



## Impacts of dissolved oxygen on the behavior and physiology of bonefish: Implications for live-release angling tournaments

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### ABSTRACT

Saltwater tournaments for bonefish (*Albula* spp.) often retain fish in livewells for up to 8 h to allow fish to recover from the physiological disturbances associated with angling. During livewell confinement, oxygen concentrations may fall due to elevated biomass of fish, coupled with low exchange of water. Some anglers use oxygen infusion systems, potentially exposing fish to water that is supersaturated with oxygen. Currently, the effects of differing levels of oxygen on bonefish recovery are unknown. Because physiological disturbances related to angling can influence the probability of post-release predation in bonefish, livewell conditions that maximize recovery rates without imparting additional negative consequences need to be defined. The objective of this study was to assess the behavior, physiological response (i.e., blood chemistry), and metabolic rates of bonefish recovered in hypoxic, normoxic, or hyperoxic seawater after exercise (i.e., a simulated angling event). Behavioral experiments consisted of placing bonefish in one of three dissolved oxygen concentrations and monitoring gill ventilation rates. For blood sampling and metabolic rates, bonefish were exercised and then recovered in different dissolved oxygen concentrations, replicating an angling event coupled with different livewell holding conditions. Both hypoxic and hyperoxic conditions caused bonefish to experience behavioral and physiological disturbances, compared to fish in the normoxic treatment. In addition, bonefish used more energy when recovered in hyperoxic seawater and fish in the hypoxic treatment were unable to remove lactate compared to fish in the normoxic treatment. These results indicate that anglers and tournament organizers should recover angled bonefish in normoxic seawater. To achieve these conditions, dissolved oxygen concentrations should be monitored with a commercially available meter and maintained between 4–8 mg/L by circulating fresh seawater into livewells.

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### 1. Introduction

Live-release angling tournaments for saltwater fishes are a popular leisure activity that can have economic benefits for local communities (Schramm et al., 1991; Oh et al., 2006). Indeed, catch-and-release angling and recreational tournaments for bonefish (*Albula* spp.) are worth a significant amount in Florida (Humston, 2001) and The Bahamas (Fedler, 2010). By releasing fish alive at the conclusion of these events, anglers, managers and organizers hope that fish will survive to reproduce and/or be caught again (Cooke and Schramm, 2007). Nonetheless, angling can result in fish mortality due to a number of different factors that include stress and/or hooking damage

(Arlinghaus et al., 2007), and post-release predation of bonefish can occur due to angling-induced disturbances (Danylchuk et al., 2007a,b). Facilitating recovery from angling so that bonefish return to the water with reduced physiological disturbances will maximize survival after release (Cooke and Philipp, 2004, 2008) and ensure minimal impact of angling tournaments on marine communities.

A previous study has shown that many of the physiological disturbances induced by angling normalize within approximately 4 h of recovery time if bonefish are placed in ambient seawater (Suski et al., 2007a). However, we currently do not know whether additional practices can be employed that would allow physiological parameters to return to resting values more quickly, thus reducing the duration of the recovery period and further reducing the possibility of predation after release. Past work on trawl-caught Pacific salmon, for example, has demonstrated that recovery times were reduced if fish were forced to swim slowly against a gentle current during recovery rather than remaining in static water (Farrell et al., 2001). Similarly, studies with

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largemouth bass have shown that variation in dissolved oxygen concentrations, ammonia concentrations, and temperature impairs the recovery processes relative to fish recovered in ambient water conditions (Suski et al., 2007a; Suski et al., 2006). There are two different scenarios where anglers often maintain fish in livewells for extended periods of time. During tournaments fish can remain in livewells until weigh-in process (James et al., 2007), and recreational anglers may retain bonefish in livewells to allow them to recover from angling and potentially avoid post-release predation (Dallas et al., 2010). Determining species-specific guidelines for the recovery of bonefish in different water quality environments will allow for the development of best management practices that could be implemented (or avoided) to ensure the fastest recovery possible for this important recreational species (Cooke and Suski, 2005).

One critical environmental parameter that has the potential to reduce the time required for bonefish to recover from exercise is dissolved oxygen. Previous work with other fish species has documented that recovery from exercise requires oxygen (Perry and Wood, 1989), indicating that holding fish in low oxygen environments will impair the recovery process (Suski et al., 2006). However, there has to date been no formal study performed that describes the oxygen requirements/limits of bonefish, or whether hypoxic/hyperoxic water facilitates or impairs recovery from exercise relative to normoxic water. It is therefore difficult to make recommendations to tournament organizers and anglers about minimum/maximum dissolved oxygen requirements that should be maintained to optimize recovery conditions for angled bonefish.

The objective of this paper was to quantify the physiological and behavioral impacts of dissolved oxygen on bonefish, with particular emphasis on recovery from exhaustive exercise. To achieve this goal, a series of complimentary experiments were performed that included behavioral assessments, blood sampling for physiological parameters, and metabolic rate analysis under different dissolved oxygen concentrations.

## 2. Methods

### 2.1. Behavioral assessments

The behavioral portion of the study was conducted in October, 2008 at the Cape Eleuthera Institute (CEI) on the island of Eleuthera, The Bahamas (N 24°50'05" W 76°20'32"). All research was conducted in accordance with the policies of the University of Illinois Institutional Animal Care and Use Committee (Protocol # 08135). Wild bonefish, *A. vulpes* (434 ± 6 mm total length; mean ± SE; range = 400–470 mm) were captured from nearby tidal creeks on an outgoing tide using a large seine net (0.6 cm mesh, 46 m long; 1.3 cm mesh, 46 m long; 3.2 cm mesh, 76 m long; 7.0 cm mesh, 61 m long), and transported to CEI using protocols described by Murchie et al. (2009). Once at CEI, bonefish were transferred to a large, aerated holding tank (3.7 m diameter × 1.25 m height, 13,180 L) that was continuously supplied with fresh seawater at a rate of approximately 1800 L/h (Murchie et al., 2009). A low-pressure aeration pump (Sweetwater model S41; 15 V; 3450 rpm; Aquatic Ecosystems, Apopka, FL, USA) provided aeration for the tank, and dissolved oxygen concentrations during holding averaged 5.7 ± 0.1 mg/L SE and temperatures averaged 26.6 ± 0.3 °C SE (YSI 55, Yellow Springs Incorporated, Yellow Springs, OH). Fish were given a minimum of 24 h to acclimate to laboratory conditions before any experiments were initiated.

Following the acclimation period, bonefish were netted from the common holding tank and transferred to individual, darkened holding containers (approximately 80 L) that were aerated and continuously supplied with flow-through seawater. Bonefish were given approximately 24 h to acclimate to the individual containers, during which time lids remained on the containers and fish were undisturbed. During this time, dissolved oxygen concentrations averaged 5.1 ± 0.3 mg/L SE and

temperature averaged 28.7 ± 0.7 °C SE. After the 24 h acclimation period, the lids to the containers were removed and bonefish were given 3 additional hours to acclimate to the open containers. Following this third acclimation period, bonefish were assigned at random to one of three different dissolved oxygen treatments: normoxia, hyperoxia or hypoxia. Fish in the hypoxic treatment had pure nitrogen gas bubbled into their containers through an airstone to reduce dissolved oxygen concentrations to 3 mg/L (dissolved oxygen concentration = 2.7 ± 0.6 mg/L; mean ± SE; range = 0.8–2.83 mg/L). Less than 2 min were required to lower dissolved oxygen concentrations, and fish were held at this reduced dissolved oxygen concentration for 60 min. Each fish was observed for 1–2 min every 15 min during this 60 min period, during which time ventilation rates (opercular beats per minute) were measured and recorded (Suski et al., 2007a). The hyperoxic treatment replicated this procedure, except that oxygen concentrations in individual containers were adjusted to 14 mg/L (dissolved oxygen concentration = 16.5 ± 0.7 mg/L; mean ± SE; range = 6.8–20 mg/L) using oxygen from a generating system (OGSI, OG-20, North Tonawanda, NY) that was pumped through the airstones. Bonefish in the normoxic treatment continued receiving low-pressure air from the aeration pump without any manipulation of oxygen concentration, and ventilation rates were also observed (dissolved oxygen concentration = 5.3 ± 0.5 mg/L; mean ± SE; range = 4.2–6.6 mg/L).

### 2.2. Blood sampling for physiological parameters

Between December 14 and December 17, 2008 wild bonefish (415 ± 5.8 mm total length; mean ± SE; range = 270–495 mm) were again captured from small tidal creeks and transported to CEI as described above. Dissolved oxygen concentrations in the holding tanks averaged 5.8 ± 0.2 mg/L SE and temperature averaged 22.0 ± 0.2 °C SE. Fish were given at least 24 h to acclimate to laboratory conditions.

To measure control (resting) physiological values, bonefish were netted from the common holding tank and transferred to darkened, individual holding chambers containing an air stone (approximately 100 L volume) continuously supplied with fresh sea water. During holding in individual chambers, dissolved oxygen concentration was 5.9 ± 0.1 mg/L SE and water temperature was 22.4 ± 0.1 °C SE. After 24 h of undisturbed holding, bonefish were quickly netted from the holding chamber and non-lethally sampled for blood without the use of anesthetic. During this procedure, fish were restrained by hand in a foam-lined trough containing sufficient seawater to completely submerge their gills. Blood was drawn from the caudal vessels using a 21-gauge needle and a sterile 3 ml vacutainer containing lithium heparin (BD vacutainer blood collection tube; Becton, Dickinson and Company; Franklin Lakes, NJ, USA). The time required to draw blood was typically less than 45 s, and previous work has shown that this technique is effective at collecting blood quickly enough that samples accurately represent resting conditions (Suski et al., 2007b). Hematocrit values for whole blood (% packed cell volume, PCV) were determined on-site (LW Scientific zipocrit, model # ZO-1, 10,000 r/min). The remainder of the collected blood was spun in a centrifuge (Clay Adams Compact II Centrifuge) at 10,000 × gravity (g) for 3 min to separate plasma from red cells. Following centrifugation, plasma was removed from red cells with a pipette, placed in a labeled microcentrifuge tube, and immediately placed in a dry shipper charged with liquid nitrogen. Samples were returned to an ultracold freezer (<−75 °C) and samples did not thaw or warm between being placed in the charged dry shipper and transfer to the ultracold freezer.

To generate physiological disturbances through exercise, bonefish were netted from the common holding tank and transferred to smaller treatment tank (approximately 2.5 m diameter × 0.5 m height; 500 L) containing fresh seawater where they were chased by tail grabbing for 4 min. Previous work has shown that tail grabbing induces a number of physiological disturbances through anaerobic metabolism that are representative of changes that arise during a typical angling event

(Wood, 1991; Wang et al., 1994; Kieffer, 2000; Suski et al., 2007b). Furthermore, a 4-minute exercise protocol is representative of angling events for bonefish (Cooke and Philipp, 2004; Danylchuk et al., 2007a,b). Following 4 min of exercise, one group of bonefish were removed from the exercise tank and immediately sampled for blood as described above to quantify disturbances associated with exercise. To quantify the impacts of dissolved oxygen concentration on the ability of bonefish to recover from exercise, six additional groups were exercised for 4 min and then transferred to the individual holding chambers where they were left to recover in individual darkened recovery chambers for either 2 h or 6 h at different oxygen concentrations. These recovery periods were chosen because Suski et al. (2007b) reported that blood chemistry levels for exercised bonefish returned to baseline levels after 4 h. By allowing bonefish to recover for less and more than 4 h, we were able to ascertain initial recovery status at 2 h and any delay in recovery at 6 h due to different dissolved oxygen concentrations. The oxygen concentrations used were (1) 3 mg/L for 2 h (mean oxygen concentration =  $2.95 \pm 0.1$  mg/L; mean  $\pm$  SE; range = 1.9–3.6 mg/L), (2) 3 mg/L for 6 h (mean oxygen concentration =  $3.0 \pm 0.6$  mg/L; mean  $\pm$  SE; range = 1.5–4.2 mg/L), (3) 10 mg/L for 2 h (mean dissolved oxygen concentration =  $10.2 \pm 0.2$  mg/L; mean  $\pm$  SE; range = 7.2–12.5 mg/L), (4) 10 mg/L for 6 h (mean oxygen concentration =  $10.3 \pm 0.2$  mg/L; mean  $\pm$  SE; range = 8.3–14.7 mg/L), (5) 6 mg/L for 2 h (mean dissolved oxygen concentration =  $5.5 \pm 0.2$  mg/L; mean  $\pm$  SE; range = 4.5–6.8 mg/L), and (6) 6 mg/L for 6 h (mean dissolved oxygen concentration =  $5.5 \pm 0.2$  mg/L; mean  $\pm$  SE; range = 2.9–6.2 mg/L). During holding the average water temperature for all individual chambers was  $23.5 \pm 0.1$  °C SE. After designated recovery times, fish were quickly netted from the recovery chambers and non-lethally sampled for blood without the use of anesthetic as described above.

### 2.3. Metabolic rates

The effects of ambient oxygen concentration on metabolic rate and post-exercise recovery were determined using intermittent-flow respirometry. The respirometry system consisted of four perspex chambers (746 mm length  $\times$  140 mm wide) outfitted with fiber optic oxygen probes immersed in a tank (3.09 m length  $\times$  0.65 m width  $\times$  0.17 m height) of aerated sea water at ambient temperatures. Each chamber was connected to two aquarium pumps (Eheim 1046A, 5 L/min), one for re-circulating water within the chamber, and one for flushing ambient, oxygenated water into the chamber. The total volume per set up, including the glass chamber, two pumps, and all associated tubing was 11.48 L. Oxygen consumption in each individual chamber with 26 min cycles consisted of a 10 min measurement phase during the recirculation period, a 15 min flush period to replace water in each chamber, and a 1 min wait period following each flushing prior to commencing measurements. During each measurement period, water from the chambers was continually re-circulated over the fiber optic oxygen probes to ensure adequate mixing and dissolved oxygen concentration was recorded every 2 s. The change in oxygen concentration ( $\alpha$ ) for each chamber was calculated as slope ( $\Delta O_{2\text{saturation}}/\Delta t$ ), and oxygen consumption rate ( $MO_2$ , mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) for each fish was calculated as:

$$MO_2 = \alpha V_{\text{resp}} \beta M_b^{-1}$$

where  $V_{\text{resp}}$  is the volume of each glass chamber minus the volume of the fish (L),  $\beta$  is oxygen solubility (adjusted daily for both temperature and barometric pressure), and  $M_b$  is the fish mass (kg) measured before placing in the respirometer chamber (Steffensen, 1989). The coefficient of determination ( $r^2$ ) for all slope measurements was  $>0.95$  during each trial. All calculated dissolved oxygen values were corrected for background oxygen consumptions generated for each specific fish and chamber prior to commencing experiments. Calibration of the fiber

optic oxygen probes occurred with oxygen-free water and fully saturated water regularly through the experiments, and data were recorded with AutoResp software (Version 1.4, Steffensen, 1989; Schurmann and Steffensen, 1997).

Bonefish used for metabolic rate experiments were collected from nearby tidal creeks (as described above) and transported to the holding facility at CEI between February 7, 2009 and February 12, 2009. Fish were acclimated to tank conditions for a minimum of 48 h and were not fed 24 h prior to experimentation. Fish were loaded into individual chambers at approximately 16:00 hours, and standard metabolic rate values (SMR) were calculated as the average of six lowest values recorded between 20:00 and 06:00 (Schurmann and Steffensen, 1997; Gingerich et al., 2010). After 06:00, individual fish were removed from their chambers, exercised in a manner identical to that describe above, and then returned to their chamber where maximal metabolic rate (MMR) in either normoxic sea water ( $5.5 \pm 0.2$  mg/L; mean  $\pm$  SE; range = 5.0–6.3 mg/L) or hyperoxic sea water ( $10.2 \pm 1.0$  mg/L; mean  $\pm$  SE; range = 7.5–13.6 mg/L) was measured over a 6 h period. A 6 h recovery period was selected as bonefish have been found to require 4 h to return to baseline blood chemistry values following exhaustive exercise (Suski et al., 2007b). MMR was determined as the highest value recorded during the 6 h recovery period, and metabolic scope was calculated by subtracting SMR from MMR. Previous work has used swimming respirometry and the continuous monitoring of recovering fish over time to generate data on MMR and to quantify oxygen consumption following exercise (Jain et al., 1998; Farrell, 2007). In the current study, post-exercise oxygen consumption was calculated in a manner that allows a relative comparison of the metabolic scope devoted to recovery following exhaustive exercise for each dissolved oxygen environment. This approach yielded an approximation of post-exercise oxygen consumption, and it was not possible to calculate the area under the curve (as per Farrell, 2007; Redpath et al., 2010; Murchie et al., 2011). The size of the fish used in the normoxic treatment averaged  $655 \pm 84$  g SE, range 355–910 g while the fish in the hyperoxic treatment were  $879 \pm 44$  g SE, range 780–1100 g.

### 2.4. Laboratory analyses

Plasma cortisol was quantified using a commercially available kit (Kit # 900–071, Assay Designs, Ann Arbor, MI). Sink et al. (2008) confirmed the accuracy and precision of this kit compared to conventional radioimmunoassay techniques, and recommended its use for cortisol detection. Moreover, the product has low cross-reactivity with other hormones produced by fishes that could interfere with binding in fish (Assay Designs Kit #900-071 Insert). Plasma sodium and potassium concentrations were determined using a flame photometer (Model 2655–00, Cole-Parmer Instrument Company, Chicago, IL) and plasma chloride concentrations were determined using a chloride titrator (Model 4435000, Labconco Corporation, Kansas City, MO). Plasma glucose and lactate concentrations were determined enzymatically following the methods of Lowry and Passonneau (1972) in a 96-well microplate with a commercially available spectrophotometer (Spectra Max Plus 384, Model # 05362, Molecular Devices, Union City, CA).

### 2.5. Statistical analyses

Changes in ventilation rates and metabolic rates during the 6 h monitoring period were assessed with a two-way repeated-measure (mixed model) analysis of variance (RMANOVA), with time period and oxygen concentration entered as fixed effects, and individual fish entered as a random effect (Sokal and Rohlf, 1995; SAS Institute Inc., 2005). Differences in plasma-based parameters and total lengths of fish across treatment groups were assessed using a one-way analysis of variance (ANOVA) followed by a Tukey–Kramer HSD test when appropriate. A  $t$ -test was used to assess differences in SMR, MMR, and recovery scope between bonefish recovered in different oxygen

concentrations after exercise. An ANOVA was used to assess difference in the weights of fish between treatments. All analyses were performed using JMP 7.0.1 (SAS Institute Inc., 2005), all means are reported as ± SE where appropriate, and the level of significance ( $\alpha$ ) for all tests was 0.05.

### 3. Results

#### 3.1. Behavioral assessments

After 15 min exposure to hypoxic (3 mg/L) or hyperoxic (14 mg/L) seawater, the rate of opercular beats of bonefish did not change relative to fish held in normoxic water (6 mg/L) (Fig. 1). Following 30 min exposure to these oxygen concentrations, however, there was a two-fold increase in the rate of opercular beats for bonefish exposed to hypoxic water relative to the normoxic treatment (Fig. 1). The rate of opercular beats remained elevated for fish in this treatment group after 45 and 60 min. Bonefish exposed to hyperoxic sea water experienced approximately a 50% reduction in opercular beats relative to the normoxic treatment after 60 min (two-way, mixed model repeated measures ANOVA: time  $F_4=10.9$ ,  $p<0.0001$ ; treatment  $F_2=16.6$ ,  $p<0.0001$ ; time × treatment  $F_8=35.8$ ,  $p<0.0001$ ; Fig. 1). The size of fish was not different among treatment groups ( $F_{2, 14}=0.16$ ,  $p=0.85$ ).

#### 3.2. Physiological recovery

No significant differences were observed for cortisol or glucose concentrations within a recovery period or between recovery periods and controls or exercised bonefish (Table 1; Fig. 2). Similarly, plasma  $Cl^-$  values for bonefish did not vary significantly from control or exercise concentrations within or across any sampling point (Table 1; Fig. 3A). Recovery of bonefish in hypoxic (3 mg/L) sea water resulted in an increase in plasma  $Na^+$  concentrations that were approximately 50% greater than resting values and 20% greater than fish recovered in normoxic sea water at 2 h post-exercise. Concentrations of  $Na^+$  in plasma remained elevated for the hypoxic treatment group relative to the controls and exercised fish even after 6 h of recovery. In contrast, bonefish recovered in the hyperoxic (10 mg/L) or normoxic (6 mg/L) sea water did not experience changes in plasma  $Na^+$  concentrations relative to controls or exercised values (Table 1; Fig. 3B). No differences were observed in plasma  $K^+$  concentrations relative to control or exercise concentrations at either 2 or 6 h post-exercise. At

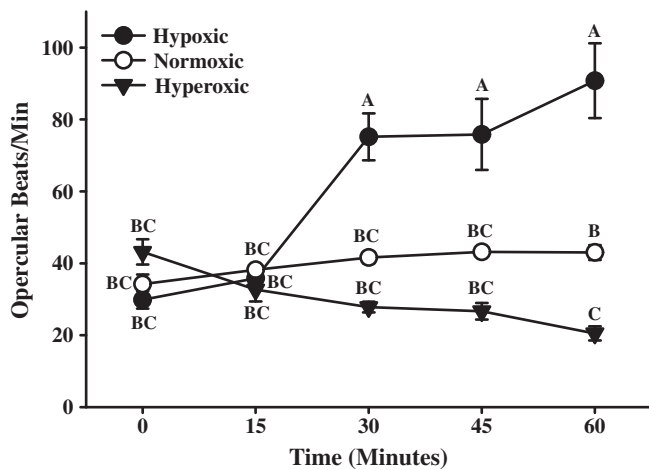


Fig. 1. The number of opercular beats per minute performed by bonefish held in hypoxic (3 mg/L), normoxic (6 mg/L) and hyperoxic (14 mg/L) sea water over a 60 min period. Sample sizes are: hypoxic from 0–30 min n = 6, 45 min n = 5, and 60 min n = 4, control n = 6 and hyperoxic n = 6.

Table 1

One-way ANOVA results examining the effects of dissolved oxygen concentrations on blood-based physiological parameters in bonefish following 4 min of exercise and recovery in hypoxic (3 mg/L), normoxic (6 mg/L), and hyperoxic (10 mg/L) sea water. Recovery was for either 2 or 6 h.

Variable	Source	d.f.	SS	F	P
Plasma cortisol (ng/ml)	Whole model	7	15,708.5	1.8	0.10
	Error	50	61,518.3		
	Total	57	77,226.8		
Plasma glucose (mmol/L)	Whole model	7	45.1	2.1	0.06
	Error	51	157.9		
	Total	58	203		
Plasma $Cl^-$ (meq/L)	Whole model	7	6,157.9	0.9	0.53
	Error	49	49,208.7		
	Total	56	55,366.6		
Plasma $Na^+$ (meq/L)	Whole model	7	145,889.2	6.2	<0.0001
	Error	51	172,340.6		
	Total	58	318,229.7		
Plasma $K^+$ (meq/L)	Whole model	7	52.4	3.3	0.0058
	Error	55	126.3		
	Total	62	178.6		
Plasma lactate (mmol/L)	Whole model	7	1,948.1	7.5	<0.0001
	Error	49	1,818.7		
	Total	56	3,766.8		
Hematocrit (% PCV)	Whole model	7	256.5	5.3	0.0002
	Error	48	355.3		
	Total	55	591.9		

both 2 h and 6 h post-exercise, however, bonefish recovering in hyperoxic water experienced nearly a two-fold increase in plasma  $K^+$  concentrations relative to bonefish recovered in normoxic water conditions (Table 1; Fig. 3C).

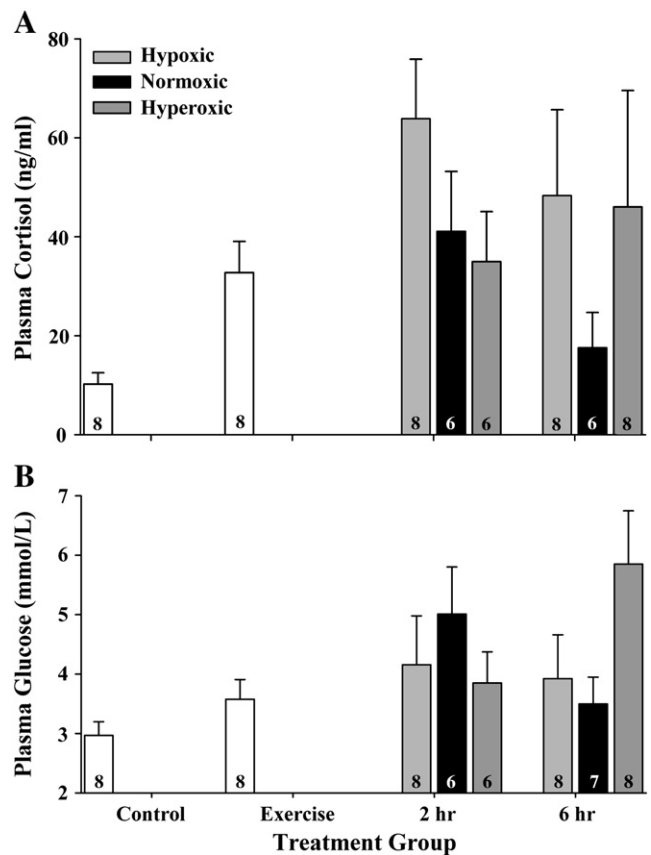
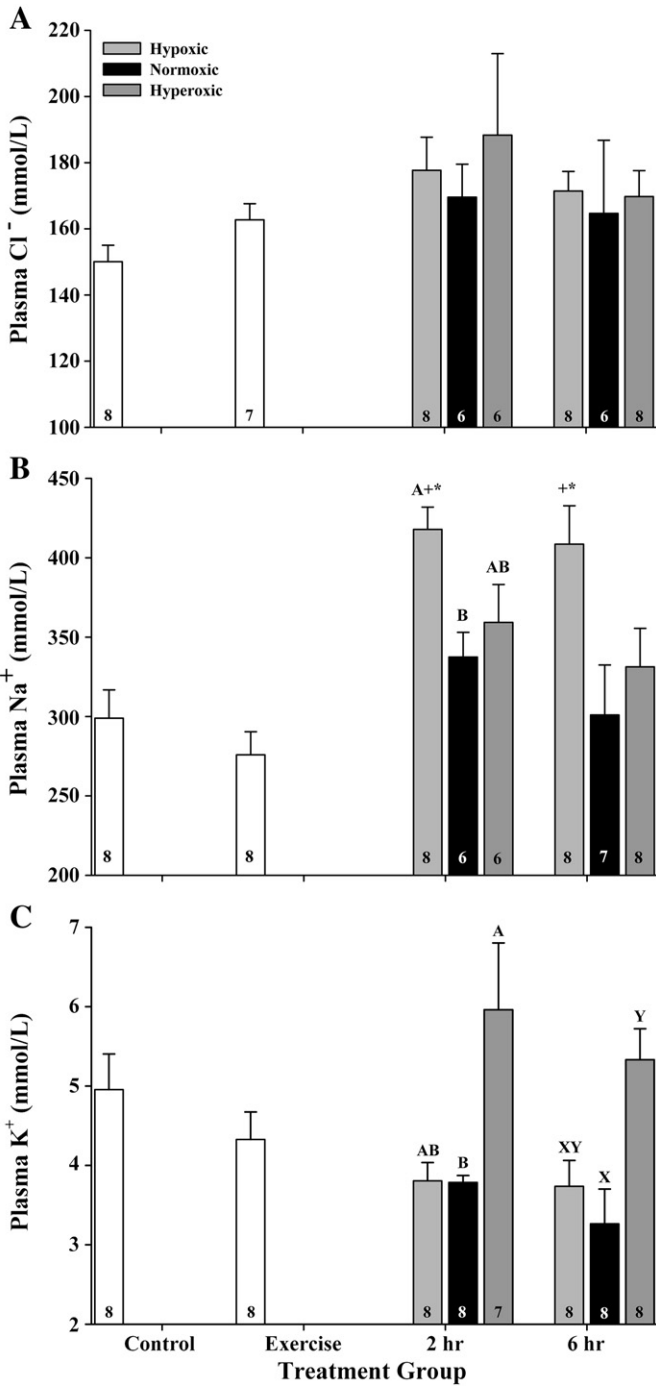


Fig. 2. Concentrations of plasma cortisol (A) and glucose (B) for bonefish exercised for 4 min and allowed to recover in hypoxic (3 mg/L), normoxic (6 mg/L) and hyperoxic (10 mg/L) sea water for different durations of time. No statistically significant differences were observed across treatments and sample sizes are shown on individual bars.





**Fig. 3.** Concentrations of plasma chloride (A) sodium (B) and potassium (C) for bonefish exercised for 4 min and allowed to recover in hypoxic (3 mg/L), normoxic (6 mg/L) and hyperoxic (10 mg/L) sea water for different durations of time. Different symbols denote significant differences between recovery periods and controls (+) or fish exercised for 4 min (\*). Dissimilar letters denote significant differences within a recovery period. Sample sizes are shown on individual bars.

Plasma lactate values increased by approximately 20-fold relative to resting for bonefish exercised for 4 min and recovered in hypoxic water for 2 h relative to control and exercised fish. Plasma lactate levels remained elevated after 6 h for fish recovered in hypoxic sea water while concentrations of lactate for bonefish in normoxic or hyperoxic water had returned to control values by 6 h post-exercise. Within the 2 h recovery period, plasma lactate values increased over 10-fold for bonefish exposed to hypoxic water conditions than fish

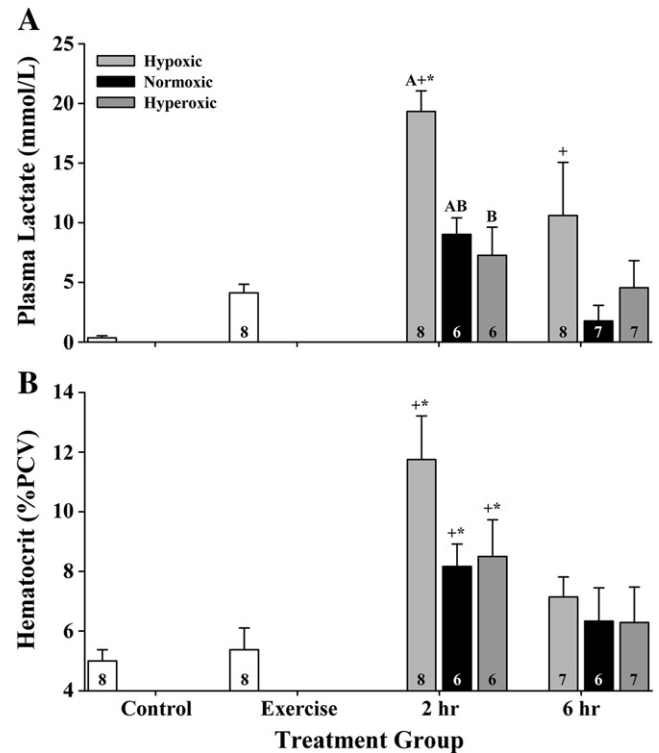
recovered in hyperoxic water conditions (Table 1; Fig. 4A). Bonefish exposed to hypoxic, normoxic, and hyperoxic sea water for 2 h following 4 min of exercise had a significant increase in hematocrit values relative to controls and fish exercised for 4 min. After 6 h all hematocrit values had returned to control levels (Table 1; Fig. 4B). The total length of bonefish did not differ statistically across treatment groups ( $F_{7, 48} = 1.17, p = 0.85$ ).

### 3.3. Metabolic rate

Prior to exercise, the SMR of bonefish exercised and recovered in normoxic conditions was not significantly different from fish recovered in hyperoxic seawater (Table 2). Following 4 min of forced exercise, the MMR of bonefish recovered in hyperoxic sea water was 70% greater than fish recovered in normoxic sea water (Table 2). In addition, the recovery scope (i.e. MMR–SMR) was nearly 60% greater for bonefish recovered in hyperoxic water conditions than fish recovered in control conditions (Table 2). Bonefish that recovered from exercise in hyperoxic water conditions expended 25% more energy on average than did fish recovered in normoxic water. Recovery was delayed when fish were exposed to hyperoxic water conditions while fish in normoxic water demonstrated a reduction in their metabolic rates over the 6 h period (two-way, mixed model repeated measures ANOVA: time  $F_1 = 26.3, p < 0.0001$ ; treatment  $F_1 = 12.0, p < 0.0007$ ; time  $\times$  treatment  $F_1 = 0.1, p = 0.71$ ; Fig. 5). The weight of bonefish in the hyperoxic group was 37% greater than fish in the normoxic treatment ( $t = 2.28, d.f. = 10, p = 0.045$ ).

## 4. Discussion

Following a bout of exhaustive exercise, bonefish recovered in hyperoxic water demonstrated a host of behavioral, energetic and



**Fig. 4.** Plasma lactate (A) and hematocrit (B) values for bonefish exercised for 4 min and allowed to recover in hypoxic (3 mg/L), normoxic (6 mg/L) and hyperoxic (10 mg/L) sea water for different durations of time. Different symbols denote significant differences between recovery periods and controls (+) or fish exercised for 4 min (\*). Dissimilar letters denote significant differences within a recovery period. Sample sizes are shown on individual bars (plasma lactate control, n = 7).

**Table 2**

Standard metabolic rate (SMR,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ), maximum metabolic rate (MMR,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ), estimated recovery scope (MMR–SMR) and statistical comparisons for laboratory-acclimated bonefish exercised for 4 min and recovered in normoxic ( $5.5 \pm 0.2 \text{ mg/L}$  SE, range = 5.0–6.3 mg/L) and hyperoxic ( $10.2 \pm 1.0 \text{ mg/L}$  SE, range = 7.5–13.6 mg/L) sea water over a 6 h period. Data were generated using 7 fish in each treatment and means are shown  $\pm$  SE.

	Normoxic	Hyperoxic	t	d.f.	P
SMR	149 $\pm$ 18	141 $\pm$ 9	0.36	7.49	0.64
MMR	290 $\pm$ 17	419 $\pm$ 27	–4.00	10.13	<b>0.0012</b>
Recovery scope	154 $\pm$ 13	277 $\pm$ 25	–4.38	9.03	<b>0.0009</b>

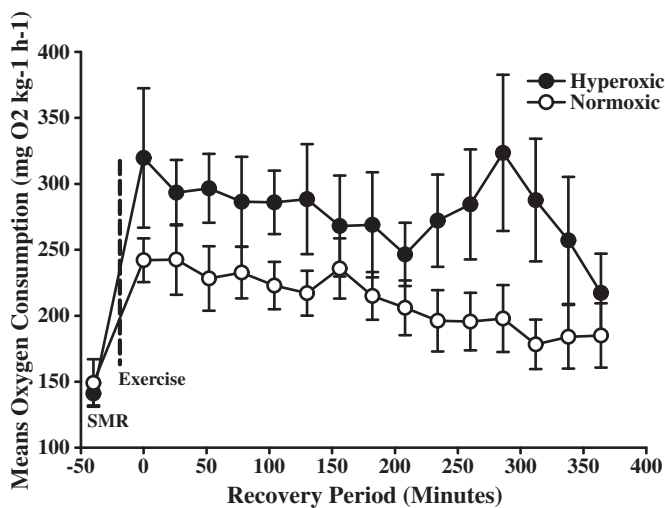
physiological disturbances that impaired recovery relative to fish recovered in normoxic water. More specifically, recovery in hyperoxic water caused bonefish to display reduced ventilation rates and to absorb potassium from their surroundings, relative to fish recovered in normoxic sea water. This potassium disturbance persisted for 6 h after exercise. In addition, holding bonefish in hyperoxic water resulted in the consumption of approximately 25% more energy during recovery relative to fish recovered in ambient sea water. In fish, ambient oxygen stimulates ventilation and the passage of water over the gills, with low oxygen conditions resulting in gill ventilation (Dejours, 1973; Smith and Jones, 1982). Similar to studies on other teleost fishes (e.g., Perry and Wood, 1989; Gilmour and Perry, 1994), placing bonefish in highly oxygenated water reduces this stimulus and results in reduced ventilation rates, as demonstrated here. The most serious consequence of reduced ventilation is an inability of fish to excrete wastes, mainly carbon dioxide ( $\text{CO}_2$ ). Although not measured in the current study, this accumulation of  $\text{CO}_2$  can result in an internal acidosis for fish (Dejours, 1973; Perry and Wood, 1989; Gilmour and Perry, 1994; Gilmour, 2001) and a suite of other disturbances such as changes to ion concentrations (Wood, 1991). Together, the results from the current study demonstrate that hyperoxic water induces physiological disturbances and elevated energy consumption not present in fish recovered in ambient water, impairing recovery from exercise (e.g. an angling event).

Similar to recovery in hypoxia, forcing bonefish to recover from exercise in hypoxic water resulted in prolonged recovery times and significant physiological disturbances relative to fish recovering in normoxic water. Due to a lack of oxygen, bonefish in hypoxic water elevated ventilation rates relative to fish in normoxic water in an effort to pass more water over their gills to obtain oxygen. Similarly,

largemouth bass that were maintained in a hypoxic environment demonstrated an increase in ventilation rates that resulted in ionic disturbances (Vanlandeghem et al., 2010). This lack of oxygen also caused significant elevation of plasma sodium relative to the normoxic treatment at 2 h post-exercise, and also a 50% increase in hematocrit values for hypoxia fish relative to fish in normoxic waters (although differences in hematocrit were not statistically significant). More importantly, at 2 h post-exercise, the lack of oxygen in the water prevented bonefish from clearing accumulations of lactate, essentially slowing recovery times relative to fish in normoxic water. A similar study on largemouth bass has shown that hypoxic water conditions result in an accumulation of lactate (Vanlandeghem et al., 2010). The buildup of lactate results in a reduction in the fish's ability to burst swim (Kieffer, 2000). Together, the results from the current study demonstrate that low oxygen environments (hypoxia) prolong recovery times and induce significant physiological disturbances for bonefish that have been exercised.

During a live-release angling tournament, bonefish that are to be weighed are typically held in livewells on board boats. Similarly, recreational anglers may hold bonefish after an angling event to ensure physiological recovery and reduce the potential for post release predation (Dallas et al., 2010). The length of time that fish are held in livewells is variable, but can range from 1–8 h. During this time, bonefish that have been angled (exercised) begin the process of physiological recovery that can take in excess of 4 h for fish recovered in normoxic seawater (Suski et al., 2007b). The water quality conditions of livewells can be variable and will either facilitate or impair recovery, which can have implications for the condition of bonefish following release. Previous work with species such as largemouth bass has shown that dissolved oxygen concentration in livewells can decline to near-hypoxic levels due to high biomass of fish in a small, confined space with relatively low water exchange (Hartley and Moring, 1993). While dissolved oxygen concentrations in livewells were not measured in the current study, there is potential for oxygen levels to fall during bonefish tournaments (a) if the biomass of fish in a livewell consume oxygen faster than the flush pump can deliver fresh water, (b) if water temperatures rise, (c) if flush pumps are not run as frequently as needed, or (d) a combination of these factors. Should dissolved oxygen concentrations fall, results from the current study show that this low oxygen water will not only cause physiological disturbances for bonefish, but also impaired recovery from exercise. In an attempt to facilitate recovery from exercise and improve holding conditions for bonefish, tournament anglers have employed oxygen infusion systems that release oxygen gas from a bottle into livewell water (Suski et al., 2006). Use of these infusion systems, without knowledge of the dissolved oxygen concentration in a livewell, has the potential to generate hyper-oxygenated water through continuous input of oxygen that can persist for extended periods. As shown in the current study, should infusion systems be used without knowledge of the concentration of oxygen in the water and hyperoxic conditions develop, there is potential to have negative consequences for bonefish, resulting in increased energy consumption and physiological disturbances. Together, results from this study indicate that deviations in oxygen concentration, either higher or lower than ambient sea water can result in physiological disturbances and impaired recovery times for bonefish held in livewells during angling tournaments.

Bonefish may experience low oxygen environments during the summer when water temperatures in flats and tidal creek ecosystems are the highest. The current study has shown that recovery from exercise in hypoxic sea water has negative consequences for bonefish recovery. Physiological disturbance associated with angling can impair burst swimming capabilities, predominantly the buildup of lactate after exercise (Kieffer, 2000). In the current study, bonefish were unable to remove the buildup of lactate after 2 h when recovered in hypoxic sea water. This is particularly important for



**Fig. 5.** Oxygen consumption of bonefish exposed to normoxic (6 mg/L) and hyperoxic (10 mg/L) sea water over a 6 h recovery period after 4 min of exercise. Standard metabolic rate (SMR) for each treatment group is shown to the left of time zero and the dashed line represents 4 min of exercise. Each treatment group has an  $n=7$ .

bonefish that may need to avoid predators such as great barracudas (*Sphyrna barracuda*) and lemon sharks (*Negaprion brevirostris*) after being captured (Cooke and Philipp, 2004). Future research should address the recovery and survivorship of bonefish released in high temperature/low oxygen environments compared to fish released in cooler, well oxygenated environments.

## 5. Conclusions

Results from the current study demonstrate that, during holding of bonefish in the context of live-release angling tournaments, proper water oxygenation must be maintained to maximize the rate of physiological recovery for bonefish that are to be released. It is therefore recommended that the dissolved oxygen concentrations during holding do not deviate from that of ambient sea water, which is typically 6 mg/L. Currently it is believed that continuous use of livewell flush pumps and/or the continuous addition of fresh seawater to holding tanks should be sufficient to maintain this concentration (based on current practices of holding a single bonefish during an angling tournament), although future studies should verify this assumption. Should changes in dissolved oxygen occur, either due to elevated fish biomass in livewells or due to continuous use of oxygen infusion systems, it is recommended that dissolved concentrations remain between 4 mg/L and 8 mg/L to minimize disturbances. While the current study only examined the impact of a single high and a single low oxygen concentration (3 mg/L and 10 mg/L), results of impaired recovery at these concentrations were evident, and should be avoided.

We also recommend that tournament organizers (and possibly anglers) purchase, maintain, and use dissolved oxygen meters to accurately determine dissolved oxygen concentration during bonefish holding. Similar studies on live-release bass tournaments have recommended that anglers and tournament organizers monitor dissolved oxygen concentrations to maintain sufficient levels of dissolved oxygen for recovery (Suski et al., 2006; Furimsky et al., 2003; Suski et al., 2003). The proper use of these oxygen meters in bonefish tournaments will remove any doubt or uncertainty regarding oxygen concentration, and will ensure adequate water quality during holding. At present, the use of dissolved oxygen meters could significantly improve fish care, change the way these tournaments are run, and become an essential tool in the saltwater tournament circuit.

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