



## Physiological and behavioural consequences of cold shock on bonefish (*Albula vulpes*) in The Bahamas



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### ABSTRACT

The effects of cold shock on fish have rarely been assessed in sub-tropical regions despite the fact that such events can occur as a result of upwelling, storms, or other climactic events that are expected to increase as a result of global environmental change. Here, the sub-lethal physiological and behavioural consequences of cold shock on bonefish (*Albula vulpes*) were assessed using four treatment groups (control, handled control, 7 °C below ambient, and 14 °C below ambient conditions; n = 8 fish/treatment). The control and handled control remained at ambient temperature (~24 °C) while other treatments were exposed to a 2 h cold shock. Handled controls were treated with the same handling procedures as cold shock treatments, while control fish were less disturbed. Ventilation rate and reflex indicators (e.g., equilibrium, response to stimuli) were recorded throughout the experiment, and swimming ability was evaluated 2 h after cold shock exposure. A blood sample was taken after the cold shock exposure and 2 h following the swimming assessment to quantify glucose and lactate concentrations, hematocrit and pH in whole blood, and cortisol titers from spun plasma. Bonefish exposed to 14 °C below ambient temperature experienced elevated lactate concentrations and high reflex impairment, with the majority of fish losing equilibrium <30 min into exposure. During cold shock exposure, ventilation rates were significantly lower in the 7 °C below ambient treatment than in the handled control. After cold shock exposure, swimming ability decreased as the magnitude of cold shock increased, however, it was also depressed in the handled control relative to unhandled controls. Exposure to 14 °C below ambient temperatures had significant physiological and behavioural impacts on bonefish, while 7 °C below ambient had little effect. Abrupt declines in water temperature in shallow tropical or sub-tropical seas >7 °C due to storm events or upwelling have the potential to cause physiological and behavioural impairment that could lead to mortality in this species.

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### 1. Introduction

Temperature is one of the most important abiotic variables affecting fish and fish populations (Beitinger and Fitzpatrick, 1979; Brett, 1971; Magnuson et al., 1979). Because fish are ectothermic, temperature plays a dominant role in regulating enzymatic and metabolic processes (Hazel, 1984; Shaklee et al., 2005), bioenergetics (Fry, 1971) and growth (Meekan et al., 2003; Pepin, 1991). Temperature also affects behavioural responses such as swimming, migration, predator avoidance and foraging (Beamish, 1974; Fischer et al., 1987; Green and Fisher, 2004). The thermal biology of fish has been generally well studied. Early work focused on identifying thermal thresholds (e.g., critical thermal maxima; reviewed

in Beitinger and Bennett, 2000) and understanding the manifold influence of temperature on biological processes (Brett, 1971; Fry, 1971). More recently, there has been a renewed interest in studying the thermal biology of fish in the context of global climate change (Roessig et al., 2004). Fish are adapted to specific temperature regimes, and changes in these temperatures can have adverse effects on their physiology or behaviour (Genner et al., 2004; Jones et al., 2004).

Most research on thermal stress in fish has focused on the consequences of chronically increasing temperatures (e.g., Bevelhimer and Bennett, 2000; Elliott, 1991; Kammerer and Heppell, 2013), while relatively few studies have examined the physiological or behavioural consequences of acute temperature changes, especially acute decreases in temperature. Cold shock is defined as an acute drop in temperature as a result of abnormally cold ambient conditions (Donaldson et al., 2008). Cold shock often induces physiological stress (e.g. accumulation

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of lactate, ionic imbalance, consumption of tissue energy stores) in fish that can lead to behavioural impairments (e.g., reductions in swimming ability), which may compromise their survival due to increased predation, poor foraging decisions, or stress-related mortality (reviewed in Donaldson et al., 2008).

Anthropogenic disturbances such as effluent discharge from manufacturing facilities and power generation plants likely represent the dominant causes of cold shock in fish (Cherko et al., 1976; Horne et al., 2004; Ryan and Witzel, 1993; Smythe and Sawyko, 2000). These point-sources for thermal pollution often discharge warm effluent, attracting fish that subsequently become acclimated to higher than usual temperatures; if effluent discharge ceases, fish often experience cold shock (Coutant, 1970; Hubbs, 1991; Miller, 1977). Cold shock can also occur naturally through severe weather disturbances and differences in the position of the thermocline and seiches, upwelling or other hydrodynamic processes (Donaldson et al., 2008). Weather and other physically driven disturbances have the potential to affect fish over broader spatial scales than point-source thermal effluent. Given that climate change is expected to not only increase water temperature, but also incur more dynamic thermal conditions as a result of frequent storms (Ellison and Farnsworth, 1996), cold shock may be a relevant stressor in this context. Although cold shock has been studied in temperate regions where seasonal variation is large (e.g., Ash et al., 1974; Barton and Peter, 1982; Lemoine and Smith, 1980; Tanck et al., 2000; Wilde, 1988), it is poorly understood in tropical and sub-tropical regions, where it is also pertinent (Coutant and Talmadge, 1977).

In January 2010, Florida, USA, experienced their coldest 12-day period since 1940 (NOAA, 2010). Water temperatures in Butternut Key, Florida declined 11.2 °C from 19.3 °C on January 1 to 8.1 °C on January 12 at a depth of 1.5 m (NOAA, 2010). Associated with this anomaly was a mass fish kill that affected a variety of species including several important recreational fish species such as bonefish (*Albula vulpes*), common snook (*Centropomus undecimalis*), and Atlantic tarpon (*Megalops atlanticus*). The exact number of fish that died was uncertain (a common trait of fish kills; La and Cooke, 2011), but estimates were on the order of hundreds of thousands of individuals (Aaron Adams, Bonefish and Tarpon Trust, Personal Communication; FWCC, 2010). Other such fish kills of various scales have also been reported to occur around the globe when weather-driven cold shock occurs in tropical and sub-tropical zones (e.g., Bohnsack, 1983; Cyrus and McLean, 1996; Lamadrid-Rose and Boehlert, 1988). Surprisingly, cold shock has rarely been considered in studies of tropical fish (Donaldson et al., 2008) aside from largely anecdotal accounts of cold-induced mortality. Indeed, we are unaware of any studies that have examined the sub-lethal consequences of cold shock on fish in sub-tropical or tropical regions.

Bonefish (*Albula* spp.) were heavily affected by the Florida cold shock event (Larkin, 2011), and are ecologically and economically important in Florida and throughout the Caribbean (Adams et al., 2008; Danylchuk et al., 2008). Bonefish primarily occur in shallow tidal flats where they feed on benthic organisms such as crustaceans and molluscs (Crabtree et al., 1998). These expansive shallow marine ecosystems are often subject to rapid fluctuations in temperature. For example, temperatures at the site bonefish were collected from in the present study can fluctuate up to 11.4 °C over a 24 h period (Murchie et al., 2011). On almost a daily basis, bonefish travel between the flats and tidal creeks, and therefore experience routine fluctuations in temperature (Murchie et al., 2011, 2013). However, associated with a severe weather event, these fluctuations may occur in greater magnitude, occur more quickly and remain over a longer period of time, which may elicit impairment or mortality (Donaldson et al., 2008) in fish found in near-shore habitats. If a cold shock event occurs rapidly over a large spatial scale it may render the fish unable to avoid (i.e., swim away from) the cold water temperatures, and consequently may be exposed for long enough to show sub-lethal or lethal effects, particularly in expansive shallow flats where it is harder to find deeper water refuge. While Murchie et al. (2011) identified critical thermal maxima ( $CT_{Max}$ ) of bonefish ( $36.5 \pm 0.5$  °C

when acclimated to 27.4 °C); their lower thermal tolerances are still unknown. Given the relevance of cold shock to this ecologically and economically valuable species, bonefish were a good candidate to explore the physiological and behavioural effects of cold shock on a sub-tropical fish.

The objective of this research was to quantify physiological and behavioural responses of bonefish to ecologically relevant cold shock exposures. To this end, we exposed wild-caught bonefish (*A. vulpes*), acclimated to ambient thermal conditions during early summer (i.e., ~25 °C), to either a 7 °C or 14 °C acute reduction in water temperature for up to 2 h, and used blood physiology, ventilation rates, reflex impairments and swimming abilities as endpoints to quantify the response to these challenges. These responses were compared to fish in both a control group, as well as a handled control group to isolate the effects of handling on physiological and behavioural responses. Collectively, this work will provide an understanding of the mechanistic basis of cold shock mortality of bonefish, and, more generally, provide insight into the thermal biology of sub-tropical marine fish.

## 2. Methods

The study was conducted in south Eleuthera, The Bahamas (N 24°50' 05" and W 76°20'32") at the Cape Eleuthera Institute (CEI) from May 9 to May 20, 2012, in accordance with the policies of Canadian Council on Animal Care as administered by the Carleton University Animal Care Committee (Protocol B12-03). Thirty-two adult bonefish (mean =  $403 \pm 10$  SE fork length; 340–465 mm) were collected from three tidal creeks (Kemps Creek, Broad Creek, and Starved Creek; see Fig. 1 in Murchie et al., 2009) and transported to the laboratory facilities at CEI following capture, handling, and transport methodologies outlined by Murchie et al. (2009). Previous genetic analyses on bonefish from the study area indicated that all specimens were likely exclusively *A. vulpes* (Danylchuk et al., 2007a). Upon arrival at CEI, bonefish were transferred to a large (3.7 m diameter  $\times$  1.25 m height; 13,180 L) circular holding tank that was aerated and continuously supplied with fresh seawater (1,800 L/h) at ambient temperatures. Fish were given a minimum of 48 h to acclimate to laboratory conditions prior to experimentation.

Each of the four cold shock exposure trials included eight fish, two from each treatment group (control, 25.4 °C mean  $\pm$  1.7 SE; handled control, 24.7 °C  $\pm$  1.0; 7 °C below ambient temperature, 18.6 °C  $\pm$  1.1; and 14 °C below ambient temperature, 11.8 °C  $\pm$  1.3). Trials were run on separate days starting at 900 h. Twenty-four hours prior to experimentation, bonefish were dip netted from the holding tank and placed in individual opaque plastic containers (53 cm length  $\times$  32 cm width  $\times$  25 cm height; 42 L) supplied with fresh flowing seawater and an air stone. Containers were covered and fish were left undisturbed until experimentation. Fish were held outdoors, so the locations of treatments were randomized each day to account for potential variation in conditions due to container location.

### 2.1. Ventilation rate & reflex action mortality predictors (RAMP)

At the start of experimentation, resting ventilation rate was recorded by visually counting the number of opercular beats for a 30 sec period (as per Shultz et al., 2011; Table 1). Immediately after recording ventilation rate, reflex action mortality predictors (RAMP) were evaluated in < 10 sec in the containers, following the same methodology as Raby et al. (2012). RAMP scores provide a rapid evaluation of the level of reflex impairment in fish that are predictive of mortality (Davis, 2010; Raby et al., 2012), and the five reflex tests used here [i.e., equilibrium, body flex, vestibular-ocular response (VOR), tail grab, and head complex] are good predictors of bonefish behavioural impairment (Brownscombe et al., 2013). A positive response (score of 0) for equilibrium was characterized by the fish righting itself within 3 sec when held upside down; body flex was assessed at the

**Table 1**

Time period (hr into trials) of data collection for ventilation rate, RAMP, and blood physiology data from bonefish in Control (C), Handled control (H), 7 °C below ambient temperature (7), and 14 °C below ambient temperature (14) treatments.

Time period (hr)	Ventilation rates	RAMP	Blood physiology
0 (pre exposure)	H 7 14	H 7 14	-
0.5–2 (during exposure)	H 7 14†	-	-
2 (post exposure)	C	C H 7 14†	C H 7 14†
4 (post chase to exhaustion)	H 7 14	H 7 14	-
6 (2 h post chase)	H 7 14	-	H 7 14
26 (post chase to exhaustion)	C	C	-
28 (2 h post chase)	C	-	C

† Seven of eight fish in the 14 °C below ambient treatment lost equilibrium within the first 30 min, at which point ventilation data collection ceased, and RAMP and blood physiology were measured at the 30 min time point.

same time, with a positive response being the fish flexing its body in an attempt to escape the grip of the handler. VOR was also assessed at the same time as equilibrium when the fish was on a lengthwise axis, with a positive response being characterized as the fish rolling its eye to track the handler and maintain pitch. A positive response for tail grab was generated by an immediate burst-swim when the fish's tail was pinched. A positive response for head complex was characterized by regular opercular beats (opening and closing of the lower jaw) when observed for 5 sec. Higher RAMP scores indicated greater impairment.

## 2.2. Cold shock exposure trials

Following ventilation rate and RAMP assessments, fish were transferred by dip net into a covered, insulated cooler, partitioned into two equal sized compartments (45 cm length × 36 cm width × 36 cm height; 58 L) for each of the two fish in each treatment group, except the control group, which remained in their original containers. For cold shock treatments, water in the cooler was adjusted to 7 °C and 14 °C below ambient temperature using sealed bags of ice, which were removed from the cooler immediately before receiving a fish. A consistent temperature was maintained between the two sides of the cooler and both sides were provided with an air stone. This same netting and transport procedure was followed for the handled controls, except that water in the coolers remained at ambient temperature. Ventilation rates were recorded every 30 min during the 2 h treatment periods (Table 1). Given that the focus of this study was on sub-lethal effects, cold shock exposure was terminated if the fish lost equilibrium.

## 2.3. Blood sampling

Following the 2 h treatment period, fish from all treatments were individually transported to a sloped trough continuously supplied with fresh, ambient seawater for blood sampling (Suski et al., 2007). However, in the event that fish lost equilibrium, the affected fish was removed from the exposure and the post exposure blood sample was taken (Table 1). For blood sampling, fish were carefully restrained in a water-filled trough and non-lethally sampled for 1.5 ml of blood by caudal venipuncture using an 18 g syringe and 3 ml Vacutainer® (lithium heparin). Blood samples were taken within 3 min to avoid sampling-induced stress, inverted several times and immediately put in an ice slurry until analysis (less than 1 h; Clark et al., 2011). Immediately following blood sampling, fish were transported back to their opaque ambient containers.

## 2.4. Standardized chase to exhaustion

For handled and cold shock treatments, after 2 h rest at ambient temperature post-treatment period, individual fish were transferred to a circular tank (1.22 m diameter × 15 cm depth; 175 L), divided into four equal quadrants by black lines on the bottom of the tank, and the center was blocked with a circular plastic container (0.46 m diameter)

to create an annular swim flume (Portz, 2007). Each bonefish was chased by hand until exhaustion (i.e., no response after three consecutive tail grabs). The time taken to reach exhaustion and the number of lines crossed was recorded for each individual fish. Immediately following the exhaustion trial, ventilation rate and RAMP scores were assessed. Individual fish were then returned to their ambient containers to rest for 2 h. Following rest, ventilation rate and RAMP score were assessed and another 1.5 mL blood sample was taken following the above methodology. Fish were given 1 h to recover in their ambient containers before being released into the wild. For the control treatment, the same methodology for the chase to exhaustion was completed 26 h into the trial period (Table 1).

## 2.5. Blood analysis

Blood was processed within an hour of collection. Glucose, lactate, pH and hematocrit were all measured using whole blood, while cortisol was determined from blood plasma. Glucose and lactate concentrations (mM/L) were measured on site using glucose (ACCU-CHEK glucose meter; Roche Diagnostics; Basel, Switzerland) and lactate (Lactate Pro LT-1710 portable lactate analyser; Arkray Inc.; Kyoto, Japan) meters, two devices previously validated for use on teleost fish (Cooke et al., 2008; Venn Beecham et al., 2006) while pH was measured using a hand-held pH meter (IQ128 miniLab Elite Pocket pH Meter; IQ Scientific Instruments; Carlsbad, CA, U.S.A.). Hematocrit (%PCV) was determined using heparinized micro-capillary tubes (Drummond Scientific®; Broomall, PA, U.S.A.) which were spun for 5 min at 10,000 RPM (LW Scientific® ZIPocrit; Lawrenceville, GA, U.S.A.). The remaining blood in the Vacutainer was centrifuged for 5 min at 3,200 RPM (Clay Adams Compact II centrifuge; Corning, NY, U.S.A.) and the resulting plasma was stored at -20 °C in a cryo vial for later laboratory analysis. Cortisol (ng/mL) was measured using the methods outlined in Gingerich et al. (2010) with commercially available kits with proper intra- and inter-assay controls as per manufacturer recommendations (Enzo Life Sciences, Kit # ADI-900-071, Farmingdale, NY, USA).

## 2.6. Statistical analysis

Linear mixed effects models (LME) were used to determine if there was a significant effect of treatment (control, handled control, 7 °C below ambient, 14 °C below ambient), time, or interaction between treatment and time on bonefish ventilation rates and blood physiology (lactate, glucose, pH, hematocrit) with individual fish as a random factor. Variance structures were added to correct for variance heterogeneity between treatments and time periods (Pinero and Bates, 2000). Tukey HSD post-hoc analyses were used to determine which treatments and time periods were significantly different. Two LME models were applied to ventilation rates: one to compare all treatments before exposure, after exposure, and after the chase to exhaustion, and a second LME model to compare ventilation rates every 30 min during cold shock exposure to resting and post-treatment values for the handled control and 7 °C below ambient treatments. The 14 °C below ambient treatment was not included in the latter analysis because equilibrium was lost during exposure, which was terminated for that treatment. Cortisol data could not be fit with an LME and were analyzed using mixed-measures ANOVA with treatment as a fixed effect and time as a repeated measure. Bonferroni-corrected pairwise t-tests were used to determine which treatments and time periods were significantly different. One-way ANOVAs were used to compare the time chased and number of lines crossed between treatments during the standardized chase to exhaustion. Data were checked for assumptions of normality and homogeneity prior to analysis and were log<sub>10</sub>-transformed where necessary. The level of significance was  $p \leq 0.05$  and all analyses were conducted using R-Studio (R Core Team, 2012).

### 3. Results

#### 3.1. Ventilation rates

During the cold shock exposure period, ventilation rates (VR) decreased from resting values for both handled control and 7 °C below ambient treatment (Table 2). There were no VR data collected during the exposure period for control fish, and the majority of fish lost equilibrium in 14 °C below ambient treatment within the first 30 min of exposure, and therefore no VR were recorded. Comparing handled control to the 7 °C below ambient treatment from resting, post-exposure, and every 30 min during exposure, there was a significant effect of treatment, time, and an interaction between the two (Table 3). Fish exposed to 7 °C below ambient had significantly lower VR 1, 1.5, and 2 h into the cold shock period than resting and post-treatment (Tukey HSD;  $p < 0.05$ ), while there were no significant differences in the handled controls over time. Comparing VR in all treatments across resting, post-exposure, and post-chase time periods, there was also a significant effect of treatment, time and an interaction (Table 4). There were no significant differences between treatments within time periods, nor did VR increase significantly from resting to post-exposure in any treatment, except for control fish ( $p < 0.01$ ). After the chase to exhaustion, VR were significantly higher than resting values for all treatments ( $p < 0.05$ ). In handled control, 7 °C and 14 °C below ambient treatments, VR were also significantly higher post-chase than post-treatment ( $p < 0.05$ ), but not control fish.

#### 3.2. RAMP

Bonefish showed little sign of impairment (RAMP score of zero) in all treatment groups prior to exposure and 2 h following the standardized chase (Table 4). Similarly, handled control and 7 °C below ambient treatments did not experience any RAMP impairment after the 2 h exposure period. Conversely, bonefish that were exposed to the acute cold shock of 14 °C below ambient experienced several RAMP impairments (Table 4). All but one fish lost equilibrium < 30 min into the cold shock exposure, at which point cold shock exposure was terminated. After the standardized chase to exhaustion, all fish had RAMP scores of one due to a lack of response to tail grabbing, which was intrinsic to the chase to exhaustion process.

#### 3.3. Blood physiology

The combination of acute cold shock and an exhaustive chase period resulted in significant changes to blood-based physiological parameters in bonefish. Blood lactate concentrations were higher in the 14 °C below ambient treatment than other treatments both post-exposure and post-chase to exhaustion; however, lactate rose by 3-fold following the chase to exhaustion in control fish (Table 5). Treatment had a significant effect on lactate values ( $F_{3,24} = 9.5$ ,  $p = 0.003$ ; Table 3). The 14 °C below ambient treatment had significantly higher lactate values than control and 7 °C below ambient treatments after cold shock exposure (Tukey HSD;  $p < 0.001$ ), but not the handled control ( $p = 0.08$ ). Blood cortisol was lower in the control treatment when compared to all other

**Table 3**

Statistical outputs from linear mixed effects models or mixed-measures ANOVA (cortisol) comparing bonefish blood physiology and ventilation rates across cold shock and control treatments. P-values of significant parameters are italicized.

Variable	Parameter	DF	F-value	p-value
Lactate	Treatment	3,24	9.5	<i>0.003</i>
	Time	1,24	1.4	0.25
	Treatment × Time	3,24	2.3	0.10
Cortisol	Treatment	3,24	0.19	0.90
	Time	1,24	0.03	0.87
	Treatment × Time	3,24	4.4	<i>0.01</i>
Glucose	Treatment	3,24	0.78	0.52
	Time	1,24	0.45	0.51
	Treatment × Time	3,24	1.0	0.40
pH	Treatment	3,24	0.26	0.85
	Time	1,24	1.34	0.26
	Treatment × Time	3,24	0.22	0.88
Hematocrit	Treatment	3,24	0.81	0.50
	Time	1,24	0.35	0.56
	Treatment × Time	3,24	6.7	<i>0.002</i>
Ventilation during	Treatment	3,24	8.2	<i>0.01</i>
	Time	1,24	6.0	<i>&lt;0.001</i>
	Treatment × Time	3,24	3.0	<i>0.02</i>
Ventilation outside	Treatment	3,24	15.2	<i>&lt;0.001</i>
	Time	1,24	144.2	<i>&lt;0.001</i>
	Treatment × Time	3,24	3.5	<i>0.005</i>

treatments, and increased nearly 3-fold 2 h after the chase to exhaustion (Table 5). There was also a significant interaction between treatment and time when comparing cortisol values ( $F_{3,24} = 4.4$ ,  $p = 0.01$ ; Table 3). Control fish had significantly higher cortisol 2 h after the chase to exhaustion than post-treatment period ( $p = 0.007$ ). There were no significant effects of treatment or time, or their interaction for either blood glucose or pH following cold exposure (Table 3). There was a significant interaction between treatment and time in hematocrit values ( $F_{3,24} = 6.7$ ,  $p = 0.002$ ; Table 3), which were significantly higher in the 7 °C below ambient treatment 2 h after the chase to exhaustion than post treatment ( $p = 0.02$ ).

#### 3.4. Standardized chase to exhaustion

During the standardized chase to exhaustion, fish in the control treatment crossed the most lines and were chased for the most amount of time ( $128 \pm 24$  and  $147 \pm 24$ , respectively) while the 14 °C below ambient treatment crossed the fewest lines and were chased for the least amount of time before reaching exhaustion ( $21 \pm 5$  and  $37 \pm 10$ , respectively; Fig. 1). There was a significant effect of treatment on the amount of time chased (one-way ANOVA:  $F_{3,25} = 8.5$ ,  $p < 0.001$ ). The control group was chased significantly longer than the handled control group ( $49 \pm 13$ ; Tukey HSD:  $p = 0.001$ ), 7 °C below ambient ( $56 \pm 10$ ;  $p = 0.002$ ), and 14 °C below ambient treatments ( $37 \pm 10$ ;  $p = 0.001$ ; Fig. 1). Treatment also had a significant effect on the number of lines crossed (one-way ANOVA:  $F_{3,25} = 8.7$ ,  $p < 0.001$ ). The control group crossed significantly more lines than the handled control group ( $36 \pm 14$ ;  $p = 0.001$ ), 7 °C below ambient ( $38 \pm 11$ ;  $p = 0.001$ ), and the 14 °C ambient treatment ( $21 \pm 5$ ;  $p < 0.001$ ; Fig. 1).

**Table 2**

Ventilation rates of bonefish at rest, during the cold shock exposure, after the standardized chase to exhaustion and 2 h following the chase for all treatment groups ( $\pm$  SE).

Treatments	Ventilation rates (opercular beats per 30 s)						
	Resting	0.5 h	1.0 h	1.5 h	2.0 h	After standardized chase	2 h following standardized chase
Control (n = 7)	19 ± 1	-	-	-	-	38 ± 4	35 ± 3
Handled control (n = 7)	22 ± 1	22 ± 1	21 ± 1	21 ± 1	21 ± 2	33 ± 1	24 ± 1
7 °C below ambient (n = 8)	26 ± 2	19 ± 2	17 ± 1	18 ± 1	18 ± 1	38 ± 2	24 ± 2
14 °C below ambient (n = 8)	21 ± 1	5 ± 3	17 ± 0†	15 ± 0†	14 ± 0†	35 ± 2	26 ± 2

† Only one fish of eight in the 14 °C below ambient temperature treatment group maintained equilibrium past the 30 min ventilation check.

**Table 4**

Reflex Action Mortality Predictor (RAMP) values for bonefish in control and cold shock treatments during the resting period, directly after exposure, after the standardized chase to exhaustion and two hours following the chase to exhaustion ( $\pm$ SE).

Treatment	Resting RAMP	RAMP After Exposure	RAMP After Chase	RAMP 2 h After Chase
Control	0 $\pm$ 0	n/a	1 $\pm$ 0	0 $\pm$ 0
Handled control	0 $\pm$ 0	0 $\pm$ 0	1 $\pm$ 0	0 $\pm$ 0
7 °C below ambient	0 $\pm$ 0	0 $\pm$ 0	1 $\pm$ 0	0 $\pm$ 0
14 °C below ambient	0 $\pm$ 0	4.13 $\pm$ 0.35	1 $\pm$ 0	0 $\pm$ 0

#### 4. Discussion

The rapid temperature declines tested here (7 and 14 °C) had both physiological and behavioural impacts on bonefish. Physiological impacts were characterized by increased blood lactate concentrations in the 14 °C below ambient treatment relative to all other treatments, indicating anaerobic respiration (Kieffer, 2000). Indeed, during the 14 °C below ambient exposure fish exhibited periods of hyperactivity prior to losing equilibrium, and had low ventilation rates 30 min into exposure compared to other treatments. However, it is surprising that there were no significant differences in cortisol, hematocrit, or pH values between treatments post exposure period (although hematocrit and cortisol were higher in the 14 °C below ambient treatment than both controls). It is important to note that since 7 of the 8 fish in the 14 °C below ambient treatment lost equilibrium the cold shock was terminated to prevent lethal consequences. Since these fish underwent a very short (albeit intense) cold shock, it can be speculated that their swimming ability was less affected, therefore not significantly worse than the handled control or the 7 °C below ambient treatment. Bonefish in the control group also exhibited elevated lactate and cortisol concentrations 2 h after the chase to exhaustion. This was likely due to the fact that control fish swam significantly longer and further during the chase to exhaustion, which is surprising given that blood chemistry was similar across all treatments post-treatment (aside from 14 °C below ambient). Indeed, blood chemistry values were similar in our control and cold shock treatments to baseline in bonefish (see Suski et al., 2007), while peak values in controls 2 h after the chase to exhaustion were similar to those found by Suski et al. (2007) 2 h after similar exhaustive physical exercise.

Behavioural responses of bonefish to cold shock were generally characterized by decreased ventilation rates for the 7 °C below ambient treatment with little reflex impairment, and extreme behavioural and reflex impairment in the 14 °C below ambient treatment. Fish in the latter treatment exhibited varying periods of hyperactivity followed by impaired or no swimming ability, reduced responsiveness, and the loss of equilibrium, which are all common traits of cold shock exposures (Fuiman and Batty, 1997; Muir et al., 1994; Smith and Hubert, 2003). Less than 30 min into the cold shock exposures seven of the eight fish in the 14 °C below ambient treatment lost equilibrium. These findings are very similar to Friedlander et al. (1976), who found that goldfish (*Carassius auratus*) exhibited uncoordinated swimming 13–14 °C below ambient conditions, and lost equilibrium at 15 °C below ambient

(Friedlander et al., 1976). Loss of equilibrium could result in bonefish being unable to escape colder, near shore habitats during extreme weather events, and may leave them more susceptible to predation (Danylchuk et al., 2007b).

Bonefish swimming abilities were impacted by treatment during cold shock experiments; however, it was the control fish that had significantly stronger swimming abilities than all other treatments, while there was no difference between the handled control treatment and the cold shock treatments. This indicates that handling likely had the greatest impact on swimming abilities, not the cold shock exposure. Despite the fact that bonefish in the 14 °C below ambient treatment had almost complete reflex impairment during the exposure and sustained high blood lactate concentrations than other treatments, post-exposure swimming abilities were similar to handled control fish. This suggests that although fish become highly behaviourally impaired at colder temperatures, if they are able to escape to more suitable conditions, swimming abilities quickly return and they are unlikely to experience further fitness consequences due to behavioural impairment (e.g. higher predation risk).

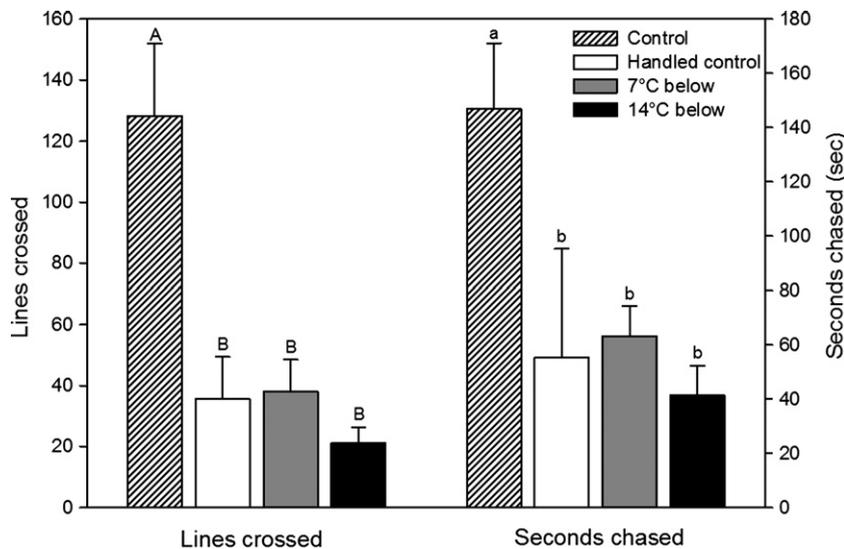
Sub-tropical and tropical systems are more sensitive to temperature changes as a result of severe weather events (Clusella-Trullas et al., 2011; Knutson et al., 2010). These severe weather events are projected to not only increase in frequency, but also in the magnitude of the storm events due to changing climate (Clusella-Trullas et al., 2011). Further, bonefish and other sub-tropical fish species (Atlantic tarpon, common snook, etc.) are expected to expand their ranges northward due to warming ocean temperatures (Shenker, 2009), where cold shock may be a prevalent stressor. Bonefish commonly occupy tidal flats where the water is rarely deeper than 1 m (Murchie et al., 2013), and if caught in these areas during a cold snap event, fish may be exposed to cold temperatures outside of their thermal tolerance. Another near-shore dwelling fish species, the blacktip reef shark (*Carcharhinus limbatus*) showed the innate ability to sense an incoming storm event and evacuate near-shore areas in Florida (Heupel, 2003). Many fish, especially those living in tidal areas have a keen sense for hydrostatic pressure, which helps guide movement related to tidal fluctuations (Gibson, 1970, 1984). However, if changes in temperature and pressure occur very quickly, fish may still become stranded in shallow near-shore areas, as was likely the case in Florida during the 2010 cold snap. This possibility may be exacerbated by behavioural impairments due to exposure to cold temperatures, leaving fish unable to escape to deeper refuge habitat.

Exposing bonefish to 14 °C below ambient temperatures resulted in physiological and behavioural impairments, and a prolonged exposure of this magnitude could have fitness impacts for this species. However, short-term exposure to 7 °C below ambient had little physiological or behavioural impacts. As the magnitude of severe weather events is expected to increase due to climate change, it can be speculated that bonefish and other important species in shallow marine systems will experience more severe cold shock events, similar to the 14 °C below ambient temperature treatment in our experiment. Indeed, climate change is projected to have physiological impacts on individual animals which could negatively influence population-level processes (Pörtner

**Table 5**

Bonefish blood physiology for all treatments directly after exposure and 2 hours after the standardized chase to exhaustion ( $\pm$ SE). Letters indicate significant differences between treatments and time periods within each parameter.

Treatment	Glucose (mM/L)		Lactate (mM/L)		pH		Hematocrit (%PCV)		Cortisol (ng/mL)	
	After Exposure	2 h After Chase	After Exposure	2 h After Chase	After Exposure	2 h After Chase	After Exposure	2 h After Chase	After Exposure	2 h After Chase
Control	4.2 $\pm$ 0.3 <sup>a</sup>	3.4 $\pm$ 0.4 <sup>a</sup>	1.5 $\pm$ 0.2 <sup>a</sup>	5.0 $\pm$ 1.9 <sup>ab</sup>	7.7 $\pm$ 0.1 <sup>a</sup>	7.8 $\pm$ 0.1 <sup>a</sup>	22.7 $\pm$ 2.1 <sup>a</sup>	28.9 $\pm$ 1.6 <sup>ab</sup>	13.8 $\pm$ 3.6 <sup>a</sup>	54.6 $\pm$ 18.9 <sup>b</sup>
Handled Control	4.2 $\pm$ 0.4 <sup>a</sup>	4.4 $\pm$ 0.2 <sup>a</sup>	2.4 $\pm$ 0.5 <sup>ab</sup>	1.6 $\pm$ 0.4 <sup>a</sup>	7.7 $\pm$ 0.1 <sup>a</sup>	7.7 $\pm$ 0.04 <sup>a</sup>	28.7 $\pm$ 1.3 <sup>ab</sup>	27.0 $\pm$ 0.9 <sup>ab</sup>	22.1 $\pm$ 3.1 <sup>ab</sup>	21.6 $\pm$ 4.3 <sup>ab</sup>
7 °C below ambient	4.5 $\pm$ 0.3 <sup>a</sup>	4.4 $\pm$ 0.4 <sup>a</sup>	1.4 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.3 <sup>a</sup>	7.8 $\pm$ 0.1 <sup>a</sup>	7.8 $\pm$ 0.03 <sup>a</sup>	23.8 $\pm$ 1.3 <sup>a</sup>	34.2 $\pm$ 2.9 <sup>b</sup>	26.2 $\pm$ 6.0 <sup>ab</sup>	25.2 $\pm$ 3.4 <sup>ab</sup>
14 °C below ambient	4.2 $\pm$ 0.4 <sup>a</sup>	4.3 $\pm$ 0.4 <sup>a</sup>	4.6 $\pm$ 0.6 <sup>b</sup>	4.0 $\pm$ 1.1 <sup>ab</sup>	7.7 $\pm$ 0.1 <sup>a</sup>	7.7 $\pm$ 0.1 <sup>a</sup>	30.4 $\pm$ 3.8 <sup>ab</sup>	25.6 $\pm$ 1.6 <sup>ab</sup>	26.0 $\pm$ 6.0 <sup>ab</sup>	22.7 $\pm$ 3.0 <sup>ab</sup>



**Fig. 1.** Lines crossed and seconds chased  $\pm$  SE by bonefish in all treatment groups during the standardized chase to exhaustion. Uppercase letters indicate statistical differences among treatments for the number of lines crossed, and lowercase letters indicate statistical differences among treatments for the number of seconds chased.

and Farrell, 2008). Our research could thus serve as a model for tropical and sub-tropical fish response to projected temperature fluctuations. However, the acute and abrupt exposure used here may not perfectly represent how bonefish may experience thermal variation in the wild. As such, this exposure may represent a short-term “worst case scenario”, particularly given that experiments were conducted in the summer when fish would be acclimated to the highest water temperatures. We also examined a relatively narrow size range of bonefish in this study, and future research should aim to examine the influence of fish size on vulnerability to cold shock. Juvenile stages of fish are typically more sensitive to cold shock (Donaldson et al., 2008) and would thus be a worthwhile direction of research. There are many facets that have yet to be explored as this research was the first attempt to understand the sub-lethal consequences of cold shock on these sub-tropical fish species. Our research only considered swimming ability as a proxy to understand predation risk in the wild. Future research may focus on determining whether the fish experience compromised disease resistance, poor foraging decisions, changes to fecundity or altered developmental stages. The combination of a changing climate and the economic importance of bonefish throughout the Caribbean warrant more research to be conducted on this species and their responses to an array of changes to ambient conditions.

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### Disclosure:

Petra Szekeres contributed to experimentation, data analysis, and preparation of this manuscript. Jacob Brownscombe contributed to experimental design, data analysis, and manuscript preparation. Felicia Cull and Karen Murchie contributed to experimental design, experimentation, and manuscript preparation. Andy Danylchuk, Aaron Shultz, Cory Suski, and Steven Cooke contributed to experimental design and manuscript preparation. [SS]

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