



Moving from Measuring to Predicting Bycatch Mortality: Predicting the Capture Condition of a Longline-Caught Pelagic Shark

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Incidental fisheries capture has been identified as having a major effect on shark populations throughout the world. However, factors that contribute to the mortality of shark bycatch during fisheries capture are not fully understood. Here, we investigated the effects of capture duration, sea surface temperature, and shark total length (snout to the tip of the upper caudal lobe) on the physiology and condition of longline-caught bronze whalers, *Carcharhinus brachyurus*. Plasma lactate and potassium concentration had a positive linear relationship with capture duration, indicating that this species experiences increasing physiological challenges while on fishing gear. Additionally, we used stereotype logistic regression models to determine variables that could predict the capture condition of sharks (categorized as “healthy,” “sluggish,” or “moribund or dead”). In these models, elevated plasma lactate concentration, plasma potassium concentration, and capture duration increased the likelihood of *C. brachyurus* being captured in a “sluggish” condition or in a “moribund or dead” condition. After plasma lactate concentration exceeded 27.4 mmol/L, plasma potassium concentration exceeded 8.3 mmol/L, or capture durations exceeded 293 min, the majority of captured sharks (>50%) were predicted to be “moribund or dead.” We recommend that a reduction in the amount of time longlines are left fishing (soak time) will reduce immediate and post-release mortality in *C. brachyurus* bycatch and that our methods could be applied to identify causes of fisheries-induced mortality in future studies. The identification of operational, environmental, and biological variables contributing to poor condition will be necessary to implement conservation strategies that reduce mortality during capture.

Keywords: shark, longline, biochemistry, post-release mortality, capture duration, capture condition

INTRODUCTION

Commercial fisheries are one of the greatest threats to shark populations worldwide (Dulvy et al., 2014). Many sharks have slow life history strategies and their populations are inherently susceptible to the effects of fishing (Hoenig and Gruber, 1990; Smith et al., 1998). However, a large number of sharks caught by commercial fisheries are not retained, due to low commercial value, excessive physical damage, or fisheries regulations (Bonfil, 1994; Oliver et al., 2015). Sharks that are not

retained may die before they are brought onto the vessel (immediate mortality) or after release (post-release mortality) due to physical injuries or physiological trauma caused by capture (Davis, 2002; Skomal, 2007; Dapp et al., 2015). Immediate and/or post-release mortality may be substantial in some species and can have detrimental effects on shark populations (Alverson et al., 1994; Dapp et al., 2015). Despite this, the variables influencing the likelihood of shark mortality during fisheries capture are poorly understood. Because longline fisheries constitute a large portion of the world's discarded shark catch (Bonfil, 1994; Oliver et al., 2015), additional investigation into variables contributing to mortality during longline capture is warranted.

Shark mortality studies should consider both immediate and post-release mortality (Carruthers et al., 2009; Dapp et al., 2015), but, post-release mortality studies require logistically challenging and/or expensive programs (e.g., tag and release, satellite tracking, or captive holding (Hueter et al., 2006; Campana et al., 2009; Frick et al., 2010)). Consequently, these types of studies are limited to only a few shark species (Musyl et al., 2011; Dapp et al., 2015). In the absence of traditional post-release mortality estimation techniques, the externally visible condition (sometimes termed "vitality") of the animal when removed from fishing gear (e.g., "healthy," "sluggish," and "moribund") has been used as a proxy for post-release mortality (Hueter et al., 2006; Benoit et al., 2012; Braccini et al., 2012). The identification of variables that contribute to poor capture condition will allow researchers to better understand causes of post-release mortality during capture.

Ultimately, mortality studies should be used to design models that can predict species-specific mortality given operational, environmental, biological, and/or physiological measurements (Davis, 2002). Predictive models can then be used to make recommendations to fisheries managers that reduce the likelihood of immediate and post-release mortality in discarded species. For example, if the likelihood of mortality increases with increasing sea surface temperature, temporal restrictions on fishing during some months could be enacted to reduce bycatch mortality. Predictive models can also be used to identify physiological thresholds at which immediate and post-release mortality are likely to occur (Moyes et al., 2006; Hight et al., 2007; Frick et al., 2010).

The aim of the present study was to determine how shark capture condition is influenced by capture duration, sea surface temperature, total body length, and physiological variables. To achieve this aim, we took blood samples from longline-caught bronze whalers, *Carcharhinus brachyurus*, to measure hematological disruptions caused by capture. Hematological measurements (coupled with recordings of capture duration, sea surface temperature, and shark total length) were used to predict the capture condition of longline-caught *C. brachyurus*. Predictive models were applied to identify physiological thresholds that were likely to result in deteriorating shark condition when exceeded. The effect of non-physiological variables on capture condition was examined to make recommendations that can be used to reduce *C. brachyurus* mortality during and following longline capture.

MATERIALS AND METHODS

Fishing Experiment

Longline fishing was conducted onboard research vessels within Gulf St Vincent, South Australia, Australia (−34.836801, 138.256073). The longline was set just before dusk and remained set until dawn, resulting in soak times of 12–14 h. Lines were checked approximately every 2 h throughout the night by slowly driving a boat parallel to the longline and checking for sharks caught on fishing gear. Moribund or dead sharks (defined in the capture condition indexing section) were removed from the line immediately after identification. In some instances, after a healthy or sluggish shark (defined in the capture condition indexing section) was identified on the main line, it was left there to reach a specific capture duration.

All hooks (16/0 circle hooks attached to 2 m stainless steel gangions; baited with Australian salmon, *Arripis* spp.) were fitted with a time-depth recorder (TDR; LAT 1100, Lotek Wireless, Newmarket, ON, Canada) or a hook timer (HT; 600, Lindgren-Pitman, Pompano Beach, FL, USA). Capture duration was determined by changes in depth recorded by the TDR or by triggered hook timers. However, small sharks (typically <1000 mm total length) were usually not powerful enough to trigger hook timers, so capture duration could not be determined for those animals. All fieldwork conducted was approved by the Animal Ethics Committee of Flinders University (prior to October 2014; E360) or Monash University (during and after October 2014; BSCI/2014/22).

Sampling

Once captured, sharks were brought onboard the vessel and restrained. A bilge pump hose placed in the shark's mouth passed water over its gills to ensure continued oxygenation. Blood (~2.5 ml) was collected via the caudal vein with a heparinized 18-gauge × 1.5-inch needle and 3-ml syringe. The sample was immediately placed on crushed ice and stored for laboratory analysis. Shark total length (snout to the tip of the upper caudal lobe) and sea surface temperature were recorded. The entire sampling process, from the onset of aerial exposure until the shark was returned to the water, was completed in less than 5 min.

Upon reaching the laboratory, blood samples were mixed using a vortex mixer (model SVM1, Selby Biolab, Clayton, VIC, Australia). Whole blood (~75 µl) was drawn into a microcapillary tube and centrifuged at 10,000 rpm for 10 min to determine hematocrit. The remaining blood sample was centrifuged for 10 min at 10,000 rpms to separate the plasma and red blood cells. Once separated, plasma was extracted and stored at −20°C for chemical analysis at a later date. Concentrations of plasma glucose, lactate, potassium, chloride, and calcium were subsequently measured using blood gas analyzers (ABL 800 and ABL 80 Flex, Radiometer Pacific, Mt Waverley, VIC, Australia).

There was some variation in the number of blood variable measurement records. Because ABL 80 Flex analyzers are designed to operate on human blood, seven plasma chloride and three plasma calcium concentrations were out of measurement range. After initial tests, not enough sample remained to dilute and retest plasma chloride and calcium concentrations. Logistical

difficulties caused two samples to be destroyed prior to glucose and lactate analysis. Although, 52 sharks were captured in total, blood was only acquired from 51 sharks, because needles used were too small to collect blood from one captured shark that was approximately 3 m in total length.

Capture Condition Indexing

We used a qualitative capture condition indexing system to categorize shark condition upon capture (Manire et al., 2001; Enever et al., 2009; Benoît et al., 2010; Braccini et al., 2012; Mandelman et al., 2013). Condition categories were adapted from Benoît et al. (2010), by combining moribund and dead sharks into a single category to strengthen statistical analyses (Table 1). This is a valid approach because obligate ram-ventilating sharks, such as *C. brachyurus*, released in a “very poor” condition (defined as limited or no swimming observed when released) have high percentages of post-release mortality (e.g., *Carcharhinus limbatus* and *Sphyrna tiburo*, 82% and 79% post-release mortality, Hueter et al., 2006). *C. brachyurus* released in a moribund condition required long revival periods alongside the boat (>15 min), could not effectively swim after revival, and were observed sinking with no tail movements upon release. Although, stationary respiring elasmobranchs have been able to effectively recover from a moribund condition (Laptikhovskiy, 2004), it is unlikely that an obligate ram-ventilating shark would have the same capacity to physiologically recover if unable to move.

Additionally, combination of these categories was warranted because continuous line checks throughout the night ensured that most “moribund” and “dead” sharks were identified within 2 h. Those sharks that did progress from a “sluggish” to a “dead” category within the 2 h checking interval were likely to have died shortly before sampling.

Lastly, we performed *t*-tests to ensure that physiological measurements examined did not significantly differ between sharks categorized as moribund and dead. No significant differences were noted in concentrations of plasma lactate, potassium, chloride, calcium, or hematocrit percentage (Table 2). Plasma glucose concentrations in dead sharks were significantly lower than moribund sharks (Table 2), so glucose could not be examined across three categories. Glucose was not used to predict capture condition in subsequent analyses because combining moribund and dead sharks was necessary to achieve an adequate category sample size for regression models.

TABLE 1 | Capture condition descriptions adapted from Benoît et al. (2010).

Capture condition	Description
“Healthy”	Vigorous body movements.
“Sluggish”	Weak body movements. Responds to touching and prodding.
“Moribund or Dead”	No body movements. No response to touching or prodding. Movements of the operculum noted (moribund) or no movements of the operculum noted (dead).

Physical injury was not included in capture condition descriptions because, excluding minor hook damage, no captured sharks had visible wounds.

Statistical Analysis

We used regression analysis to investigate how each physiological variable was affected by capture duration (chloride, $n = 35$; calcium, $n = 38$; lactate and glucose, $n = 39$; hematocrit and potassium, $n = 40$; only sharks with a known capture duration included). For variables which conformed to a normal distribution, we fitted first (linear) order equations to the data (statistical significance designated as $p < 0.05$). Plasma glucose was not normally distributed, so capture duration was assigned to 100 min bins and its effect on glucose concentration was analyzed using non-parametric Kruskal-Wallis tests (statistical significance designated as $p < 0.05$).

To relate capture condition to capture duration ($n = 40$), sea surface temperature ($n = 52$), total length of shark ($n = 52$), and physiological variables (lactate and glucose, $n = 49$; potassium and hematocrit, $n = 51$; chloride, $n = 44$; calcium, $n = 48$), we used stereotype logistic regression models (Anderson, 1984). These models were chosen over ordered logistic regression models because our data violated the proportional odds ratio assumption (Long and Freese, 2006). Models were run using a Bayesian statistical philosophy (Markov chain Monte Carlo estimation—three chains with 500,000 iterations per chain; burn-in length = 1000, thinning rate = 100, uninformative prior distribution). DIC (deviance information criterion) values were used to determine the most appropriate models and variables to predict capture condition and 95% credibility intervals were used to determine statistically significant predictor variables. The final models used (including derived coefficients) can be found in the Supplementary Material Section.

Statistical data analysis was conducted using the “R Project for Statistical Computing” version 2.14.2 (R Development Core Team, 2013) and “Just Another Gibbs Sampler” version 3.4.0 (<http://mcmc-jags.sourceforge.net/>).

TABLE 2 | Physiological comparisons between sharks classified as “moribund” and “dead.”

	Mean (mmol/L)	Range (mmol/L)	Sample size	<i>p</i> -value
Lactate				0.45
Moribund	32.3	19.5 – 42.0	8	
Dead	35.4	25.5 – 41.2	5	
Glucose				0.049*
Moribund	4.6	2.2 – 7.8	8	
Dead	2.7	1.8 – 4.2	5	
Potassium				0.17
Moribund	8.6	6.3 – 11.6	9	
Dead	9.5	8.5 – 11.0	5	
Chloride				0.31
Moribund	224.6	211 – 244	9	
Dead	220.5	219 – 224	4	
Calcium				0.47
Moribund	2.8	2.2 – 3.2	9	
Dead	2.7	2.3 – 3.1	5	
Hematocrit				0.93
Moribund	25%	16 – 36%	9	
Dead	26%	15 – 43%	5	

p-values were derived from student *t*-tests comparing these condition categories. * is used to denote statistically significant results (*p*-value less than 0.05).

RESULTS

In total, 52 *C. brachyurus* were captured and 51 were blood sampled. Of those caught, 40 *C. brachyurus* had known capture durations, because small sharks (mean: 883 mm, range: 740–1170 mm) were not physically strong enough to trigger hook timers. Capture duration ranged from 5 to 402 min (mean = 167 min \pm 17.2 SE). Of sharks with known capture durations, 16, 14, and 10 *C. brachyurus* were in “healthy,” “sluggish,” and “moribund or dead” conditions, respectively.

Regression models showed that capture duration had a significant effect on plasma lactate ($p < 0.05$, $r^2 = 0.27$, **Figure 1A**) and plasma potassium ($p < 0.05$, $r^2 = 0.49$, **Figure 1D**). Plasma lactate and potassium increased as capture duration increased. There was no significant effect of capture duration on hematocrit (**Figure 1C**), glucose concentration (**Figure 1B**), calcium concentration (**Figure 1E**), or chloride concentration (**Figure 1F**).

Predictive models incorporating plasma lactate had the lowest DIC values and this variable significantly effected capture condition (**Table 3**). Increasing lactate resulted in a higher probability of sharks being caught in a “sluggish” condition or in a “moribund or dead” condition. When plasma lactate concentration in *C. brachyurus* was <18 mmol/L, nearly all sharks boated (removed from fishing gear) were predicted to be in a “healthy” (32%) or “sluggish” (62%) condition (**Figure 2**).

After plasma lactate concentrations exceeded 18 mmol/L, there was a sharp increase in the probability of sharks boated “moribund or dead” (**Figure 2**). From 18 to 23 mmol/L an increase in 1 mmol/L of plasma lactate resulted in a mean 2.9% increase in sharks boated in a “moribund or dead” condition (**Figure 2**). From 23 to 28 mmol/L, 1 mmol/L lactate increment increases enhanced chances of boating a shark “moribund or dead” by an average of 7.1% (**Figure 2**). Once plasma lactate concentrations exceeded 28 mmol/L, the majority of sharks captured were predicted to be “moribund or dead” (55%) (**Figure 2**). Our lactate-only models predicted that 50, 75, and 90% of longline-caught *C. brachyurus* were “moribund or dead” at plasma lactate concentrations of 27.3, 30.8, and 34.8 mmol/L respectively (**Figure 2**). No single or additive combination of variables was significantly better at predicting capture condition than a model incorporating only plasma lactate.

Capture condition also deteriorated with increasing plasma potassium concentration (**Table 3**; **Figure 3**). When plasma potassium concentration was <6.0 mmol/L, only 5% of *C. brachyurus* were predicted to be in a “moribund or dead” condition (**Figure 3**). When plasma potassium concentration exceeded 6.0 mmol/L, minor increases in potassium were indicative of greatly increased chances of capturing sharks “moribund or dead” (**Figure 3**). From concentrations of 6.0–7.5 mmol/L of plasma potassium, a 0.5 mmol/L increase in plasma potassium concentration resulted in a 6.9% increase in

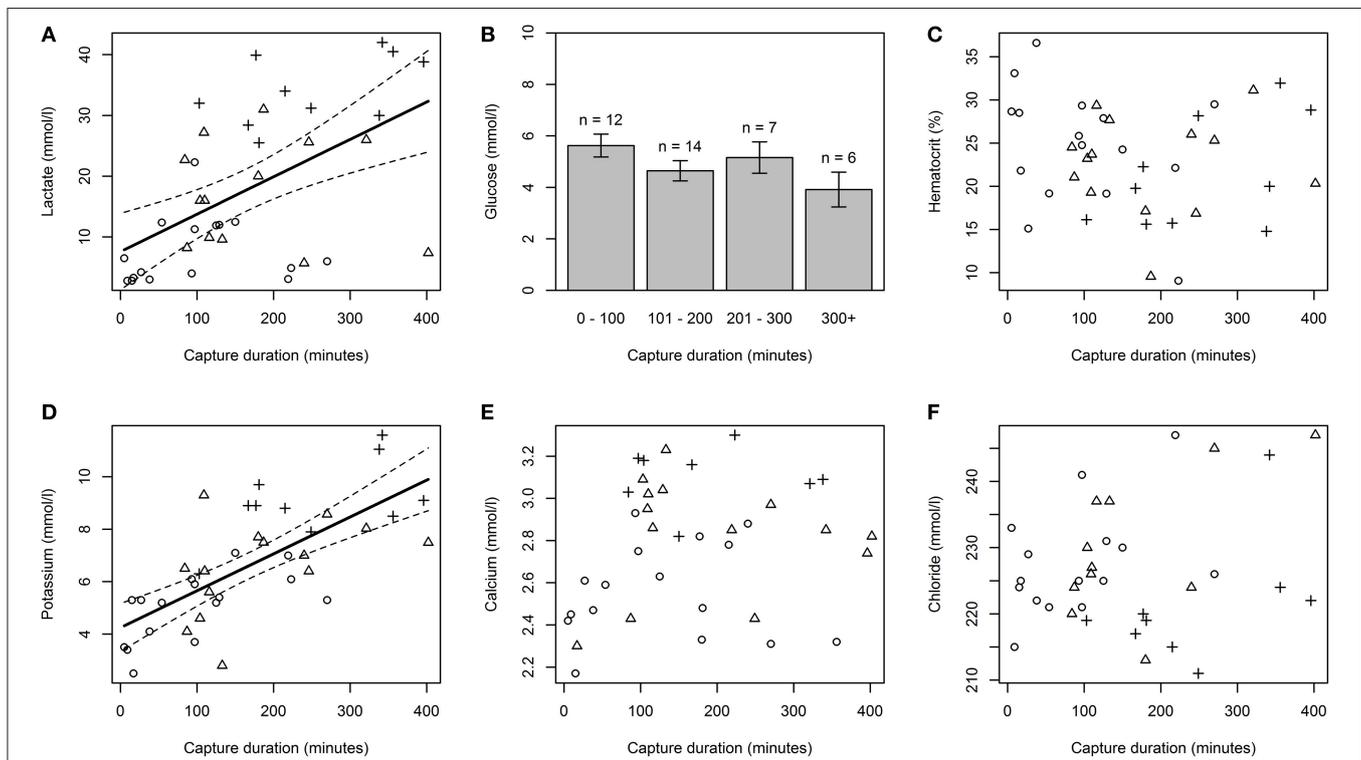


FIGURE 1 | The effect of capture duration on physiological response variables. Physiological variables examined include plasma lactate (**A**), plasma glucose (**B**), hematocrit (**C**), plasma potassium (**D**), plasma calcium (**E**), and plasma chloride (**F**). Solid lines in plasma lactate and potassium plots represent a statistically significant linear relationship between capture duration and the physiological variable examined. Dashed lines represent 95% confidence intervals. Glucose was binned using 100 min intervals because the variable deviated significantly from a normal distribution. Circles, triangles, and crosses symbolize sharks caught in a “healthy,” “sluggish,” and “moribund or dead” condition respectively.

TABLE 3 | DIC and β derived from stereotype logistic regression models.

Variable	Model DIC (Reduced)	β
Hook time	66.9	0.019 (0.008–0.033)*
Total length	78.0	0.031 (–0.002–0.068)
Sea surface temperature	79.0	–0.107 (–0.480–0.256)
Lactate	41.1	0.539 (0.291–0.877)*
Potassium	56.0	1.726 (0.920–2.786)*
Chloride	76.3	–0.074 (–0.184–0.023)
Calcium	77.8	1.130 (–1.314–3.489)
Hematocrit	76.5	–1.312 (–10.643–7.833)

β values were calculated using the full model (e.g., for lactate, all samples for which lactate readings were available) and are presented with 95% credibility intervals. The effect of each predictor variable in models was considered statistically significant (represented by *) if β 's 95% credibility intervals did not cross 0. DIC values were calculated using a reduced data set, that had only data points which measured all variables. These DIC values were calculated for model comparison purposes.

C. brachyurus being boated in a “moribund or dead” condition (Figure 3). Models predicted that the probability of being boated in a “moribund or dead” condition increases by 14.7% per 0.5 mmol/L increase in plasma potassium at plasma potassium concentrations between 7.5 and 9.0 mmol/L (Figure 3). Other physiological variables examined (hematocrit, plasma chloride concentration, and plasma calcium concentration) had no significant effect on capture condition (Table 3).

Of the non-physiological variables examined, capture duration significantly affected capture condition, while, sea surface temperature and total body length did not (Table 3). After 180 min of capture, only 31% of *C. brachyurus* were predicted to be boated in a “healthy” condition (Figure 4). By 266 min of capture, our model predicted that it is more likely to catch *C. brachyurus* in a “moribund or dead” condition than a “sluggish” condition (Figure 4). When capture durations exceed 270 min, only 12% of *C. brachyurus* captured are predicted to be in a “healthy” condition (Figure 4). Between 230 and 270 min capture durations, an increase in 5 min on fishing gear resulted in a mean of 1.1% increased probability of sharks being boated “moribund or dead.”

DISCUSSION

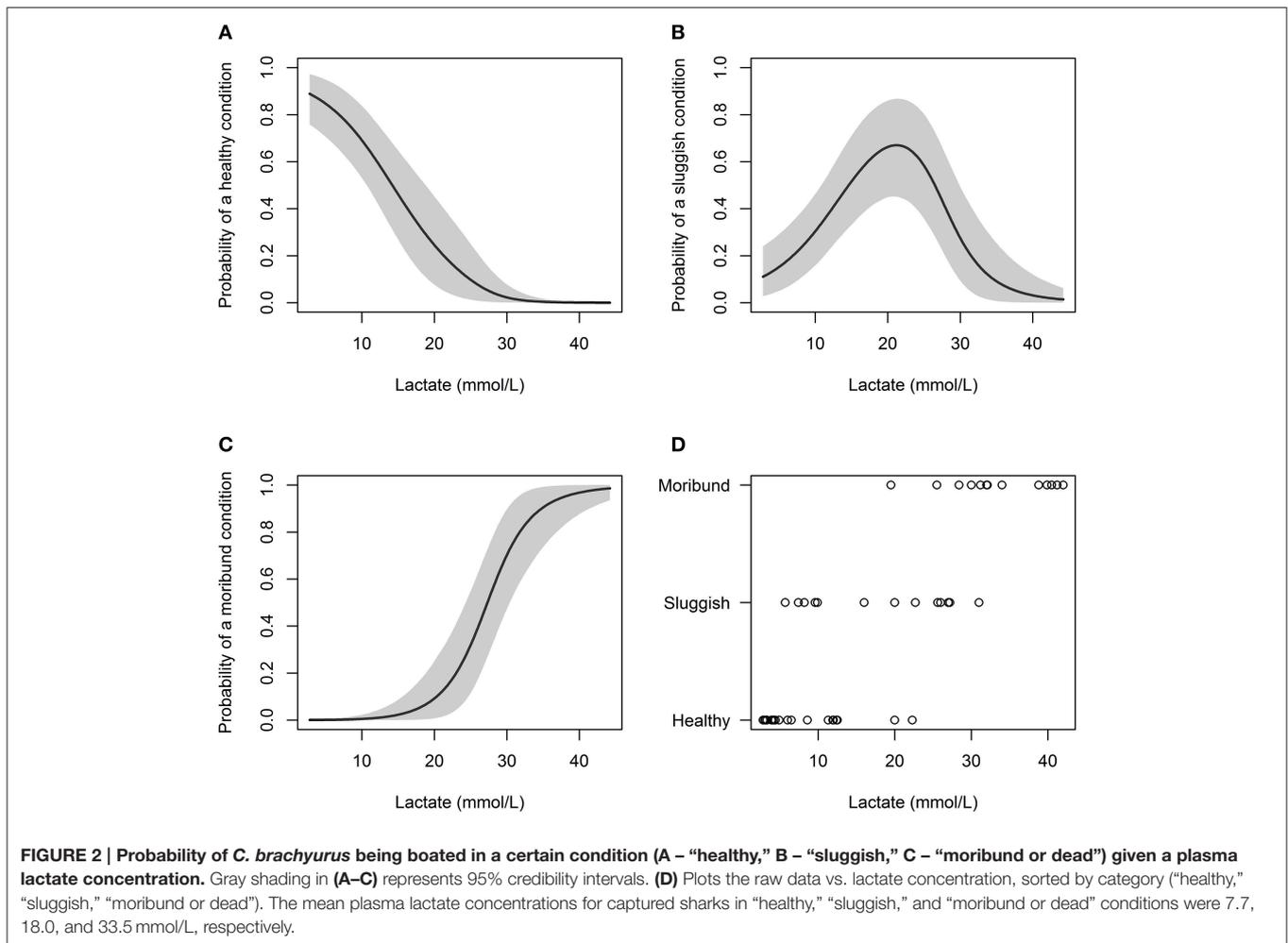
Our results demonstrated that the physiology of sharks during capture is correlated to capture duration and that plasma lactate concentration, plasma potassium concentration, and capture duration can influence a shark's capture condition. Plasma lactate and potassium concentrations in *C. brachyurus* had a positive linear relationship with capture duration. Some species capable of stationary respiration (e.g., *Mustelus antarticus*, *Heterodontus portusjacksoni*, and *Carcharhinus perezi*) exhibit a physiological response characterized by plasma lactate concentration peaking at 0–6 h after longline capture (Frick et al., 2010; Brooks et al., 2012). Following this peak, plasma lactate concentration decreases as physiological recovery begins while captured on fishing gear (Frick et al., 2010; Brooks et al., 2012). In our study, obligate ram-ventilating *C. brachyurus* did not begin to recover by 402 min of capture. Instead, plasma lactate and potassium

concentrations increased with increasing capture duration. Due to the movement-restrictive nature of longline capture (Morgan and Burgess, 2007), obligate ram-ventilating sharks would be unlikely to achieve the minimal swimming speed needed to pass water over the gills to oxygenate their tissues (Gruber and Keyes, 1981; Carlson et al., 2004). Dapp et al. (2015) estimated that post-release mortality is more likely in obligate ram-ventilating sharks than stationary respiring sharks, and our results indicate that obligate ram-ventilating sharks may have a diminished ability to recover from physiological disturbance and other fishery-induced stress compared to species capable of stationary respiration.

Capture duration elicits varying responses in several of the variables examined. Captured sharks can experience significant changes in plasma chloride concentration, plasma glucose concentration, plasma calcium concentration, and hematocrit percentage (Cliff and Thurman, 1984; McDonald and Milligan, 1992; Heberer et al., 2010). However, these measurements are unaffected by capture duration in some species (Brooks et al., 2012; Kneebone et al., 2013; Butcher et al., 2015). In the present study, we observed no significant effect of capture duration on *C. brachyurus* plasma chloride concentration, plasma calcium concentration, plasma glucose concentration, and hematocrit percentage.

The probability that *C. brachyurus* were caught in a “sluggish” condition or a “moribund or dead” condition increased with increasing potassium. Several studies have suggested that hyperkalemia (elevated plasma potassium) can cause neuromuscular interference and muscle tetany in physiologically stressed sharks (Cliff and Thurman, 1984; Frick et al., 2010; Skomal and Mandelman, 2012). These conditions are thought to be one of the primary causes of shark mortality during and following fisheries capture (Martini, 1974; Cliff and Thurman, 1984; Frick et al., 2010). Several *C. brachyurus* captured in a “moribund or dead” condition during our study had stiffened muscles, indicating tetany. This symptom was not observed in any sharks in a “healthy” or “sluggish” condition (pers. obs). Given the importance of potassium as a predictor of condition and qualitative observations in the field, our results provide further evidence for the role of hyperkalemia in fisheries-induced mortality.

It is unlikely that increased plasma lactate is the proximate cause of death in physiologically stressed sharks because, in isolation, the lactate ion is relatively benign to fish (Jonas et al., 1962). However, in previous shark stress studies, plasma lactate has been used as an indicator of metabolic acidosis (Frick et al., 2010). Along with respiratory acidosis, metabolic acidosis can cause depressions in blood pH (Skomal and Mandelman, 2012). Metabolic acidosis can then compromise the integrity of cellular membranes, causing a leakage of inorganic ions (e.g., potassium, magnesium, and calcium) into the blood stream, resulting in intracellular acidosis (Cliff and Thurman, 1984). Intracellular acidosis is thought to be the primary cause of hyperkalemia in sharks during capture (Cliff and Thurman, 1984). Consequently, lactate was a good predictor of mortality in our study, not because it is toxic, but probably because it is an indicator of physiological processes that are detrimental to the health of sharks.

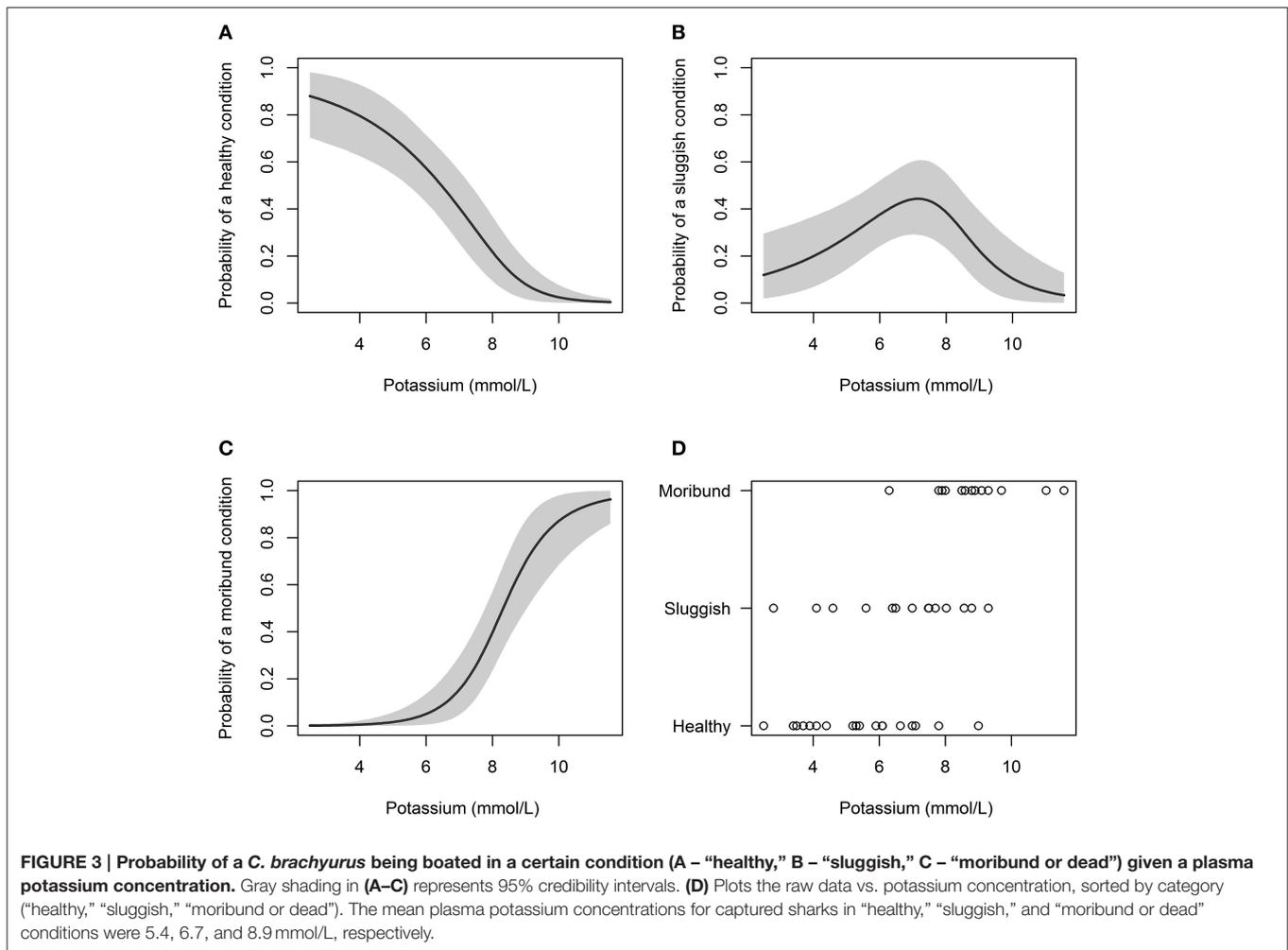


In addition to predicting when *C. brachyurus* are likely to be boated in a “moribund or dead” condition, it is crucial to estimate when sharks are more likely to be boated in a “sluggish” condition than a “healthy” condition. Forty-two percent (22 of 52) captured sharks were deemed to be in a “healthy” condition in our study. Previous studies have estimated that “healthy” sharks have low post-release mortality percentages (e.g., *Prionace glauca*–0%, Campana et al., 2009), so these sharks were likely to survive subsequent release. In contrast, sharks released “sluggish” are estimated to have post-release mortality percentages between 19% (*Sphyrna tiburo*) and 34% (*Carcharhinus limbatus*) (Hueter et al., 2006). Twenty-nine percent (15 of 52) of *C. brachyurus* we captured were in a “sluggish” condition and it is likely that some of these animals may have experienced post-release mortality.

Our models predicted that the percentage of *C. brachyurus* boated in a “sluggish” condition begins to exceed the percentage of *C. brachyurus* boated in a “healthy” condition at a plasma lactate concentration of 14.4 mmol/L, plasma potassium concentration of 6.9 mmol/L, and capture duration of 148 min. Peak percentages when *C. brachyurus* are most likely to be captured in a “sluggish” condition occur at a plasma lactate concentration of 21.2 mmol/L (67% of *C. brachyurus* captured

“sluggish”), plasma potassium concentration of 7.2 mmol/L (44% of *C. brachyurus* captured “sluggish”), and capture duration of 216 min (46% of *C. brachyurus* captured “sluggish”). At these peaks, only 20%, 37%, 22% of *C. brachyurus* caught are predicted to be caught in a “healthy” condition, respectively, and are susceptible to immediate or post-release mortality. Future studies are necessary to determine the likelihood of post-release mortality for *C. brachyurus* released in a “sluggish” condition.

Given our results, we consider plasma lactate concentrations of about 18 mmol/L and 23 mmol/L to be threshold values in *C. brachyurus*. These threshold values were established because *C. brachyurus* below an 18 mmol/L plasma lactate concentration are unlikely to be boated in a “moribund or dead” condition and sharks exceeding a 23 mmol/L concentration are likely to die with minor further plasma lactate concentration increases. Similarly, threshold values can be established for plasma potassium concentrations. Few *C. brachyurus* with a plasma potassium concentration <6.0 mmol/L were predicted to be in a “moribund or dead” condition and the likelihood of sharks being boated in this condition greatly increased after plasma potassium concentration exceeded 7.5 mmol/L. Thus, captured animals with plasma lactate or potassium approaching these threshold values



are at risk of tipping over into a condition with a significantly increased risk of an adverse outcome.

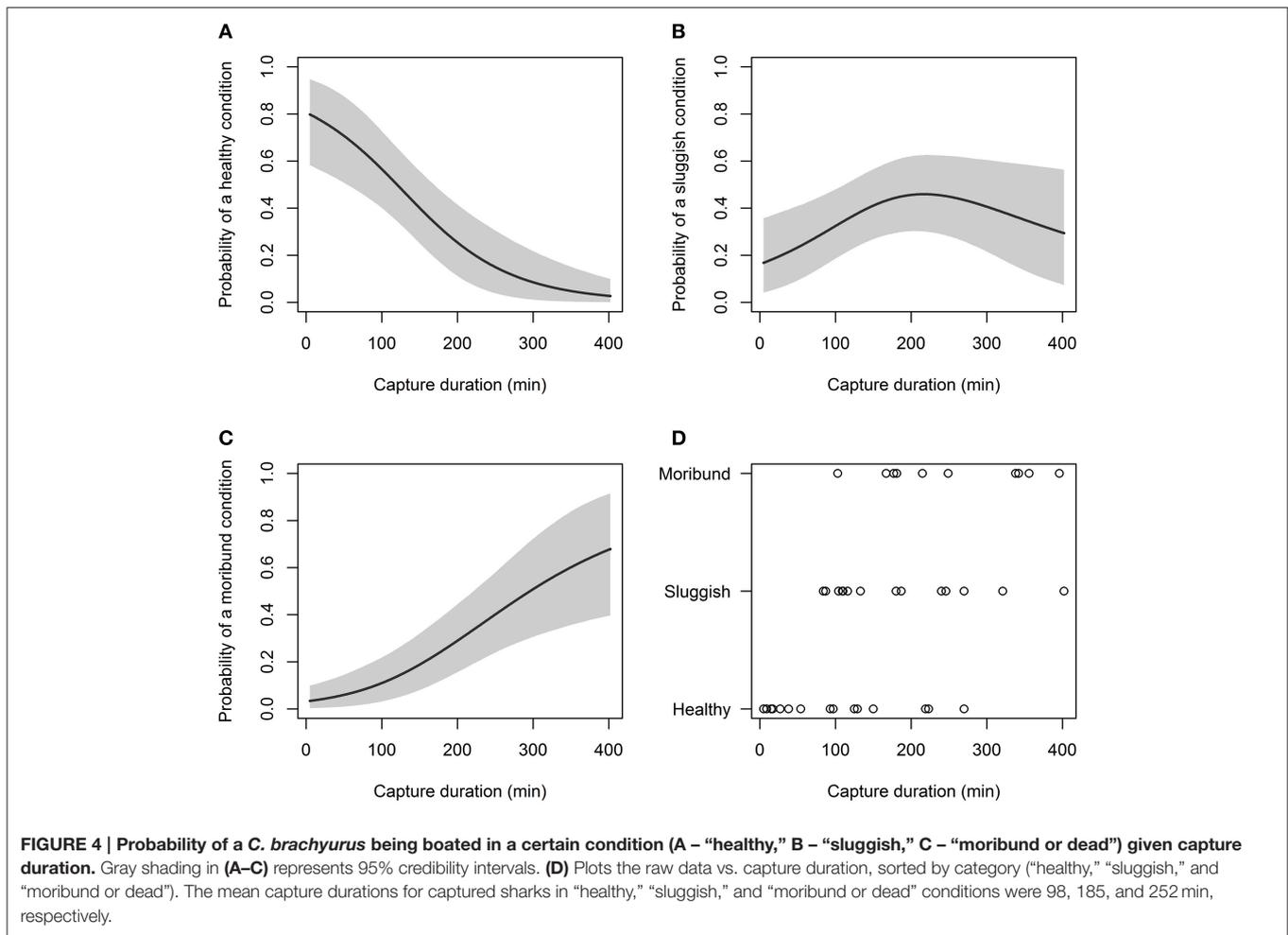
The likelihood of boating a shark in a “moribund or dead” condition increases gradually immediately after capture starts and, unlike physiological variables, there is no range of capture durations that result in a rapid increase in the likelihood this condition. Accordingly, we consider capture duration threshold values in *C. brachyurus* to be 94, 205, 293, and 402 min. At these capture durations 10, 30, 50, and 68% are predicted to be in a “moribund or dead” condition, respectively. It is also important to consider that by 293 min, few (9%) of sharks are captured in a “healthy” condition.

Ideally, maximum soak times of 94 min could be implemented to ensure that the majority of *C. brachyurus* caught are “healthy” and that few (predicted 10%) are caught “moribund or dead.” However, regulations reducing soak time to 94 min may be unrealistic because it can take up to 3 h to haul longline gear (although haul times can be reduced by reducing the number of hooks set) and may not be economically viable for fishers (Butcher et al., 2015). Butcher et al. (2015) recommended that a reduction in allowable longline soak time to 300 min would reduce the mortality of shark bycatch. Such regulations would

benefit *C. brachyurus*, because few sharks would be caught dead, if we assume an average capture duration of 150 min, resulting in 40% predicted “healthy,” 41% predicted “sluggish,” and 19% predicted “moribund or dead.”

Models used could not predict a capture duration that caused all sharks caught to be “moribund or dead” and the maximum capture duration (402 min) used in our study only predicted that 68.3% of *C. brachyurus* captured were in this condition. This result may be because capture durations used in our study were shorter than commercial fishing operations. Only two of the sharks we captured were on fishing gear for more than 6 h and 73% of the sharks had capture durations less than 4 h. Morgan and Carlson (2010) noted that peak increases in immediate mortality percentage occur at capture durations of 6 and 10 h in blacktip, *Carcharhinus limbatus*, and sandbar, *Carcharhinus plumbeus*, sharks respectively. Although, our models predicted that sharks may still be alive after 402 min, only 1% of sharks captured at this duration were in a “healthy” condition and most sharks caught live at this duration are predicted to be “sluggish.”

One caveat in our study is that, ideally, blood lactate concentrations should be measured immediately following capture using a portable analyzer (e.g., i-Stat – Frick et al.,



2012; Accutrend Plus – Butcher et al., 2015) or blood samples should be centrifuged immediately to separate plasma (Frick et al., 2010). Although, we had access to an i-Stat portable blood gas analyzer, it was consistently out of temperature range and, thus, unreliable for our purposes. Blood samples were not immediately centrifuged due to logistical difficulties onboard a rocking vessel. In humans, lactate concentrations in whole blood stored on ice increase minimally (approximately 0.1 mmol/L after 1 h; Toffaletti et al., 1992); unknown effect in shark blood) and the maximum time before centrifuging in our study was approximately 12 h. Although, several previous elasmobranch stress studies have measured and reported plasma lactate concentrations after whole blood was left on ice or frozen prior to centrifuging (Hoffmayer and Parsons, 2001; Manire et al., 2001; Brill et al., 2008; Hoffmayer et al., 2012), we recommend that this caveat should be taken into consideration when interpreting our lactate results.

To our knowledge, ours is the first study to predict capture condition under varying capture duration, sea surface temperature, shark total length, and physiological measurements. Previous studies have shown that assessing condition upon capture is valuable because it can be a reliable predictor of

post-release mortality (Hueter et al., 2006; Benoît et al., 2012). In the absence of tagging studies, capture condition indexing can be applied on a broad scale and to multiple species to estimate post-release mortality. The methods used in our study can be readily applied to future research examining operational, biological, physiological, and environmental variables such as ganglion length, capture depth, air temperature, wounding, and time spent struggling on fishing gear. Methods used are especially well-suited to observer program data sets because observers can be trained to correctly categorize capture condition and could collect data across a broad spatial and temporal range.

Future, physiological studies could include additional physiological measurements, such as intramuscular lactate and plasma catecholamine, to predict shark capture condition. Although, measuring these two variables was out of the scope of the present study, intramuscular lactate and plasma catecholamine may be good predictors of shark mortality following capture (Hight et al., 2007; Frick et al., 2012). Additional information on how these variables can influence capture condition can be used to determine the biological systems that are most detrimentally affected by fisheries capture and can help to design effective management strategies.

For example, if high intramuscular lactate concentrations (indicative of struggling and/or insufficient oxygenation) result in decreasing capture condition, then it can be determined that intense struggling behavior and/or insufficient oxygenation during capture likely have a detrimental effect on sharks. In this scenario, management measures that reduce movement restriction (e.g., longer gangions) may reduce struggling, enhance oxygenation ability, and reduce mortality percentages in sharks.

CONCLUSION

Plasma lactate and potassium concentrations had a positive linear relationship with capture duration in *C. brachyurus*, indicating that obligate ram-ventilating sharks may not have the same ability to recover during capture as stationary respiring species. The best physiological predictors of *C. brachyurus* capture condition we examined were plasma lactate and potassium. Previous studies have suggested that elevations in plasma lactate and potassium are indicative of metabolic and intracellular acidosis, and our results support the notion that these conditions are associated with deteriorating capture condition in sharks. It is possible that the extent of these conditions can be decreased during capture by reducing aerial exposure times (Cicia et al., 2012; Heard et al., 2014), avoiding fishing during hot seasons or sea surface temperatures (Hoffmayer et al., 2012), and/or reducing gear soak times (present study). We recommend that regulations reducing maximum allowable soak time to 300 min will decrease immediate and post-release mortality in longline-caught *C. brachyurus* bycatch.

Our study used capture condition indexing and stereotype logistic regression models to predict the capture condition of sharks. In the absence of logistically challenging and expensive tagging studies, we recommend that these methods could be applied to immediate mortality studies to predict causes of immediate mortality and to estimate post-release mortality.

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Although, we focused on *C. brachyurus*, these methods can be applied to any elasmobranch, bony teleost, turtle, or marine mammal during fisheries capture. Use of these methods will allow future studies to go beyond simply recording mortality and will allow fisheries scientists to predict the best management strategies to reduce the mortality of bycatch.

AUTHOR CONTRIBUTIONS

Experimental design: DD, RR, CH, and TW, Data collection: DD, CH, and MD, Data processing: DD, Data analysis: DD, Manuscript writing: DD, RR, TW, CH, and MD, Final draft approval: DD, CH, TW, MD, and RR.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmars.2015.00126>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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