Insights into the origin and magnitude of capture and handling-related stress in a coastal elasmobranch *Carcharhinus limbatus*

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Abstract: A suite of blood chemistry parameters (including acid-base indicators and plasma electrolytes) was serially measured in blacktip sharks (*Carcharhinus limbatus*), captured via rod-and-reel, to gain a more thorough understanding of the physiological stress response to recreational capture. Sharks were caught both from the shore and from fishing vessels, and experienced varying degrees of air exposure during handling. While all captured sharks exhibited a metabolic acidosis during the fight on the line (increasing lactate and decreasing pH and bicarbonate), the observed acidosis was compounded by a respiratory component (increasing pCO_2) in sharks removed from the water during handling. Vessel-caught sharks handled in the water exhibited significantly greater increases in lactate and glucose (0.73 ± 0.21 mmol^{-1} min^{-1} and 0.81 ± 1.07 mg dL^{-1} min^{-1}, respectively), than sharks handled out of water (0.21 ± 0.17 mmol^{-1} min^{-1} and -0.32 ± 1.05 mg dL^{-1} min^{-1}; $p < 0.001$ and $p < 0.05$, respectively). These findings provide insights into how differences in recreational capture methods and air exposure can mediate the origin and magnitude of capture-related stress, and highlight the importance of considering both sampling time (time from capture to phlebotomy) and sampling location (in water versus out) in studies conducted on capture-related stress.

Keywords: stress physiology, air exposure, catch and release, acidosis, recreational fishery, post-release mortality

Introduction

Reported declines in elasmobranch populations (sharks, skates, and rays) have traditionally been attributed to commercial fisheries (Dulvy *et al.*, 2014). However, the popularity of recreational fishing is increasing worldwide (Arlinghaus and Cooke, 2008; Press *et al.*, 2016) and has been shown to negatively affect fish populations (Coleman *et al.*, 2004). In the United States alone, an estimated 24.5 million elasmobranchs were caught by recreational anglers in 2018, 97% of which were estimated to be released alive (National Marine Fisheries Service, 2019a). While the practice of catch-and-release is widely advocated by fisheries managers (Arlinghaus *et al.*, 2007), the acute stress associated with capture has been shown to elicit a suite of potentially lethal physiological changes (e.g. Manire *et al.*, 2001; Skomal, 2007), with rates of post-release mortality varying widely among elasmobranch species (Ellis *et al.*, 2017). In order to develop methods to reduce capture stress and resultant mortalities, a more thorough understanding of the pathophysiology of capture-related stress is necessary (Hyatt *et al.*, 2012).

The physiological effects of a capture event are influenced by a range of both abiotic (e.g. water temperature, concentration of dissolved oxygen) and biotic factors (e.g. age, size, maturity) that can be specific to the individual and/or to the type of capture (e.g. gear type, duration of capture/handling; Skomal and Mandelman, 2012; Heard *et al.*, 2014). The stress experienced by captured sharks has traditionally been quantified through an assessment of the acid-base status of the blood (Mandelman and Skomal, 2009; Heberer *et al.*, 2010; Marshall *et al.*, 2012), which is based on the notion that exhaustive exercise causes a decrease in blood pH, known as acidemia, due to both metabolic (lactate-driven) and respiratory (CO_2-driven) acidoses (Skomal, 2007). During metabolic acidosis due to lactate accumulation, the production of lactic acid results in excess metabolic protons, which are then depleted by bicarbonate ions – leading to a decrease in

the concentration of bicarbonate in the blood (Frick *et al.*, 2012). Conversely, during respiratory acidosis, insufficient gas exchange results in an accumulation of CO_2 in the blood (Frick *et al.*, 2012). Changes in plasma electrolyte concentrations, driven by cellular fluid shifts that can result in haemoconcentration and disruptions to ionic and osmotic homeostasis (Wood, 1991; Skomal and Mandelman, 2012), have also been used as indices of the stress response (e.g. Marshall *et al.*, 2012; French *et al.*, 2015). More recently, the use of a heat shock protein (Hsp70) as an indicator of the stress response at the molecular level has been investigated (Moyes *et al.*, 2006; Marshall *et al.*, 2012; French *et al.*, 2015). Heat shock proteins (Hsp) are expressed in cells to maintain protein–protein interactions and can be induced in response to both thermal and oxidative stressors (Iwama *et al.*, 2006). The role of Hsp in mediating and/or recovering from the stress associated with struggling on a fishing line, however, remains relatively unknown. Because interspecific differences in dealing with capture-related stress may be linked to the metabolic scope and physiology of the species in question (Skomal and Mandelman, 2012), screening for a suite of blood chemistry parameters may allow for a more thorough understanding of the intraspecific effects of capture, while providing potential insights into interspecific differences (Skomal, 2007; French *et al.*, 2015).

While capture-related stress is relatively well studied in coastal shark species commonly caught in recreational fisheries (e.g. Heberer *et al.*, 2010; Kneebone *et al.*, 2013; Whitney *et al.*, 2017), previous studies have often focused on the stress experienced by captured sharks during the time on the line (i.e. fight time). Few studies have investigated potential differences in the stress experienced by captured sharks during the fight time and that experienced during handling by anglers (e.g. through air exposure), as doing so requires performing more than one phlebotomy (blood draw) (but see Hyatt *et al.*, 2012; Heard *et al.*, 2014). Moreover, some blood

stress indicators do not reach peak levels until hours post-capture (e.g. lactate; Heard *et al.*, 2014), suggesting that repeated blood sampling can more accurately assess physiological stress in elasmobranchs (Frick *et al.*, 2009; Van Rijn and Reina, 2010). Understanding the physiological effects of air exposure during handling is becoming increasingly important, as a specialized method of recreational fishing targeting large coastal sharks from beaches, known as shore-based or land-based shark angling, has been receiving increasing attention in recent years (Ajemian *et al.*, 2016; Shiffman *et al.*, 2017). Sharks caught from the shore are often brought out of the water and onto the sand for hook removal and photographs, and may be subject to increased handling stress and exposure to air, reducing the shark's ability to exchange oxygen and carbon dioxide (Casselman, 2005). Moreover, captured sharks are often removed from the water by fisheries researchers during routine sampling procedures, though the potential physiological consequences of such remain largely unknown.

The blacktip shark *Carcharhinus limbatus* (Müller and Henle, 1839) is a relatively large (maximum total length 202 cm; Castro, 1996), coastal species with a circumglobal distribution in temperate coastal waters (Compagno, 1984; Castro, 2011). The blacktip is a migratory species that inhabits shallow coastal waters and surface offshore waters, and preys primarily on small bony fishes and small elasmobranchs (Castro, 2011). Blacktip sharks are currently the most commonly landed large coastal shark species in the U.S. Atlantic and Gulf of Mexico (National Marine Fisheries Service, 2019b); were the most frequently captured shark reported by users of a shore-based shark angling forum in Florida (Shiffman *et al.*, 2017); and were one of the most frequently landed sharks in a Texas shore-based shark fishery (Ajemian *et al.*, 2016). Previous research conducted on the stress response of blacktip sharks caught on longlines indicates that the species may exhibit a strictly respiratory response to capture (i.e. an acidosis driven by the

accumulation of carbon dioxide; Mandelman and Skomal, 2009), and it has been suggested that blacktip sharks could lack the mechanisms (e.g. splenic red blood cell ejection and red blood cell swelling via Na^+–H^+ exchangers, Nikinmaa, 1992; Brill *et al.*, 2008) responsible for maintaining or increasing oxygen delivery during strenuous activity (Mandelman and Skomal, 2009). Moreover, Mohan *et al.* (2020) suggested that out-of-water handling and air exposure likely contributed the greatest to the increased post-release mortality rate of recreationally caught blacktip sharks in the western Gulf of Mexico (30%). However, more recent research has documented elevated blood lactate concentrations with increasing fight times in blacktip sharks caught on rod-and-reel (Whitney *et al.*, 2017; Mohan *et al.*, 2020; Weber *et al.*, 2020), suggesting that recreational capture results in a blood acidosis that is at least partially metabolic in origin.

Given the documented differences in the physiological stress response of blacktip sharks to capture, a more thorough understanding of capture-related stress, and how such stress may be influenced by the capture method used and air exposure during handling, is warranted. The present study utilized serial blood sampling to investigate the metabolic and respiratory origins and magnitude of capture and handling-related stress in blacktip sharks caught by recreational anglers both from the shore and from fishing vessels. In particular, the objectives of the present study were (1) to quantify changes in the blood chemistry of blacktip sharks caught both from the shore and from fishing vessels; and (2) to determine the potential continued effects of air exposure during handling on the blood chemistry of captured sharks.

Materials and methods

Sampling design

Blacktip sharks were captured with rod-and-reel by participating recreational anglers and South Carolina Department of Natural Resources (SCDNR) biologists from the shore and from fishing vessels. Recreational anglers used their personal fishing equipment, which varied in size and strength (e.g. reel type [spinning versus conventional level-wind], maximum reel drag capacity [35 to 66 lb], line strength [50 to 200 lb]), while SCDNR biologists used equipment that mimicked that commonly used by the recreational anglers. Sampling was conducted in the coastal waters of South Carolina and Florida from May 2017 to September 2019. Sea-surface temperature (°C) was recorded during each angling trip using a General Purpose Blue Spirit Thermometer (1.5°C accuracy; VWR International, LLC).

Once a shark was hooked, the 'fight time,' defined as the time from the initial strike until the time the shark was secured by the angler, was recorded to the nearest second. All sharks caught from shore were brought out of the water and onto the sand. Sharks caught from fishing vessels were either left in the water (secured alongside and parallel to the vessel using both the leader and a tail rope, with gills submerged to enable ventilation) or brought onto the deck of the vessel (out of water). Blood was drawn via caudal venipuncture immediately after the shark was secured and again immediately before release. For vessel caught sharks that remained in the water during the handling procedure, the caudal fin was lifted out of the water during both phlebotomies, while the gills remained submerged to enable ventilation. In between phlebotomies, sharks were measured to the fork length (cm) and sexed, and the recreational anglers completed their routines (which often included hook removal, measurement, and photographs). Additionally, electronic tags (V16-4H acoustic transmitters, Vemco, Bedford, Canada and PSATLifes, Lotek Wireless Inc., Newmarket, Canada) were affixed to a subset of the sharks caught by recreational anglers in the present study, as part of a different study on post-

release mortality in the blacktip shark (Weber *et al.*, 2020). The 'handling time,' defined as the time from when the shark was initially secured (i.e. brought onto the sand, secured alongside the vessel with gills submerged, or brought onto the deck of the vessel) to the release of the shark, was recorded to the nearest second. To further investigate the results obtained from sharks caught from fishing vessels, an additional subset of sharks were immediately brought onto the deck of the vessel (out of water); a blood sample was drawn, and sharks were then returned to the water with gills submerged for 3–5 min, after which a second blood sample was drawn (while the shark remained in the water, with gills submerged).

Research was completed under the South Carolina Code of Law section 50-5-20 which authorizes the South Carolina Department of Natural Resources to conduct research in state waters. Additionally, research was conducted in accordance with the College of Charleston Institutional Animal Care and Use Committee (IACUC) through protocol no. IACUC-2017-007.

Blood biochemistry

Blood collection and acid–base indicators

Blood (3 mL) was drawn via caudal venipuncture, using 18-gauge sterilized needles with 5 mL heparin-rinsed syringes, and samples were immediately injected into 10 mL sodium heparin vacutainers (Becton, Dickinson and Co.). To avoid compromising blood gas accuracy after venipuncture (Whitney *et al.*, 2017), a subsample of whole blood (90 μL) was immediately (within 30 s) analyzed for pH, lactate, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), percent oxygen saturation of hemoglobin (sO_2), and bicarbonate (HCO_3^-) using an i-STAT portable blood analyzer (Abaxis Inc., Union City CA) with a CG4+ cartridge. As puncture of the caudal vein in the hemal arch of fishes often results in puncture of the caudal artery, it cannot be ensured that blood samples drawn were purely arterial or venous (Mandelman

and Skomal, 2009). Thus, all samples are assumed to be a mix of arterial and venous blood. Additionally, while use of the i-STAT system for the analysis of whole blood pH has been validated in elasmobranchs (e.g. sandbar shark, *Carcharhinus plumbeus*, Harter *et al.*, 2015) recent research suggests that relationships between blood gas pressures and temperature are species-specific (Gallagher *et al.*, 2010), but validated conversion factors are not available for the blacktip shark. Therefore, all changes in blood chemistry parameters described herein are assumed to be relative to each other (i.e. first blood sample drawn is relative to the second, as samples were drawn from the same individual) and are not intended to represent true *in vivo* conditions (Kneebone *et al.*, 2013).

Blood metabolic acid load was estimated following Milligan and Wood (1986):

(1) $\Delta H^{+}_{m} = [HCO_3^{-}]_1 - [HCO_3^{-}]_2 - \beta(pH_1 - pH_2)$

where $[HCO_3^{-}]_1$ and $[HCO_3^{-}]_2$ represent the concentration of blood bicarbonate observed at the time that sharks were secured and immediately before release, respectively; pH_1 and pH_2 represent the whole blood pH observed at the time that sharks were secured and immediately before release, respectively; and β represents the whole blood non-bicarbonate buffer capacity. The whole blood non-bicarbonate buffer capacity (β) for spiny dogfish (*Squalus acanthias*) was used (-7.13 mmol pH unit^{-1} L^{-1}), as published values for the blacktip shark are not available (Gilmour *et al.*, 2002; Frick *et al.*, 2012).

Hematocrit and plasma electrolytes

A separate subsample of whole blood (0.2 mL) was simultaneously placed on ice (within 30 s) for hematocrit analysis. The remaining whole blood was centrifuged (E8 Portafuge, LW Scientific Inc., Lawrenceville GA) for 5 min at 3,500 RPM, to separate the plasma and the red blood cells. Subsamples of plasma (3 × 0.5 mL) and red blood cells (3 × 0.1 mL) were frozen

immediately in liquid nitrogen and, subsequently, stored at –80 °C. At the time of hematocrit analysis (completed within 4 h of capture; Manire *et al.*, 2001), whole blood samples (n = 3 per individual) were transferred into microcapillary tubes and centrifuged for 5 min at 10,000 RPM. Hematocrit was determined as the percentage of total blood volume comprised of red blood cells. At the time of plasma electrolyte analysis, plasma samples were thawed, diluted 2:3 with deionized water (plasma:dH_2O), and approximately 55 μL of the diluted samples were injected into a Critical Care Xpress (CCX, Nova Biomedical, Waltham MA) benchtop analyzer to quantify Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, and glucose. All concentrations were within the detection limits of the instrument.

Heat shock protein 70

The concentration of a 70 kDa heat shock protein (Hsp70) in the red blood cells was determined through semi-quantitative near-infrared western blotting. Prior to blotting, the total concentration of protein in the red blood cells was determined through a protein assay (Micro BCA Protein Assay, Thermo Scientific). Proteins (9 μg total protein per lane) were then separated by size via SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. Equal protein loading was determined using a separate gel for coomasie staining, and loading was adjusted based on arithmetic mean weighting of the total protein signal. Lane-to-lane variation in total protein loading and transfer efficiency was further controlled by staining total protein (REVERT^{TM} Total Protein Stain, LI-COR Biosciences, Lincoln, NE, USA). The presence of Hsp70 immunoaffinity on the membrane was detected through a primary antibody (AS05-083, Agrisera Antibodies, Vännäs, Sweden) diluted 1:2,500 and specific against Hsp70, followed by an infrared-labeled secondary antibody (IRDye® 800CW, LI-COR Biosciences, Lincoln NE,

USA) diluted 1:20,000. Semi-quantitative results were determined using the LI-COR Odyssey Fc Imaging System (LI-COR Biosciences, Lincoln NE, USA).

To avoid signal saturation, a preliminary blot was run using different amounts of protein per lane (0.5, 1, 2, 4, 8, 17, 35 μg). Signal intensity was plotted and examined for linearity. Conditions that exhibited a linear response were then selected for semi-quantification. Additionally, to control for non-specific signals, a secondary only control was run where only secondary antibody was utilized. Because elasmobranch heat shock protein is not commercially available, antibody competition (pre-adsorbed) controls were not possible.

Statistical analysis

Shapiro-Wilk tests and F-tests were used to assess for normality and homogeneity of variances, respectively. Lactate and sO_2 values were square root transformed, while pO_2 values were inverse transformed, to obtain normality and homogeneity of variances. Linear regressions were used to determine if the fight time or total disturbance time (fight time + handling time) had an effect on the blood chemistry parameters for either recreational capture method (shore-based versus vessel-based) or vessel-based handling method (in water versus out of water). Analyses of covariance (ANCOVA) were used to determine if the blood chemistry parameters differed between capture methods or between vessel-based handling methods, where ANCOVAs were used to account for extraneous variability due to differences in fight time and/or handling time between capture or handling methods. Differences in the concentration of Hsp70 across binned sea-surface temperatures were analyzed using a one-way Analysis of Variance (ANOVA) followed by a Tukey's post-hoc test.

To assess for the effects of the continued stress response during handling by anglers, blood chemistry parameters were compared between the first and second blood samples using

paired *t*-tests. Additionally, to compare the stress response during handling between capture methods, and to standardize the effect of handling due to varied handling times, the rates of change of the blood chemistry parameters were calculated as:

(2) $$\Delta X / \Delta T = \frac{X_2 - X_1}{T_2 - T_1}$$

where X_1 and X_2 refer to a blood chemistry parameter value from the first and second blood samples, respectively, and T_1 and T_2 refer to the time of the first and second blood samples, respectively (Hyatt *et al.*, 2012). The rate of change was compared between capture methods and between vessel-based handling methods using *t*-tests. All analyses were conducted using the R programming language (version 3.6.1; R Core Team 2019).

Results

Capture characteristics

Blood chemistry data were collected from 94 blacktip sharks: 43 caught from the shore and 51 caught from fishing vessels. Of the 51 sharks caught from fishing vessels, 32 were brought out of the water during handling; 16 were left in the water during handling; and 3 were initially taken out of water but then returned to the water for 3–5 min with gills submerged before the second blood sample was drawn (Table 1). Mean fight times were 5.09 ± 2.82 min and 4.65 ± 1.94 min (mean ± *SD*) for sharks caught from shore and from fishing vessels, respectively, while mean handling times (i.e. time between phlebotomies) were 3.33 ± 1.16 min and 3.63 ± 1.15 min (mean ± *SD*), respectively. There were no significant differences in fork length, sex ratio, fight time, handling time, or sea-surface temperature between capture methods (i.e. shore versus fishing vessel) (Table 1). However, for sharks caught from fishing vessels, fight times (*t*-test, $p = 0.008$) and handling times (*t*-test, $p < 0.001$) were significantly longer for sharks handled in the water than those handled out of the water (Table 1). Additionally, there was no significant

difference in handling time between sharks that were tagged with electronic tags as part of a different study (Weber *et al.*, 2020) and those that were not tagged (*t*-test, $p = 0.160$).

Shore-based versus vessel-based

Acid base indicators

The fight time and total disturbance time (i.e. fight time + handling time) had significant effects on numerous blood chemistry parameters, including pH, lactate, pCO_2, pO_2, sO_2, and HCO_3^- (linear regressions; Fig. 1; Table 2). Blood pCO_2 increased more rapidly with increasing total disturbance time for sharks caught from shore, while lactate, pO_2, and sO_2 increased more rapidly for sharks caught from fishing vessels (ANCOVAs; Fig.1; Table 3). During handling by anglers, both lactate and the metabolic acid load increased more rapidly in sharks caught from fishing vessels (mean ± *SD*: 0.42 ± 0.37 mmol L^{-1} min^{-1} and 0.32 ± 0.27 mmol L^{-1} min^{-1}, respectively), when compared to sharks caught from shore (mean ± *SD*: 0.12 ± 0.14 mmol L^{-1} min^{-1} and 0.16 ± 0.21 mmol L^{-1} min^{-1}, respectively). The partial pressure of CO_2 (pCO_2) increased more rapidly in sharks caught from shore (mean ± *SD*: 1.14 ± 1.11 $mmHg^{-1}$ min^{-1} versus 0.12 ± 0.98 $mmHg^{-1}$ min^{-1} vessel-based; Table 4). Additionally, pO_2 and sO_2 decreased significantly during handling by shore-based anglers (mean ± *SD*: –3.62 ± 5.71 $mmHg^{-1}$ min^{-1} and –6.67 ± 8.62 % min^{-1}, respectively), while both pO_2 and sO_2 exhibited slight increases during handling by vessel-based anglers (mean ± *SD*: 2.55 ± 5.34 $mmHg^{-1}$ min^{-1} and 0.82 ± 6.56 % min^{-1}, respectively; Table 4).

Plasma electrolytes, hematocrit, and glucose

For sharks caught from shore, hematocrit increased significantly with increasing fight time, while plasma potassium increased significantly with increasing fight time and total disturbance time (Table 2). Potassium increased more rapidly with increasing fight time and total disturbance

time for sharks caught from shore, when compared to sharks caught from fishing vessels (Table 3). Glucose increased significantly with increasing fight time for sharks caught from shore, and with increasing total disturbance time for sharks caught from vessels (Table 2).

Heat shock protein 70

For sharks caught from fishing vessels, the concentration of Hsp70 in the red blood cells decreased significantly between blood samples drawn at the time the shark was secured and samples drawn immediately before release (Table 5). The sea-surface temperature at the location of capture had a significant effect on the concentration of Hsp70 in the red blood cells for all sharks combined (ANOVA, $p = 0.019$). In particular, the concentrations of Hsp70 at sea-surface temperatures of 26–29 °C and 29–32 °C were higher than those at 23–26 °C (Tukey's post-hoc, $p = 0.032$ and $p = 0.033$, Fig. 2).

Vessel-based: handled in water versus out of water

Acid-base indicators

Sharks caught from fishing vessels and handled in the water exhibited significant increases in the rate of change of pO_2 (mean ± *SD*: 3.57 ± 3.73 $mmHg^{-1}$ min^{-1}) and sO_2 (mean ± *SD*: 2.32 ± 4.31 % min^{-1}), and decreases in the rate of change of pCO_2 (mean ± *SD*: –0.21 ± 0.50 $mmHg^{-1}$ min^{-1}) and HCO_3^- (mean ± *SD*: –0.25 ± 0.16 mmol L^{-1} min^{-1}) during handling, while sharks handled out of the water exhibited significant increases in the rate of change of pCO_2 (mean ± *SD*: 0.57 ± 0.89 $mmHg^{-1}$ min^{-1}; Fig. 3; Fig. 4; Table 4). Notably, the concentration of lactate increased 3.5 times more rapidly in sharks handled in the water, when compared to sharks handled out of the water (mean ± *SD*: 0.73 ± 0.32 mmol L^{-1} min^{-1} versus 0.21 ± 0.17 mmol L^{-1} min^{-1}; Fig. 3; Fig. 4; Table 4). The three individuals that were removed from the water for an average of 35 s (± 19 s) before initial phlebotomy exhibited blood pCO_2 values (mean ± *SD*: 14.76 ± 3.84 mmHg) that

were more than twice as high as that of individuals left in the water during phlebotomy (mean ± *SD*: 8.29 ± 1.38 mmHg).

Plasma electrolytes, hematocrit, and glucose

Sharks caught from fishing vessels and handled in the water exhibited significant increases in the rate of change of glucose (mean ± *SD*: 0.81 ± 1.07 mg dL^{-1} min^{-1}), while those handled out of the water exhibited decreases in glucose (mean ± *SD*: –0.32 ± 1.05 mg dL^{-1} min^{-1}; Table 4).

Discussion

The origin of the blood acidosis experienced by captured blacktip sharks differs between recreational capture methods, and this result is driven largely by whether or not captured sharks remain in the water during handling. During metabolic acidosis, bicarbonate ions deplete excess metabolic protons, leading to a decrease in the concentration of bicarbonate in the blood (Frick *et al.*, 2012). Conversely, during respiratory acidosis, blood bicarbonate temporarily increases, due to an acute accumulation of CO_2 as a result of insufficient gas exchange (Frick *et al.*, 2012). In the present study, sharks caught from fishing vessels and handled in the water exhibited a blood acidosis that was primarily metabolic in origin, as indicated by an increase in blood lactate and decrease in bicarbonate (Mandelman and Skomal, 2009; Frick *et al.*, 2012). However, sharks caught both from shore (all of which were brought out of the water and onto the sand during handling) and sharks caught from fishing vessels but removed from the water during handling exhibited a blood acidosis that was further compounded by a respiratory component, as indicated by a significant increase in pCO_2 and decreases in pO_2 and sO_2. No significant difference in blood pH was observed between capture methods or between vessel-based handling methods, suggesting that while the origin of the acidosis may differ, the magnitude of the acidosis does not. Specifically, while sharks handled out of water exhibited a metabolic acidosis that was

further compounded by a respiratory component (i.e. pCO_2 accumulation), the relatively greater metabolic component (i.e. increase in lactate) observed in sharks handled in the water could explain the lack of difference in pH observed.

It is widely accepted by fisheries researchers that fish removed from the water during handling by anglers experience increased stress due to air exposure (Casselman, 2005; Cook *et al.*, 2015). However, in the present study, the concentration of blood lactate increased 3.5 times more rapidly for vessel-caught sharks that were left in the water during handling, when compared to those handled out of the water. Additionally, blood glucose increased drastically for sharks that remained in the water during handling, while glucose decreased for sharks handled out of the water. During air exposure, teleost fishes have been shown to exhibit significant decreases in cardiac output (Cooke *et al.*, 2001), with drastic decreases in heart rate (bradycardia; Cooke *et al.*, 2001; Cooke *et al.*, 2003; Cook *et al.*, 2015). Additionally, both the epaulette shark (*Hemiscyllium ocellatum*) and shovelnose ray (*Aptychotrema rostrata*) have been shown to exhibit decreases in heart rate, cardiac output, and dorsal aortic blood pressure when exposed to hypoxic waters (Speers-Roesch *et al.*, 2012). A decrease in cardiac output and aortic blood pressure in sharks brought out of the water could decrease the rate of clearance of lactate and glucose out of the muscle tissue, a phenomenon that has been proposed to explain relatively low lactate concentrations observed during anesthesia-associated decreases in blood pressure in gummy sharks (*Mustelus antarcticus*; Frick *et al.*, 2012). This could explain the lower concentrations of lactate and glucose observed in sharks removed from the water in the present study. Alternatively, the blacktip shark may exhibit whole-animal metabolic rate depression when exposed to air – a phenomenon that has been proposed to explain the relative lack of accumulation of lactate in white muscle and plasma in epaulette sharks exposed to hypoxic

conditions (Speers-Roesch *et al.*, 2012). With respect to glucose, catecholamines (e.g. epinephrine and norepinephrine) stimulate glucose release from the liver during exercise (DeRoos and DeRoos, 1978; Sherwin *et al.*, 1980; Sheridan, 1988), and may continue to stimulate this response in sharks left in the water, driving elevated concentrations of blood glucose.

The elevated lactate and glucose concentrations observed in sharks left in the water during handling are inconsistent with the results of many previous studies conducted on elasmobranchs. For instance, Heard *et al.* (2014) found that sparsely spotted stingarees (*Urolophus paucimaculatus*) exposed to four different trawl treatments, including air exposure and crowding, exhibited maximal lactate concentrations and increased glucose when exposed to air. Similarly, Van Rijn (2009) found that air exposure is the primary cause of physiological stress in captured Australian swellsharks (*Cephaloscyllium laticeps*). Moreover, Mohan *et al.* (2020) suggested that increased lactate concentrations observed in blacktip sharks caught via rod-and-reel could be attributed to increased handling times and air exposure. However, the increases in lactate observed by Mohan *et al.* (2020) could be a continued effect of the fight on the line, and cannot be decoupled from that potentially experienced during handling and air exposure, as only one phlebotomy was performed. Further investigation into the effects of air exposure on the physiological status of elasmobranchs, and how such effects may relate to cardiac output and/or decreased rates of metabolite clearance, is required in order to gain a better understanding of the consequences of capture and handling for elasmobranch homeostasis. Moreover, how such physiological effects of air exposure are influenced and potentially alleviated by ventilation during handling (e.g. through maintaining water flow over the gills using a hose), a common practice among fisheries researchers, remains unknown.

The results of the present study highlight the importance of considering both sampling time (i.e. time from capture to phlebotomy) and sampling location (i.e. in water versus out of water during phlebotomy) in studies conducted on capture-related stress. For instance, the drastic increase in lactate observed during handling by anglers (mean increase of 61% over 3.4 min) suggests that small differences in sampling time (i.e. phlebotomy) can potentially affect comparative studies on acid-base status. Additionally, the drastic increases in pCO_2 observed in the three blacktip sharks that were removed from the water for an average of 35 s (± 19 s) before phlebotomy (mean 14.8 ± 3.8 mmHg versus mean 8.3 ± 1.4 mmHg for those left in the water), suggests that rapid increases in pCO_2 can occur when exposed to air for < 1 minute. Such increases in pCO_2 may explain the differences in observed acid-base disturbances previously documented in the blacktip shark. Specifically, in the present study, all sharks exhibited a blood acidosis that was at least partially metabolic in origin during the time on the line (as indicated by increasing lactate and decreasing pH and bicarbonate with increasing fight times), irrespective of the capture method used. These results are consistent with those reported by Gallagher *et al.* (2014) for blacktip sharks captured on experimental drumlines, and with those reported by Whitney *et al.* (2017) for blacktip sharks captured via rod-and-reel in the Florida recreational fishery. However, Mandelman and Skomal (2009) found that blacktip sharks captured via longlines exhibited a blood acidosis that was largely respiratory in origin (i.e. driven by an accumulation of pCO_2), and suggested that the blacktip shark may lack mechanisms for maintaining oxygen delivery during capture (e.g. splenic red blood cell ejection and red blood cell swelling via Na^+–H^+ exchangers; Nikinmaa, 1992; Brill *et al.*, 2008). Interestingly, Gallagher *et al.* (2014) and Whitney *et al.* (2017) left captured blacktip sharks in the water (partially submerged) during phlebotomy, while Mandelman and Skomal (2009) brought

captured blacktip sharks onto the deck of the boat for phlebotomy. Thus, while discrepancies between studies in the observed acid-base status could be a result of the capture method used (i.e. drumline, Gallagher *et al.*, 2014; rod-and-reel, Whitney *et al.*, 2017; and longline, Mandelman and Skomal, 2009), the results of the present study indicate that Mandelman and Skomal (2009) were likely to document a rapid increase in pCO_2 due to air exposure (irrespective of capture method), that likely would not have been observed by Gallagher *et al.* (2014) or Whitney *et al.* (2017). In a recent review on the best practices for blood sampling of fishes, Lawrence *et al.* (2020) recommend completing phlebotomy within 3 min of the time that the fish is first handled, in order to minimize impacts on the welfare of the fish. However, the results of the present study indicate that drastic changes in blood chemistry (e.g. lactate and pCO_2) can occur within that time frame, and must be accounted for when assessing capture-related stress.

Plasma potassium generally increases during exercise (Medbo and Sejersted, 1990), and can be a result of several factors, including a release of potassium from muscle cells due to increased electrical activity (Fenn, 1938; Sejersted and Sjogaard, 2000) and a decrease in plasma water, due to increased intracellular lactate levels which cause a net fluid shift from extracellular to intracellular compartments (Van Dijk and Wood, 1988; Wood, 1991). However, rhabdomyolysis, a syndrome characterized by muscle necrosis and the release of intracellular electrolytes, often due to muscle trauma associated with intense exercise, can also lead to elevated plasma potassium concentrations (Keltz *et al.*, 2013). In the present study, plasma potassium increased with increasing fight time and total disturbance time for sharks caught from shore, but not for sharks caught from fishing vessels. As shore-based fishermen typically drag captured sharks onto the beach by their caudal fin, it is possible that this handling technique causes tissue damage and muscle lysis, potentially compounded by the weight of gravity on the

internal organs, and could drive elevated concentrations of plasma potassium. Increases in plasma potassium in response to a capture event have been reported in several previous studies (e.g. Manire *et al.*, 2001; Mandelman and Farrington, 2007; Frick *et al.*, 2010), and Marshall *et al.* (2012) found that blacktip sharks captured on longlines had higher plasma potassium concentrations than 10 other pelagic and coastal shark species caught on longlines. The origin of the high potassium concentrations in sharks caught from shore could simply be a normal response to exercise, but conditions such as rhabdomyolysis cannot be excluded. Moreover, while previous studies have documented increases in Na^+, Cl^-, Ca^{2+}, and Mg^{2+} in response to capture events (e.g. Moyes *et al.*, 2006; Mandelman and Farrington, 2007; Brill *et al.*, 2008; Frick *et al.*, 2010), few changes in the aforementioned ion concentrations were observed in the present study, suggesting that blacktip sharks captured via rod-and-reel do not experience a significant intracellular acidosis over the capture and handling durations observed.

The relative concentration of Hsp70 in the red blood cells was not affected by the fight time or total disturbance time, and did not differ between capture methods, suggesting that recreational capture does not induce an increase in Hsp70 protein abundance in the blacktip shark over the relatively short time frames assessed. Previous research has demonstrated that the concentration of Hsp70 mRNA in the red blood cells can be used to distinguish survivors from non-survivors in blue sharks (*Prionace glauca*) caught on longlines (Moyes *et al.*, 2006). Additionally, Heberer *et al.* (2010) found that relative levels of Hsp70 are elevated up to five times in thresher sharks hooked in the tail, when compared to levels of Hsp70 in blood that has recovered from the capture event (i.e. incubated *in vitro* for 24 h post-capture at 4–6°C). These increases in the concentrations of Hsp70 likely reflect some degree of capture-related cellular stress, as fish erythrocytes (unlike mammalian erythrocytes) possess nuclei and have the capacity

to rapidly induce Hsp70 gene expression in response to cellular stress (Currie *et al.*, 1999; Moyes *et al.*, 2006; Skomal and Bernal, 2010). In the present study, the concentration of Hsp70 increased with increasing sea-surface temperature at the location of capture. This increase was not related to the capture event, suggesting that increases in the concentration of Hsp70 can be observed across relatively small increases in sea-surface temperature (e.g. 3°C in the present study), as sharks are likely inducing Hsp70 in order to delay or prevent potential thermal damage to cellular proteins (Currie, 2011). Future research into the potential role of Hsp70 during capture events could improve our understanding of the stress response at the molecular level, though the results of the present study suggest that increases in Hsp70 protein abundance are not likely to be observed over short capture and handling durations (e.g. <20 min).

Overall, the documented effects of air exposure during handling on captured blacktip sharks (including increasing pCO_2 and decreasing pO_2) highlight the importance of leaving captured sharks in the water, or if removal is necessary, returning sharks back to the water as quickly as possible. However, the documented increases in lactate in blacktip sharks left in the water during handling are concerning, as increased lactate concentrations have been routinely used as indicators of increased stress in elasmobranchs (e.g. Skomal and Mandelman, 2012; Heard *et al.*, 2014; Gallagher *et al.*, 2014), and have been associated with post-release mortality events in some (e.g. Mohan *et al.*, 2020) but not all studies conducted on post-release mortality in the blacktip shark (e.g. Whitney *et al.*, 2017; Weber *et al.*, 2020). Moreover, the mean lactate concentration observed at the time of release in vessel-caught sharks handled in the water (5.69 ± 1.84 mmol L^{-1}) was similar to that associated with immediate mortality in blacktip sharks caught on rod-and-reel in the Gulf of Mexico recreational charter fishery (median: 5.90 mmol L^{-1}; Mohan *et al.*, 2020). While the results presented herein are specific to recreational fisheries,

captured sharks are commonly removed from the water by fisheries researchers during sampling procedures, and the results presented herein suggest that the context in which sampling procedures are conducted (e.g. in water versus out of water) has implications for both the physiological status of the individual being sampled, and the results obtained. A more thorough understanding of how air exposure during handling influences recovery times and rates of post-release mortality (e.g. through the joint assessment of changes in blood chemistry and the deployment of electronic tags) is warranted – as accurate estimates of post-release mortality are critical to the determination of total mortality estimates in stock assessment models, and thus directly influence the management of fisheries. Moreover, how intraspecific physiological responses to capture and handling are potentially influenced by sex-specific metabolic differences remains largely unknown.

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Author contributions

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Figure 1. Linear regressions fitted to blood chemistry data from blood samples collected both at the time that blacktip sharks (*C. limbatus*) were secured by anglers (i.e. fight time, solid circles) and immediately before release (i.e. handling time, open circles), including (**A**) pH, (**B**) lactate, (**C**) pCO_2, (**D**) pO_2, (**E**) sO_2, and (**F**) HCO_3^-. Colors represent the recreational capture method used. Continuous lines indicate regression model predictions for blood samples drawn at the time that sharks were secured, and dashed lines indicate regression model predictions for blood samples drawn immediately before release. Summary statistics for regression model predictions, including *p*-values and adjusted R^2 values are provided in the table.

Figure 2. Effect of sea-surface temperature at the location of capture on the concentration of Hsp70 in the red blood cells of blacktip sharks (*C. limbatus*). The concentration of Hsp70 at both 26–29°C and 29–32°C was higher than that at 23–26°C (ANOVA followed by Tukey's post-hoc). Horizontal bars represent the median; lower and upper hinges correspond to the 25th and 75th percentiles; whiskers extend from the hinge to the highest value within 1.5 times the inter-quartile range of the hinge. Significance (*) indicates $p < 0.05$.

Figure 3. Changes in acid-base indicators from the first blood samples, drawn at the time that blacktip sharks (*C. limbatus*) were secured by vessel-based anglers (i.e. fight time, solid circles), to the second blood samples, drawn immediately before release (i.e. handling time, open circles), including (**A**) pH, (**B**) lactate, (**C**) pCO_2, (**D**) pO_2, (**E**) sO_2, and (**F**) HCO_3^-. Colors represent the vessel-based handling location (in water versus out of water).

Figure 4. Rates of change of acid-base indicators during handling of blacktip sharks (*C. limbatus*) by vessel-based anglers, calculated to standardize the effect of handling due to varied handling times. Horizontal bars represent the median; lower and upper hinges correspond to the

25th and 75th percentiles; whiskers extend from the hinge to the highest value within 1.5 times the inter-quartile range of the hinge. Rates were compared between vessel-based handling locations using *t*-tests, where “ns” indicates non-significance; * indicates $p < 0.05$; ** indicates $p < 0.01$; and *** indicates $p < 0.001$.

Table 1. Capture characteristics for blacktip sharks (*C. limbatus*) caught via rod-and-reel from the shore and from fishing vessels. Sharks caught from fishing vessels are further divided into groups according to the handling location. Values are reported as mean ± *SD*.

	Capture characteristics						
Capture method	*n*	Handling location	Fork length (cm)	Sex ratio (M:F)	Fight time (min)	Handling time (min)	Sea-surface temp (°C)
Shore	43	Out of water	124.5 ± 24.4	1:3.3	5.09 ± 2.82	3.33 ± 1.16	27.7 ± 2.6
Vessel	51	-	117.7 ± 22.8	1:2.9	4.65 ± 1.94	3.63 ± 1.15	26.8 ± 2.5
Vessel	16	In water	115.2 ± 27.5	1:7.0	5.78 ± 2.15	4.49 ± 1.09	26.0 ± 2.5
Vessel	32	Out of water	120.8 ± 20.5	1:1.9	4.04 ± 1.56	3.19 ± 0.97	27.3 ± 2.5
Vessel	3	Out & in water	98.3 ± 9.6	0:3.0	5.13 ± 2.19	3.80 ± 0.82	26.0 ± 0.0

Table 2. Results from linear regressions, used to determine if the fight time (time on the line) or total disturbance time (fight time + handling time) had an effect on blood chemistry parameters measured in blacktip sharks (*C. limbatus*) caught from shore and from fishing vessels, as well as for vessel-based handling methods (in water versus out of water). Lactate, pO_2, and sO_2 values were transformed prior to regression analysis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non-significant.

		Model parameter													
	Fight time	pH	Lactate	pCO_2	pO_2	sO_2	HCO_3^-	Hct	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Glucose	Hsp70
Shore		**	***	ns	***	**	***	**	ns	ns	*	ns	ns	*	ns
Vessel		*	***	ns	*	ns	**	ns	ns	ns	ns	ns	ns	ns	ns
Vessel: in water		ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vessel: out of water		***	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
	Total time	pH	Lactate	pCO_2	pO_2	sO_2	HCO_3^-	Hct	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Glucose	Hsp70
Shore		***	**	ns	ns	ns	**	ns	ns	ns	*	ns	ns	ns	ns
Vessel		*	***	*	**	**	***	ns	ns	ns	ns	ns	ns	**	ns
Vessel: in water		ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Vessel: out of water		**	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 3. Results from ANCOVAs, used to determine if the blood chemistry parameters measured in captured blacktip sharks (*C. limbatus*) differed between recreational capture methods (shore versus vessel) or between vessel-based handling methods (in water versus out of water). ANCOVAs were used to account for extraneous variability due to differences in fight time and/or handling time between capture or handling methods. Lactate, pO_2, and sO_2 values were transformed prior to running ANCOVAs. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non-significant.

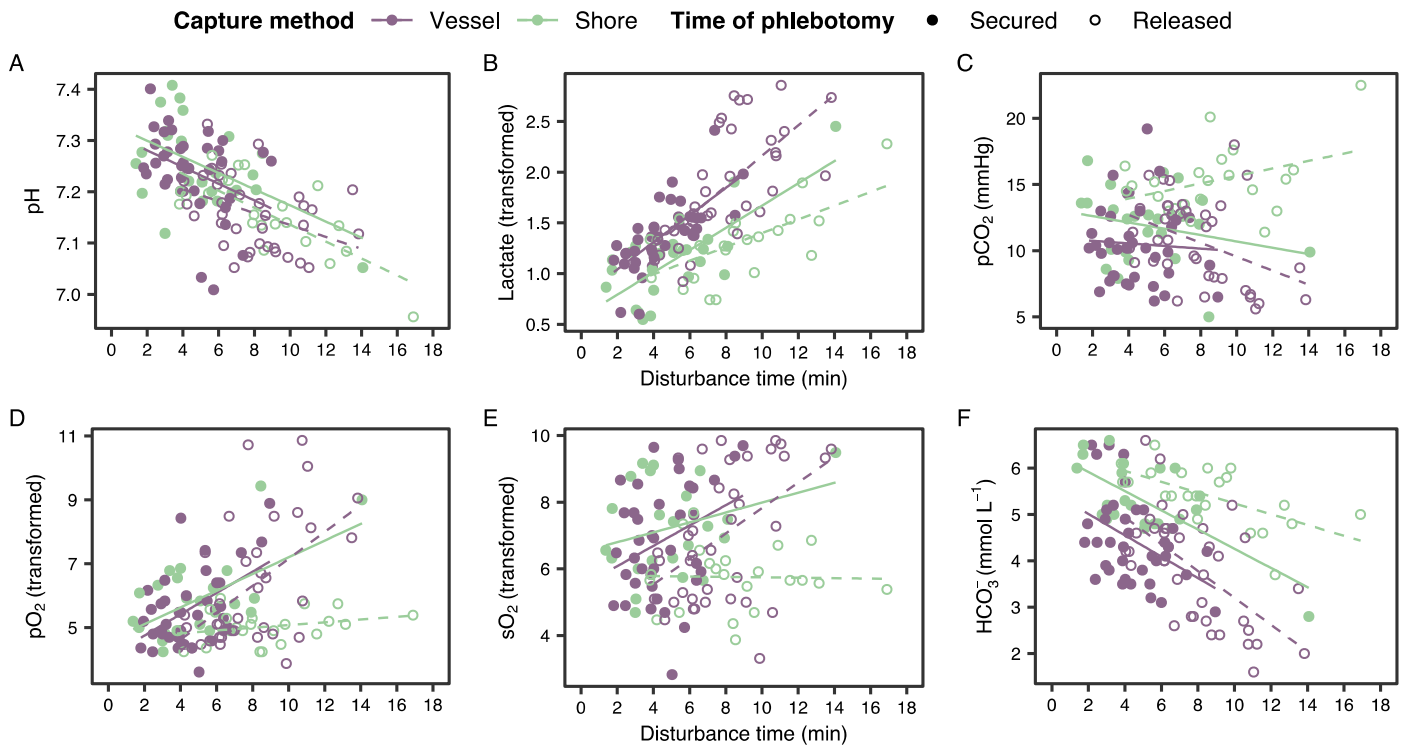
	Model parameter													
Fight time	pH	Lactate	pCO_2	pO_2	sO_2	HCO_3^-	Hct	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Glucose	Hsp70
Shore vs. vessel	ns	ns	*	ns	ns	**	ns	ns	ns	**	ns	ns	ns	ns
Vessel: in water vs. out of water	**	*	***	***	***	***	ns	**	*	ns	ns	ns	ns	ns
Total time	pH	Lactate	pCO_2	pO_2	sO_2	HCO_3^-	Hct	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Glucose	Hsp70
Shore vs. vessel	ns	***	***	**	*	***	ns	ns	ns	***	ns	ns	**	ns
Vessel: In water vs. out of water	ns	***	***	***	***	***	ns	ns	ns	ns	ns	ns	ns	ns

Table 4. Rates of change of blood chemistry parameters measured in captured blacktip sharks (*C. limbatus*), expressed as the change per min and calculated in order to compare the stress response during handling and to standardize the effect of handling due to varied handling times. The rate of change was compared between capture methods (shore versus vessel) and between vessel-based handling methods (in water versus out of water) using *t*-tests, where bold values indicate significant differences between capture methods and/or between vessel-based handling methods. Values are reported as mean ± *SD*.

Capture method	pH (min^{-1})	Lactate ($mmol\ l^{-1}\ min^{-1}$)	pCO_2 ($mmHg\ min^{-1}$)	pO_2 ($mmHg\ min^{-1}$)	sO_2 ($\%\ min^{-1}$)	HCO_3^- ($mmol\ l^{-1}\ min^{-1}$)	ΔH^+_m ($mmol\ l^{-1}\ min^{-1}$)	Hct ($\%\ min^{-1}$)	Na^+ ($mmol\ l^{-1}\ min^{-1}$)	Cl^- ($mmol\ l^{-1}\ min^{-1}$)	K^+ ($mmol\ l^{-1}\ min^{-1}$)	Ca^{2+} ($mmol\ l^{-1}\ min^{-1}$)	Mg^{2+} ($mmol\ l^{-1}\ min^{-1}$)	Glucose ($mg\ dL^{-1}\ min^{-1}$)	Hsp70 (min^{-1})
Shore	– 0.03	**0.12**	**1.14**	**– 3.62**	**– 6.67**	**0.05**	**0.16**	0.20	0.45	0.34	– 0.01	0.01	0.00	– 0.23	0.00
	± 0.02	**± 0.14**	**± 1.11**	**± 5.71**	**± 8.62**	**± 0.24**	**± 0.21**	± 0.67	± 1.36	± 1.06	± 0.26	± 0.03	± 0.01	± 1.27	± 0.02
Vessel	– 0.02	**0.42**	**0.12**	**2.55**	**0.82**	**– 0.16**	**0.32**	0.14	– 0.06	– 0.43	0.12	– 0.01	0.00	– 0.02	0.00
	± 0.03	**± 0.37**	**± 0.98**	**± 5.34**	**± 6.56**	**± 0.23**	**± 0.27**	± 0.90	± 2.87	± 2.57	± 0.23	± 0.04	± 0.02	± 1.16	± 0.02
Vessel handling location	pH (min^{-1})	Lactate ($mmol\ l^{-1}\ min^{-1}$)	pCO_2 ($mmHg\ min^{-1}$)	pO_2 ($mmHg\ min^{-1}$)	sO_2 ($\%\ min^{-1}$)	HCO_3^- ($mmol\ l^{-1}\ min^{-1}$)	ΔH^+_m ($mmol\ l^{-1}\ min^{-1}$)	Hct ($\%\ min^{-1}$)	Na^+ ($mmol\ l^{-1}\ min^{-1}$)	Cl^- ($mmol\ l^{-1}\ min^{-1}$)	K^+ ($mmol\ l^{-1}\ min^{-1}$)	Ca^{2+} ($mmol\ l^{-1}\ min^{-1}$)	Mg^{2+} ($mmol\ l^{-1}\ min^{-1}$)	Glucose ($mg\ dL^{-1}\ min^{-1}$)	Hsp70 (min^{-1})
In water	– 0.02	**0.73**	**– 0.21**	**3.57**	**2.32**	**– 0.25**	0.42	0.17	1.31	0.97	0.08	0.01	0.01	**0.81**	– 0.01
	± 0.02	**± 0.32**	**± 0.50**	**± 3.73**	**± 4.31**	**± 0.16**	± 0.17	± 0.15	± 3.90	± 2.15	± 0.13	± 0.05	± 0.02	**± 1.07**	± 0.01
Out of water	– 0.03	**0.21**	**0.57**	**0.08**	**– 1.67**	**– 0.06**	0.25	0.14	– 0.57	– 0.94	0.14	– 0.02	0.00	**– 0.32**	– 0.01
	± 0.03	**± 0.17**	**± 0.89**	**± 2.76**	**± 6.21**	**± 0.23**	± 0.31	± 1.06	± 2.31	± 2.56	± 0.25	± 0.04	± 0.02	**± 1.05**	± 0.02

Table 5. Results from paired *t*-tests on blood samples drawn immediately after blacktip sharks (*C. limbatus*) were secured by anglers and blood samples drawn immediately before release. Lactate, pO_2, and sO_2 values were transformed prior to running paired *t*-tests. For blood chemistry parameters showing significant differences between the two blood samples, plus signs indicate an increase from the first blood sample to the second, while minus signs indicate a decrease. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non-significant.

	Blood chemistry parameter													
Capture method	pH	Lactate	pCO_2	pO_2	sO_2	HCO_3^-	Hct	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Glucose	Hsp70
Shore	***	***	***	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
	(–)	(+)	(+)	(–)	(–)									
Vessel	***	***	ns	ns	ns	***	ns	ns	ns	*	ns	ns	ns	*
	(–)	(+)				(–)				(+)				(–)
Handling location	pH	Lactate	pCO_2	pO_2	sO_2	HCO_3^-	Hct	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Glucose	Hsp70
Vessel: in water	***	***	ns	**	ns	***	ns	ns	ns	ns	ns	ns	ns	ns
	(–)	(+)		(+)		(–)								
Vessel: out of water	***	***	**	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
	(–)	(+)	(+)								(–)			



	A	B	C	D	E	F
Vessel (Secured)	p = 0.018; R sq. = 0.09	p < 0.001; R sq. = 0.45	p = 0.782; R sq. = 0.00	p = 0.022; R sq. = 0.09	p = 0.083; R sq. = 0.04	p = 0.007; R sq. = 0.12
Vessel (Released)	p = 0.012; R sq. = 0.14	p < 0.001; R sq. = 0.45	p = 0.015; R sq. = 0.13	p = 0.002; R sq. = 0.23	p = 0.003; R sq. = 0.20	p < 0.001; R sq. = 0.35
Shore (Secured)	p = 0.001; R sq. = 0.21	p < 0.001; R sq. = 0.35	p = 0.958; R sq. = 0.00	p < 0.001; R sq. = 0.34	p = 0.003; R sq. = 0.19	p < 0.001; R sq. = 0.31
Shore (Released)	p < 0.001; R sq. = 0.54	p = 0.002; R sq. = 0.36	p = 0.082; R sq. = 0.10	p = 0.177; R sq. = 0.04	p = 0.922; R sq. = 0.00	p = 0.002; R sq. = 0.35

Figure 1.

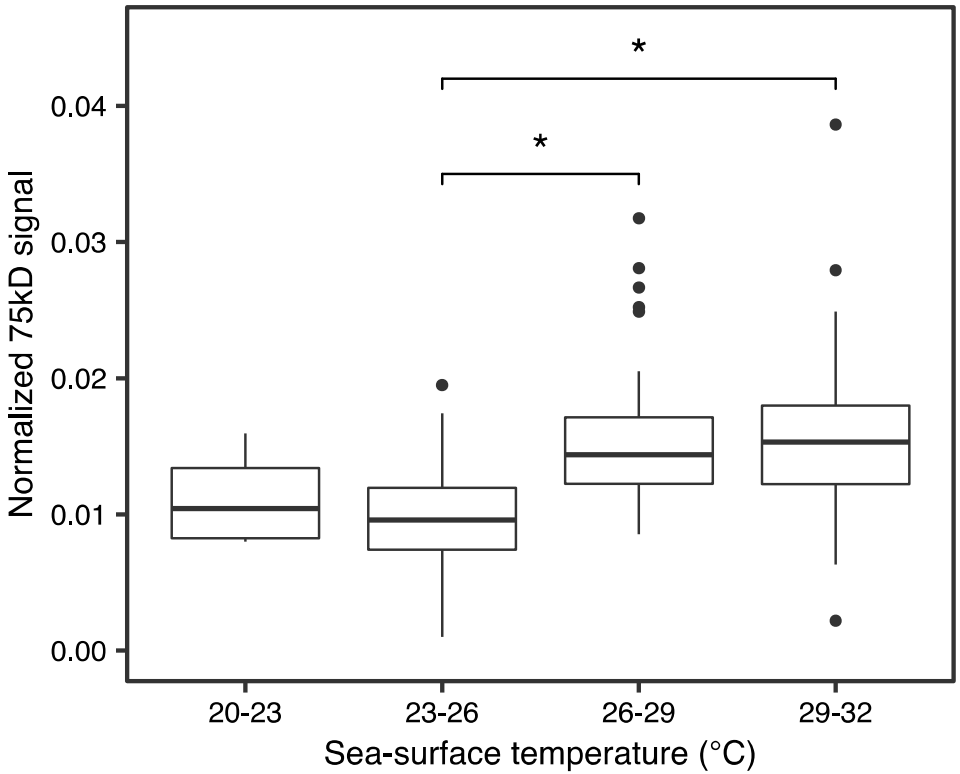

*
*
0.04
0.03
0.02
0.01
0.00
Normalized 75kD signal
20-23
23-26
26-29
29-32
Sea-surface temperature (°C)

Figure 2.

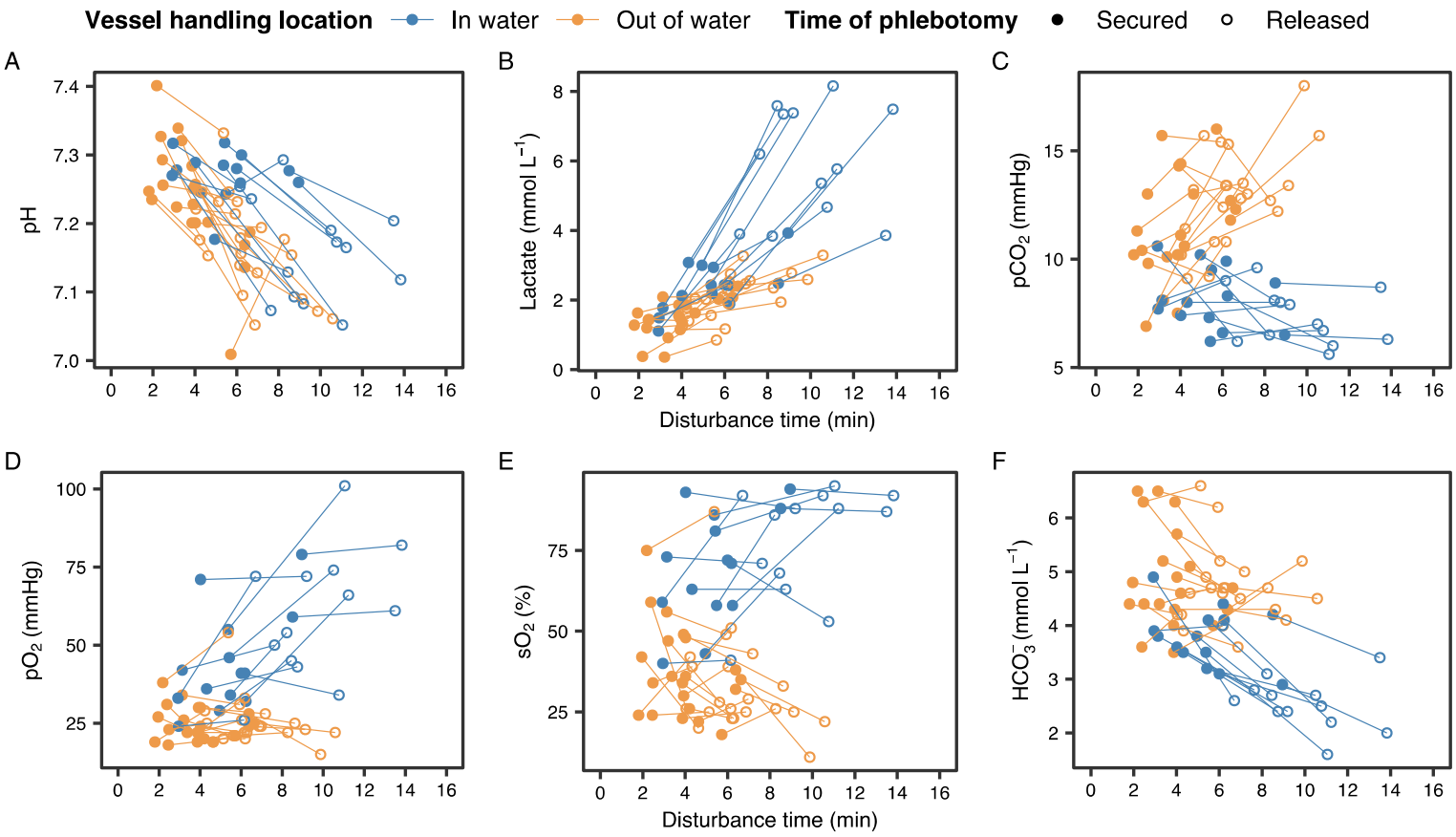

Vessel handling location
In water
Out of water
Time of phlebotomy
Secured
Released
A
B
C
D
E
F
pH
Lactate (mmol L^{-1})
pCO$_2$ (mmHg)
pO$_2$ (mmHg)
sO$_2$ (%)
HCO$_3^-$ (mmol L^{-1})
Disturbance time (min)
Disturbance time (min)

Figure 3.

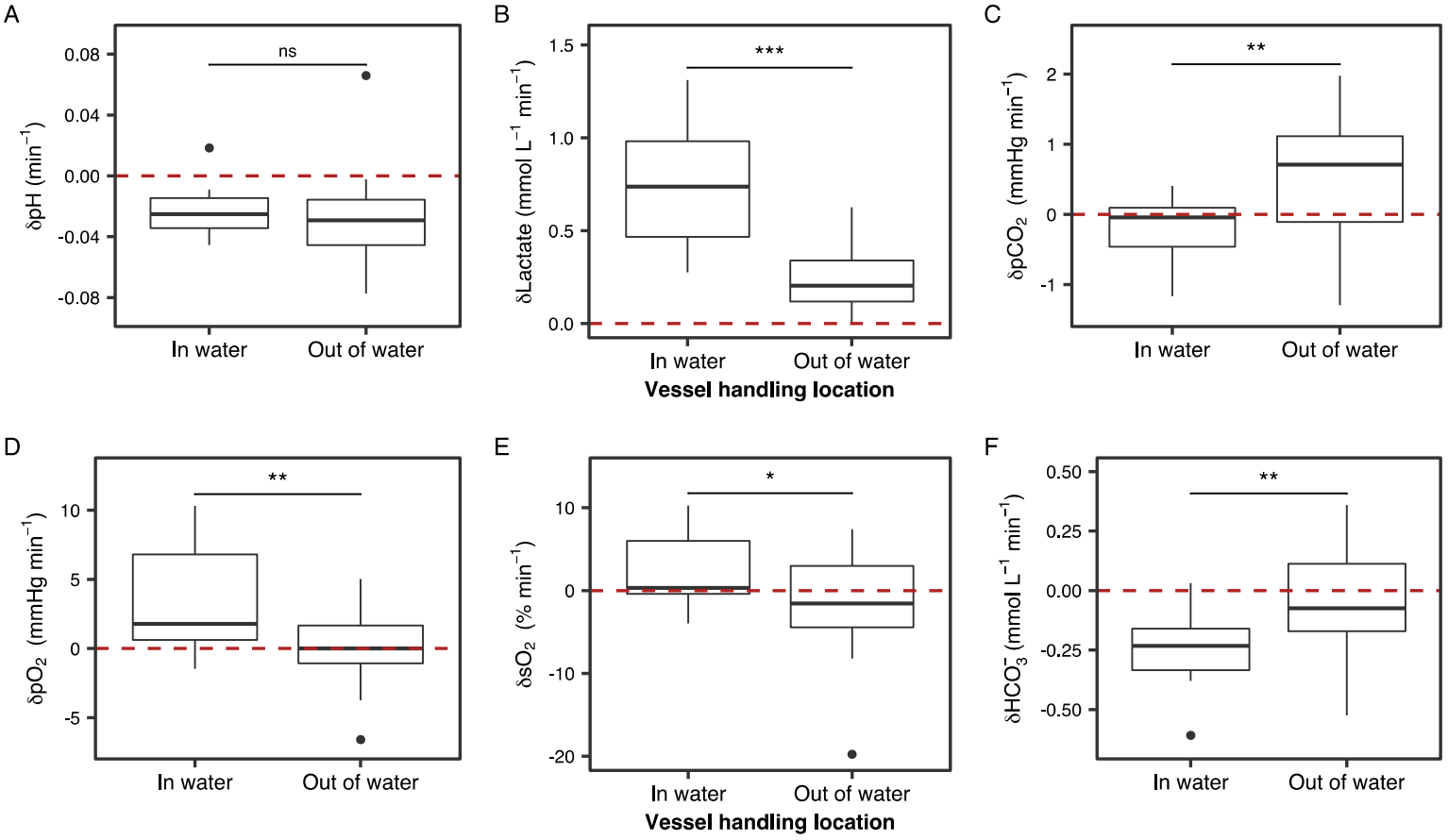

A
ns
δpH (min^{-1})
0.08
0.04
0.00
-0.04
-0.08
In water
Out of water
B
***
δLactate (mmol L^{-1} min^{-1})
1.5
1.0
0.5
0.0
In water
Out of water
Vessel handling location
C
**
δpCO$_2$ (mmHg min^{-1})
2
1
0
-1
In water
Out of water
D
**
δpO$_2$ (mmHg min^{-1})
10
5
0
-5
In water
Out of water
E
*
δsO$_2$ (% min^{-1})
10
0
-10
-20
In water
Out of water
Vessel handling location
F
**
δHCO$_3^-$ (mmol L^{-1} min^{-1})
0.50
0.25
0.00
-0.25
-0.50
In water
Out of water

Figure 4.