



FINAL REPORT

POST-RELEASE SURVIVAL OF CAPTURED MAKO SHARKS: CONTRIBUTING TO DEVELOPING BEST-PRACTISE FOR CATCH AND RELEASE GAME FISHING

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Executive summary

The shortfin mako shark (*Isurus oxyrinchus*) is a species commonly targeted by commercial and recreational anglers in many parts of the developed world. In Australia, the species is targeted by recreational anglers only, with an assumption that most of the sharks are released and populations remain minimally impacted. If released sharks do not survive, the current management strategy may need to be revised. Shortfin mako sharks are commonly subjected to long fight times; however, their unique physiology may provide an advantage over other fishes when recovering from exercise. This study assessed the post-release survival of recreationally caught shortfin mako sharks using Survivorship Pop-up Archival Transmitting (sPAT) tags and examined physiological indicators of capture stress from blood samples as well as any injuries that may be caused by hook choice. Survival estimates were based on 30 shortfin mako sharks captured off the south-eastern coast of Australia. Three mortalities were observed over the duration of the study, yielding an overall survival rate of 90%. All mortalities occurred in sharks with fight times less than 30 minutes. Sharks experienced significant increases in blood plasma levels of the compound lactate when subjected to longer fight times and higher sea surface temperatures (SSTs). Increased concentrations of plasma glucose were also found when sharks were captured in warmer waters, whereas the concentrations of heat shock protein 70 (facilitates recovery of protein structure when cells experience protein-damaging stress) and β -hydroxybutyrate (plays an important role in the supply of energy for recovery after exercise) were recorded in lower concentrations from sharks caught in warmer waters. Long fight times did not impact survival. Circle hooks significantly reduced foul hooking when compared with J hooks. Under the conditions of this study, we found that physical injury associated with hook type contributed to an increased likelihood of mortality, whereas the unique physiology of the species probably enabled it to cope with long fight times and the associated physiological responses to capture. As a consequence, as long as the shark has not sustained injuries, recreational capture and release of mako sharks results in high survivorship and as such is likely to have minimal impact on this species populations.

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1. Introduction

Recreational fishing is a popular pastime in many parts of the developed world (Post et al., 2002) and while the negative impacts of fishing on global populations has typically been attributed to commercial fisheries, it is becoming more commonly accepted that the recreational sector also contributes to many of these impacts (Mc Phee et al., 2002; Post et al., 2002; Coleman et al., 2004; Cooke and Cowx, 2004; Arlinghaus et al., 2005; Lewin et al., 2006). For decades, catch and release fishing methods have been advocated by fisheries managers and recreational fishing organisations in an attempt to promote the sustainable use of fisheries resources (Policansky, 2002; Arlinghaus et al., 2007). However, it is recognised that not all individuals are likely to survive once released, with post-release survival rates being highly variable among species (Muoneke and Childress, 1994; Bartholomew and Bohnsack, 2005; Skomal and Mandelman, 2012; Gallagher et al., 2014). This highlights the need to assess post-release survival on a species-by-species basis.

Like many elasmobranchs, the shortfin mako shark (*Isurus oxyrinchus*) is vulnerable to fishing pressure due to its life history characteristics (Hoenig and Gruber, 1990; Stevens, 2008; Semba et al., 2011). It is an endothermic species with one of the highest metabolic rates recorded for any pelagic shark; this implies a high aerobic scope that could be an advantage when dealing with physiological disturbances (Sepulveda et al., 2007). The shortfin mako shark is a popular target species for game fishers and a substantial portion of the bycatch in commercial longline fisheries targeting tuna and billfish (Stevens, 2008). In Australia, the shortfin mako shark was listed as a protected species under the Environment Protection and Biodiversity Conservation (EPBC) Act in 2010, following listings by the International Union for Conservation of Nature (IUCN) and Convention on the Conservation of Migratory Species (CMS), as ‘vulnerable’ and ‘migratory’ respectively. A controversial political debate around the protection of the species in Australia resulted in the shortfin mako shark remaining available to be targeted by recreational anglers only, under the assumption that the retained catch is relatively low and that populations remain minimally impacted by the fishery. There is, however, little information on post-release survival rates for recreationally caught shortfin mako sharks, and thus uncertainty as to the efficacy of the current management strategy.

In many instances angling mortality can be linked to physical injuries associated with the gear used and the handling of the animal (Muoneke and Childress, 1994; Cooke and Hogle, 2000; Cooke and Suski, 2004; Bartholomew and Bohnsack, 2005; Campana et al., 2009; Carruthers et al., 2009; Burns and Froeschke, 2012). Capture related physiological disruptions exceeding a fish’s ability to return to homeostasis can also result in mortality of released individuals (Kieffer, 2000; Moyes et al., 2006; Hight et al., 2007; Frick et al., 2010; Frick et al., 2012). Additionally, physiological disruptions can influence the behaviour of released fish, resulting in increased vulnerability to predation during the recovery period (Brownscombe et al., 2014; Raby et al., 2014).

Physiological responses to stressors can be observed through changes in blood chemistry and it has been observed that the magnitude of the stress response in sharks and other fishes can be linked to environmental factors such as water temperature (Kieffer et al., 1994; Manire et al., 2001). These responses include the anaerobic breakdown and mobilization of energy reserves such as glucose and glycogen to meet energetic demands and the associated accumulation of lactate (La⁻) and metabolic protons (H⁺) leading to lacticacidosis (Skomal and Mandelman, 2012). Changes in plasma ion concentrations can also result from lacticacidosis and drive cellular fluid shifts that result in haemoconcentration and disruptions to osmotic homeostasis (Skomal and Mandelman, 2012). A cellular stress response utilising heat shock proteins (HSPs) may also be present if cellular proteins are negatively impacted by the stress (Roberts et al., 2010; Currie,

2011). Recovery from these impacts is an aerobic process fuelled partly by the oxidation of ketones (Richards et al., 2003) and it has been suggested that interspecific differences in dealing with capture stress may be linked, in part, to the metabolic scope and thermal physiology of the target species (Skomal and Bernal, 2010; Skomal and Mandelman, 2012). Therefore, in understanding the implications of capture on subsequent survival it is necessary to screen for a suite of physiological and cellular markers (Skomal, 2007).

Post-release survival itself can be problematic to assess, particularly in large migratory species, where controlled experiments are not possible and conventional tag recapture studies may be limited by dispersal (Moyes et al., 2006; Skomal, 2007). Satellite tags are one way of addressing survivorship in large migratory animals (Graves et al., 2002; Stokesbury et al., 2011); however, the cost of tags often precludes large sample sizes (Donaldson et al., 2008). The recent development of specialised survivorship tags provides researchers with a more cost effective solution to this problem (Hutchinson et al., 2015).

This study aimed to quantify the post-release survival rate for recreationally caught shortfin mako sharks with consideration of the nature and magnitude of the physiological response to capture. Given the high metabolic rate and aerobic scope associated with this species' thermal strategy, we hypothesised a high post-release survival rate that is independent of the level of physiological stress experienced during recreational capture.

2. Methods

2.1. Capture and sampling

Shortfin mako sharks were caught in south-eastern Australian waters off the coast of Tasmania (Tas), South Australia (SA) and New South Wales (NSW) using gears and methods commonly utilised by Australian game fishers when targeting this species. Sharks were attracted to the boat using chum and offered a baited hook once sighted. Each shark was allowed to take the bait and swim away from the boat before the hook was set. Gear used included 15, 24 and 37 kg rated monofilament line, joined to a ~130 kg monofilament wind-on leader and 1.6 mm stainless-steel wire trace. Terminal tackle alternated between non-offset Shogun 9/0 stainless J (straight shank) hooks and non-offset Eagle Claw 13/0 circle hooks. Once boat-side, 26 sharks were left in the water and restrained by looping a thick, soft rope around the body posterior to the pectoral fins. Fight time (time from hook-up to restraint) was recorded to the nearest minute. The boat was kept in gear and the shark moved slowly forward facilitating ventilation of the gills. Seven sharks were manually lifted through a dive door for handling on deck; one where the tag applicator would not penetrate the skin, and six times where small (< ~50 kg) sharks either became tangled in gear or where it was deemed more efficient to handle them without the use of rope. In such instances, animals were not ventilated as to replicate game fishing conditions as closely as possible; no restraint on deck was necessary.

Once restrained or on deck, a pre-heparinised 5 mL syringe fitted with a 16 G needle was used to take a ~4 mL blood sample via caudal puncture. The sharks were measured to the nearest cm (FL) and sex and hooking location (Table 1) noted. Sharks were then tagged adjacent to the dorsal fin with a Survivorship Pop-up Archival Transmitting (sPAT) tag (Wildlife Computers) fitted with a Domeier umbrella anchor. Where possible the hook was removed before release; if this was not possible the trace was cut as close to the hook as possible. Each shark was examined for physical damage associated with hooking and substantial bleeding (free flowing blood that was not obviously slowed by natural haemostasis) noted. Handling time (time from restraint to release) was recorded to the nearest minute and the general condition upon release and the vigour of the shark as it swam off were also categorised (Table 1).

Table 1. Detailed definitions of variables recorded from each shark at capture.

Hooking locations		
	Jaw	Hooked around the jaw directly, including gums.
	Throat	Hook set behind teeth to oesophagus, excluding gill arches or filaments. Hook still visible.
	Gills	Hook set internally in gill arches or gill filaments.
	Gut	Hook set in deep oesophagus (beyond vision), and further down alimentary canal.

	Body	Hook is set in any external surface of the shark, excluding jaw.
Condition at capture	Good	Active and responsive shark with no damage beyond the hook puncture
	Average	Shark appears exhausted, is not very responsive or has sustained superficial injuries.
	Poor	Shark appears dead or dying (moribund) or has sustained heavy injuries/heavy bleeding.
Swimming vigour at release	Strong	Vigorous or high energy swimming
	Well	Regular pre-capture like swimming
	Slow	Exhausted, sluggish or buoyancy troubled
	Lifeless	No active swimming at all, drifted away

2.2. Post-release survival

Post-release survival was determined using data from the sPAT tags as per Hutchinson et al. (2015). These tags are pre-programmed to release and report survival after 30 days at liberty, or report prematurely if mortality occurs. Each tag summarises data in situ and transmits daily minimum and maximum temperature and depth, light change (day / night transitions) and attachment pin status (whether or not the tag has separated from the anchor). These data, along with the final pop-up location, are transmitted via Argos satellites once the tag reaches the surface and are used to determine whether the shark was actively swimming and alive at the time of release. If no movement (no depth change) is detected over 24 h or the tag exceeds 1700 m in depth, the tag will release prematurely. The fate of each tag, and therefore each shark, will fall into one of four categories; completed deployment, sinker, sitter or floater. A completed deployment refers to a tag still attached to a swimming animal 30 days after deployment; from this, survival is inferred as recovery from any physiological disturbances associated with the capture experience is expected to have occurred well within this deployment period (Frick et al., 2010). Sinker is assigned to tags that surpass 1700 m in depth, it can be assumed the tag is

attached to a shark that has died and is sinking; this depth is well beyond the maximum reported for shortfin mako sharks (Abascal et al., 2011). Floater refers to a tag that remains floating on the surface for 24 h; this may indicate attachment failure or possible fishing mortality. Sitter refers to a tag remaining at a constant depth that is shallower than 1700 m for 24 h, inferring the shark has died, sank and is resting on the ocean floor.

2.3. Biochemical analyses

Whole blood glucose and lactate were quantified immediately with the use of handheld meters (Accu-Chek Active blood glucose meter (Roche); Lactate Pro (Arkray)). Spun haematocrit (Hct) was determined by centrifuging blood for 5 min at Relative Centrifugal Force (RCF) 4,400 g in duplicate 75 mm mylar-coated capillary tubes plugged with Critoseal clay (ZIPocrit portable haematocrit centrifuge, LW scientific). The remaining blood was then centrifuged at RCF 2,800 g for 5 min (ZIPspin microcentrifuge, LW scientific) to separate plasma and red blood cells (RBCs), and immediately placed in liquid nitrogen for later analysis using the procedures detailed below. Long-term storage was at -80°C.

Protein levels of HSP70

Soluble protein was extracted from RBCs as per (LeBlanc et al., 2012). Each sample was diluted in 200 µL of shark saline (in mM: 280 NaCl; 7 KCl; 10 CaCl₂; 4.9 MgCl₂; 8 NaHCO₃; 1 NaH₂PO₄; 0.5 Na₂SO₄. pH 7.8, modified from Villalobos and Renfro (2007)) before DNA was sheared. This saline was free of urea, trimethylamine oxide (TMAO) and glucose for analysis purposes, as these compounds were measured as part of the experimental protocol. The resulting supernatant was diluted 1:200 in shark saline and assayed (BioRad) at 750 nm using a VERSAMAX microplate reader.

HSP70 was analysed based on methods developed for spiny dogfish (*Squalus acanthias*) (Kolhatkar et al., 2014). 30 µg of soluble protein was run alongside a four point standard curve (5, 15, 45 and 135 ng) of HSP70/72 standard (SPP-758, Enzo Life Sciences). The primary antibody AS05-083A diluted 1:4,000 in ECL (Global anti-HSP70, Agrisera, recognizing both constitutive and inducible isoforms of HSP70). Imaging was captured in a VERSADOC™ imaging system (MP 4000, BioRad) with Quantity One software. Image Lab ® software (BioRad) was used to quantify band signal against the standard curve.

Plasma lactate

As Lactate Pro is designed for use on human samples, we used a plasma lactate assay to validate the results obtained from the hand meter. Plasma lactate was quantified using a NADH-linked spectrophotometric assay as described in (Currie et al., 1999). Samples were incubated for 30 min after the addition of the glycine buffer before concentrations were read at 340 nm using a VERSAMAX microplate reader.

The values obtained from the Lactate Pro meter were tested for agreement against lactate assay values using Bland-Altman analysis (Bland and Altman, 1995, 1999; Krouwer, 2008). As a number of assay samples were compromised by equipment malfunction, this procedure allowed the use of a larger, more accurate data set with respect to lactate concentrations. Data were log transformed to account for normality and proportional error of differences during the analysis. Transformed Lactate Pro values underrepresented the assay values by a mean difference of -0.4119 (p=.007; n =30). These values were adjusted accordingly by adding 0.4119 and converted

back to reflect untransformed values. The adjusted lactate values are used in all further statistical analyses.

Plasma ions

Na⁺, Cl⁻ and K⁺ were quantified by diluting plasma samples 1:2 with double distilled H₂O and analysing with a Diamond Diagnostics SmartLyte electrolyte analyser.

Osmolytes

RBC and plasma urea were measured in accordance with Kolhatkar et al. (2014). The saline described above was used for dilutions. Plasma TMAO was analysed by diluting samples 1:5 in cold acetone before analysis using a quadrupole linear ion trap (LTQ) mass spectrometer as described in MacLellan et al. (2015). 5 µL of sample was diluted in 495 µL of 50/49.9/0.1 (v/v/v) methanol/water/formic acid; this solution was then laced with 5 µL of d₉-TMAO (0.01M) to give a final concentration of 0.1 mM. 10 µL of sample was injected into the mass spectrometer in triplicate. Plasma TMAO concentrations were determined by comparing the signal strengths of both endogenous and labelled TMAO and applying the appropriate dilution factor.

β-hydroxybutyrate

Plasma β-hydroxybutyrate (β-OHB) was quantified using a colorimetric assay kit (Cayman Chemical Company IN: 700190) with a VERSAMAX microplate reader as per manufacturer's instructions.

2.4. Statistical analyses

The 95% confidence interval associated with the survival estimate was calculated using the Release Mortality version 1.1.0 software developed by Goodyear (2002) and based on 10,000 simulations with no error sources or natural mortality incorporated, as described in Kerstetter and Graves (2006).

All other statistical analyses were carried out using SPSS (IBM) and R (R Core Team, 2014). A Kruskal-Wallis H test was used to determine if the size distributions of sharks differed between sampling regions (NSW, Tas, SA), and whether concentrations of blood parameters differed between sharks with fight times below 70 min (the bulk of the data) and four sharks with fight times that exceeded this limit (122 – 513min). The non-parametric tests were chosen as a Shapiro-Wilk test of normality indicated non-normal distributions were present in these data. Adjusted p values are presented. The association between hook type and the occurrence of foul hooking (throat, gut, body and gill locations combined) versus jaw hooking was investigated using a chi-square test. Fisher's exact test was used to determine if two uncontrolled components of our handling procedure contributed to mortality, i.e. bringing sharks on board and not removing hooks. Line class was not tested as a factor in the analyses as drag weight was not standardised.

Generalized additive models

Generalized additive models (GAMs) were used to investigate which factors (FL, SST and hooking location) influenced the length of fight time and to test the relationship between the characteristics of capture (namely: SST, fight time, handling time, whether sharks were handled on deck, hook type and hooking location) and blood-based dependent variables representing the physiological stress response.

Cleveland dotplots and boxplots were visually inspected to check for outliers in accordance with methods recommended by Zuur et al. (2010). Covariates were selected based on correlation matrices, Pearson's coefficients and variance inflation factors. Final selection of covariates for the model was made logically within these constraints (Zuur et al., 2009; Zuur et al., 2010; Zuur, 2012). Extreme data need to be removed prior to analysis in order to reduce the likelihood of type 1 and 2 errors. As such, sharks with fight times over 70 min (122 - 513 min) were omitted from GAMs to prevent the clustering caused by extreme values contributing to statistical errors; beyond this time point, relationships become based on too few data points to be considered reliable (plots of the full data are included in Supplementary Figure 1 to illustrate this point).

Beginning with a fully factored model for each response variable, a stepwise, backwards elimination method was used to drop predictor variables from the model based on statistical significance and relevance until only significant predictors remained (Ambelu et al., 2014). A smoothing function was applied to the primary non-linear predictor (fight time or FL) and the number of knots (inflection points) adjusted so that the spline did not indicate over-fitting (Ambelu et al., 2014). All GAMs were run using the Gaussian family algorithm and Identity link function.

3. Results

Thirty-three shortfin mako sharks ranging between 110 – 265 cm FL (equating to 13.4 kg and 191.5 kg based on the length-weight conversion presented by Stevens (1984)) were sampled; twenty-three sharks were caught adjacent to Tas, three adjacent to SA and seven adjacent to NSW. There was no significant difference between the size frequency compositions of sharks from each region (Kruskal-Wallis test: $H = 2.190$, d.f. = 2, $n = 33$, $P = 0.335$).

Fight times ranged from one to 513 min. A significant relationship between fight time and fork length ($F = 15.862$, $P < 0.0004$) and fight time and SST existed ($F = 4.166$, $P = 0.027$; $n = 29$, $GCV = 169.43$, $Adj R^2 = 0.496$, $Dev. Exp. = 54.8\%$). Larger sharks had longer fight times, and these times were extended further at warmer SSTs (Fig. 1).

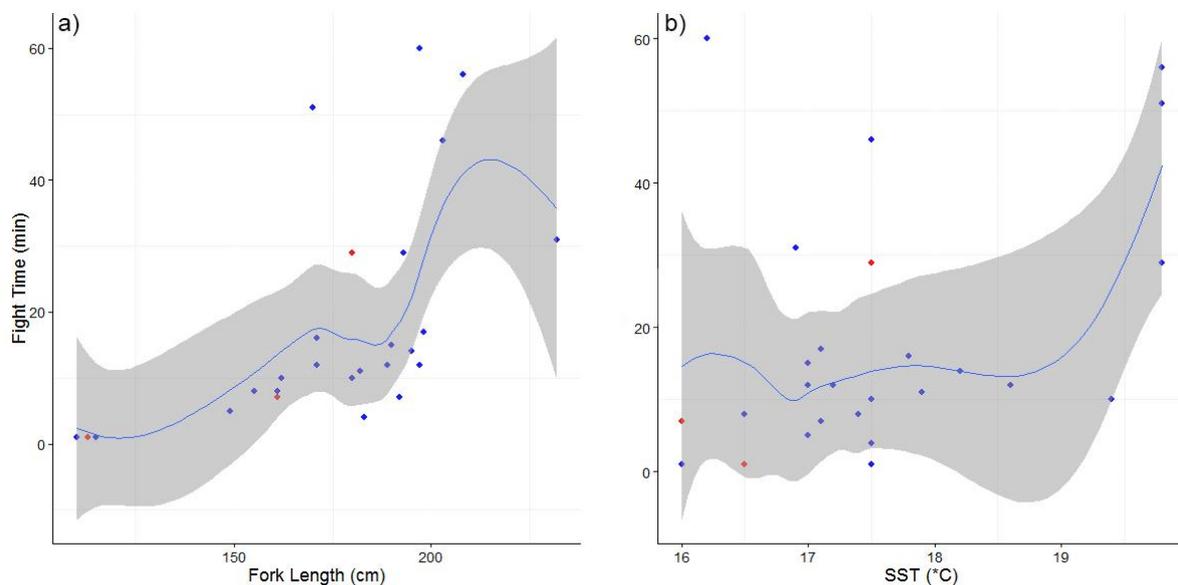


Figure 1. Loess smoothing functions (blue line) showing the relationship and 95% confidence intervals (grey shading) between a) fork length (cm) and fight time (minutes) and b) sea surface temperature (°C) and fight time for all sharks with fight times up to 70 minutes ($n = 29$). Tagged individuals ($n = 26$) are overlaid on the function with blue dots representing survivors and red dots indicating mortalities.

3.1. Hook type and hooking location

Of the sharks sampled, 18 (54.5%) were caught using circle hooks and 15 (45.5%) using J hooks. The majority of sharks caught using circle hooks were jaw hooked (83.3%), whereas using J hooks resulted in more variable hooking locations and a significantly lower proportion of sharks hooked in the jaw (20%) ($\chi^2 = 13.237$, d.f. = 1, $P = .0001$; Table 2). Only one shark was observed to have substantial bleeding; it was caught using a J hook which was lodged in the gills. We were able to remove hooks from 12 sharks before release (Table 3).

Table 2. Summary of anatomical hooking locations for 33 shortfin mako sharks caught on two types of terminal tackle. J hooks are 9/0 stainless steel “Shogun” hooks, Circle hooks are 13/0 “Eagle Claw”. Numbers and percentages are shown.

	J hook (% of hook type in location)	Circle hook (% of hook type in location)
Jaw	3 (20%)	15 (83.3%)
Throat	5 (33.3%)	2 (11.1%)
Gut	4 (26.7%)	0 (0%)
Body	0 (0%)	1 (5.6%)
Gills	3 (20%)	0 (0%)
Total	15 (100%)	18 (100%)

3.2. Post-release survival

Thirty sharks were tagged with sPAT tags, twenty-seven of which survived for the full duration of the 30 day tag deployment (Table 3), equating to a survival rate of 90% (95% Confidence Interval: 80 – 97%). The three mortalities occurred within 24 h of release and were all categorised as ‘sitters’, meaning that min and max daily depth were the same, and remained constant for over 24 hours. In all instances these resting depths corresponded with bathymetry, confirming the shark was resting on the seabed. With the exception of three individuals, the sharks were generally in good condition when captured, and most swam off well (Table 3). Three individuals were, however, in poor condition at release; two appeared moribund and lifeless and the other exhibited severe bleeding; only the latter of these three did not survive. A blood sample was available for only one of the sharks that died, and thus we were unable to investigate the relationship between physiological stress and post-release mortality. Physiological parameters for this shark were, however, well within the ranges of all surviving individuals (Figs 2 -5). Although two of the three mortalities were sharks that were brought on board, this handling practise did not have a significant impact on mortality ($P = 0.094$), nor did failure to remove hooks before release ($P = 0.672$).

Table 3. Capture variables for all caught and released shortfin mako sharks. Sharks were caught on either 9/0 stainless steel “Shogun” J hooks or 13/0 “Eagle Claw” Circle hooks. Bleeding unknown is for deep hooked sharks where puncture location was not visible. Displacement is the distance in km from the release location after 30 days. # indicates sharks that were omitted from GAMs. * indicates sharks were brought on deck. † indicates hooks were removed before release.

Shark	Size FL (cm)	Weight (kg)	Sex	Fight time (min)	Handling time (min)	Hook Type	Hook Location	Catch Condition	Bleeding	Swim Off	Displacement (km)	Survived	Blood Sample
M007	190	49.8	M	15	5*	J	Throat	Good	No	Slow	1042.0	Yes	No
M008	180	59.2	F	29	6*	J	Throat	Good	No	Strong	22.8	No (Sitter)	No
M009	192	50.7	M	7	8	Circle	Jaw†	Good	No	Well	1597.0	Yes	Yes
M010	189	66.4	M	12	5*	Circle	Throat†	Good	Unknown	Strong	1386.0	Yes	Yes
M012	161	42.2	F	7	4	Circle	Jaw†	Good	No	Well	21.3	No (Sitter)	No
M013 [#]	197	77.8	F	266	2	J	Gut	Poor	Unknown	Lifeless	1350.0	Yes	Yes
M014	170	49.8	M	51	3	Circle	Body	Good	No	Well	121.0	Yes	Yes
M015	180	59.2	F	10	2	Circle	Jaw	Good	No	Well	1260.0	Yes	Yes
M016	208	91.8	N/A	56	4	Circle	Jaw	Good	No	Strong	1818.0	Yes	Yes
M017	193	74.3	M	29	3	Circle	Jaw	Good	No	Strong	52.5	Yes	Yes
M018	171	50.7	F	12	7	Circle	Jaw†	Good	No	Strong	1671.0	Yes	Yes
M019 [#]	240	141.7	F	122	12	Circle	Jaw†	Good	No	Strong	47.9	Yes	Yes
M020	149	33.4	F	5	2*	Circle	Jaw	Good	No	Strong	344.1	Yes	Yes
M021	110	13.4	F	1	2*	Circle	Jaw†	Good	No	Strong	132.5	Yes	Yes
M022	183	62.2	M	4	2	J	Throat	Good	No	Strong	79.1	Yes	Yes
M023	115	15.3	F	1	2	J	Throat	Good	No	Strong	427.6	Yes	Yes
M024	162	43.0	F	10	2	J	Jaw	Good	No	Strong	285.9	Yes	Yes
M025	161	42.2	M	8	2	J	Gut	Good	Unknown	Well	392.8	Yes	Yes
M026	113	14.5	F	1	4*	J	Gills	Poor	Yes	Strong	0.4	No (Sitter)	Yes

M027 [#]	182	61.2	F	160	4	J	Throat	Poor	No	Lifeless	498.3	Yes	Yes
M028 [#]	265	191.5	F	513	12	J	Throat	Good	No	Well	1711.0	Yes	Yes
M029	110	13.4	F	4	3*	Circle	Jaw†	Good	No	Well	No Tag	Unknown	Yes
M030	232	127.8	F	31	10	J	Gills	Average	No	Well	1128.0	Yes	Yes
M031	197	77.8	F	12	3	Circle	Throat	Good	Unknown	Well	1918.0	Yes	Yes
M032	198	79.0	M	17	3	Circle	Jaw	Good	No	Well	756.8	Yes	Yes
M033	120	17.4	M	2	2	J	Jaw†	Good	No	Well	No Tag	Unknown	Yes
M034	197	77.8	F	60	4	Circle	Jaw†	Good	No	Strong	194.9	Yes	Yes
M035	203	85.2	M	46	5	Circle	Jaw†	Good	No	Strong	360.6	Yes	Yes
M036	182	61.2	M	11	3	J	Gills	Average	No	Slow	341.9	Yes	Yes
M037	132	23.2	F	1	1	Circle	Jaw†	Good	No	Well	No Tag	Unknown	Yes
M038	171	50.7	F	16	2	Circle	Jaw†	Good	No	Slow	318.3	Yes	Yes
M039	195	75.4	F	14	2	J	Jaw	Good	No	Well	530.4	Yes	Yes
M040	155	37.6	M	8	2	J	Gills	Good	No	Well	1652.0	Yes	Yes

3.3. Physiological response to capture

Twenty-seven of the tagged sharks were blood sampled, along with three non-tagged sharks. All thirty blood samples were analysed with field meters at time of capture, with thirteen of the frozen blood samples further analysed in the laboratory. None of the tested physiological variables (Table 4) were significantly related to handling time, handling on deck, hooking location or hook type.

Table 4. Physiological parameters measured in blood of shortfin mako sharks. All parameters measured in mmol L⁻¹ with the exception of Hct and RBC HSP70. *Values as a proxy for plasma lactate calculated from Lactate Pro values. ** Values are reported in fmol of HSP70 per µg of soluble protein from RBCs.

	Min	Max	Mean	SE	N
<i>La⁻</i> (mM)*	0.6	33.8	8.4	1.5	29
<i>Glucose</i> (mM)	4.1	8.7	6.0	0.2	29
<i>Hct</i> (%)	22.5	40	33.8	0.8	26
<i>Na⁺ Plasma</i> (mM)	242	272	252.6	2.7	11
<i>K⁺ Plasma</i> (mM)	3.4	4.4	3.9	0.1	11
<i>Cl Plasma</i> (mM)	222	240	230.1	1.4	11
<i>Urea Plasma</i> (mM)	306.7	399.6	353.1	9.1	11
<i>Urea RBC</i> (mM)	237.1	337.5	284.5	9.1	12
<i>TMAO Plasma</i> (mM)	97.5	195.5	139.9	7.3	11
<i>Ratio Urea : TMAO</i>	1.7 : 1	3.5 : 1	2.6 : 1	.15	11
<i>β-OHB</i> (mM)	0.212	0.910	0.567	.06	11
<i>RBC HSP70</i> (fmol.µg ⁻¹)**	3.05	49.23	36.6	3.9	12

For sharks with fight times up to 70 min, there were significant positive relationships between plasma lactate and increasing fight time (Fig. 2a) and SST (Fig. 2b) and plasma glucose and increasing SST (Fig. 3). There were significant negative relationships between both RBC HSP70 (Fig. 4) and plasma OHB (Fig. 5), and increasing SST (Table 5).

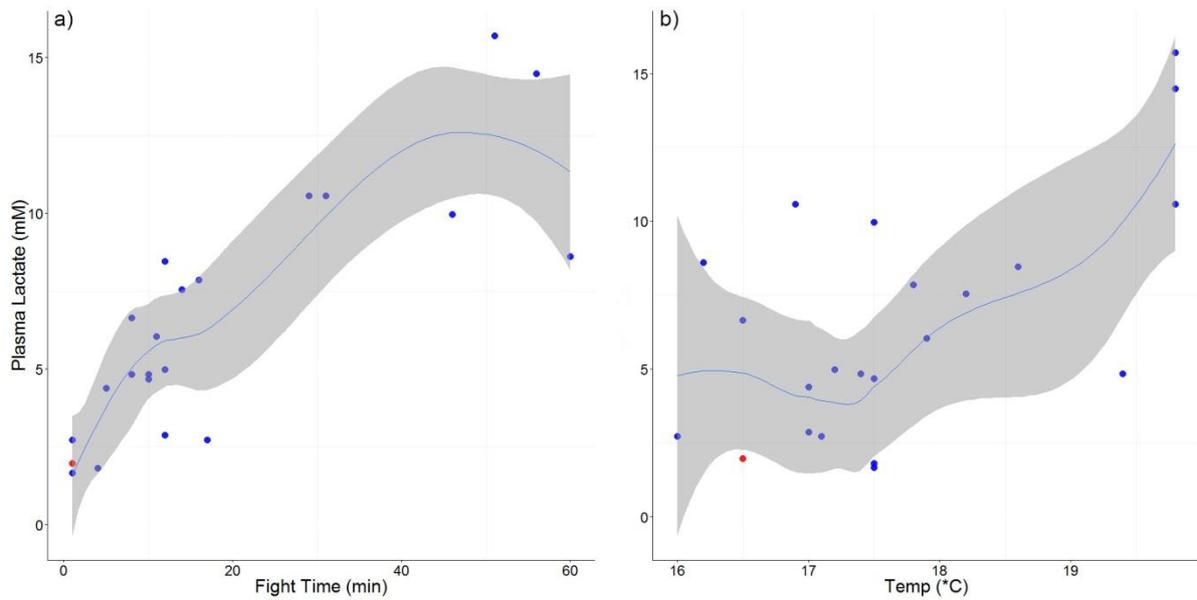


Figure 2. Loess smoothing functions (blue lines) showing the relationship and 95% confidence intervals (grey shading) between calculated plasma lactate (millimolar) with fight time ($n = 25$; in minutes; a) and calculated plasma lactate (millimolar) with sea surface temperature (in degrees Celsius; $n = 25$; b). Tagged individuals ($n = 22$) are overlaid on the smoothing function, with blue dots representing survivors and red dots indicating mortalities.

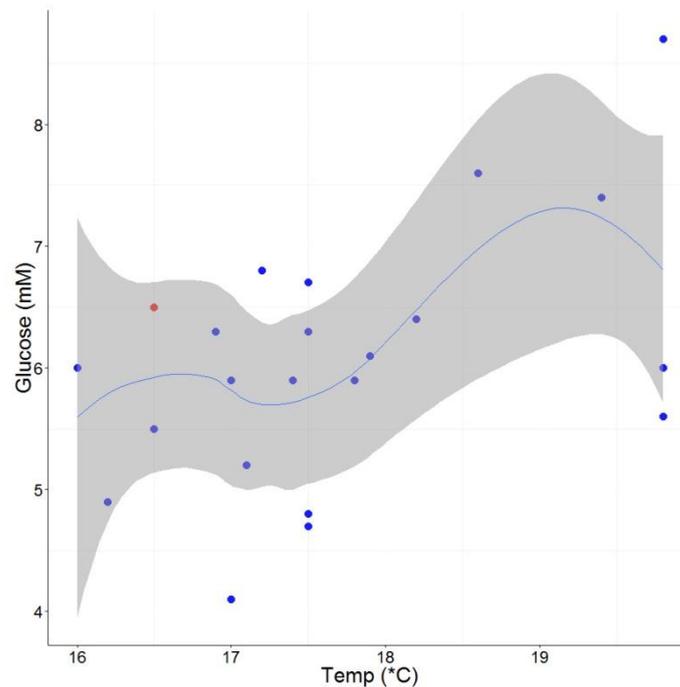


Figure 3. Loess smoothing function (blue line) showing the relationship and 95% confidence intervals (grey shading) between plasma glucose (millimolar; $n = 25$) and sea surface temperature (in degrees Celsius) for all sharks with fight times up to 70 min. Tagged individuals ($n = 22$) are overlaid on the smoothing function, with blue dots representing survivors and red dots indicating mortalities.

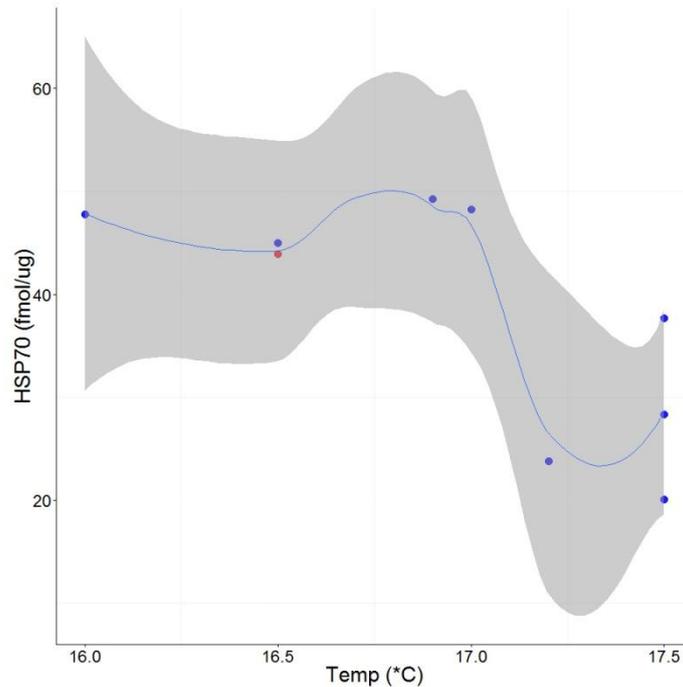


Figure 4. Loess smoothing function (blue line) showing the relationship and 95% confidence intervals (grey shading) between red blood cell heat shock protein 70 (HSP70; in femtomoles per microgram) and sea surface temperature (in degrees Celsius; $n = 10$) for all sharks with fight times up to 70 min. Tagged individuals ($n = 9$) are overlaid on the smoothing function, with blue dots representing survivors and red dots indicating mortalities.

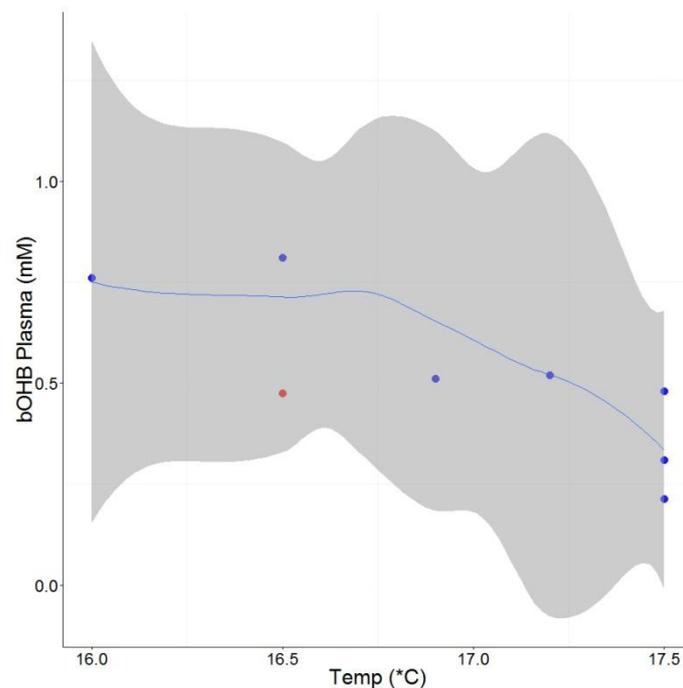


Figure 5. Loess smoothing function (blue line) showing the relationship and 95% confidence intervals (grey shading) between plasma β -hydroxybutyrate (β -OHB; millimolar) and sea surface temperature (in degrees Celsius; $n = 9$) for all sharks with fight times up to 70 min. Tagged individuals ($n = 8$) are overlaid on the smoothing function, with blue dots representing survivors and red dots indicating mortalities.

Table 5. Results from GAMs examining the physiological response in sharks with fight times up to 70 mins. Only best significant models resulting from the backwards elimination approach are presented. GCV = generalised cross validation score. edf = estimated degrees of freedom. "s" indicates a smoothing function is applied to the predictor variable. Value after "k" is the number of knots used in the smoothing function.

Model	Predictors (Best Model)	GCV	R2 Adjusted	Deviance Explained %	n	edf	F	P
Plasma Lactate	s (FightTime, k3) s(SST, k3)	3.423	0.820	84.1	25	1.717 1.000	32.210 9.502	<0.0001 0.005
Glucose	s (SST, k3)	0.935	0.141	17.7	25	1.000	4.954	0.035
HSP70	s (SST, k3)	79.526	0.486	56.6	10	1.390	6.701	0.023
β - OHB	s (SST, k3)	0.036	0.474	54.0	9	1.000	8.220	0.023

Hct and concentrations of plasma Na⁺, plasma K⁺ or plasma Cl⁻, urea (plasma and RBC), plasma TMAO and the ratio between plasma urea and plasma TMAO were not explained by any of the factors tested in our models.

Although not included in GAMs, sharks with fight times in excess of 70 min had significantly higher plasma lactate (H = 4.904, P = 0.026) and plasma Na⁺ (H = 4.541, P = 0.033) concentrations than sharks caught within 70 min. No other blood parameters differed significantly between the two fight time groups.

4. Discussion

This is the first study to directly assess post-release survival and capture stress physiology for shortfin mako sharks in a recreational fishery. Overall, post release survival rate is 90% when shortfin mako sharks are caught on rod and reel and subjected to fight times up to 513 min and handled for up to 12 min. The high survival rate supports the efficacy of catch and release as a strategy promoting responsible fishing for this species. Plasma lactate concentrations indicate that substantial anaerobic activity was associated with resisting capture; however, no sign of disruption to ionic or osmotic or energetic homeostasis was observed with fight times up to 70 min. The limited number of sharks with fight times in excess of this threshold precludes conclusions being made about the stress response to longer angling events; although, sharks with long fight times did have significantly higher plasma La⁻ and plasma Na⁺ concentrations. These individuals also survived, implying some degree of resilience to the increased physiological impacts of longer fight times. HSPs were elevated in cooler SSTs; a phenomenon that we believe may be linked to the thermal strategy of this species. Additionally, changes in plasma glucose and plasma β -OHB concentrations were noted with varying SSTs. Three mortalities occurred after short fight times which were not expected to provoke a strong stress response; however, two of these sharks were foul hooked. Taking this into account with the apparent resilience to capture stress, it is most likely that physical injury associated with hook choice had the greatest impact on survival in this study.

4.1. Physiological response to stress

We report an increase in plasma lactate with both fight time and SST; a relationship that has been previously noted in rainbow trout (Kieffer et al., 1994; Meka and McCormick, 2005) but until now, not for elasmobranchs, nor in any aquatic endothermic species. Increases in lactate concentration with fight time alone have been observed in game fish by numerous authors and represent the most consistently reported physiological marker of exhaustive anaerobic activity (Hoffmayer and Parsons, 2001; Heberer et al., 2010; Gallagher et al., 2014).

Increased blood lactate is one of many physiological changes associated with exhaustive exercise, although is not thought to be directly linked to survival (Wood et al., 1983; Frick et al., 2010). Nevertheless, some authors have observed significant differences in blood lactate concentrations between sharks that they have classed as either moribund or survivors (Hight et al., 2007; Marshall et al., 2012). Marshall et al. (2012) recorded significantly higher lactate concentrations in longline-caught (soak time 4-12 h) shortfin mako sharks that they classed as moribund (34.3 ± 5 mM) versus those that were classed as survivors (16.7 ± 12 mM); however, did not examine post-release survival directly, nor qualify how they classed animals as moribund. Hight et al. (2007) classed long-line caught sharks as moribund based on physical appearance and responsiveness. Similarly, we report lactate concentrations in sharks that are comparable to those assessed as moribund by Hight et al. (2007) and Marshall et al. (2012), and note that, despite these lactate concentrations and the moribund appearance of sharks boat-side, these individuals survived. For example, the highest recorded lactate value (33.8 mM) was taken from a shark that was retrieved after a fight of over 4 h; this individual became tangled in the line and was retrieved tail first, appearing lifeless boat-side with no active swimming observed at release. This individual recovered and the tag was detected 30 days later, 1350 km from its deployment location. These findings demonstrate that neither plasma lactate concentration nor subjective physical measures (appearance, responsiveness) are reliable predictors of actual mortality.

Concentrations of plasma ions and Hct were comparable to those of longline-caught shortfin mako sharks (Marshall et al., 2012). Changes in osmolarity and subsequent increases in

haematocrit may accompany the onset of acidosis (Turner et al., 1983; Cliff and Thurman, 1984; Skomal and Mandelman, 2012); however, we found no relationship between the variables included in our models and Hct or the concentrations of plasma ions. The results relating to the relationship between fight time and plasma ions are likely limited by the sample size of sharks used in the GAMs ($n = 9$) and the four sharks with fight times over 70 min being omitted from this analysis. This is supported by the significantly higher distribution of plasma Na^+ concentrations that we observed in these four sharks relative to the others and suggests that longer fight times may still provoke an ionic response to capture stress in this species.

The osmolytes urea and TMAO are a key component of the elasmobranch osmoconformation strategy (Yancey and Somero, 1979; Treberg et al., 2006). This is the first study to examine TMAO in an endothermic shark species, with plasma concentrations in the shortfin mako shark ($139.9 \pm 7.3 \text{ mM}$) at similar levels to those measured in ectothermic species such as Port Jackson sharks (*Heterodontus portusjacksoni*) (mean = 121 mM; Cooper and Morris (1998)). Our values for plasma urea ($353 \pm 9.1 \text{ mM}$) were comparable to other reports for this species following angling (322 mM; $n = 2$; (Wells et al., 1986)) and levels reported for ectotherms such as Port Jackson (394 mM) and gummy sharks (*Mustelus antarcticus*) (377 mM; (Frick et al., 2010)). There have been reports of significant decreases in plasma urea in response to otter-trawl capture and transport in spiny dogfish (Mandelman and Farrington, 2007) and gillnet capture in gummy sharks (Frick et al., 2010), which are likely attributed to a stress-induced increase in gill surface area and urea permeability (Evans and Kormanik, 1985). However, we did not observe any relationship between fight time and either osmolyte; nor did we find a significant difference in the concentrations of these osmolytes between sharks included in our model and those with long fight times. These findings support those of Brill et al. (2008) who reported no difference between the plasma urea levels of control and exercise-stressed sandbar sharks (*Carcharhinus plumbeus*). The utility of urea and TMAO as indicators of the stressed state in elasmobranchs remains unclear as also concluded by Skomal and Mandelman (2012).

The ketone, β -OHB, plays an important role in the supply of energy for exercise recovery; its oxidation supplying about 20% of the ATP required by *S. acanthias* (Richards et al., 2003). It is also an important energy source for the heart and red muscle in elasmobranchs (Ballantyne, 1997) and therefore plays an integral role in exercise physiology. We observed no relationship between plasma β -OHB concentrations and fight time which may be explained by the white muscle uptake of plasma ketones occurring at the same rate as their supply from the liver (Richards et al., 2003). We did find a significant negative relationship between β -OHB and SST for sharks caught within 70 min; however, the reason for this relationship is uncertain. β -OHB concentrations in this study ($0.567 \pm 0.06 \text{ mM}$) are comparable with those previously reported for mako sharks (0.978 mM (Watson and Dickson, 2001)), and the lack of exceptionally high values ($\sim 5 \text{ mM}$) would suggest that none of the sharks in this study were affected by starvation events (Walsh et al., 2006; Wood et al., 2010). Assuming starvation can contribute to poor health and negatively impact the energy available for metabolic processes such as swimming and recovery; our β -OHB data suggest that the sharks in this study were not starved or energy-depleted. We also observed a positive relationship between blood glucose and SST, with increased blood glucose possibly reflecting an increase in metabolic rate associated with the warmer SSTs and potentially an increase in feeding frequency that is necessary to sustain these increased energetic demands (Hoffmayer et al., 2012).

When all cells experience protein-damaging stress, HSPs are up-regulated within minutes to facilitate recovery of protein structure by guiding refolding, preventing protein aggregation and targeting irreparable proteins for destruction (Roberts et al., 2010; Currie, 2011). We examined the impacts of fight times of up to 70 min on cellular function by quantifying one of the most highly conserved of the stress proteins, HSP70 (Roberts et al., 2010; Currie, 2011). No

relationship between RBC HSP70 and fight time was found, although interestingly, we found a significant negative relationship between RBC HSP70 and SST. This is a surprising relationship and one that conflicts with what is known about the heat shock response in teleosts; specifically, that HSP70 usually increases at warmer temperatures (Currie, 2011). Given that the shortfin mako is an endothermic elasmobranch (Bernal et al., 2001) capable of maintaining body temperatures 7-10°C above ambient (Carey and Teal, 1969), the higher RBC HSP70 levels in cooler waters may be a response to a larger temperature difference experienced by the blood as it circulates from the cool periphery to the warm core of the fish. Additionally, increased HSP70 expression may reflect an elevated metabolic rate in response to the cold, as observed in the splenic tissue of Pacific Bluefin tuna (*Thunnus orientalis*) (Mladineo and Block, 2009). Skomal and Bernal (2010) present data showing HSP70 expression in shortfin mako sharks with 30 – 45 min fight times to be six times higher than in those on the line for < 1 min; however, the use of relative HSP70 levels and the absence of temperature data presented by these authors prevent any direct comparisons being made with our findings. It is possible that the strong relationship we observed between RBC HSP70 and SST dominated any effects that fight time may have had under a constant temperature. Further research is certainly needed to understand how endothermic fishes utilise HSPs during stress events and under varying environmental conditions.

4.2. Effect of fight time

Decreased survival associated with long fight times was not observed in this study, differing from previous reports on pelagic sharks in both commercial (Campana et al., 2009; Gallagher et al., 2014) and recreational fisheries (Heberer et al., 2010). Growth, digestion and exercise recovery all require the delivery of oxygen and metabolic substrate to the tissues at rates above those required by routine activities (Brill, 1996). Hence, elasmobranchs with a high aerobic scope should be capable of supplying more oxygen to tissues to deal with multiple aerobic demands (such as swimming and recovering from stress) simultaneously. This may also enable them to cope with a greater magnitude of physiological disruption from exercise and recover faster from this relative to their less active counterparts (Priede, 1985; Brill, 1996; Sepulveda et al., 2007; Skomal and Bernal, 2010).

Heberer et al. (2010) identified fight time as a significant predictor of survivorship for tail hooked common thresher sharks (*Alopias vulpinus*), with all sharks on the line ≥ 85 min succumbing to mortality. However, the capture method used by Heberer et al. (2010) involved pulling sharks backward, preventing effective ram ventilation and, in turn, limiting the aerobic capacity of the common thresher sharks. In contrast, our results indicate that all individuals with fight times over 85 min ($n = 4$, max 513 min) survived. As it is unlikely that respiration was inhibited by our capture method, oxygen delivery was not limited and it is probable that the shortfin mako's ability to cope with multiple aerobic demands was not compromised. Moreover, the three mortalities that we did observe had fight times < 30min, suggesting that mortalities in this study were not likely to be a direct consequence of the physiological impacts of fight time and indicate that this species may be more vulnerable to physical damage resulting from gear use and handling.

4.3. Effect of gears

Our data indicate a much higher occurrence of foul hooking associated with the use of J hooks compared with circle hooks. Foul hooking has been shown to significantly increase mortality rate in a number of species (Bartholomew and Bohnsack, 2005; Reeves and Bruesewitz, 2007; Campana et al., 2009; Epperly et al., 2012; Kneebone et al., 2013) and for the shortfin mako in particular, foul hooked sharks were over four times more likely to be retrieved from longlines dead than jaw hooked sharks (Epperly et al., 2012). Two of the three mortalities

observed in this study were foul hooked sharks caught using J hooks. One was gill hooked, with the associated bleeding almost certainly the cause of death; the other was deep hooked, possibly sustaining internal injuries or bleeding. Necropsy has shown foul hooking to be associated with hook penetration of the pericardium (Kneebone et al., 2013) and vital organs such as the heart, liver and parts of the lower alimentary canal (Caruso, 2000; Borucinska et al., 2001; Borucinska et al., 2002). Retained hooks can also lead to mortality over longer periods by causing systemic diseases (Borucinska et al., 2001; Adams et al., 2015). The significant reduction in foul hooking that we observed with circle hook use is in agreement with the findings of many other authors (Caruso, 2000; Cooke and Suski, 2004; Bartholomew and Bohnsack, 2005; Mapleston et al., 2008; Pacheco et al., 2011). It should be noted that although circle hooks are better for fish welfare in the majority of instances, offsetting circle hooks can counteract their conservation benefits by increasing deep hooking and subsequent mortality (Cooke and Suski, 2004; Epperly et al., 2012; Rice et al., 2012). The circle hooked shark that died in this study was hooked in the jaw and appeared to be in good health; however, no blood sample was taken, therefore we cannot speculate on the reasons for this mortality beyond the increased risk of predation during recovery.

4.4. Resilience to capture stress

Other survival estimates published for this species relate to individuals mostly taken on longlines and are based on tag-recapture data, 79% (Wood et al., 2007) and estimates from quantifying catecholamines at release, 80% (Hight et al., 2007). The lower survival estimates presented by these studies likely reflect the differences in capture and handling techniques between commercial and recreational fisheries; a finding consistent with blue shark (*Prionace glauca*) hooking mortality between the two sectors (Campana et al., 2006). When compared to other active sharks that exhibit a physiological response to capture of similar magnitude, the shortfin mako, and other lamnids, have an apparently high level of survivorship (Marshall et al., 2012) indicating a resilience to the physiological stresses of capture.

Previous work suggests that the activity level and ecological classification of elasmobranchs will affect the magnitude of their response to capture (Marshall et al., 2012). Taking into account the data presented by Marshall et al. (2012) and the results of this study, we suggest that the mako shark, a species renowned for its high activity, is resilient to capture stress due to the metabolic rate and aerobic scope attributed to its endothermy. Other active species, lacking the aerobic scope of endotherms, may differ in their ability to recover from intense exercise whilst simultaneously performing other necessary aerobic processes. As a result, these species may exhibit high mortality rates associated with their limited aerobic capacity, for example: blacktip shark (*Carcharhinus limbatus*, 88% mortality) and dusky shark (*C. obscurus*, 81% mortality (Marshall et al., 2012)). The data presented by Marshall et al. (2012) also suggest that some less active species have relatively high survivorship (e.g.: tiger shark, (*Galeocerdo cuvier*)) and do not appear to become as physiologically perturbed as active sharks (Marshall et al., 2012; Gallagher et al., 2014). This suggests that less active sharks do not require the aerobic scope associated with endothermy to deal with capture stress; rather, these species avoid substantial physiological perturbation altogether, suggesting divergent strategies in dealing with capture stress between active and less-active species.

4.5. Summary

Fight time did not impact shortfin mako shark survival, despite elevated plasma La- and plasma Na+ after long fight times indicating pronounced metabolic acidosis. This highlights the species' resilience to capture stress and likely reflects the aerobic capabilities associated with endothermy. No other physiological responses were found to be related to the duration of the capture event. Fight times reported in this study represent those that would be imposed by

recreational fishers and give merit to the use of catch and release fishing as a conservation method for shortfin mako. Post-release survival in this species is most likely to be impacted by hooking injuries which can be reduced through the adoption of circle hooks. If sharks are deep hooked, our results indicate that leaving hooks in may be beneficial, rather than risk further internal injury trying to remove them (Bartholomew and Bohnsack, 2005; Kneebone et al., 2013). Furthermore, sharks that appeared moribund when boat-side were observed to make a complete recovery after release which is an important factor to take into consideration when conducting survivorship studies and when making a decision about whether or not to release an individual. Recent studies have highlighted the highly interspecific nature of the stress response in sharks (Marshall et al., 2012; Renshaw et al., 2012) and species can differ greatly in their ability to cope with physiological disruptions (Renshaw et al., 2012); this may particularly apply when comparing ectothermic and endothermic species. Additionally, the need for fishery-specific assessments may be as equally important as species-specific assessments where gears and handling techniques are expected to vary between users.

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