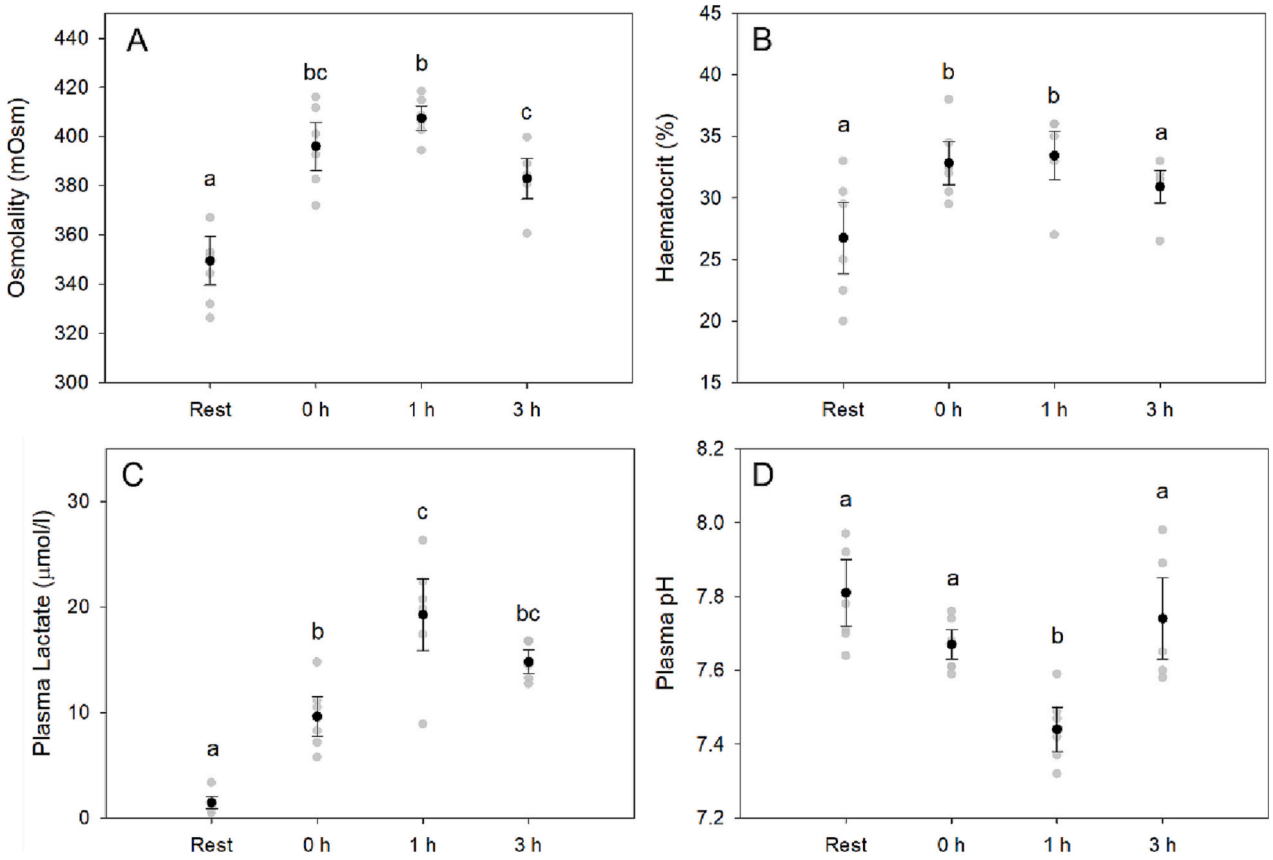

Fig. 4. The effects of exhaustive exercise and time course of recovery on blood stress indices in large slot size class red drum ($N = 24$; 50.8–73.7 cm total length; 3.0 ± 0.4 kg body mass). The mean and SEM are denoted by the black dots and error bars and individual raw data points are represented by the gray dots. Rest denotes the non-exercised control fish and the time points represent the duration of post-exercise recovery. The specific exercise stress indicators in the blood are as follows: A) plasma osmolality ($N = 5$–6 per time point), B) haematocrit ($N = 6$), C) plasma lactate ($N = 5$–6) and D) plasma pH ($N = 5$–6). Significant differences are noted by the different letters ($P \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

showed evidence of full recovery – as demonstrated by significant differences from the 0 h time point and non-significant differences from resting values – in muscle lactate, ATP and pH_i by 3 h post-exercise. The PCr values were statistically similar to both resting and exhausted values at 3 h post-exercise, but had risen significantly above the 0 h time point by 6 h post-exercise. Note that the PCr trends should be viewed with some caution owing to the very low resting values in small sized red drum (e.g. Gingerich and Suski, 2012; Milligan, 1996). Larger red drum did not significantly recover white muscle ATP during the 3 h recovery window, and neither muscle lactate nor pH_i had fully recovered to resting values during the experimental period. PCr showed a qualitatively similar pattern between size classes as the values at 3 h post-exercise were not significantly different from values at rest or immediately post-exercise.

In the blood, plasma lactate did not rise above resting levels until 30 min post-exercise. This response peaked at 1 h post-exercise and did not return to within range of resting values until 6 h post-exercise. The majority of lactate produced in the muscle of fishes is retained and directly recycled to glycogen during recovery (Milligan and Wood, 1986b; Turner and Wood, 1983; Turner et al., 1983; Wardle, 1978). The plasma lactate release following exercise represents a relatively small pool relative to that in the muscles, and is generally considered to be the result of leak as opposed to active clearance. Interestingly, the scope of the lactate leak into the plasma was greater in the larger fish, as evident by the significant elevation found immediately post-exercise in larger fish. From a white muscle recovery perspective the lactate found in the plasma is equivalent to lost energetic substrate, as plasma lactate is thought to be cleared by uptake into highly oxidative tissues (e.g. the heart and liver)(Milligan and Girard, 1993). The plasma of larger red drum exhibits a greater concentration of lactate as a consequence of exercise, which when combined with the 20-fold higher absolute blood volume suggests that these fish may require greater external (i.e. non-white muscle) replenishment of carbohydrate stores than smaller fish. Unfortunately, we did not measure muscle glycogen in this study so it is difficult to say whether the greater lactate loss from white muscle is physiologically meaningful.

Three additional blood indicators of exercise stress were also evaluated. The well-established increase in plasma osmolality following exhaustive exercise has been attributed to intracellular homeostatic processes that alter intra- and extracellular fluid balance (Wang et al., 1994) as well as the osmorespiratory compromise that states exercise-induced hyperventilation would increase water loss across the gills of marine fishes (Byrne et al., 1972; Ern and Esbaugh, 2018). Regardless of the cause, exercise resulted in an immediate significant elevation in plasma osmolality in both size classes of fish, but again the larger fish had a more prolonged recovery period. In contrast, the patterns observed for haematocrit and plasma pH were generally similar between the two size classes, with the exception that significant increases in haematocrit were observed immediately post-exercise in larger fishes. The exercise induced decrease in plasma pH is the result of a mixed systemic acidosis caused by the exercise-induced increase in blood CO_2 and the release of metabolically produced intracellular H^+ into the plasma (Milligan and Wood, 1986a, 1986b; Wood, 1991). The extracellular acidification subsequently impacts haematocrit when the red blood cell protects pH_i through Na^+ H^+ exchange causing cell swelling (Nikinmaa et al., 1990). As such, it is not surprising that the time course

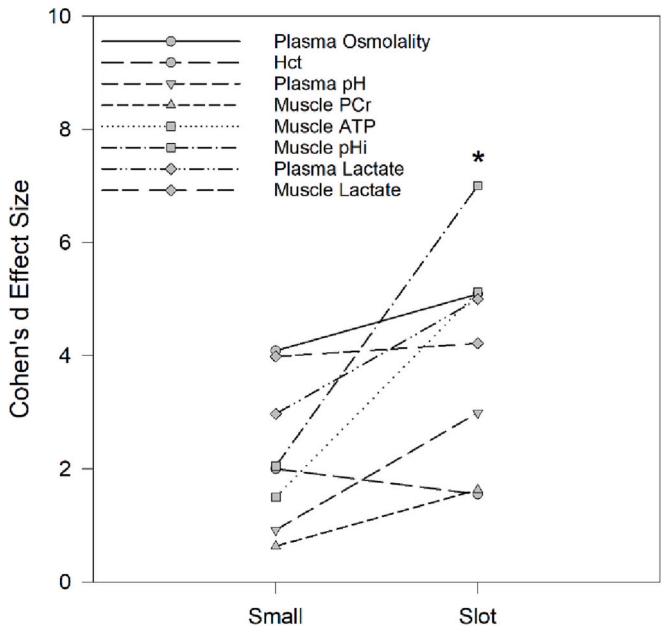

Fig. 5. The calculated Cohen's d effect size of all exercise stress indicators between the rest and peak response for small (N = 48; 20.3–30.5 cm total length; 159.1 ± 26.1 g body mass) and large slot-sized red drum (N = 24; 50.8–73.7 cm total length; 3.0 ± 0.4 kg body mass). A significant difference in the cumulative effect sizes for all stress indicators is denoted by an asterisk as determined by a paired t-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of plasma pH and haematocrit post-exercise recovery mirror each other.

Several previous studies have suggested that teleosts may exhibit allometric relationships with exhaustive exercise and subsequent recovery. Early work in rainbow trout first demonstrated that longer fish produced higher intracellular lactate and H^+ loads following a 5-min exercise challenge (Ferguson et al., 1993). The white muscle anaerobic energy expenditures (formula = 1.5*Δlactate + ΔATP + ΔPCr) associated with exhaustive exercise was higher in larger sized fishes for Atlantic salmon, brook trout, and rainbow trout. Data for largemouth bass are more equivocal as studies have shown both a similar trend to that of salmonids (Gingerich and Suski, 2012) or no effect of size (Kieffer et al., 1996), and hematological studies in sturgeon following exercise also showed no effect of size (Beyea et al., 2005).

Several lines of evidence from our work support the notion of an allometric relationship for exhaustive exercise and recovery in red drum. First, the larger red drum showed either earlier onset or a more prolonged response in several stress indicators including muscle and plasma lactate, muscle ATP, muscle pH_i, plasma osmolality, and haematocrit. The second piece of evidence is apparent when the peak exercise responses for each stress indicator are evaluated cumulatively using Cohen's d effect size. It is clear that the effect size (i.e. size of the exercise-induced response) was significantly greater in larger fish than smaller fish (Fig. 5). A similar conclusion can be drawn if the cumulative mean responses within the muscle are interpreted as anaerobic energy expenditures, whereby small fish exhibit expenditures of 39.6 μmol/g while larger fish exhibit values of 66.3 μmol/g. It is important to acknowledge that these pieces of evidence can be equally affected by differences in resting phenotype as by exercise and recovery. In fact, statistical analysis demonstrated that all differences noted in the muscle are derived from changes in resting phenotype (Table 1). The fact that comparable sampling procedures were used for both size classes may indicate that these resting phenotypic differences are physiologically relevant, although we acknowledge the resting PCr values are particularly suspect in relation to the literature (e.g. Milligan, 1996) likely owing to the labile nature of PCr in vivo. Furthermore, estimates of white muscle lactate clearance rates that are independent of resting phenotypes also demonstrate a difference between large and small fish. Over the first hour post-exercise the small fish exhibit a lactate clearance rate nearly double that of the larger fish (18.08 vs 10.45 μmol/g, respectively), which provides additional support that the larger fish are more greatly impacted by exhaustive exercise than smaller fish.

Overall, the results of this study demonstrate that red drum conform to typical patterns of exhaustive exercise and recovery. Importantly, this work illuminated the allometric differences in anaerobic exercise and recovery factors across a 20-fold size range in red drum. From an applied perspective, red drum are typically managed both through slot size limitations (e.g. 51–71 cm total length in Texas) and daily catch limits, which means that CAR protocols and recommended best-practices are important tools for managing the ecological impact of recreational angling. Importantly, many anglers pursue trophy "bull" red drum that exceed slot size limitations and are exclusively limited to CAR practices. These trophy individuals are a crucial component of the spawning stock biomass, and as such represent an important segment of the population for fisheries management. Assuming the allometric trends reported here extend to larger size fishes (e.g. >28 cm length) our work suggests that such trophy targets may be the most at risk to the ancillary effects of CAR that result from slow recovery, such as predation (Danylchuk et al., 2007). Our work would suggest that best CAR practices for oversized red drum should strive to minimize the anaerobic energy expenditure by minimizing play times and post-catch air-exposure.

Funding

Primary funding for this work was provided by the University of Texas Marine Science Advisory Council with contributions from Dr. Robert Dickey, George Hillhouse, Clint Bybee, as well as the Port Aransas Rod and Reel Club. Additional funding was provided through a National Science Foundation grant (#2002549) to AJE. BNJ was supported by National Science Foundation Graduate Research Fellowship Program (#1610403).

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding this work.

Data availability

Data will be made available on request.

Acknowledgements

We thank Austin Richards and Angelina Dichiera for assisting in field collections and experimental sampling and also thank all citizen volunteers that contributed to red drum collection events.

References

Ackerly, K.L., Esbaugh, A.J., 2020. The additive effects of oil exposure and hypoxia on aerobic performance in red drum (Sciaenops ocellatus). Sci. Total Environ. 737, 140174.
Ackerly, K.L., Esbaugh, A.J., 2021. The effects of temperature on oil-induced respiratory impairment in red drum (Sciaenops ocellatus). Aquat. Toxicol. 233, 105773.
Arlinghaus, R., Cooke, S.J., Lyman, J., Policansky, D., Schwab, A., Suski, C., Sutton, S.G., Thorstad, E.B., 2007. Understanding the complexity of catch-and-release in recreational fishing: an integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. Rev. Fish. Sci. 15, 75–167.
Arlinghaus, R., Klefoth, T., Cooke, S.J., Gingerich, A., Suski, C., 2009. Physiological and behavioural consequences of catch-and-release angling on northern pike (Esox lucius L.). Fish. Res. 97, 223–233.
Beyea, M.M., Benfey, T.J., Kieffer, J.D., 2005. Hematology and stress physiology of juvenile diploid and triploid shortnose sturgeon (Acipenser brevirostrum). Fish Physiol. Biochem. 31, 303.
Booth, R.K., Kieffer, J.D., Tufts, B.L., Davidson, K., Bielak, A.T., 1995. Effects of late-season catch and release angling on anaerobic metabolism, acid–base status,

survival, and gamete viability in wild Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 52, 283–290.
Byrne, J.M., Beamish, F.W.H., Saunders, R.L., 1972. Influence of salinity, temperature, and exercise on plasma osmolality and ionic concentration in Atlantic Salmon (Salmo salar). J. Fish. Res. Board Can. 29, 1217–1220.
Card, J.T., Hasler, C.T., 2021. Physiological effects of catch-and-release angling on freshwater drum (Aplodinotus grunniens). Fish. Res. 237, 105881.
Cooke, S.J., Cowx, I.G., 2004. The role of recreational fishing in global fish crises. Bioscience 54, 857–859.
Cooke, S.J., Suski, C.D., 2005. Do we need species-specific guidelines for catch-and-release recreational angling to effectively conserve diverse fishery resources? Biodivers. Conserv. 14, 1195–1209.
Cooke, S.J., Donaldson, M.R., O'connor, C.M., Raby, G.D., Arlinghaus, R., Danylchuk, A. J., Hanson, K.C., Hinch, S.G., Clark, T.D., Patterson, D.A., Suski, C.D., 2013. The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation and relevance to stakeholders. Fish. Manag. Ecol. 20, 268–287.
Danylchuk, S.E., Danylchuk, A.J., Cooke, S.J., Goldberg, T.L., Koppelman, J., Philipp, D. P., 2007. Effects of recreational angling on the post-release behavior and predation of bonefish (Albula vulpes): the role of equilibrium status at the time of release. J. Exp. Mar. Biol. Ecol. 346, 127–133.
Danylchuk, A.J., Suski, C.D., Mandelman, J.W., Murchie, K.J., Haak, C.R., Brooks, A.M. L., Cooke, S.J., 2014. Hooking injury, physiological status and short-term mortality of juvenile lemon sharks (Negaprion bevirostris) following catch-and-release recreational angling. Conserv. Physiol. 2.
Donaldson, M.R., Hinch, S.G., Jeffries, K.M., Patterson, D.A., Cooke, S.J., Farrell, A.P., Miller, K.M., 2014. Species- and sex-specific responses and recovery of wild, mature pacific salmon to an exhaustive exercise and air exposure stressor. Comp. Biochem. Physiol. A. 173, 7–16.
Ern, R., Esbaugh, A.J., 2018. Effects of salinity and hypoxia-induced hyperventilation on oxygen consumption and cost of osmoregulation in the estuarine red drum (Sciaenops ocellatus). Comp. Biochem. Physiol. a-Mol. Integrat. Physiol. 222, 52–59.
Ern, R., Norin, T., Gamperl, A.K., Esbaugh, A.J., 2016. Oxygen-dependence of upper thermal limits in fishes. J. Exp. Biol. 219, 3376–3383.
Esbaugh, A.J., Perry, S.F., Gilmour, K.M., 2009. Hypoxia-inducible carbonic anhydrase IX expression is insufficient to alleviate intracellular metabolic acidosis in the muscle of zebrafish, Danio rerio. Am. J. Phys. Regul. Integr. Comp. Phys. 296, R150–R160.
Ferguson, R.A., Kieffer, J.D., Tufts, B.L., 1993. The effects of body size on the acid-base and metabolite status in the white muscle of rainbow trout before and after exhaustive exercise. J. Exp. Biol. 180, 195–207.
Gingerich, A.J., Suski, C.D., 2012. The effect of body size on post-exercise physiology in largemouth bass. Fish Physiol. Biochem. 38, 329–340.
Gingerich, A.J., Cooke, S.J., Hanson, K.C., Donaldson, M.R., Hasler, C.T., Suski, C.D., Arlinghaus, R., 2007. Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released. Fish. Res. 86, 169–178.
Goolish, E.M., 1991. Aerobic and anaerobic scaling in fish. Biol. Rev. 66, 33–56.
Khursigara, A.J., Johansen, J.L., Esbaugh, A.J., 2018. Social competition in red drum (Sciaenops ocellatus) is influenced by crude oil exposure. Aquat. Toxicol. 203, 194–201.
Khursigara, A.J., Johansen, J.L., Esbaugh, A.J., 2021. The effects of acute crude oil exposure on growth and competition in red drum, *Sciaenops ocellatus*. Sci. Total Environ. 751, 141804.
Kieffer, J.D., 2000. Limits to exhaustive exercise in fish. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 126, 161–179.
Kieffer, J.D., 2010. Perspective — exercise in fish: 50+years and going strong. Comp. Biochem. Physiol. A. 156, 163–168.
Kieffer, J., Currie, S., Tufts, B., 1994. Effects of environmental temperature on the metabolic and acid-base responses of rainbow trout to exhaustive exercise. J. Exp. Biol. 194, 299–317.
Kieffer, J.D., Ferguson, R.A., Tompa, J.E., Tufts, B.L., 1996. Relationship between body size and anaerobic metabolism in brook trout and largemouth bass. Trans. Am. Fish. Soc. 125, 760–767.
Killen, S.S., Adriaenssens, B., Marras, S., Claireaux, G., Cooke, S.J., 2016. Context dependency of trait repeatability and its relevance for management and conservation of fish populations. Conserv. Physiol. 4, cow007.
Landsman, S.J., Wachelka, H.J., Suski, C.D., Cooke, S.J., 2011. Evaluation of the physiology, behaviour, and survival of adult muskellunge (Esox masquinongy) captured and released by specialized anglers. Fish. Res. 110, 377–386.
Lowry, O.H., Passonneau, J.V., 1972. A Flexible System of Enzymatic Analysis. Academic Press, New York.
Milligan, C.L., 1996. Metabolic recovery from exhaustive exercise in rainbow trout. Comp. Biochem. Physiol. A Physiol. 113, 51–60.
Milligan, C.L., Girard, S.S., 1993. Lactate metabolism in rainbow trout. J. Exp. Biol. 180, 175–193.
Milligan, C.L., Wood, C.M., 1986a. Intracellular and extracellular acid-base status and H + exchange with the environment after exhaustive exercise in the rainbow trout. J. Exp. Biol. 123, 93–121.
Milligan, C.L., Wood, C.M., 1986b. Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. J. Exp. Biol. 123, 123–144.
National Marine Fisheries Service, 2018. Fisheries economics of the United States, 2016, in: N.T. US Department of Commerce (Ed.), Memo, 243.
Negrete Jr., B., Ackerly, K.L., Dichiera, A.M., Esbaugh, A.J., 2022. Respiratory plasticity improves aerobic performance in hypoxia in a marine teleost. Sci. Total Environ. 849, 157880.
Nikinmaa, M., Tiihonen, K., Paajaste, M., 1990. Adrenergic control of red cell pH in salmonid fish: roles of the sodium/proton exchange, Jacobs-Stewart cycle and membrane potential. J. Exp. Biol. 154, 257–271.
O'Toole, A.C., Danylchuk, A.J., Suski, C.D., Cooke, S.J., 2010. Consequences of catch-and-release angling on the physiological status, injury, and immediate mortality of great barracuda (Sphyraena barracuda) in the Bahamas. ICES J. Mar. Sci. 67, 1667–1675.
Pan, Y.K., Ern, R., Esbaugh, A.J., 2016. Hypoxia tolerance decreases with body size in red drum Sciaenops ocellatus. J. Fish Biol. 89 (2), 1488–1493.
Portner, H.O., Boutilier, R.G., Tang, Y., Toews, D.P., 1990. Determination of intracellular pH and PCO_2 after metabolic inhibition by fluoride and nitrilotriacetic acid. Respir. Physiol. 81, 255–273.
Schmidt-Nielsen, K., 1972. Locomotion: energy cost of swimming, flying, and running. Science 177, 222–228.
Suski, C.D., Cooke, S.J., Danylchuk, A.J., O'Connor, C.M., Gravel, M.A., Redpath, T., Hanson, K.C., Gingerich, A.J., Murchie, K.J., Danylchuk, S.E., Koppelman, J.B., Goldberg, T.L., 2007. Physiological disturbance and recovery dynamics of bonefish (Albula vulpes), a tropical marine fish, in response to variable exercise and exposure to air. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 148, 664–673.
Turner, J.D., Wood, C.M., 1983. Factors affecting lactate and proton efflux from pre-exercised, isolated-perfused rainbow trout trunks. J. Exp. Biol. 105, 395–401.
Turner, J.D., Wood, C.M., Clark, D., 1983. Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 104, 247–268.
Wakefield, A.M., Cunjak, R.A., Kieffer, J.D., 2004. Metabolic recovery in Atlantic salmon fry and parr following forced activity. J. Fish Biol. 65, 920–932.
Wang, Y., Heigenhauser, G.J., Wood, C.M., 1994. Integrated responses to exhaustive exercise and recovery in rainbow trout white muscle: acid-base, phosphogen, carbohydrate, lipid, ammonia, fluid volume and electrolyte metabolism. J. Exp. Biol. 195, 227–258.
Ward, T.D., Algera, D.A., Gallagher, A.J., Hawkins, E., Horodysky, A., Jorgensen, C., Killen, S.S., McKenzie, D.J., Metcalfe, J.D., Peck, M.A., Vu, M., Cooke, S.J., 2016. Understanding the individual to implement the ecosystem approach to fisheries management. Conserv. Physiol. 4, cow005.
Wardle, C.S., 1978. Non-release of lactic acid from anaerobic swimming muscle of plaice Pleuronectes platessa L.: a stress reaction. J. Exp. Biol. 77, 141–155.
White, A.J., Schreer, J.F., Cooke, S.J., 2008. Behavioral and physiological responses of the congeneric largemouth (Micropterus salmoides) and smallmouth bass (M dolomieu) to various exercise and air exposure durations. Fish. Res. 89, 9–16.
Wood, C.M., 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. J. Exp. Biol. 160, 285–308.