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SEDAR77-DW09

Received: 11/30/2021

Please cite this document as:

Prohaska, Bianca K., Heather Marshall, R. Dean Grubbs, Bryan S. Frazier, John J. Morris, Alyssa Andres, Karissa Lear, Robert E Hueter, Bryan A Keller, Nicholas M Whitney. 2021. Stress physiology of scalloped and great hammerhead sharks from a bottom longline fishery. SEDAR77-DW09. SEDAR, North Charleston, SC. 34 pp.

Stress physiology of scalloped and great hammerhead sharks from a bottom longline fishery

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ABSTRACT

The scalloped hammerhead *Sphyrna lewini* and the great hammerhead *Sphyrna mokarran* are large, coastal to semi-oceanic shark species common to waters of the U.S. east coast where they are regularly taken in commercial and recreational fisheries, particularly the bottom longline fishery. High rates of hooking mortality and low rates of population growth are believed to have caused severe declines in the U.S. Atlantic populations of these species. The objective of this study was to determine the physiological stress induced by bottom longline capture in both *S. lewini* and *S. mokarran*. Physiological stress was quantified using the blood biochemical indicators glucose, lactate, pH, hematocrit, sodium, potassium, calcium, chloride, and magnesium, which have been demonstrated to indicate physiological stress in elasmobranchs. Each shark captured was assigned a condition factor, which was compared with the stress parameters and time on hook to quantify stress induced by different longline hook times. The physiological stress parameters lactate and pH were found to scale negatively with hook time and condition factor in both species. For both species, possible predictors of mortality include hook time, lactate, potassium, and pH. These data will be useful for estimating post-release mortality of *S. mokarran* from measurements taken at the time of capture and the physiological stress response to longline capture in both species to the Atlantic bottom longline fishery.

INTRODUCTION

The scalloped hammerhead *Sphyrna lewini* and great hammerhead *Sphyrna mokarran* are large, coastal to semi-oceanic species that are distributed throughout warm temperate and tropical oceans of the world including the nearshore and pelagic waters of the U.S. east coast (Compagno, 1984, Castro, 2011). Because of their broad range of habitats in U.S. Atlantic waters, these species are regularly caught in both inshore and offshore fisheries in this region, such as the Atlantic Directed Shark Bottom Longline Fishery (Morgan et al. 2009) and the U.S. Atlantic Pelagic Longline Fishery (Miller et al. 2013). Based on recent stock assessments, the effect of these fisheries is believed to have resulted in significant declines in the northwest Atlantic populations of both species (Hayes et al. 2009, Jiao et al. 2011). For example, the US Atlantic *S. lewini* population is believed to have been depleted by over 80% of their virgin stock biomass since the early 1980s (Hayes 2009). Comparable declines have also been reported for US Atlantic *S. mokarran* populations but are less certain because of species misidentification (Beerkircher et al. 2002, 2004) and naturally lower densities of this species resulting in low sample sizes. New federal stock assessments for both species in US Atlantic and Gulf of Mexico waters are currently underway (https://sedarweb.org/sedar-77).

Because of high fishery exposure, it is important to obtain information on capture induced stress and post-release mortality rates, particularly for *S. lewini* as they have been shown to experience high hooking mortality in commercial fisheries (Morgan & Burgess 2007, Morgan et al. 2009, Gulak et al. 2015). Morgan et al. (2009) reported >98% total mortality rate for both *S. lewini* and *S. mokarran* based on fishery observer data collected aboard commercial longline vessels targeting sharks. This rate ranged from an at-vessel mortality of 60% to 100% and 91 to 100% in *S. lewini* and *S. mokarran*, respectively, depending on soak time. This has been used to

suggest that limiting soak time may be an important mechanism for decreasing hooking mortality in numerous species, including *S. lewini* (Morgan et al. 2009). More recently, Gulak et al. (2015) specifically examined time-on-hook (not only overall gear soak time) and reported at vessel mortality of 62.9% for *S. lewini*, with 50% mortality predicted at 3.5 hrs. About 50% of the *S. lewini* were captured in the first two hours of a soak and most of these were alive upon capture. However, the probability of their survival if released is unknown, and the effectiveness of applying shorter soak times in lowering fishing mortality on *S. lewini* depends entirely on the post-release survival.

The magnitude of physiological stress from capture experienced in elasmobranchs (sharks, skates, and rays) is thought to be most influenced by the capture method, hook time, and the metabolic scope of the species (i.e. low metabolic scope-benthic/sluggish versus high metabolic scope-pelagic/continuously swimming) (Skomal 2006, 2007; Mandelman & Skomal 2009; Skomal & Mandelman 2012). Differences in stress induced by capture method can result from varying degrees of physical trauma and respiratory inhibition (Skomal & Mandelman 2012). Longer hook times are generally related to increased stress and mortality (Morgan et al. 2009, Morgan & Carlson 2010), although some species have been found to recover after release even with long hook times (Brooks et al. 2012, Marshall et al. 2012). Stress is examined in most wild animals by quantifying stress hormones; however, at this time there is not a validated assay to quantify the primary stress hormone in elasmobranchs (Anderson 2012). An alternative way to examine stress in these organisms is to quantify the secondary stress response, which can include investigating blood glucose, pCO_2, lactate, bicarbonate, pH, sodium, potassium, calcium, chloride, magnesium and hematocrit. Skomal and Mandelman (2012) provide a detailed review of the secondary stress response in marine elasmobranchs.

With these points in mind, our aims were to determine longline capture induced physiological stress in both *S. lewini* and *S. mokarran*. Our specific objectives were to: 1) quantify how secondary stress parameters fluctuate in relation to time on the hook, the shark's length, and the water temperature at capture, 2) identify characteristic secondary stress parameters in relation to release condition, 3) determine if any blood parameters could potentially be used as predictors of mortality.

MATERIALS AND METHODS

Survey

Sphyrna lewini and *S. mokarran* were captured and sampled during an ongoing Florida State University (FSU) fishery-independent longline survey which targets elasmobranchs, as well as separately and concurrently through contracted bottom longline fishing efforts with commercial fishermen. The FSU longline consists of a 4.0 mm monofilament mainline that was anchored on each end and marked with a surface buoy bearing the permit numbers. Each mainline set was approximately 750 m long. A standard set included 50 gangions consisting of a stainless-steel tuna clip with an 8/0 stainless steel swivel attached to 2.5 m of 360 kg monofilament that was doubled in the terminal 25 cm and attached to 16/0 circle hook. Hooks were baited with ladyfish *Elops saurus* or Spanish mackerel *Scomberomorus maculatus*. Since January 2017, each gangion included an in-line HT-600 hook timer (Lindgren-Pitman, Pompano Beach, FL). Depth (m), turbidity (cm), water temperature (°C), salinity and dissolved oxygen (mg/l) were recorded from the surface to the bottom for all sets made in depths less than 10 m, and bottom water temperature (°C) was recorded for those deeper than 10 m. Two sets were typically soaked concurrently. Soak times for the first set were 1 h to minimize mortality, and all

lines were set during daylight hours. Soak times for the second set varied depending on the haul duration of the first set, but were typically less than three hours.

The concurrent contracted fishing effort was done in collaboration with commercial bottom longline fishermen aboard their vessels. Gear specifications were similar to the FSU longline, with the following differences: up to 260 gangions were suspended on 3-6 NM of 4.0 mm monofilament mainline. Each gangion was approximately 3 m long of 3.5 mm monofilament attached to an 18/0 circle hook, and also integrated with HT-600 hook timers, and soak times ranged 2-18 hours. Oceanographic conditions were measured at each fishing location using a hand-held meter (YSI model Pro Plus, Yellow Springs, OH, USA). For both fishing efforts, the line was hauled in the order and direction it was set and hammerheads were sampled as they were caught during retrieval. Areas sampled included the Atlantic side of the Florida Keys from Key West to Islamorada and inside Everglades National Park in Florida Bay, as well as state- and federal-waters of the eastern Gulf of Mexico (specifically near Madeira Beach and Key West, FL, USA).

Sampling

Hooked sharks were brought alongside the vessel and, in most cases, were brought onboard the deck of the boat or a swim step where they were restrained by hand. As soon as a shark was restrained, a 1–5 ml blood sample was collected, in ~30-120 sec or less, using a 16-18-gauge needle attached to a heparinized syringe (Lithium heparin #374858, Sigma-Aldrich, St. Louis, MO, USA). Blood samples were obtained via caudal venipuncture either from the ventral or lateral surface of the caudal region. The lateral caudal puncture was employed more throughout the study as a means to reduce on-deck time, because the blood sample and tagging could happen concurrently (with shark sitting in ventral side). The lateral puncture technique

proved to be quick, and involved inserting the needle from the side of the individual's caudal region, targeting the hemal arch. Given that all blood samples were from the hemal arch, it is not expected this sampling approach would impact blood values.

After blood sampling, sharks were measured (precaudal length (PCL), fork length (FL), stretch total length (STL)), sex was determined, and sharks were externally tagged with an identification tag or one of various electronic tags for a separate study. Upon release, each shark was assigned a condition score using a 5-point scale: 1 = vigorous, excellent condition, 2 = normal swimming, good condition, 3 = labored or disoriented swimming, fair condition, 4 = nictitating membrane response, slow movement, poor condition, and 5 = at-vessel mortality (AVM) or moribund.

Blood analyses

To assess pH and lactate, a small aliquot of blood was immediately (in some cases, within an acceptable 15 minute window) loaded into a CG4+ cartridge and then inserted into a VetScan i-STAT 1 point of care device (Abaxis Inc., Union City, CA), which has been validated for use in elasmobranchs (Mandelman & Farrington 2007, Mandelman & Skomal 2009, Gallagher et al. 2010). Because of variability in additional blood gas data, pCO_2 and bicarbonate data are not being reported here (Harter et al., 2015). A subset of the blood samples were centrifuged entirely onboard the fishing vessels. These samples were first spun (n=4 microcapillary tubes per individual) in a portable hematocrit centrifuge (Zipocrit, LW Scientific, Lawrenceville, GA, USA) for 5 minutes. The remaining blood for these samples was then centrifuged so that plasma could be separated from the red blood cell pellet, and both were immediately frozen using a liquid nitrogen dry shipper. A separate subset of samples had glucose measured on board using an Accu-Chek glucose meter (Roche Diagnostics, Basel, Switzerland),

157 which has been validated for use on fishes (Cooke et al. 2008). These blood samples were not
158 centrifuged in the field and were placed on ice in a cooler (4°C) for up to 12 h and processed
159 later.

160 Upon returning to land, blood samples that were not centrifuged on the boat were
161 processed in the laboratory. Hematocrit was measured in in a hematocrit centrifuge at 15,000 g
162 for 5 min. Hematocrit levels were determined by calculating the red blood cell percentage of the
163 whole blood volume. The remaining whole blood was then centrifuged at 1,800 g for 5 min
164 (Unico, Dayton, NJ). The separated plasma was stored at –20°C. The plasma layer from blood
165 samples that were centrifuged and frozen at sea was stored at -80°C until thawed for analysis of
166 lactate, glucose, and ions (i.e., potassium, sodium, chloride, magnesium, and calcium) using
167 benchtop Critical Care Xpress and pHOx blood analyzers (Nova Biomedical, Waltham, MA,
168 USA).

169 *Statistical analyses*

170 Stress physiology data for pH were temperature corrected to water temperature
171 measurements at the time of capture (Mandelman & Skomal 2009, Gallagher et al. 2010), and
172 are indicated with the subscript "TC" from this point forward. Because hematocrit is represented
173 as a percentage, these data were arcsine transformed prior to analyses.

174 To investigate if there were any patterns between the blood stress parameters and hook
175 time, water temperature or FL, an interactive model was fit for each stress parameter in each
176 species. No fully interactive models, or additive models were a better fit over single variable
177 models, so we further investigated the fluctuations in stress parameters with hook time, water
178 temperature and FL in each species by stress parameter using linear and polynomial regressions.
179 If both linear and polynomial regressions were significant the models were statistically

compared. If the higher order model was not a statistically better fit, the reduced model was kept. To investigate direct differences in the stress parameters between the two species, *S. lewini* and *S. mokarran*, a T-test, a Welch's T-test or a Wilcoxon sum rank test were conducted depending if the data were normally distributed and homoscedastic.

To identify blood parameters that not only define the stress response, but also may be used as predictors of mortality, binomial logistic regressions with a logit link function were performed (R version 3.3.3, with package car) with the response variable set as live (=0) versus dead (=1). Model fit was verified by visual inspection of null and residual deviance values. For those parameters that tested significant, the logistic regression function equation was used to generate parameter values at a 50%-mortality threshold. To satisfy the assumption of homogeneity of variances between live and dead groups, Levene's Tests were performed prior to the logistic regressions.

Stress parameters were statistically compared between release condition groups in each species using a one-way analysis of variance (ANOVA), a Welch's ANOVA or a Kruskal-Wallis test, depending if data were normally distributed and homoscedastic. When ANOVAs or their corresponding test were significant, a Tukey HSD or Wilcoxon rank sum test was conducted to identify significant pairwise differences. Because each shark was captured at a unique hook time a one-way analysis of co-variance (ANCOVA) was conducted prior to the aforementioned analyses to investigate differences in the stress parameters between release condition groups, while controlling for the covariate hook time; however, in all cases but one hook time was not significant in the model, and in the one case that it was there were non-overlapping covariate ranges for the stress parameter and hook time, making an ANCOVA an inappropriate analysis

for these data. Unless otherwise indicated, all statistical analyses were conducted using R version 3.0.3 (R-Core Development, 2014). All tests were considered significant at $\alpha = 0.05$.

RESULTS

Blood samples were collected from 86 *S. lewini* and 85 *S. mokarran* with mean (± S.E.) FLs of 156 ± 4 cm and 180 ± 4 cm respectively. Known hook times for *S. lewini* ranged from 6 to 382 minutes, and 5 to 538 minutes for *S. mokarran* (Figure 1).

No significant change over time on the hook was observed in *S. lewini* for the stress parameters glucose, hematocrit, sodium, chloride or magnesium, or in *S. mokarran* for the stress parameters glucose, calcium, chloride or magnesium (Figure 2 & 3; Table 1). In both *S. lewini*, and *S. mokarran* a significant increase in lactate and potassium as well as a significant decrease in pH were observed with time on the hook (Figure 2 & 3; Table 1). Additionally, in *S. lewini* calcium showed a positive relationship with hook time (Figure 3C; Table 1), and in *S. mokarran* sodium showed a positive relationship with hook time (Figure 3A; Table 1) while hematocrit showed a negative relationship (Figure 2D; Table 1).

The only stress parameter which showed a significant difference as a function of water temperature in either species was magnesium in *S. mokarran* (Table 2). A significant increase in lactate and decrease in pH were found as a function of FL in *S. lewini* (Table 3).

In *S. lewini*, binomial logistic regressions revealed that mortality could be predicted by hook time ($p = 0.003$, Standard Error = 0.004, 50% mortality probability = 306 min), lactate ($p = 0.001$, SE = 0.14, 50% = 17 mmol l^{-1}), calcium ($p = 0.023$, SE = 2.9, 50% = 3.3), and pH_{TC} ($p = 0.006$, SE = 5.4, 50% = 6.8). Whether potassium was a significant predictor of mortality in *S. lewini* could not be calculated as this model did not converge because the values for potassium

were quite different between live (i.e., mean ± standard deviation: 4.6±0.7 mmol l^{-1}, range 3.3-5.9 mmol l^{-1}) and dead (8.6±1.2 mmol l^{-1}, range 7.1-9.8 mmol l^{-1}) individuals with no overlap of ranges. *S. mokarran* mortality was predicted by hook time (p = 0.021, SE = 0.008, 50% = 230 mins), potassium (p = 0.009, SE = 0.37, 50% = 9.3 mmol l^{-1}), lactate (p = 0.0007, SE = 0.04, 50% = 28.7 mmol l^{-1}), and pH_{TC} (p = 0.003, SE = 1.9, 50% = 6.7).

The stress parameters glucose, sodium, chloride and magnesium did not vary significantly between release conditions in either species (Figures 4 & 5; Tables 4 & 5). However, in both *S. lewini* and *S. mokarran* lactate and potassium significantly increased, while pH significantly decreased with decreasing release condition (Figures 4 & 5; Tables 4 & 5). Additionally, in *S. lewini* calcium significantly increased with decreasing release condition (Figures 4 & 5; Tables 4 & 5), while in *S. mokarran* hematocrit significantly decreased with decreasing release condition (Figure 4D; Tables 4 & 5).

Direct comparisons of the stress parameters between the two species revealed significant differences in lactate, pH, hematocrit, and potassium. Lactate (t-test: t = -4.03, df = 160, p < 0.001) and potassium (t-test: t = -3.08, df = 51, p = 0.003) were significantly higher in *S. mokarran* relative to *S. lewini*, while pH (t-test: t = 2.98, df = 160, p = 0.003) and hematocrit (Welch's t-test: t = 6.56, df = 149, p = < 0.001) were significantly lower. No significant differences in glucose, sodium, calcium, chloride or magnesium were found between the two species.

DISCUSSION

Both *S. lewini* and *S. mokarran* are known to be sensitive to fishing capture based on previously observed high secondary stress responses and hooking mortality (e.g Morgan et al.

2009, Gulak et al. 2015; Jerome et al., 2017). In this study we examined the secondary stress response of both species in relation to their time on the hook, body length, the water temperature where they were captured, and their release condition to identify factors affecting the stress response.

Both hammerhead species clearly showed release condition deteriorating as hook time increased (Figure 1). Mortality thresholds determined from predictive logistic regression in this study indicated 50% at-vessel mortality thresholds at ~306 and ~260 minutes of longline capture in *S. lewini* and *S. mokarran*, respectively. These results signal the relative increased sensitivity of *S. mokarran* compared to *S. lewini*. Several previous studies have also highlighted the high stress disruption characteristic of *S. mokarran* compared to several sympatric carcharhinid sharks, where *S. mokarran* tends to show the highest levels of mortality or disruption of commonly caught species (Gallagher et al. 2014, Jerome et al. 2017). For example, a previous capture stress study on drumline captured sharks observed some moribund *S. mokarran* after only 24-minute hook time (Gallagher et al. 2014). Conversely, in a study by Gulak et al. (2015) most longline captured *S. lewini* were alive at 2 hours, and they observed about 50% at-vessel mortality at 3.5 hour hook time.

Increases in metabolic stress, as well as overall acidosis, were observed in both *S. lewini*, and *S. mokarran* with increasing hook time. These results indicate that upon capture these species are likely using burst swimming behavior to escape, which triggers anaerobic respiration in the muscle, and a resulting buildup of acidic metabolic byproducts like lactic acid, as indicated by increasing lactate (e.g. Black 1958; Cliff & Thurman 1984; Wood 1991). Shark baseline lactate levels have been found to be ~1.3 mmol l^{-1} (Spargo, 2001), and baseline pH values ~7.4-8.0 (Spargo, 2001; Mandelman and Skomal, 2009). With prolonged capture stress, we see

several of these metabolic blood parameters becoming indicative of mortality, with 50% mortality predicted for *S. lewini* at ~17 mmol l^{-1} lactate and ~6.8 pH, and ~28.7 mmol l^{-1} lactate and ~6.7 pH for *S. mokarran*. Similar increases in lactate with hook time have been observed in longline-caught Caribbean reef sharks *Carcharhinus perezi* (Brooks et al. 2012), bronze whalers *Carcharhinus brachyurus* (Dapp et al. 2016), and several other species (e.g., Whitney et al., 2021). This potential burst escape behavior, which is likely contributing to metabolic stress, has been previously documented in *S. mokarran* by analyzing data collected from accelerometers attached to fishing gear (Gallagher et al. 2017). Contrasting behavior has been documented in the gummy shark *Mustelus antarcticus*, and nurse shark *Ginglymostoma cirratum*, suggesting these sharks rest on the bottom after capture, and thus no significant increases in lactate (*M. antarcticus*), or increasing pH (*G. cirratum*) were noted with increasing hook time (Guida et al. 2016; Bouyoucos et al., 2018). Disparate results have also been observed in smalltooth sawfish *Pristis pectinata*, which were captured using identical methods to the sharks in this study. Sawfish exhibited a lower stress response for all of the parameters investigated, likely as a result of their fairly calm behavior post hooking and their low metabolic scope (Prohaska et al. 2018). Although pCO_2 could not reliably be measured and reported in this study, the overall declines in blood pH in both species could be affected not only by metabolic acidosis (as indicated with rising lactate levels), but respiratory acidosis as well. The longline capture method likely contributes to reduced respiration, as commercially-used gangion lengths in bottom longline fisheries (~2.5m) can limit movement and potentially compromise respiration in ram-ventilating species (Morgan & Burgess 2007). Given that incidental commercial capture of sharks occurs frequently by bottom longline (2.5-3.0 m gangion lengths) and pelagic longline gear (10-20 m

293 gangion lengths) (Beerkircher et al. 2002, Lewison et al. 2004, Gilman et al. 2008), it is
294 important to consider the respiratory limitations that gangion length can have on these species.
295 Ionic disruptions were apparent in both species, with *S. lewini* experiencing increased
296 calcium and potassium values with longer hook times, and *S. mokarran* experiencing increases in
297 potassium and sodium. *Sphyrna lewini* were predicted to have 50% mortality with calcium
298 values ~3.3 mmol l^{-1}, but the model for potassium could not converge despite clear separation
299 between live (K < 6 mmol l^{-1}) and dead (K > 7 mmol l^{-1}) individuals. *S. mokarran* were predicted
300 at 50% mortality with blood potassium values ~9 mmol l^{-1}. Taken together, these blood
301 indicators all represent fluctuations in ions that are important in key homeostatic and metabolic
302 pathways. Previous work has also highlighted stress-induced increase in potassium and
303 associations with mortality events (e.g., Moyes et al., 2006; Mandelman and Skomal, 2009; Frick
304 et al., 2010; Marshall et al., 2012; Dapp et al., 2016; Whitney et al., 2021), which may be due to
305 disruptions that can affect cardiac functioning and potentially muscle contraction physiology as
306 well (Skomal and Mandelman, 2012).
307 Differences in stress-induced hematocrit changes were also observed, with *S. mokarran*
308 exhibiting declines in hematocrit related to hook time and release-condition (Figures 2D & 4D).
309 Conversely, *S. lewini* did not show any stress-induced hematocrit changes and overall had
310 higher hematocrit values than *S. mokarran*. Hematocrit is an important indicator of packed red
311 blood cell volume, which can be used as a proxy for oxygen carrying capacity, but does not
312 always appear to change in response to capture stress. Some of the previous stress work in sharks
313 has shown evidence of capture-related hematocrit changes (e.g., *Squalus acanthias*, Mandelman
314 and Farrington, 2007; *C. limbatus*, Whitney et al., 2021), while others have not (e.g., *C.*
315 *brachyurus*, Dapp et al., 2016). Even though stress-induced increases in hematocrit values have

been observed in some cases, there are not enough data to suggest this is a typical response, and in cases where hematocrit does change, this may be due to cell swelling as a function of ionic disruption (resulting in hematocrit increases), or cell lysis due to such swelling (resulting in hematocrit declines) (Wells and Davie, 1985; reviewed in Brill and Lai, 2016). Here, we actually observed significant declines in *S. mokarran* hematocrit, which may be indicative of red blood cell swelling due to ionic changes, to the point of cell lysis. In this case, *S. mokarran* may experience unique limitations in oxygen carrying capacity, which could affect survival and recovery within this species.

Seasonal water temperature has been found to affect the physiological stress response to longline capture in *M. antarcticus* (Guida et al. 2016); however, similar to results observed in *C. brachyurus* (Dapp et al. 2016), the present study found no significant changes in any stress parameters with water temperature at the time of capture in *S. lewini*. In *S. mokkaran* magnesium was found to vary significantly with water temperature with magnesium decreasing with increasing water temperature. Lactate and pH, both indicators of metabolic stress, were found to vary significantly with FL in *S. lewini*. Lactate increased, while pH decreased with increasing FL, potentially indicating greater metabolic stress with increasing body length. No significant changes in any stress parameters with FL were found for *S. mokkaran*.

Despite small sample sizes for some release condition categories, both species exhibited greater metabolic stress and pH declines with worsening release condition. These results corroborate that our assessment of the overall condition of both *S. lewini* and *S. mokarran* at release is a good indicator of physiological status. No significant changes over release conditions were found for the fight or flight indicator glucose in either species.

S. lewini and *S. mokarran* showed similar at-vessel mortality rates of 15% and 12% respectively. While one stock assessment identified significant declines of over 80% of the *S. lewini* virgin biomass in the Atlantic population (Hayes 2009), an updated assessment is needed and no assessment has been published for Atlantic *S. mokarran* (Beerkircher et al. 2002, 2004). However, stock assessments in US Atlantic and Gulf waters for both species are currently underway (https://sedarweb.org/sedar-77) and our results suggest that concern should be given to post-release mortality estimates in both species.

While the results of this study will be useful in informing management on the physiological response of these species to longline capture, future work on this topic should focus on longer gear soak times to identify the stress response to greater time on the hook. However, given the high at-vessel mortality rates of these species at longer soak times, such studies would likely require a multi-year sampling effort. Furthermore, deployment of electronic tags should be used to monitor post-release survivorship in these species over a broad range of hook times. This would allow for the formation of a predictive post-release mortality model as a function of hook time and the secondary stress response. Forming better estimates of post-release mortality will be useful for estimating overall fishing mortality and for setting guidelines for the commercial longline industry. From our condition assessments at release, 92% of *S. mokarran* survived initial capture with maximum hook time of approximately 3.5 hours, and 86% of *S. lewini* survived initial capture with maximum hook time of approximately 5.5 hour. This suggests that limiting gear soak times could increase survival, given that trends in post-release survival are similar to those seen at-vessel. However, median soak times in the commercial bottom longline fishery are often greater than 12 hours (e.g., Morgan et al., 2009; Morgan and Carlson 2010) and so limiting soak times would require significant changes in the fishery.

Hammerhead shark specific time area closures may be a more direct route for decreasing mortality on these stocks. Currently, hammerhead mortality is limited by setting a small hammerhead-specific quota that acts as a control rule where, once that quota is reached, the entire bottom longline fishery is closed, thus acting as an incentive to fishers to avoid areas where high catches of hammerheads may occur. No such control rules exist for the pelagic longline fisheries. If stock assessments indicate that reduced fishery mortality is necessary for recovery of hammerhead species, resource managers will have to assess the costs and benefits of multiple approaches to achieving this goal.

Acknowledgments

We thank the collaborating commercial captains (Dave Campo, Jim Bonnell, Randy Lauser, Luke Hill) and their first mates. We thank the many graduate students and volunteers that assisted in the FSU surveys and many interns and staff at Mote Marine Laboratory. For analysis of ions and metabolites, we thank Diego Bernal (University of Massachusetts Dartmouth) for the use of the CCX, and Charles Innis, Deana Edmunds and Kerry McNally (New England Aquarium) for use of their pHOx benchtop analyzer. This work was conducted under Florida State University Animal Care and Use Committee protocol 1718 and Mote Marine Laboratory Institutional Animal Care and Use Committee protocol #13-11-NW2. Fishing activities were permitted by Special Activity License 1345 and 12-0041-SRP from the Florida Fish and Wildlife Conservation Commission, and permit #SHK-EFP-1310 issued by the National Marine Fisheries Service Highly Migratory Species Management Division.

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Table 1. Statistical results of linear regressions comparing the stress physiology parameters glucose (mmol l^{-1}), lactate (mmol l^{-1}), pH, hematocrit (%), sodium (mmol l^{-1}), potassium, (mmol l^{-1}), calcium (mmol l^{-1}), chloride (mmol l^{-1}), and magnesium (mmol l^{-1}) of *Spyrna lewini* and *Sphyrna mokarran* with hook time

		F	DF	R^2	p-value
Sphyrna lewini					
	Glucose	0.09	1, 57	0.01	0.77
	Lactate	40.4	1, 60	0.40	3.1 e -8
	pH	17.9	1, 61	0.23	8.0 e -5
	Hematocrit	0.002	1, 59	3.1 e -5	0.97
	Sodium	0.50	1, 22	0.02	0.49
	Potassium	23.56	1, 22	0.52	7.5 e -5
	Calcium	8.53	1, 22	0.28	0.008
	Chloride	0.94	1, 22	0.04	0.34
	Magnesium	1.83	1, 22	0.08	0.19
Sphyrna mokarran					
	Glucose	0.46	1, 45	0.01	0.46
	Lactate	28.2	1, 47	0.38	6.5 e -9
	pH	9.00	1, 47	0.16	0.004
	Hematocrit	4.93	1, 41	0.11	0.03
	Sodium	5.02	1, 22	0.19	0.04
	Potassium	6.51	1, 22	0.23	0.02
	Calcium	0.003	1, 22	1.2 e -4	0.96
	Chloride	0.45	1, 22	0.02	0.51
	Magnesium	0.10	1, 22	0.004	0.76

Table 2. Statistical results of linear regressions comparing the stress physiology parameters glucose (mmol l^{-1}), lactate (mmol l^{-1}), pH, hematocrit (%), sodium (mmol l^{-1}), potassium, (mmol l^{-1}), calcium (mmol l^{-1}), chloride (mmol l^{-1}), and magnesium (mmol l^{-1}) of *Sphyrna lewini* and *Sphyrna mokarran* with water temperature

		F	DF	R^2	p-value
Sphyrna lewini					
	Glucose	3.74	1, 70	0.05	0.06
	Lactate	0.03	1, 79	3.2 e -4	0.87
	pH	1.5 e -4	1, 80	1.8 e -6	0.99
	Hematocrit	0.32	1, 79	0.004	0.57
	Sodium	0.68	1, 24	0.03	0.42
	Potassium	0.01	1, 24	3.6 e -4	0.93
	Calcium	0.80	1, 24	0.03	0.38
	Chloride	0.94	1, 24	0.04	0.34
	Magnesium	0.37	1, 23	0.02	0.55
Sphyrna mokarran					
	Glucose	1.58	1, 74	0.02	0.21
	Lactate	3.28	1, 78	0.04	0.07
	pH	0.81	1, 77	0.01	0.37
	Hematocrit	0.53	1, 70	0.01	0.47
	Sodium	2.6 e -4	1, 24	1.1 e -5	0.99
	Potassium	1.91	1, 24	0.07	0.18
	Calcium	0.98	1, 24	0.04	0.33
	Chloride	0.24	1, 24	0.01	0.63
	Magnesium	6.14	1, 24	0.20	0.02

Table 3. Statistical results of linear regressions comparing the stress physiology parameters glucose (mmol l^{-1}), lactate (mmol l^{-1}), pH, hematocrit (%), sodium (mmol l^{-1}), potassium, (mmol l^{-1}), calcium (mmol l^{-1}), chloride (mmol l^{-1}), and magnesium (mmol l^{-1}) of *Sphyrna lewini* and *Sphyrna mokarran* with fork length (cm)

		F	DF	R^2	p-value
Sphyrna lewini					
	Glucose	2.04	1, 70	0.03	0.16
	Lactate	9.61	1, 79	0.11	0.003
	pH	11.4	1, 80	0.13	0.001
	Hematocrit	0.90	1, 79	0.01	0.35
	Sodium	2.66	1, 24	0.10	0.12
	Potassium	0.26	1, 24	0.01	0.61
	Calcium	0.72	1, 24	0.03	0.40
	Chloride	1.37	1, 24	0.05	0.25
	Magnesium	3.19	1, 23	0.12	0.09
Sphyrna mokarran					
	Glucose	0.13	1, 71	0.002	0.72
	Lactate	0.80	1, 76	0.01	0.37
	pH	0.42	1, 75	0.01	0.52
	Hematocrit	1.02	1, 67	0.02	0.32
	Sodium	3.18	1, 24	0.12	0.09
	Potassium	0.50	1, 24	0.02	0.49
	Calcium	0.68	1, 24	0.03	0.42
	Chloride	0.46	1, 24	0.02	0.50
	Magnesium	0.01	1, 24	4.8 e -4	0.92

582 **Table 4.** Mean (± SE) concentrations of stress physiology parameters glucose (mmol l^{-1}), lactate
583 (mmol l^{-1}), pH, hematocrit (%), sodium (mmol l^{-1}), potassium, (mmol l^{-1}), calcium (mmol l^{-1}),
584 chloride (mmol l^{-1}), and magnesium (mmol l^{-1}) in *Sphyrna lewini* and *Sphyrna mokarran* by
585 release condition category. Sample sizes in parentheses
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	Glucose (mmol[0][-1])		Lactate (mmol[0][-1])		pH		Hematocrit (%)		Sodium (mmol[0][-1])		Potassium (mmol[0][-1])		Chloride (mmol[0][-1])		Calcium (mmol[0][-1])		Magnesium (mmol[0][-1])	
Sphyrna lewini																		
Excellent	7.55 ± 0.72	(5)	2.51 ± 0.77	(5)	7.46 ± 0.04	(5)	34.97 ± 1.86	(5)	277.6 ± 8.35	(3)	5.09 ± 0.32	(3)	280.0 ± 7.22	(3)	2.68 ± 0.15	(3)	1.04 ± 0.10	(3)
Good	6.98 ± 0.69	(34)	4.81 ± 0.54	(34)	7.41 ± 0.02	(35)	34.99 ± 1.13	(36)	279.4 ± 2.75	(7)	4.59 ± 0.15	(7)	283.2 ± 2.04	(7)	2.90 ± 0.05	(7)	1.38 ± 0.07	(7)
Fair	7.66 ± 0.44	(16)	6.33 ± 1.12	(18)	7.34 ± 0.04	(18)	35.23 ± 1.67	(15)	286.4 ± 3.21	(9)	4.25 ± 0.28	(9)	287.6 ± 2.51	(9)	3.01 ± 0.07	(9)	1.61 ± 0.25	(9)
Poor	6.52 ± 0.83	(8)	12.1 ± 1.20	(11)	7.18 ± 0.09	(12)	32.69 ± 2.91	(10)	297.6 ± 3.00	(2)	5.69 ± 0.14	(2)	291.0 ± 2.40	(2)	2.82 ± 0.15	(2)	1.64 ± 0.47	(2)
AVM	7.61 ± 0.64	(9)	20.4 ± 2.42	(10)	6.70 ± 0.03	(9)	32.79 ± 2.22	(11)	276.6 ± 13.5	(5)	8.59 ± 0.53	(5)	274.6 ± 13.0	(5)	3.25 ± 0.14	(5)	3.02 ± 1.12	(5)
Spyrna mokarran																		
Excellent	5.30 ± 0.13	(6)	3.17 ± 0.48	(6)	7.38 ± 0.04	(6)	30.92 ± 4.63	(6)										
Good	7.47 ± 0.43	(22)	8.84 ± 1.17	(26)	7.24 ± 0.05	(26)	29.11 ± 1.24	(23)	281.3 ± 10.4	(5)	5.75 ± 0.35	(5)	280.8 ± 6.50	(5)	3.04 ± 0.17	(5)	1.37 ± 0.18	(5)
Fair	7.67 ± 0.35	(25)	11.6 ± 1.19	(25)	7.22 ± 0.05	(25)	28.07 ± 0.98	(23)	284.3 ± 1.87	(9)	6.08 ± 0.30	(9)	283.5 ± 1.77	(9)	3.08 ± 0.05	(9)	1.43 ± 0.07	(9)
Poor	7.68 ± 0.84	(11)	18.7 ± 2.20	(12)	7.10 ± 0.07	(12)	22.44 ± 2.02	(9)	285.8 ± 2.26	(8)	7.03 ± 0.55	(8)	283.1 ± 2.60	(8)	2.92 ± 0.10	(8)	1.30 ± 0.08	(8)
AVM	6.41 ± 0.39	(13)	22.2 ± 2.85	(11)	6.77 ± 0.07	(10)	27.93 ± 1.59	(12)	290.8 ± 1.85	(5)	9.59 ± 0.97	(5)	281.8 ± 2.40	(5)	3.16 ± 0.11	(5)	1.49 ± 0.09	(5)

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Table 5. Statistical results of one-way analysis of variance tests comparing stress physiology parameters glucose (mmol l^{-1}), lactate (mmol l^{-1}), pH, hematocrit (%), sodium (mmol l^{-1}), potassium, (mmol l^{-1}), calcium (mmol l^{-1}), chloride (mmol l^{-1}), and magnesium (mmol l^{-1}) in *Sphyrna lewini* and *Sphyrna mokarran* by release condition

				Sample Sizes				
	p	**F**	**df**	**E**	**G**	**F**	**P**	**AVM**
Sphyrna lewini								
Glucose	0.89	0.28	4, 67	5	34	16	8	9
Lactate	1.4 e-14	29.4	4, 73	5	34	18	11	10
pH	1.6 e-14	31.6	4, 64	5	35	18	12	9
Hematocrit	0.46	0.91	4, 65	5	36	15	10	11
Sodium	0.47	0.92	4, 21	3	7	9	2	5
Potassium	3.7 e-8	28.1	4, 21	3	7	9	2	5
Chloride	0.51	0.85	4, 21	3	7	9	2	5
Calcium	0.02	3.89	4, 21	3	7	9	2	5
Magnesium	0.16	1.84	4, 21	3	7	9	2	5
Sphyrna mokarran								
Glucose	0.04	2.69	4, 72	6	22	25	11	13
Lactate	2.1 e-6	13.7	4, 75	6	26	25	12	11
pH	1.1 e-7	10.3	4, 74	6	26	25	12	10
Hematocrit	0.02	3.16	4, 67	6	23	23	9	12
Sodium	0.58	0.66	3, 23		5	9	8	5
Potassium	6.2 e-4	8.35	3, 23		5	9	8	5
Chloride	0.93	0.14	3, 23		5	9	8	5
Calcium	0.41	1.00	3, 23		5	9	8	5
Magnesium	0.58	0.66	3, 23		5	9	8	5

Figure Legends

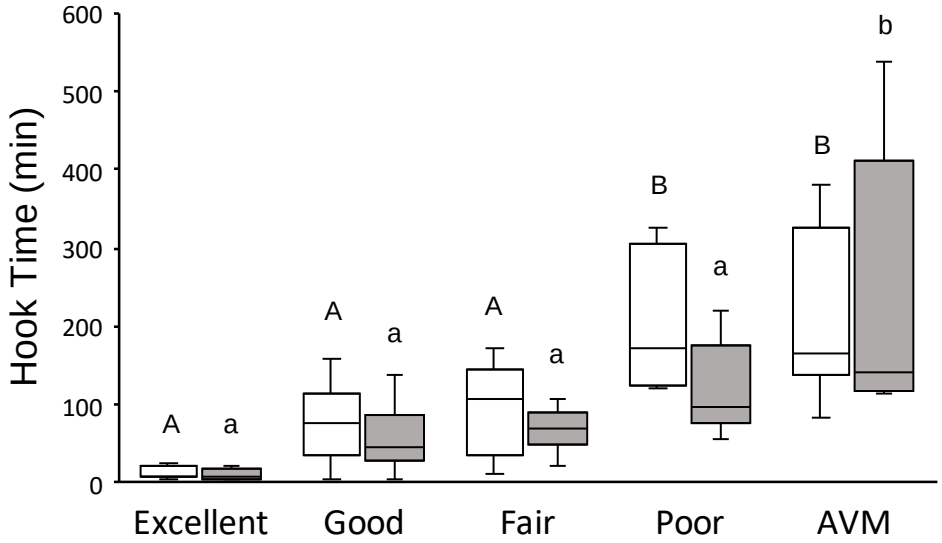
Figure 1. Box plot of *Sphyrna lewini* (white) and *Sphyrna mokarran* (grey) hook time (min) by release condition

Figure 2. Scatter plots of the stress parameters glucose (mmol l^{-1}) (A), lactate (mmol l^{-1}) (B), pH (C), and hematocrit (%) (D) over hook time in *Sphyrna lewini* (closed triangles) and *Sphyrna mokarran* (open circles). The dashed line is the regression line for *S. lewini* while the solid line is the regression line for *S. mokarran*

Figure 3. Scatter plots of the stress parameters sodium (mmol l^{-1}) (A), potassium (mmol l^{-1}) (B), calcium (mmol l^{-1}) (C), chloride (mmol l^{-1}) (D) and magnesium (mmol l^{-1}) (E) over hook time in *Sphyrna lewini* (closed triangles) and *Sphyrna mokarran* (open circles). The dashed line is the regression line for *S. lewini* while the solid line is the regression line for *S. mokarran*

Figure 4. Bar graphs of the mean (± SE) stress parameters glucose (mmol l^{-1}) (A), lactate (mmol l^{-1}) (B), pH (C), and hematocrit (%) (D) by release condition in *Sphyrna lewini* (white) and *Sphyrna mokarran* (grey). Uppercase letters above the bars indicate significant pairwise differences within *S. lewini*, while lowercase letters above the bars indicate significant pairwise differences within *S. mokarran*

Figure 5. Bar graphs of the mean (± SE) stress parameters sodium (mmol l^{-1}) (A), potassium (mmol l^{-1}) (B), calcium (mmol l^{-1}) (C), chloride (mmol l^{-1}) (D) and magnesium (mmol l^{-1}) (E) by release condition in *Sphyrna lewini* (white) and *Sphyrna mokarran* (grey). Uppercase letters above the bars indicate significant pairwise differences within *S. lewini*, while lowercase letters above the bars indicate significant pairwise differences within *S. mokarran*



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621 **Figure 1.**

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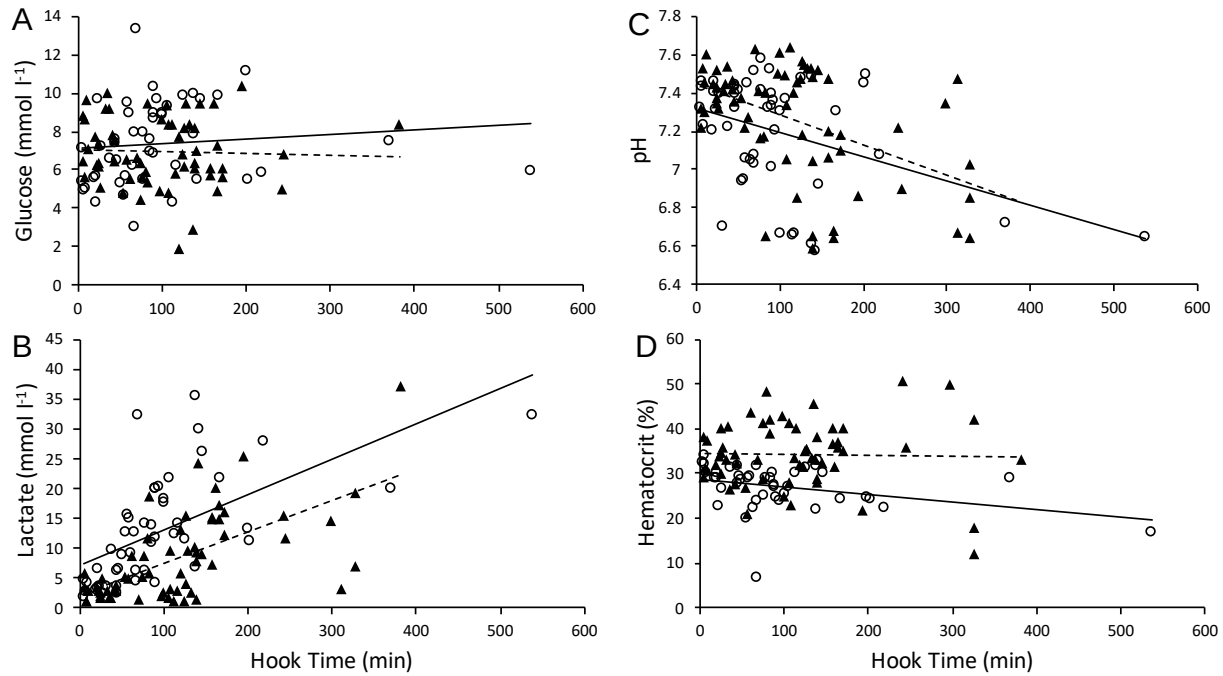

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624 **Figure 2.**

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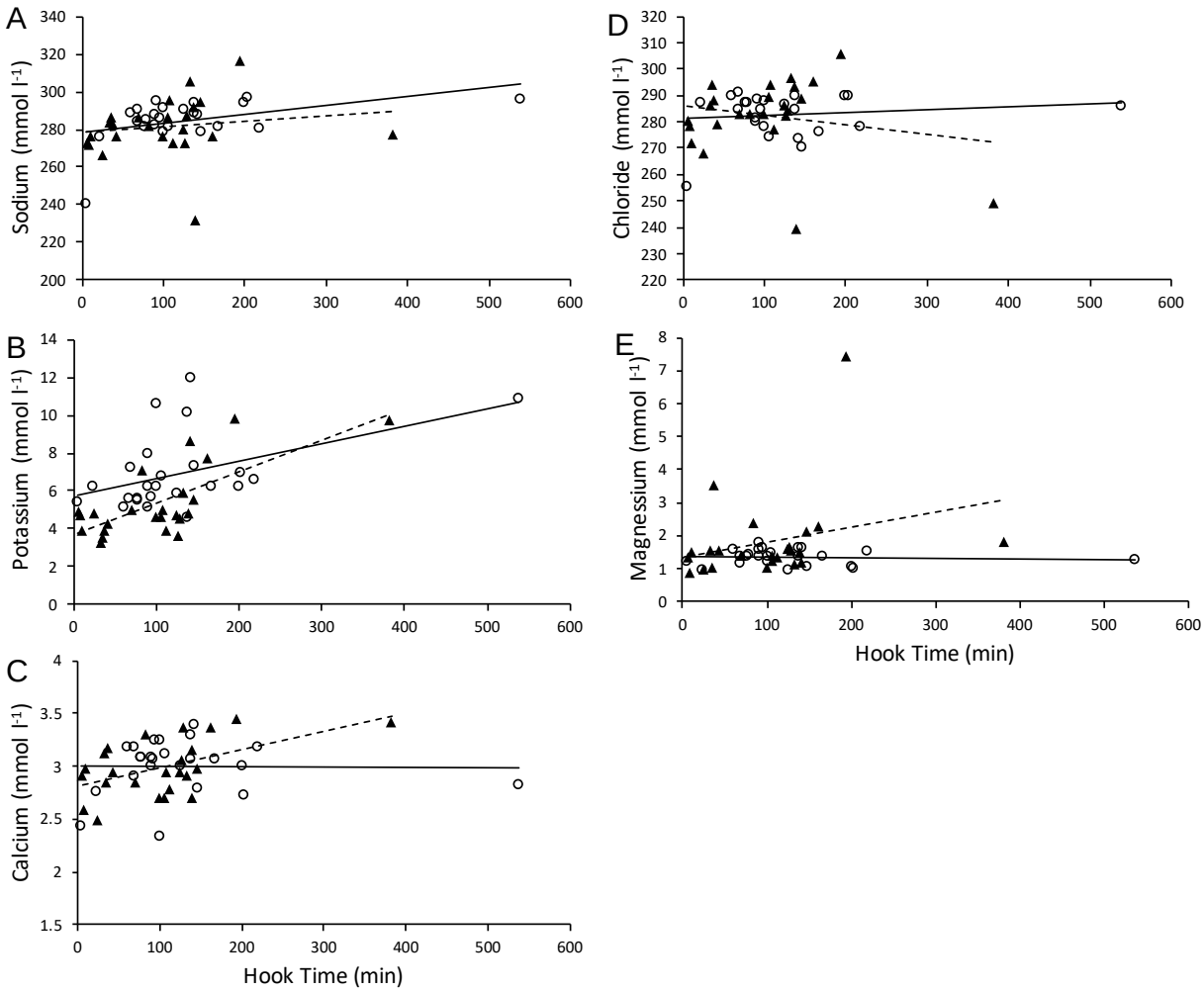

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627 **Figure 3.**

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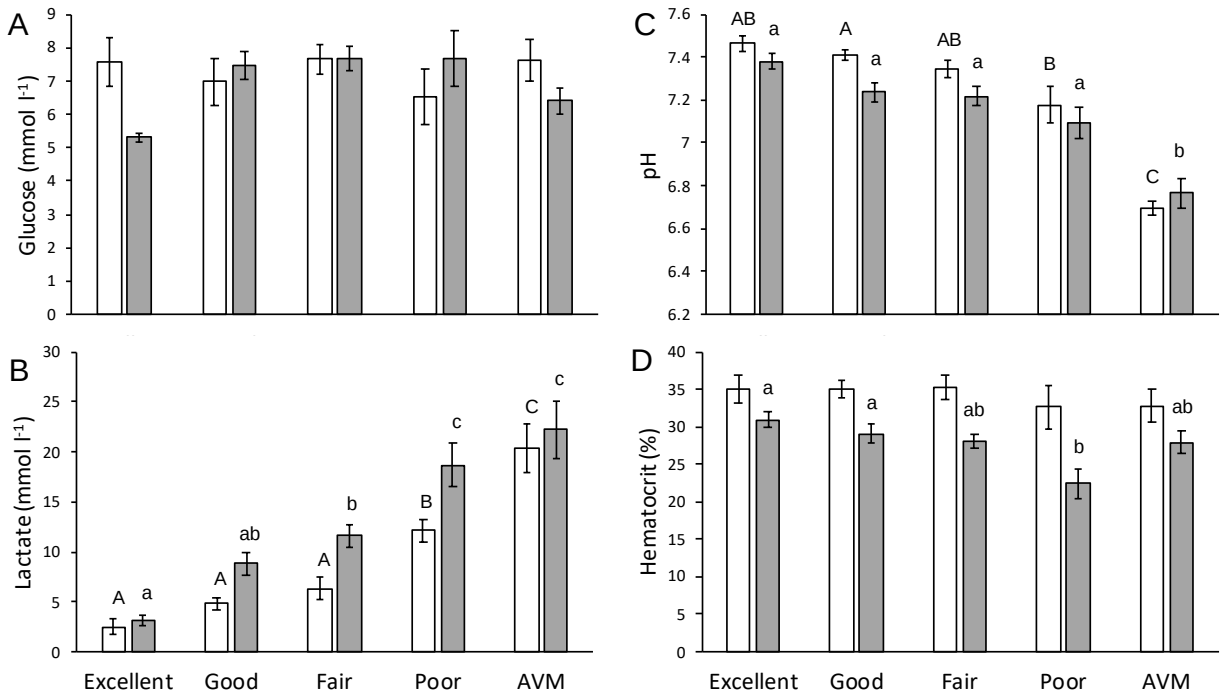

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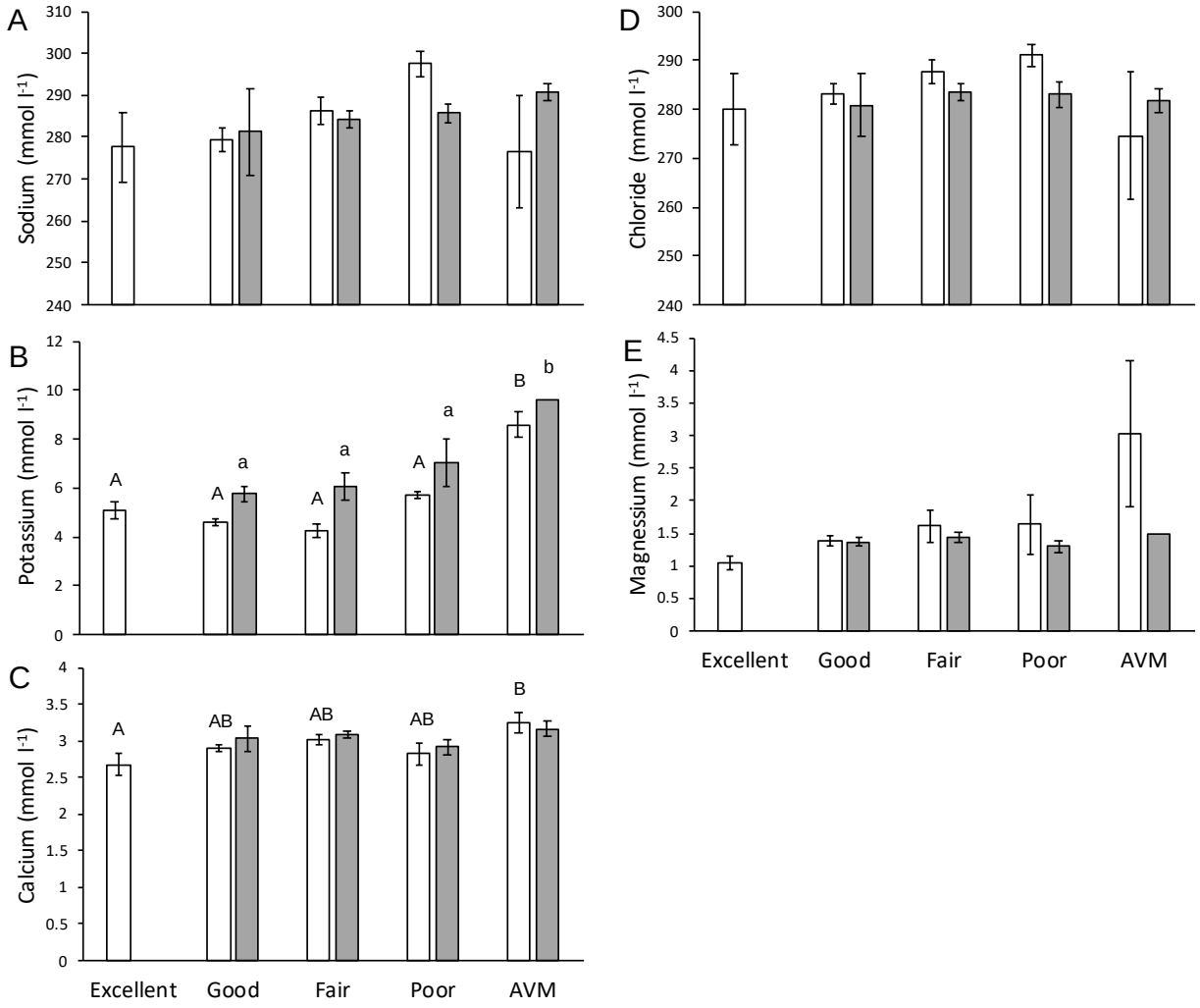
632 **Figure 4.**



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634 **Figure 5.**

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