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Physiological disturbance and recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and exposure to air

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Abstract

Current understanding of the stress response in fishes has largely come from studies of freshwater-adapted salmonids, with proportionately few comparative studies having examined marine fishes. The current study sought to quantify the magnitude of physiological disturbances, recovery dynamics, and post-exercise behaviour in bonefish (*Albula vulpes*; a tropical marine fish) exposed to several different exercise and air exposure regimens. Results showed that metabolic disturbances (lactate production, hyperglycemia) increased following exercise and exposure to air, and that the magnitude of metabolic disturbance was proportional to the duration of the stressful event. Fish required between 2–4 h to return to resting values. Exercise and exposure to air also resulted in significant increases in plasma Ca^{2+}, Cl^{-} and Na^{+}, but the magnitude of these ionic changes did not vary with exercise or exposure to air duration and required over 4-h to return to baseline levels. Mortality following exercise was observed only for fish that had been exposed to air for 3 min and not in fish that had been exposed to air for 1 min. Together, results from this study provide a physiological basis for management strategies that can improve the post-release survival of bonefish that have been caught during a catch-and-release angling event.

Keywords: Air exposure; Bonefish; Exercise; *Albula vulpes*; Ion loss; Marine; Recovery; Stress

1. Introduction

Angling induces anaerobic respiration in fish, resulting in a suite of physiological changes that include a consumption of energy stores, a production of lactate, and osmotic/ionic disruptions (Gustaveson et al., 1991; Wood, 1991; Kieffer, 2000; Suski et al., 2006). In addition, Thorstad et al. (2003) showed that angling can disrupt the upstream migration behaviour of salmonid fishes, while Danylchuk et al. (2007a) showed that handling techniques used by anglers can impact post-release behaviour of bonefish (*Albula vulpes*), which, in turn, can influence their susceptibility to predation. Furthermore, recreational angling has the potential to negatively impact both marine and freshwater fish populations through a number of different mechanisms that include size-selective harvest, disrupted spawning and individual mortality (Post et al., 2002; Coleman et al., 2004; Cooke and Cowx, 2004; Lewin et al., 2006). To help conserve fish populations while permitting

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recreational angling, managers often implement catch-and-release regulations that mandate that angled fish be returned to the water (Noble and Jones, 1999). For many species, this catch-and-release ethic has been adopted by conservation-minded anglers, even in the absence of mandated legislation. Bonefish (*Albula* spp.) are an example of a fish for which catch-and-release angling is practiced almost exclusively despite an absence of regulations (Policansky, 2002; Cooke et al., 2006; Cooke and Philipp, 2007).

The success of a catch-and-release ethic or management strategy to protect fish populations, however, depends on the assumption that released fish survive (Bartholomew and Bohnsack, 2005; Cooke and Suski, 2005). Therefore, ensuring that anglers undertake practices that minimize physiological and behavioural impairments to released fish will improve the likelihood of survival, thereby likely benefiting populations (Cooke and Schramm, 2007).

The objective of this study was to quantify physiological disturbances in bonefish (*A. vulpes*) that were exercised in a manner typical of an angling event (independent of exposure to air), and to document the time required for these disturbances to return to resting (control) conditions. Physiological disturbances, post-exercise behaviour, and post-exercise recovery times were also examined in response to different exercise regimes and durations of exposure to air designed to simulate a range of catch-and-release angling scenarios. This information can be used to design management strategies to minimize mortality for bonefish that are angled and released. Understanding the physiological impacts recreational angling has on bonefish will ultimately allow for scientifically-founded guidelines that stakeholders can employ to ensure the maintenance of bonefish populations as well as the economies that rely on bonefish-related tourism. In addition, bonefish represent a group of circumtropical, inshore marine fishes that have a poorly understood evolutionary history (including a number of morphologically indistinguishable yet genetically distinct species; Colborn et al., 2001), as well as unique life-history stages (i.e., leptocephalous larvae) (Pfeiler et al., 1990; Bishop and Torres, 1999). Furthermore, bonefish reside in dynamic coastal regions that are characterized by dramatic fluctuations in water temperature and high predator burdens. Because the stress response in fishes has been disproportionately derived from studies of salmonids (Wendelaar Bonga, 1997), our study on bonefish offers an excellent opportunity to improve our understanding of comparative fish physiology by studying a taxonomically ancient marine species (Colborn et al., 2001).

2. Materials and methods

This study was conducted between February 20th and February 24th, 2007, at the aquatic holding facility of the Cape Eleuthera Institute (CEI), Eleuthera, The Bahamas (24.54°N 76.12°W). All research was conducted in accordance with the policies of the Canadian Council on Animal Care as administered by the Carleton University Animal Care Committee (Protocol B07-03, 04). Wild bonefish (*Albula vulpes*, Albulidae) [average size=446 mm total length, standard error (SE)=5 mm, range=359–547 mm] were captured from small tidal creeks (see Danylchuk et al., 2007b for a description of sampling sites) using seine nets and transported to CEI using aerated transport tanks. Once at CEI, bonefish were transferred to a large holding tank (3.7 m diameter×1.25 m height, 13,180 L) that was aerated and continuously supplied with fresh sea water (1800 L/h). Fish were given at least 24-h to acclimate to laboratory conditions before any experiments were begun. During acclimation, ambient water temperature ranged from 21–23 °C, dissolved oxygen ranged from 5–7 mg/L, and salinity ranged from 33–35‰.

2.1. Recovery from exercise

To generate control (resting) physiological values, bonefish were netted from the common holding tank and transferred to darkened, individual holding chambers (approximately 100 L volume) continuously supplied with sea water. Fish were left undisturbed for 36 h, at which time they were quickly netted from the holding chamber, euthanized by cerebral percussion, and approximately 1.5 mL of blood was drawn from the caudal vessels using a 21 gauge needle into a 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. Blood was held in an ice water slurry prior to analyses (see below), and this holding time was typically less than 10 min.

To simulate the exercise induced by an angling event, fish were netted from the common holding tank and transferred to a smaller aerated treatment tank (1.6 m diameter×0.85 m height; 1400 L) containing sea water, where they were chased by tail grabbing for 4 min. Previous work has shown the physiological disturbances arising from tail grabbing are representative of physiological changes that arise due to angling (Wood, 1991; Wang et al., 1994; Kieffer, 2000; Suski et al., 2006). Furthermore, a 4 min exercise protocol is representative of angling events for bonefish (Cooke and Philipp, 2004; Danylchuk et al., 2007a). Following 4 min of exercise, one group of bonefish were removed from the exercise tank and immediately sampled for blood as described above, with the exception that this post-exercise blood sampling was non-lethal and blood was collected without the use of anesthetic. To obtain blood non-lethally, fish were restrained by hand in a foam-lined trough that contained sufficient sea water to completely submerge their gills, and blood was drawn as described above. Three additional groups were exercised for 4 min and then transferred to the individual holding chambers where they were left to recover for 1, 2 or 4-h. After designated recovery times, fish were quickly netted from the recovery chambers and non-lethally sampled for blood without the use of anesthetic.

2.2. Exercise and air exposure duration

To quantify the impacts of varying exercise regimes and durations of exposure to air, one additional exercise duration and two air exposure durations were used, each of which has

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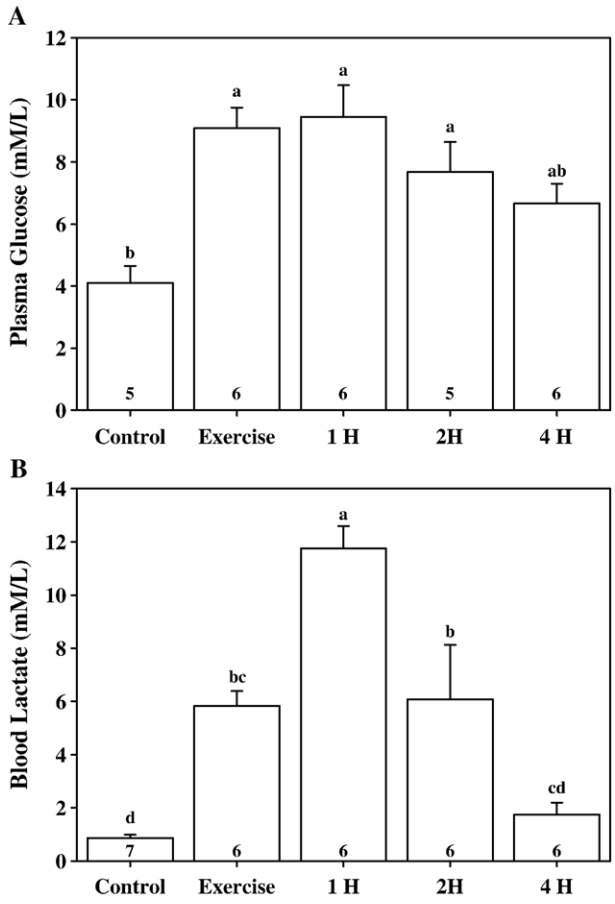

Fig. 1. Concentrations of plasma glucose (A) and blood lactate (B) for bonefish exercised 4 min and allowed to recover 4-h in sea water without additional exposure to air. Dissimilar letters above bars denote statistically significant differences across recovery times (Glucose: ANOVA, $F_{4,23}=7.1$, $P=0.0007$; Tukey–Kramer HSD test, $P<0.05$) (Lactate: ANOVA, $F_{4,26}=18.7$, $P=0.0007$; Tukey–Kramer HSD test, $P<0.05$). Error bars show ±1 standard error (SE) and sample sizes are shown on individual bars.

previously been shown to be relevant to catch-and-release angling events for bonefish (Cooke and Philipp, 2004; Danylchuk et al., 2007a,b). One group of fish was exercised 1 min and then non-lethally sampled for blood as described above, thus representing a brief angling event. Another group was exercised 4 min, exposed to air for 1 min, and then non-lethally sampled for blood. Finally, a third group was exercised 4 min, exposed to air for 3 min, and then non-lethally sampled for blood.

Previous work has shown that by 2-h after exercise fish have begun the process of recovering, but recovery is not complete (Suski et al., 2006). Therefore, by allowing individuals to recover for 2-h after a treatment that varied exercise or air exposure, it would be possible to see if certain treatments required prolonged recovery times relative to 4 min exercise with no exposure to air, or if recovery times were reduced (Suski et al., 2006). For this, a group of fish was exercised 4 min, air exposed 1 min, allowed to recover 2-h in individual recovery chambers and then non-lethally sampled for blood, and the final group of was exercised 4 min, air exposed 3 min, allowed to recover 2-h in individual recovery chambers and then non-lethally sampled for blood as described above.

2.3. Behavioural assessments

Danylchuk et al. (2007a) documented that the duration of exposure to air following angling can impact post-release predation in field-caught bonefish, and that almost all predation events for released bonefish occur in the first 20 min post-release. In this section, we sought to compare behaviours displayed by individuals following exercise and different air exposure durations. Therefore, fish were first exercised for 4 min as described above. Then, one group was air exposed for 1 min, while a second group was air exposed for 3 min. Both groups were non-lethally sampled for blood, marked with an individually colored external tag, and placed in an aerated recovery tank (identical in size to the treatment tank) continuously supplied with sea water. The time for fish to regain equilibrium after being placed in the tank was noted, and fish were monitored for 20 min after exposure to air to assess mortality rates.

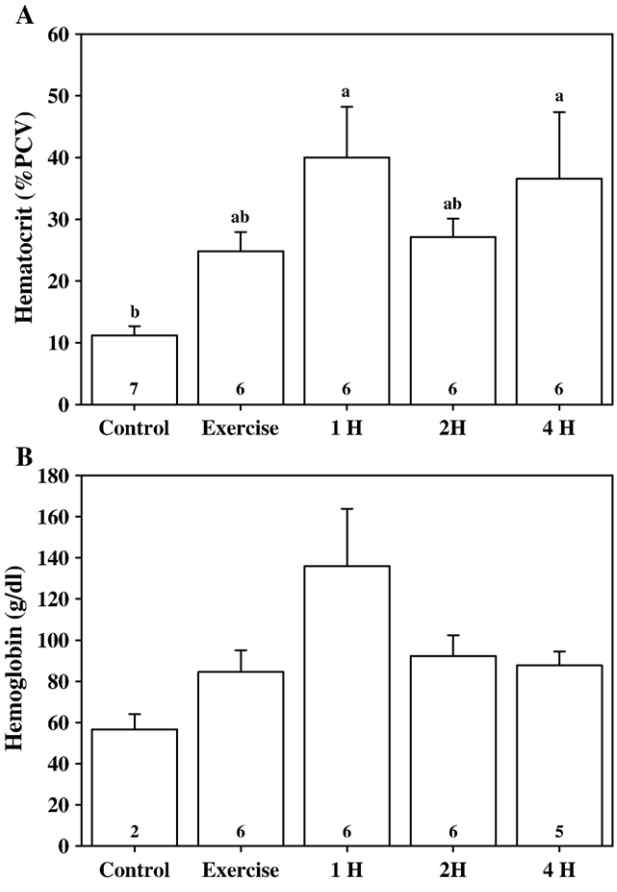

Fig. 2. Concentrations of hematocrit (A) and hemoglobin (B) for bonefish exercised 4 min and allowed to recover 4-h in sea water without additional exposure to air. Dissimilar letters above bars denote statistically significant differences across recovery times (Hematocrit: ANOVA, $F_{4,26}=3.5$, $P=0.02$; Tukey–Kramer HSD test, $P<0.05$) (Hemoglobin: ANOVA, $F_{4,20}=2.3$, $P=0.10$). Error bars show ±1 standard error (SE) and sample sizes are shown on individual bars.

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2.4. Analytical techniques

Lactate concentrations in whole blood were quantified with a commercially available handheld lactate meter (Lactate Pro LT-1710 portable lactate analyzer, Arkray Inc., Kyoto, Japan) that has been previously validated for use on fish (Venn Beecham et al., 2006). To quantify hematocrit and hemoglobin, an iStat point of care device (Heska Corporation, Fort Collins, CO, USA) was used (Harrenstien et al., 2005). For this, an aliquot of whole blood was first diluted by 25% using distilled water. Then, approximately 60 µl of diluted blood was loaded into an iSTAT E3$^+$ cartridge, and this cartridge was immediately inserted into the iSTAT unit for hematocrit and hemoglobin measurements. The remaining volume of whole blood was spun in a centrifuge (Clay Adams Compact II Centrifuge) at 10 000 ×*g* for 5 min to separate plasma from red cells. Following centrifugation, the plasma was removed from the red cells with a pipette, placed in a labeled microcentrifuge tube, and immediately placed in a dry shipper that was charged with liquid nitrogen. Samples remained at a minimum of −80 °C until further analyses. Quantifications of plasma glucose, Ca^{2+}, K^+, Na^+, and Cl^- were later performed by an animal science diagnostics laboratory (Vita-Tech, Markham, ON, Canada) that is accredited by the Veterinary Laboratory Association Quality Assurance Program and the Canadian Food Inspection Agency and is compliant with the ISO 9001:2000 Quality Management System. Plasma constituent concentrations were quantified using a Roche Hitachi 917 analyzer with the appropriate Roche reagents.

2.5. Data analyses

Differences in blood responses across treatment groups were assessed using a one-way analysis of variance (ANOVA) followed by a Tukey–Kramer HSD test (Sokal and Rohlf, 1995; Day and Quinn, 1989), and comparison of time required for fish to regain equilibrium following exercise and exposure to air was performed with a Wilcoxon rank-sum test. All analyses were performed using JMP 6.0.2 (SAS Institute, Cary, NC, USA) and the level of significance (α) for all tests was 0.05.

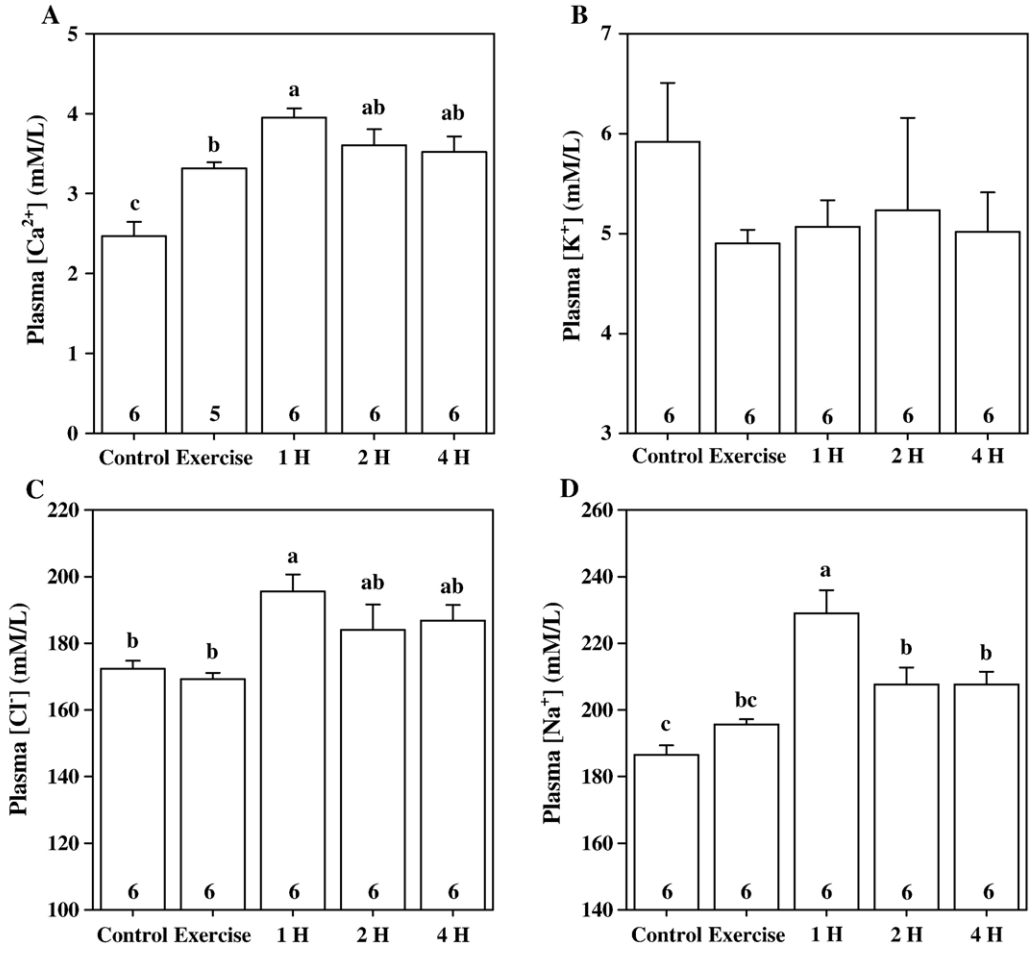

Fig. 3. Concentrations of plasma calcium (A) potassium (B) chloride (C) and sodium (D) for bonefish exercised 4 min and allowed to recover 4-h in sea water without additional exposure to air. Dissimilar letters above bars denote statistically significant differences across recovery times (Ca^{2+}: ANOVA, $F_{4,23}=12.1$, $P<0.0001$; Tukey–Kramer HSD test, $P<0.05$) (K^+: ANOVA, $F_{4,25}=0.57$, $P=0.7$) (Cl^-: ANOVA, $F_{4,25}=5.1$, $P=0.004$; Tukey–Kramer HSD test, $P<0.05$) (Na^+ : ANOVA, $F_{4,25}=13.0$, $P<0.0001$; Tukey–Kramer HSD test, $P<0.05$). Error bars show ±1 standard error (SE) and sample sizes are shown on individual bars.

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3. Results

3.1. Recovery from exercise

Immediately following 4 min of exercise, bonefish exhibited a 2-fold increase in plasma glucose concentrations, but this increase returned to control values by 4-h after exercise (Fig. 1A). Blood lactate concentrations increased over 13 fold by 1-h after exercise (Fig. 1B), but this disturbance also returned to baseline after 4-h of recovery (Fig. 1B). Hematocrit concentrations in exercised fish increased almost 4-fold by 1-h post-exercise and remained significantly higher than control values even after 4-h of recovery (Fig. 2A). Following both 4 min of exercise and 4-h recovery, plasma hemoglobin concentrations did not differ significantly from control values even though mean hemoglobin concentration was over twice control values at 1-h post-exercise. Due to technical difficulties associated with sample analyses, hemoglobin values from only two control fish could be generated possibly impacting the results of statistical analysis.

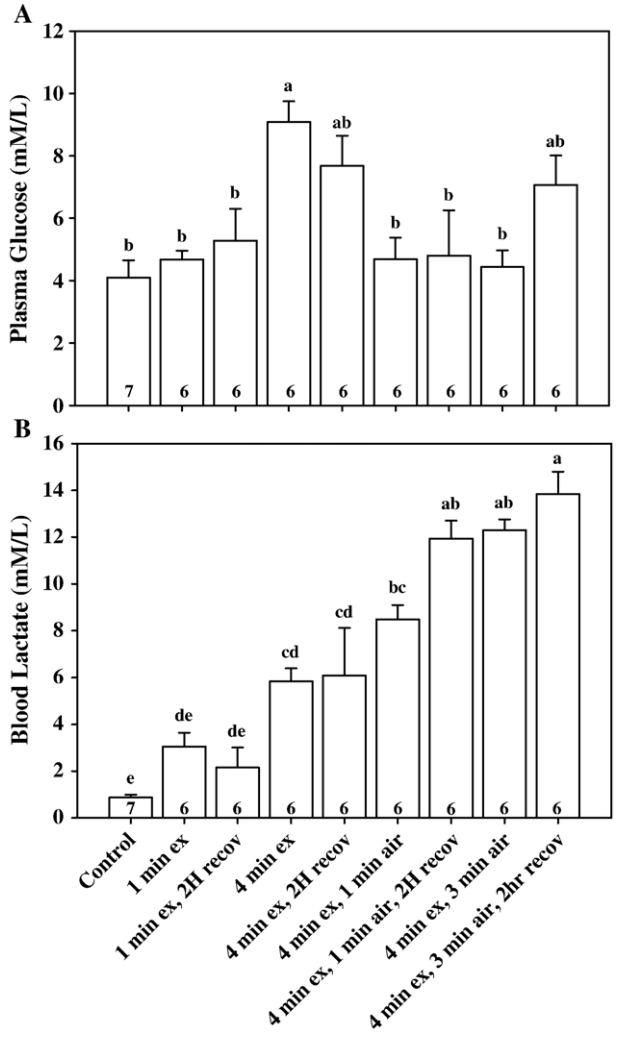

Fig. 4. Concentrations of plasma glucose (A) and blood lactate (B) for bonefish exercised for various durations, exposed to air for various durations, and allowed to recover in sea water for 2-h. Dissimilar letters above bars denote statistically significant differences across treatments (Glucose: ANOVA, $F_{8,40}=4.3$, $P<0.0001$; Tukey–Kramer HSD test, $P<0.05$) (Lactate: ANOVA, $F_{8,46}=27.9$, $P<0.0001$; Tukey–Kramer HSD test, $P>0.05$). Error bars show ±1 standard error (SE) and sample sizes are shown on individual bars.

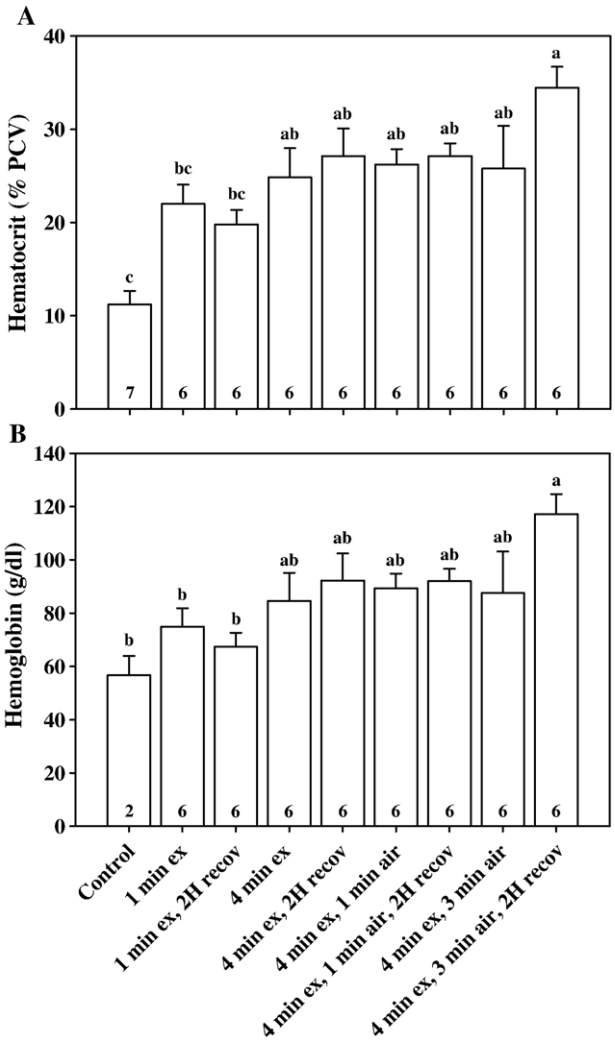

Fig. 5. Concentrations of hematocrit (A) and hemoglobin (B) for bonefish exercised for various durations, exposed to air for various durations, and allowed to recover in sea water for 2-h. Dissimilar letters above bars denote statistically significant differences across treatments (Hematocrit: ANOVA, $F_{8,46}=6.9$, $P<0.0001$; Tukey–Kramer HSD test, $P>0.05$) (Hemoglobin: ANOVA, $F_{8,41}=2.9$, $P=0.01$). Error bars show ±1 standard error (SE) and sample sizes are shown on individual bars.

Four minutes of exercise resulted in a 35% increase in plasma Ca^{2+} concentration and this disturbance had not returned to baseline levels after 4-h recovery (Fig. 3A). Plasma concentrations of K^+ did not vary across any exercise or recovery treatment (Fig. 3B). At 1-h post-exercise, fish exhibited a significant increase in plasma Cl^- concentration relative to control values but this disturbance had returned to control values by 2-h post-exercise (Fig. 3C). By 1-h post-exercise, plasma Na^+ concentrations were 23% greater than control values and remained significantly higher than control values during the entire 4-h recovery period (Fig. 3D).

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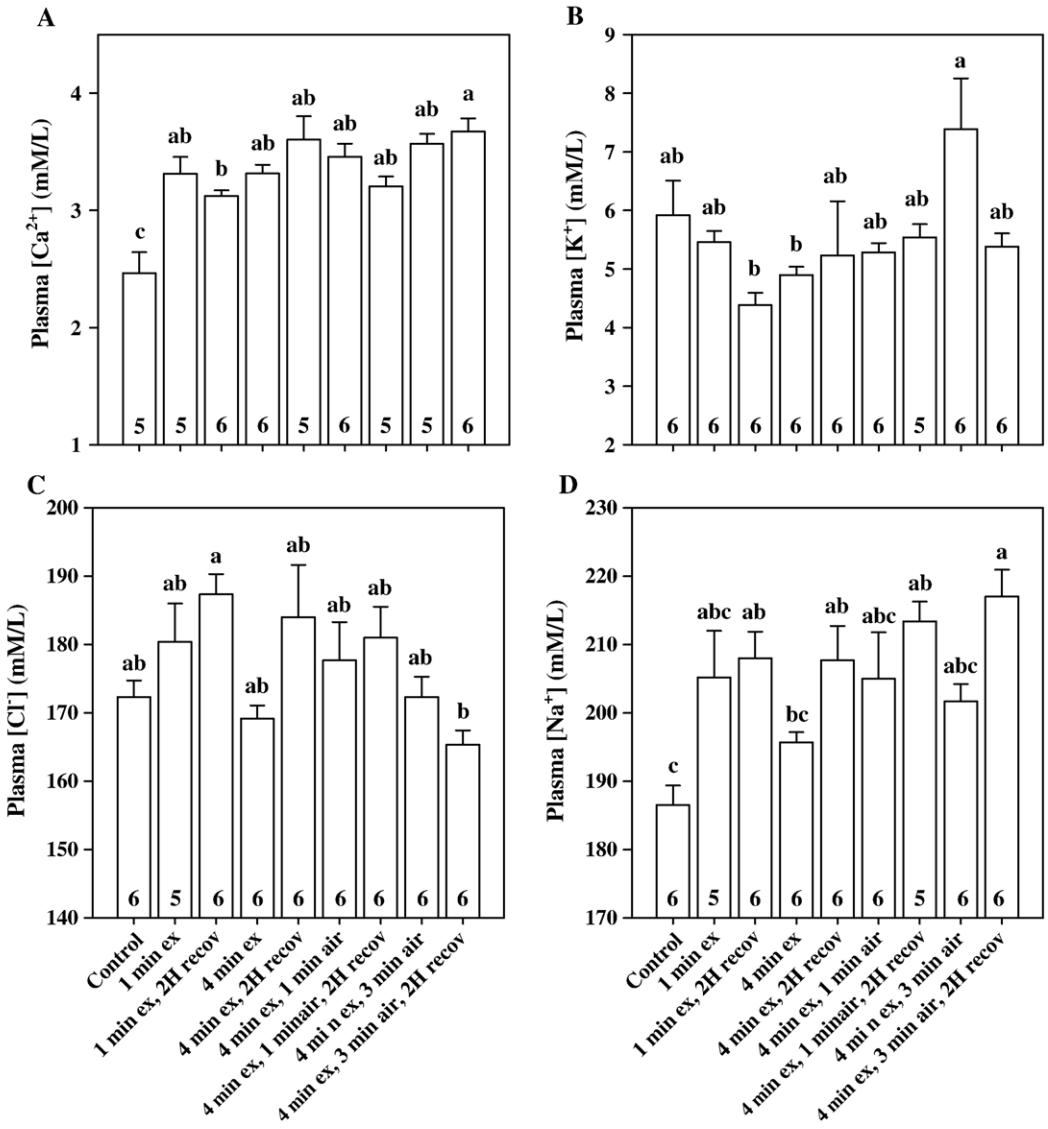

Fig. 6. Concentrations of plasma calcium (A) potassium (B) chloride (C) and sodium (D) for bonefish exercised for various durations, exposed to air for various durations, and allowed to recover in sea water for 2-h. Dissimilar letters above bars denote statistically significant differences across treatments (Ca^{2+}: ANOVA, $F_{8,40}=8.9$, $P<0.0001$; Tukey–Kramer HSD test, $P<0.05$) (K^{+}: ANOVA, $F_{8,43}=2.7$, $P<0.02$; Tukey–Kramer HSD test, $P>0.05$) (Cl^{-}: ANOVA, $F_{8,43}=2.9$, $P=0.01$; Tukey–Kramer HSD test, $P>0.05$) (Na^{+}: ANOVA, $F_{8,43}=4.5$, $P=0.0005$; Tukey–Kramer HSD test, $P<0.05$). Error bars show ±1 standard error (SE) and sample sizes are shown on individual bars.

3.2. Exercise and air exposure duration

None of the different exercise or air exposure treatments caused plasma glucose concentrations to differ significantly from control values, with the exception of the 4 min exercise treatment and this disturbance returned to control levels after 4-h recovery (Fig. 4A). Blood lactate concentrations in fish that were exercised for 1 min did not differ significantly from control values (Fig. 4B). When 4 min of exercise was combined with 1 min of exposure to air, blood lactate values were almost 10-fold greater than control values, and almost triple the blood lactate values exhibited by fish that were exercised 4 min alone (Fig. 4B). In addition, the blood lactate concentration of individuals exercised 4 min, air exposed for 1 min, and allowed to recover for 2-h was almost twice as great as fish that were exercised 4 min but not air exposed and allowed to recover for 2-h. Fish that were exercised 4 min and then air exposed for 3 min had blood lactate concentrations that were approximately 15-fold greater than control values even after 2-h recovery (Fig. 4B).

Bonefish that were exercised for 1 min did not exhibit changes in hematocrit that were significantly different from control values (Fig. 5A). However, individuals that were exercised 4 min and air exposed all showed hematocrit values that were greater than control, and values did not return to control levels by 2-h post-exercise (Fig. 5A). The different exercise and air exposure treatments resulted in significant changes to hemoglobin concentrations although only fish that were exercised 4 min, air exposed 3 min and recovered for 2-h showed concentrations that were significantly different from control treatments (Fig. 5B).

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Exercise and air exposure caused a 42% increase in plasma Ca^{2+}, which was not corrected after two hours recovery (Fig. 6A). Plasma K^+ concentrations of bonefish exposed to the different exercise and air exposure treatments differed across treatments, although differences between individual treatments could not be detected (Fig. 6B). Similarly, plasma Cl^- concentrations differed significantly across the different exercise and air exposure treatments, but trends across treatment groups could not be differentiated, and no treatment differed significantly from control values (Fig. 6C). Exercise and/or exposure to air alone did not result in significant changes to plasma Na^+ concentrations relative to control values (Fig. 6D). Individuals that were allowed to recover for 2-h following different exercise and air exposure treatments, however, all exhibited a significant increase in plasma Na^+ concentrations that were approximately 11% greater than control values, although there were no differences between the different recovery treatments (Fig. 6D).

3.3. Behavioural assessments

One of the fish that were exercised 4 min and air exposed 3 min died, while no mortality was observed in fish that were exercised 4 min and air exposed 1 min. Fish that were exercised 4 min and then air exposed 3 min required over 10 times longer to regain equilibrium (4.4 min±4.9 min, $N=5$) than fish that were air exposed 1 min (0.39 min±0.3 min, $N=6$) (Wilcoxon test, $S=42$, $Z=2.1$, $P=0.04$).

4. Discussion

Following 4 min of exercise, bonefish exhibited physiological and biochemical changes that included significant increases in plasma glucose, blood lactate, and hematocrit relative to resting values. Changes in these variables following the onset of acute stressors of this magnitude are consistent with responses exhibited by several freshwater fish species including largemouth bass (*Micropterus salmoides*) (Suski et al., 2006) and rainbow trout (*Oncorhynchus mykiss*) (Wood, 1991; Wang et al., 1994), as well as by marine fishes such as the coral trout (*Plectropomus leopardus*) (Frisch and Anderson, 2000). Lactate is produced in muscle when fish anaerobically consume energy stores such as adenosine triphosphate (ATP), phosphocreatine (PCr) and glycogen during burst exercise (Wood, 1991; Wang et al., 1994). Hyperglycemia is a common stress indicator for fishes, and glucose is released from the liver as part of the stress response to fuel metabolic demands (Wendelaar Bonga, 1997). Hematocrit is another common stress indicator for fish and is a measure of the cellular fraction of blood (Barton et al., 2002). Elevations in hematocrit can occur either due to splenic contractions that release additional red blood cells to augment oxygen transport, or to erythrocytic swelling to improve oxygen transport (Wendelaar Bonga, 1997). While changes in hemoglobin concentration during exercise and recovery were not statistically significant, hemoglobin concentrations 1-h post-exercise were more than double control values, and post-exercise changes in hemoglobin concentration were similar in magnitude to hematocrit values, suggesting that changes in hematocrit resulted from increased red blood cell number. Taken together, the results of this study suggest that the metabolic response of bonefish to forced exercise is similar to what has been documented in freshwater and marine fishes (Cooke and Philipp, 2007).

Bonefish that were forced to exercise for 4 min exhibited significant increases in plasma Na^+ , Cl^-, and Ca^{2+} concentrations at 1-h post-exercise relative to control individuals. In resting (non-exercised) marine fish, Na^+ and Cl^- concentrations are maintained when these ions exit gill epithelia down their electrochemical gradient into the surrounding water following the establishment of an electrochemical gradient by a Na^+, K^+-ATPase (Evans et al., 2005; Marshall, 2002). As well, marine fishes actively uptake Ca^{2+} from surrounding sea water by means of either a Ca^{2+}-ATPase and/or a Na^+/Ca^{2+} exchanger (Marshall, 2002). Stressors such as exercise and angling result in the secretion of stress hormones such as epinephrine (Wendelaar Bonga, 1997). The release of stress hormones serves several functions, including to increase cardiac output, increase blood pressure, recruit gill lamellae and increase gill diffusing capacity to facilitate oxygen uptake as fish attempt to meet elevated oxygen demands (Randall, 1982; McDonald and Milligan, 1997). Changes to gill morphology associated with the actions of stress hormones have been shown to facilitate ion permeability by disrupting tight junctions and decreasing the resistance to ion diffusion (Wendelaar Bonga, 1997; Gonzalez and McDonald, 1992). The end result is that exercise and stress are typically associated with a concomitant mineralization and water loss to the environment for marine fishes (Wendelaar Bonga, 1997; Wood, 1991). For example, Milston et al. (2006) noted an increase in plasma Na^+ and K^+ 1-h after a 15 min air exposure treatment in lingcod (*Ophiodon elongatus*). Similarly, Davis and Schreck (2005) showed that plasma concentrations of Na^+ increased relative to control values 1-h after a 30 min air exposure in Pacific halibut (*Hippoglossus stenolepis*). Because plasma concentrations of K^+ did not vary during exercise or recovery in the current study, elevations in ion concentrations in the plasma of exercised bonefish 1-h post-exercise likely did not result simply from water loss to the environment. Rather, the ionic disturbances observed in bonefish at 1-h post-exercise likely resulted from the passive movement of ions into the fish, with this movement being facilitated by changes to gill morphology that occurred as part of the secondary stress response (Wendelaar Bonga, 1997; Gonzalez and McDonald, 1992).

Our study indicates that 4-h of recovery for bonefish is sufficient for some physiological variables to return to baseline values (specifically blood lactate and plasma glucose), and that recovery in excess of 4-h could be required for a return of full physiological homeostasis, particularly to correct changes in ionic disturbances. The concentrations of several plasma constituents that had been disturbed by 4 min exercise had not returned to control levels even after 4-h recovery. Specifically, concentrations of blood lactate, plasma glucose, and Cl^- had all returned to control values by 4-h post-exercise, while plasma Ca^{2+}, Na^+ and hematocrit concentrations remained significantly higher than control fish 4-h after exercise.

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During recovery, fish attempt to correct ionic/osmotic imbalances, clear lactate accumulations and replenish energy stores that have been disrupted by exercise (Milligan, 1996; Suski et al., 2006). The time required for this process varies across the species examined and the variables monitored. In general, studies with rainbow trout and largemouth bass indicate that, for the metabolic variables measured in this study, recovery from exercise alone should take between 2–4-h (Milligan, 1996; Suski et al., 2006), while ionic disturbances typically take longer for full recovery, often requiring up to 12–48 h (Wood, 1991; Postlethwaite and McDonald, 1995; McDonald and Milligan, 1997). Changes to ion balance for fishes can disrupt the distribution of fluids between body compartments (Wood, 1991), electrochemical gradients necessary for the function of cardiac or skeletal muscle, cellular electroneutrality, and neural function, all of which could impact swimming and the ability of bonefish to avoid predators during the recovery period. Disruptions in Cl^- concentrations likely were corrected through either the return of excess Cl^- ions to the environment via the gills (Marshall, 2002) or else through the uptake of Cl^- ions by white muscle as part of lactate/anion exchange (Wood, 1991). Calcium ions are not actively excreted by gill epithelia (Marshall, 2002) possibly leading to their delayed recovery compared to Cl^- ions, while sodium concentrations may have remained elevated during recovery because Na^+/H^+ pumps may have been used to excrete metabolic protons, resulting in a concomitant increase in Na^+ (Wood, 1991). In summary, results from this study show that 4-h recovery is sufficient for some physiological variables to return to control values for bonefish (specifically blood lactate and plasma glucose), but, at the water temperatures used in this study, bonefish require in excess of 4-h for full physiological recovery, particularly to correct changes in ionic disturbances.

Interestingly, the different ionic and metabolic variables examined in this study showed considerable variation in their responses to the different durations of exercise and air exposure. Specifically, plasma glucose concentrations sampled after 1 min of exercise were not significantly different from control values, while plasma glucose concentrations sampled after 4 min of exercise were more than double control values. Similarly, blood lactate concentrations sampled after 1 min of exercise were not significantly different from control values, but blood lactate concentrations increased relative to control values as exercise duration and air exposure duration increased. Past studies with largemouth bass have demonstrated that the duration of exercise (or angling) correlates positively with lactate production (Gustaveson et al., 1991), while Davis and Schreck (2005) showed that lactate production in Pacific halibut was directly proportional to air exposure duration. Exposure to air for fish has been shown to prevent gas exchange at the gills, thereby forcing individuals to respire anaerobically (Ferguson and Tufts, 1992); the energetic requirements of burst activity associated with exercise or angling cannot be met aerobically, also forcing fish to generate energy anaerobically resulting in lactate production. Results from the current study show that both the duration of an exercise bout (or angling event) longer than 4 min, as well as the length of exposure to air, will result in a proportional increase in metabolic disturbances. Results from our series of behavioural experiments also show that these increased disturbances require additional time for recovery. More importantly, greater metabolic disturbances may increase the likelihood of mortality, as evidenced by the fact that we only observed mortality in the treatment group that was air exposed for 3 min after exercise (although only 1 mortality was observed). Similar results were noted by Davis and Parker (2004) who saw that mortality rate increased with air exposure duration for sablefish.

In contrast to metabolic response variables, bonefish from almost all treatment groups, regardless of the duration of exercise or exposure to air, experienced significant increases in plasma Ca^{2+} and Na^+ concentrations relative to control values, and there were almost no differences the magnitude of ionic disturbances among the different air exposure or exercise treatments. Additionally, ionic disturbances that arose due to exercise or air exposure typically were not corrected after 2-h recovery. McDonald and Milligan (1997) showed that changes in the electrolyte balance of freshwater salmonids happen almost immediately following the onset of a stressor, likely due to changes in gill characteristics associated with adrenaline secretions, and that the full restoration of osmotic imbalances after the cessation of a stressor occur slowly (often requiring 12–48 h). More importantly, McDonald and Milligan (1997) suggest that the rapid change in ionic concentrations following the onset of a stressor indicates that the degree of ionic disturbances in fish likely depends on the severity of a stressor rather than duration. Ion-specific changes in marine fishes following exercise and air exposure stressors have generated disparate results with conflicting trends emerging across species. In the current study with bonefish, the different air exposure and exercise regimes resulted in a general increase in plasma Ca^{2+} and Na^+ concentrations, with little changes observed in plasma K^+ and Cl^-. Arends et al. (1999) noted that gilthead sea bream (*Sparus aurata*) air exposed for 3 min showed no change in plasma K^+ or Ca^{2+}, and rapid increases in plasma Na^+ and Cl^-, but these changes were corrected by 1-h post-disturbance at temperatures that were comparable to those of the current study (18–22 °C). In contrast, Hur et al. (2007) showed that olive flounder (*Paralichthys olivaceus*) air exposed for 2 min at 17–18 °C showed no significant changes in plasma Na^+ concentrations relative to control individuals. Thus, results from the current study show that bonefish tend to be particularly sensitive to ionic perturbations related to exercise and exposure to air. Species-specific differences in the response of ions to stressors would be a fruitful area for future study.

The results of this study have several implications for fisheries management strategies and catch-and-release angling. First, because longer angling and air exposure times will increase metabolic disturbances for bonefish, anglers should strive to minimize angling times to reduce the degree of physiological disturbances and minimize the time required for bonefish to recover from angling. Several studies have reported significant predation of released bonefish by barracuda (*Sphyraena barracuda*) and sharks [primarily lemon shark

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(*Negaprion brevirostris*)] (Cooke and Philipp 2004; Danylchuk et al., 2007a,b). Thus, anglers should strive to land bonefish quickly to minimize metabolic disturbances and reduce the likelihood of post-release predation. Second, greater physiological disturbances associated with increased air exposure times may increase the likelihood of mortality for exercised bonefish by delaying time to regain equilibrium following release. Danylchuk et al. (2007a) found air exposure duration that often accompanies a catch-and-release angling event for bonefish was an important predictor of equilibrium loss, which, in turn, was a significant predictor of post-release mortality. In our behavioural study, initial mortality occurred only in bonefish that were exercised and exposed to air for 3 min, and the time required for bonefish to regain equilibrium and begin swimming in a tank was greater for longer air exposure durations. Therefore, reduced air exposure times during a catch-and-release angling event for bonefish can help minimize metabolic disturbances, and ultimately maximize post-release survival. Third, bonefish appear to be sensitive to ionic/osmotic disturbances following exercise. Four minutes of intense exercise resulted in significant elevations in plasma Na^+ and Ca^{2+}, and these disturbances had not resolved after 4-h of rest, indicating the slow nature of recovery for ionic variables. The impact of such ionic disturbances on subsequent swimming bouts should be further studied. Fourth, water temperatures need to be considered when interpreting results from this, and future studies of bonefish exercise and recovery. At the temperatures examined in the current study, many of the physiological variables examined had not returned to control values even after 4-h of recovery. Recent studies by both Suski et al. (2006) as well as by Galloway and Kieffer (2003) showed that reduced water temperatures will delay the time for recovery in exercised fish, likely because of reduced metabolic rate. Bonefish are widely distributed across the tropics, often inhabiting water with temperatures well in excess of 30 °C (Danylchuk et al., 2007a), which are much warmer than the temperatures of 21–23 °C in this study; warmer temperatures may impact both the magnitude of exercise-induced disturbances, as well as recovery profile dynamics.

Bonefish are a prized sportfish that are frequently targeted by catch-and-release anglers (Ault et al., 2002; Cooke and Philipp, 2007). The sustainability of this species depends on understanding the dynamics and mechanisms behind physiological changes resulting from angling (exercise) and exposure to air to provide suggestions and recommendations to fisheries managers under the auspices of "conservation physiology" (Wikelski and Cooke, 2006). Results from the current study show that the degree of metabolic disturbance for bonefish increased with the duration of an exercise stressor, and that increased disturbances require increased time to recover. Metabolic disturbances in bonefish that have been exercised 4 min appear to fully recover in 2–4-h. In contrast, ionic disturbances develop almost immediately following the onset of an exercise stressor, and the magnitude of these stressors appears to be independent of exercise duration. Certain ions appear to be more sensitive to exercise-induced disturbances than others, and recovery of ionic variables appears slow relative to metabolic variables, frequently requiring in excess of 4-h recovery time. Air exposure, particularly extended periods, further delays physiological recovery. When taken together, these results suggest that catch-and-release anglers should minimize both the duration of an angling event as well as the length of post-angling air exposure to minimize physiological disturbances in bonefish and ensure their survival.

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