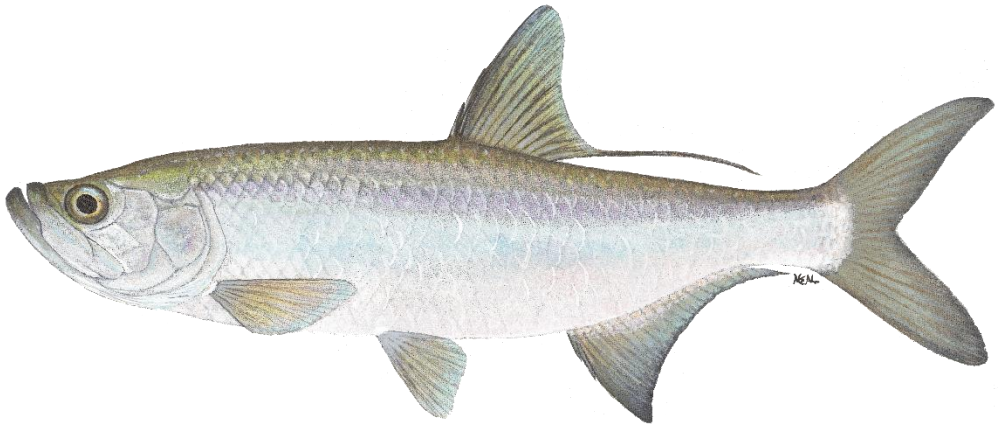
Response of Atlantic Tarpon to catch-and-release angling in Puerto Rico



Final Report for Sea Grant Project 2020-2021-007

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Table of Contents

Project Summary	**3**
Introduction and Rationale	**5**
Chapter 1: Post-release mortality of angled Atlantic tarpon in Puerto Rico	**7**
Chapter 2: Physiological effects of catch-and-release fishing on Atlantic tarpon: simulated and wild studies	**25**
Chapter 3: Effects of temperature and salinity on blood chemistry and survival of juvenile Atlantic tarpon	**47**
Products and Publications	**61**

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Project Summary

Atlantic tarpon *Megalops atlanticus* (hereafter referred to as tarpon) is a popular and economically important inshore sport fish in Puerto Rico, and the pursuit of this species by local anglers and tourists contributes to the economy. This species is managed as a no-take fishery, which aims to preserve populations by catching and releasing fish that would otherwise be subjected to harvest and removal from the population. The approach assumes minimal mortality and/or reduced fitness of released fish, yet the process of angling can produce many sub-lethal side effects or direct mortality. In this study, charter angling for tarpon in the San Juan lagoon system in Puerto Rico was examined to determine post-release mortality. Angled fish were externally tagged with acoustic transmitters and relocated periodically to determine fate after release. Post-release mortality was at least 4.5% (2 fish observed dead) and at most 18.2% (observed dead plus classified dead based on lack of movement; CI = 7.5-28.9%). Some tag loss was observed, which could have artificially elevated classified mortality estimates. Potential factors that contribute to mortality are discussed and compared to the literature. Hook type, gear action, landing procedures, and air exposure were key areas of possible improvement. Recommendations to minimize fish harm during angling include the use of heavier action gear to reduce fight time, a circle hook requirement for live bait to reduce deep hooking, maintaining fish in the water during landing and photography, and limiting air exposure to 2 minutes or less if fish are removed from the water.

Catch-and-release fishing aims to promote the sustainable use of fish populations, yet negative physiological impacts and mortality may be an unintended result to this acute stressor. Wild juvenile tarpon from the northern Gulf of Mississippi were collected and held in a controlled setting to determine the time course of physiological response to simulated angling and compared with wild tarpon sampled as a part of catch-and-release sport fishing in Puerto Rico. In the laboratory, tarpon (n = 72) were stressed using 2 intensities of simulated angling (n=72; Low: 2-min chase, 1-min air exposure; High: 6-min chase, 2-min air exposure), and blood chemistry parameters indicative of stress and air breathing frequency were monitored during pre-angling (baseline) and at 0.25, 1, 4, 8 and 24 hours post-angling. Although results for cortisol were inconclusive, there was an increase in corticosterone, and secondary indicators of physiological stress including glucose, lactate, osmolality, hematocrit, pH and air breathing frequency followed a time course of an acute stress response and a recovery period. The osmolality response was of greater magnitude following the high-intensity stressor compared to the low-intensity stressor. Larger tarpon angled in Puerto Rico (n=42) were sampled immediately following capture and had comparatively low values of secondary stress responses, possibly due to the shorter duration (generally < 0.25 h) of post-stressor sampling. Plasma lactate increased with fish length, and both lactate and osmolality increased with angling time. These results demonstrated the process of angling and handling induces a short-term physiological stress response in tarpon, similar to many other species, although additional research is needed to determine whether there are longer-term implications to health and survival.

Tarpon are highly migratory sportfish that support recreational fisheries throughout their range. In U.S. waters, juveniles can be found in coastal and estuarine habitats along the Gulf of Mexico and Atlantic seaboard, with temperature limiting their northern latitudinal distribution. Juveniles may overwinter in these areas during the first several years of life. Low temperatures are known to cause mortality in adults, but the challenges of temperature are less understood for juveniles. Furthermore, salinity, which can change dramatically in these habitats, may have a synergistic effect with temperature. To examine the physiological effects of temperature and salinity on juvenile tarpon, wild fish were acclimated to a range of conditions that potentially occur in the northern range of their estuarine habitats. The haematology of juvenile tarpon was examined in two salinity (≤2 and ≥30 ppt) and temperature (15 and 25°C) treatments, followed by a low temperature tolerance test. After two weeks in treatment conditions, blood samples were analyzed for hematocrit, pH, red blood cell concentration, haemoglobin content, and plasma osmolality. Increased plasma osmolality was observed at both low and high temperature treatments acclimated to ≥30 ppt (356 ± 3 and 343 ± 3 mmol/kg, respectively) compared to ≤2 ppt (334 ± 1 and 328 ± 3 mmol/kg, respectively). Blood pH was increased at 15°C compared to 25°C, with the highest pH at 15°C and low salinity. Haemoglobin, hematocrit, and red blood cell concentration were higher at 25°C than 15°C, with haemoglobin lowest at 15°C and low salinity. For the low temperature tolerance test, all fish were acclimated to 15°C for 2 weeks, then transferred to separate tanks where temperature was gradually decreased at 0.9 ± 0.1°C/h until fish lost equilibrium. Fish at low salinity lost equilibrium more rapidly (1 ppt, 12.65 ± 0.46°C) than fish at high salinity (30 ppt, 11.26 ± 0.14°C). Results indicate juvenile tarpon are susceptible to low temperature, which is exacerbated by low salinity, findings useful in assessment of juvenile tarpon overwintering habitat.

In conclusion, this research found that post-release mortality of tarpon is low to moderate in Puerto Rico. Factors that contribute to mortality include gear specifications, landing procedures, and air exposure, and these factors can be managed to minimize mortality. However, angling effort in the San Juan Lagoon system is intense and individual fish are being recaptured multiple times annually; thus, fish are repeatedly exposed to risk of mortality across multiple capture events. Laboratory studies indicated that tarpon recover within 24 hours from angling stressors, so it is likely that the risk of mortality is additive instead of multiplicative. When risks are additive, future capture events affect the individual independently of previous capture events. Finally, tarpon appear to be resilient at warm temperatures and elevated salinities, but are sensitive to lower temperatures, particularly at lower salinities. This has implications for juveniles of this species along the northern and southern ranges, where climate patterns are becoming more variable and estuarine conditions are becoming more volatile.

Introduction and Rationale

Marine recreational fishing is an important component of tourism and economic stability in Puerto Rico and other Caribbean islands. Atlantic tarpon *Megalops atlanticus* (hereafter referred to as tarpon) is one of the most important inshore sport fish species to this region (Garcia-Moliner et al. 2002), and charter boat enterprises are increasingly relying on this species to generate income. Tarpon fishing is a popular sport because of this species' fighting ability and large size, as well as its accessibility, as it can be captured near major population centers and within inshore and calm coastal waters.

In the past, tarpon in Puerto Rico experienced considerable fishing pressure and harvest (Zerbi 1999). Harvest of this species accounted for as much as 10,189 kg annually between 2000 and 2003, straining local populations. In February 2004, the Puerto Rico Department of Natural and Environmental Resources (DNER) imposed a catch-and-release harvest restriction on tarpon, with the objective of protecting stocks for local and tourist sport fishing. Whereas release of angled tarpon is now mandatory in Puerto Rico, there is currently a shortage of data on tarpon catch, angling effort, and fishing mortality (Guerrero Pérez et al. 2013).

In many fisheries, a substantial portion of the fish population may be captured and released (Schill et al. 1986; Nuhfer and Alexander 1992; Davis 2002). Consequently, survival of released fish can have a major impact on fishery quality. Catch-and-release angling of tarpon results in an unknown level of hooking mortality and exerts a measurable stress on all fish angled (Cooke et al. 2002). This species is very aggressive, fights with great strength and stamina, and makes acrobatic leaps into the air when hooked; these traits make it particularly susceptible to acute and chronic stress and physical damage during capture and handling (Guindon 2011).

An assumption of catch-and-release is that angled fish returned to the water will survive and help maintain sustainability of the stock (Pollock and Pine 2007). However, the process of angling can cause physical and physiological damage to fish, evoking a stress response that may have lethal or sub-lethal consequences (Mazeaud et al. 1977; Skomal 2006). Physical damages may include hook wounds, bleeding, handling damage, or predator attacks during capture, while physiological damages can include internal imbalances via interlinked cardio-vascular, respiratory, endocrine, nervous, immune, gastro-intestinal, and musculoskeletal systems (Young et al. 2006).

Tarpon angling is promoted on television, the internet, and in other popular media, and fishing for this species has become an important and growing industry in Puerto Rico and elsewhere within its range. Tarpon must be managed more intensely as recreational fishing pressure increases, and there is need to discern if Caribbean populations can withstand the current and potential increases in catch-and-release fishing pressure. Muller and Taylor (2002) reported that catch-and-release mortality in common snook *Centropomus undecimalis* in Florida approached rates as high as would be expected in a commercial fishery. If the same is true for tarpon in Puerto Rico, managers will need to account for this mortality. Further, angler education may be necessary to improve catch-and-release practices to reduce post-release mortality.

Hooking mortality can be determined, and an accepted method is to use acoustic telemetry of fish following release (Prince et al. 2002; FFWCC 2013). Furthermore, stress response can be measured using blood plasma cortisol and chemistry analyses (e.g., Sink et al. 2008; Sink and Neal 2009). Therefore, we propose the following research to elucidate the effects of catch-and-release angling on tarpon in Puerto Rico. Our objectives were to:

1) *Evaluate physiological response of tarpon to angling and recovery*,

2) *Determine short-term and delayed post-release mortality of angled tarpon*, and

3) *Explore the physiological effects of temperature and salinity on juvenile tarpon*.

This research occurred over two years in collaboration with The University of Southern Mississippi, the Puerto Rico Department of Natural and Environmental Resources, and Caribbean Fisheries Adventures, a local charter fishing outfit in the San Juan area. Results and discussion are presented in chapters that correspond to the objectives.

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Chapter 1: Post-release mortality of angled Atlantic Tarpon in Puerto Rico

The goal of catch-and-release angling is that captured fish survive to be caught again (Pollock and Pine 2007). This requires that fish are not physiologically compromised after release (Orr 2009). Despite the intent of catch-and-release practices, angling can cause acute stress and physical damage to fish, with potential lethal or sub-lethal consequences (Mazeaud et al. 1977; Skomal 2006). Visible injuries may include hooking wounds, physical deformities from repetitive hooking and handling, and bacterial/fungal infections (Meka 2004). Subsequent to capture, fish can experience respiratory issues and compromised immune systems, leading to behavioral changes (Cooke et al. 2002; Wilson et al. 2014). Other physiological impairments include damage to the reflex response, increased risk of predation, and decreased individual fitness (Campbell et al. 2010; Brownscombe et al. 2017). These stressors may act individually or synergistically to result in the death of released fish. Mortality may vary by duration and magnitude of the stressors (Meka and McCormick 2005) and are influenced by gear type (Sass et al. 2018), environmental conditions (Suski et al. 2006), and post-capture handling and release practices (Guindon 2011).

Acoustic telemetry is often used to determine *in situ* mortality over a period of hours to weeks following release (Prince et al. 2002; FFWCC 2013). For example, Red Snapper *Lutjanus campechanus* tagged with acoustic transmitters demonstrated angling mortality occurred within 72-hours following release (Eberts and Somers 2017). Results from acoustic telemetry of Bonefish *Albula vulpes* suggested angling-induced mortality often occurs within minutes of fish being released (Danylchuk et al. 2007). Furthermore, 43% of 30 Bonefish succumbed to angling-induced predation within a two-week timespan (Moxham et al. 2019). These studies utilized external attachment of transmitters to avoid intrusive surgical procedures. Although external tagging has been found to reduce growth and long-term survival, direct mortality from external tagging is rare (see review by Jepsen et al. 2015).

The tarpon is a popular inshore sport fish species, and this popularity derives from its large size, powerful fight when hooked, and accessibility, as tarpon are mainly fished inshore and in calm coastal waters (Guindon 2011). In Puerto Rico, the pursuit of this species by local anglers and island visitors contributes significantly to the economy (Garcia-Moliner et al. 2002). However, tarpon fisheries in Puerto Rico previously were prone to population declines due to angling and harvest (Zerbi et al. 2001). Between 2000 and 2003, up to 10,189 kg of tarpon were harvested annually with negative effects on population size (Guerrero Pérez et al. 2013). In response, the Puerto Rico Department of Natural and Environmental Resources (DNER) imposed a harvest prohibition on tarpon in 2004 to protect stocks for angling by locals and tourists (Guerrero Pérez et al. 2013). Currently, tarpon angling is only catch-and-release in Puerto Rico by law.

Data on catch, angling effort, and post-release mortality of tarpon have not been collected in Puerto Rico (Guerrero Pérez et al. 2013). Guindon (2011) and Edwards (1998) reported low post-release mortality in South Florida, but results may differ for fish angled in other locations. Tarpon are widely distributed (Winemiller and Dailey 2002) and environmental parameters (i.e., water quality and chemistry; Leichter et al. 2006) and varying angling techniques (Brownscombe et al. 2017) may influence post-release fate of fish. Therefore, this study evaluated catch-and-release mortality of recreationally angled tarpon in Puerto Rico using acoustic telemetry. The specific research objective was to evaluate the fate (alive or dead) of tarpon using acoustic telemetry. Factors influencing post-release fate are discussed and this information can be used to decrease the impact of catch-and-release angling on the tarpon fishery.

Methods

Study site

San José, Los Corozos, La Torrecilla, and Piñones Lagoon and associated interconnected canals lie within the metropolitan area of San Juan, Puerto Rico (Figure 1) and are collectively identified as one of the world's top tarpon angling destinations by the media. Tidal cycles influence water quality and salinity, as does rainfall input via feeder streams and canals. Lagoons are surrounded by mangrove (red *Rhizophora mangle*, black *Avicennia germinans*, and white *Laguncularia racemosa*) forests (Pool et al. 1977). Due to dredging, landfills, and other anthropogenic activities, the hydrological characteristics of the estuary have changed considerably over the past century (Villanueva et al. 2000). Hydrological characteristics that have been altered by urbanization in the San Juan Lagoon system include disconnection from San Juan Bay, sediment denitrification and fluxes of ammonium, nitrate, and phosphorus nutrients (Pérez-Villalona et al. 2015). These modifications have resulted in a relatively closed system, with a single small connection to the Atlantic Ocean through the narrow passage of Boca de Cangrejos, in the locality of Piñones.

Angling and telemetry

This research was conducted in partnership with a local charter company that specializes in San Juan Lagoon tarpon angling. The guides hosted researchers on chartered trips with clients from May to July 2021. When a client hooked a tarpon, information about the angling process was recorded from hookset until landing. Here, the angling process included fight duration, the number of jumps, rod action (light, medium, or heavy), and handling on deck (i.e., hook removal and body support for photography). We also recorded geographic location of capture. Tarpon were only captured using spinning or baitcasting gear with live bait on circle hooks during the study. To land each fish, the guide would grab the fish by the lower jaw (hand lipped), and fish were pulled into the boat over the gunwale or bow and on to the deck. Data on landing condition and procedures included hook location on the fish, observed injury such as bleeding, and handling procedure during photography. Once clients were done with their captured fish, it was

handed over to the researchers for measurement of total length (mm) and possible tag implantation prior to release. We randomly selected tarpon for tag implantation. For fish not selected, blood was drawn for another study prior to release.

Transmitter implantation and telemetry

Acoustic tag assemblies consisted of a Sonotronics (CT-82-2-E) transmitter attached to a titanium anchor (Ti Small, Wildlife Computers) with a stainless-steel leader (42.2 kg test, 0.005 cm diameter) and crimp sleeves (double barrel size 7, 0.135 cm diameter). The length of the leader was adjusted to fish size so that the transmitter did not reach to or interfere with the dorsal fin. Tag assemblies were attached to the fish's left side, two scale rows below the insert of the dorsal fin. Using forceps, two to four scales were removed from the location of tag insertion to facilitate implantation of the anchor. The tag anchor was inserted with a tag pole (comprised by AZ-TAGPOLE-004 - Swobbit Tagging Pole 16.51 cm length, AZ-DARTBUSH-001 - Dart Bushing fits 1.91 cm pole, and AZ-DARTAPP-011 - Dart Applicator 1/4-28; Wildlife Computers) within the scale removal zone through the musculature and between the pterygiophores. Fish were immediately released back to the site of capture.

Relocation efforts were conducted using active tracking once per week with a Sonotronics Manual Tracking Kit (MANTRAK; which contains USR-14 receiver, DH-4 directional hydrophone, TH-2 towed omnidirectional hydrophone, and accessories). Prior to the study, researchers established 28 listening stations, each selected to optimize lagoon coverage by utilizing a maximum line of sight distance of no more than 1 km between listening zones. During each telemetry period, the boat was maneuvered to each listening station and the omnidirectional hydrophone was lowered into the water and all frequencies were scanned. When a tagged fish was detected, approximate location was determined using the directional hydrophone. When all tag frequencies had been scanned, the boat was maneuvered to the next listening station.

Because tarpon are highly mobile (e.g., Duffing Romero et al. 2021), the lagoon system is large and complex, and time to conduct telemetry was limited to one day per week, exact locations using triangulation were not determined. Instead, the protracted telemetry period was used to confirm whether a fish was non-mobile and presumed dead, or mobile and alive. In this study, post-release fate was assessed by relocating fish weekly for up to 9 weeks. The post-release evaluation period was set at 5 days, so to classify a fish as alive, movement must be detected 5 days or more after release.

Classification rules were developed to interpret fish status based on movement (see Figure 2 for examples). Rules were based on previous studies that inferred fish status by classifying and grouping patterns of telemetry data from receivers (Heupel and Simpfendorfer 2002; Villegas- Ríos et al. 2020; Weinz et al. 2020). For a fish to be considered ***alive***, it must meet <u>all three</u> of the following criteria (Figure 2B):

1. fish was relocated at 2 or more different listening stations at least one week apart, and

2. observed movement occurred at least 5 days after release (indicating it was alive through 5 days), and
3. listening stations cannot have overlapping fields of detection (i.e., > 1 km).

For a fish to be considered ***dead***, it must meet any of the following criteria (Figure 2C):

1. during all weekly detections after day 5 with a minimum of 3 detections, fish was detected in the same area (within the 1 km receiver range), or
2. researchers recovered the dead fish, or
3. researchers received reports of a fish being dead within the 5-day evaluation period, and it was relocated in the reported location for 2 or more weekly tracking periods.

For a fish's status to be unknown and the data ***censored from analyses***, it must meet any of the following criteria (Figure 2D):

1. the fish was found just once (indicating it could have left the system, experienced tag failure, etc.), or
2. fish had only 2 detections, with the first detection occurring within the 5-day evaluation period, or
3. fish had only 2 detections in the same location or overlapping fields of detection immediately following the 5-day evaluation period on consecutive tracking days and then disappeared.

Two passive Submersible Ultrasonic Receivers (SUR, Sonotronics) were placed at the mouth of the lagoon system to determine if fish exited to the Atlantic Ocean. Before deployment, passive receiver bodies (excluding the transducer) were wrapped in black plastic and electrical tape to reduce biofouling. Mounts were constructed of PVC poles (5.08 cm diameter x 1.22 m height) inserted in concrete bases; PVC was spray painted black for concealment. Receivers were attached to PVC mounts with 550 paracord and nylon cable ties. Once deployed, SUR mounts were tethered to dock pylons to prevent lateral movement.

Data analysis

SurSoft DPCsa v1.0.8(c1) software (Sonotronics) was used to download and analyze passive receiver data. Detections from these receivers were combined with active telemetry data for the assessment of fish status. Combined fish location data were plotted in ArcMap (10.8.1) to analyze fish movement for determination of fish status. Satellite basemaps were retrieved from the ArcGIS website (ESRI - https://www.esri.com/). Confidence intervals for mortality were based on methods used by Wilde (2002) to determine confidence of status around fish using acoustic tracking.

Summary statistics included all fish caught during the study (including those not receiving transmitters). Linear regression assessed whether fish size influenced the duration of the fight. Prior to regression, the response and predictor variables were log-transformed to

correct for normality and to linearize the relationship. Regression was implemented in R (version 4.1.3).

Results

Angling process

Angling procedures were evaluated for 93 angled tarpon that ranged from 47 to 200 (mean = 88) cm in total length. Fish size directly influenced fight time (adjusted $r^2 = 0.55$, $p < 0.0001$) (Figure 3). Fight time ranged from 1 to 41 minutes but was skewed towards shorter times with a mean (± SD) of 5 (± 7) minutes. Observed jumps ranged from 0 to 14 (mean ± SD = 3 ± 2).

The charter company with whom we partnered primarily used light action rods, live bait, and circle hooks for chartered angling trips (Figure 4). With circle hooks, most fish were caught in either the corner of the mouth or upper jaw, and most (90%) were released after the hooks were removed; however, when hooking occurred in the throat or deeper, lines were cut leaving the hook in place to reduce injury. Little to no bleeding was observed among most angled fish (Figure 4). Following hook removal, clients could take photographs with the fish and choose the position to hold the fish. Horizontal photography was the most popular and encouraged by the guides to better grasp the fish (Figure 4). This practice had the client support the fish's body weight with one hand on the abdomen while controlling the fish by holding the lip with the other hand. Larger fish were often photographed lying on the deck.

Post-release mortality

Tagged tarpon moved considerably between lagoons, suggesting the classification rules based on movement are valid. Three fish were detected at the lagoon entrance, but no fish exited to the Atlantic Ocean during the study. Of the 49 tagged fish, 5 were censored from further analyses due to insufficient data to classify a fish as alive or dead. Of the remaining 44 classified fish, 36 were classified as alive based on observed movement following the 5-day evaluation period (81.8%). Conversely, 8 fish were classified as dead based on lack of movement (18.2%). One fish died soon after angling and the transmitter was retrieved; another fish was reported floating dead and, although not physically recovered, it was relocated consistently in the area reported. This yields a confirmed mortality of 4.5% (fish encountered dead), and a potential mortality of up to 18.2% estimated using fish status criteria (fish considered dead based on post-release movement, including confirmed mortality). The CI of the mortality estimate using fish status criteria was 7.5-28.9%.

Discussion

This study was the first to investigate catch-and-release mortality within the tarpon's tropical range. Mortality within 5 days of angling was at least 4.5% and at most 18.2% based on

confirmed and classified mortalities, respectively. The two confirmed mortalities occurred within 24 h of release. This suggests that post-release mortality occurs quickly, and the 5-day evaluation period was adequate to assess the effect of angling. It is possible that some classified mortalities represented tag shedding instead of fish death during the 5-day evaluation period, as the loss rate of external transmitters can be substantial and has been reported to be as high as 100% in some studies (see review by Jepsen et al. 2015). Two tarpon were reportedly captured by other charters that had scarring consistent with tag loss, and a third was captured with the tether in place but the transmitter missing, lending credence to this assertion. This study did not collect data on tag retention. Tag loss that occurred after the initial classification period (after post-release movement was confirmed) would not affect results.

The range of post-release mortality from this study was comparable to one estimate from Florida, where Guindon (2011) reported a 13% mortality rate (95% confidence interval: 6-21%) in angled tarpon (n=82). That study described predation on released tarpon by shark species to be a major vector of mortality (8.3%). Nearly 28% rates of shark predation were recorded in another large-scale satellite tagging study of tarpon (n=292) extending from North Carolina throughout the Gulf of Mexico (Luo et al 2020). Such a high predation rate by sharks may, however, be an artifact of the study, since sharks are likely attracted to the electric field generated by satellite tags, and predation rate increased in areas with high shark density (Luo et al 2020). The current study did not observe predation on tarpon and predatory shark species are not common in the lagoon complex. Another study from Florida (n=27) reported lower mortality (3.7%) of angled tarpon, and the authors attributed this low rate to angling practices, including style and size of hook, use of heavy gear and aggressive angling techniques to bring the fish to the boat as quickly as possible, and releasing the tarpon by not taking it out of the water (Edwards 1998). Angling in the current study utilized circle hooks, which reduced deep hooking. However, light action spinning rods were the primary gear, which prolonged fight times and likely increased the time of physical and physiological stress (Roth et al. 2018). Likewise, the Puerto Rico charter guides typically remove tarpon from the water for landing and photography, inducing additional stress and air exposure.

Certain factors have been identified in the literature that reduce mortality during the angling process. Hooking location can affect angling mortality in tarpon (Guindon 2011) and other species (e.g., Rainbow Trout *Oncorhynchus mykiss*, Meka 2004). Potentially lethal hooking locations can include the eye, gills, and esophagus (Ostrand et al. 2005). For example, Derbio *Trachynotus ovatus* hooked internally had an 85% mortality rate (Alós et al. 2008). Similarly, 95% and 75% of Spotted Seatrout *Cynoscion nebulosus* died when hooked in the esophagus and gills, respectively, compared to only 10% when hooked in the bony mouth (James et al. 2007). The use of circle hooks in the current study contributed to the prevalence of non-lethal hooking locations (Cooke et al. 2003). Most (n=78) tarpon were hooked in the corner of the mouth or in the upper or lower jaw, with few (n=15) fish hooked in other locations (i.e., outer head or body, inner oral cavity, or esophagus).

Most (91%) of the fish had the hook removed prior to release. Only hooks that were too deep to safely remove were left in the fish by cutting the line. When fish have deeply ingested hooks, it is often more beneficial to leave the hook in the individual as attempting to remove the hook often results in additional injuries and increased mortality (Butcher et al. 2007). In White Seabass *Atractoscion nobilis*, for example, leaving deeply embedded hooks increased survival rates, and 39% of hooks that were deeply ingested could pass through the organism's system (Aalbers et al. 2004). When looking at simulated angling in Sand Whiting *Sillago ciliate*, 23% of fish that ingested a hook died, but surviving fish were able to feed, and some fish passed ingested hooks with limited long-term physiological impacts documented (McGrath et al. 2009).

Hooking location can determine whether bleeding occurs, especially when the hook penetrates soft or highly vascularized tissues such as the gills and esophagus. Although some bleeding was observed in this study, 76% of the individuals experienced no bleeding with only 2 individuals (2%) experiencing heavy bleeding (gill hooked); 1 of which was confirmed dead. Previous studies have noted bleeding intensity with hooking location causes increased mortality (e.g., Schisler and Bergersen 1996). Conversely, hooking location did correlate to bleeding intensity but not mortality in Arctic Grayling *Thymallus arcticus* (Casselman 2005).

Roth et al. (2018) asserted longer fight times may cause physiological disturbances that can lead to a greater risk of post-release mortality. Increased fight time in Shortfin Mako Shark *Isurus oxyrinchus* did increase physiological stress factors such as lactate and glucose but did not impact survival (French et al. 2015). For Brook Trout *Salvelinus fontinalis*, mortality was independent of fight time (Kerr et al. 2017). Similarly, Guindon (2011) reported the average fight time of tarpon was 23.7 minutes for fish that survived and 16.5 minutes for fish that experienced mortality, which is contrary to Roth et al.'s (2018) hypothesis. The mean fight time in the current study (5 ± 7 min) was 3-5 times less than the Guindon (2011) study, despite angling primarily using light action gear. Rod action relates to how easily and where along the shaft a rod bends when tension is applied to the tip. Many studies previously have noted the action of the gear used but did not relate it to angling mortality (Jones 2005; Danylchuk et al. 2014; Holder et al. 2020). Catching the fish faster reduces the intensity of the fight and stress to the fish, but charter companies may prefer a longer angling experience for clients. Lowering the action creates more exciting and challenging fight for customers.

The disparity in fight time between Guindon (2011) and the current study was largely due to fish size. Although larger tarpon are captured in the San Juan lagoon system, the fishery is largely supported by smaller fish. Tarpon evaluated during the current study averaged 88 cm total length (± 27 cm), whereas fish in the Florida evaluation averaged 160.8 cm for fish that survived and 146.5 cm for fish that experienced mortality (Guindon 2011). The lack of a relationship between fish size and post-release mortality reported by Guindon (2011) was further supported by a meta-analysis of the catch-and-release literature (Bartholomew and Bohnsack 2005). This is a surprising finding because larger fish tend to have greater fight times and presumably more angling-induced stress response (Guindon 2011). Fish size was positively

correlated with fight time and handling time in the current study, and other studies support this finding (e.g., Danylchuk et al. 2014; Pinder et al. 2017). For Brook Trout, fight time was not correlated to total length (Kerr et al. 2017), suggesting the relationship of fight time and length is most likely species dependent.

Tarpon can be acrobatic and leap from the water during angling attempting to dislodge the hook (Kokomoor 2010). In a confined mangrove environment like the San Juan lagoon system, jumping behavior has potential to injure via impact with mangroves and other obstacles, or with the water surface, but this has not been empirically established (Luo and Ault 2012). In this study, tarpon averaged 3 (± 2) jumps during angling process. Schlenker et al. (2016) observed that jumping by White Marlin *Kajikia albida* increases fish exhaustion and adds additional stress to the individual (Schlenker et al. 2016). Further, jumping by angled Rainbow Trout correlated with increased risk of injury as jumping often led to deeper hook wounds and entanglement of the fish in the line (Meka 2004). It is likely jumping behavior at the very least adds to the stress response during angling.

Landing was a relatively stressful process, particularly for larger tarpon, which were dragged by the lower jaw over the edge of the gunwale or bow and into the floor or deck of the vessel. In the evaluation by Edwards (1998), Tarpon were supported in the water, thus eliminating air exposure and related injury, and survival rates were high at 96.3%. Although tarpon are facultative air breathers (Geiger et al. 2000), this raises the question of whether tarpon have greater survival when left in the water to prevent air exposure and the effects of gravity outside a liquid medium. Water supports the weight of a fish, and removal from the water can cause injury to internal organs, mandibular bone breaks and separated tongue (Danylchuk et al. 2008), vertebral separation (Gould and Grace 2009; Frawley 2015), and unexpected fish movement can lead to accidental drops and impact injuries. Increased air exposure has been correlated to increased stress in fish (Ferguson and Tufts 1992; Brownscombe et al. 2017), and increased amount of time required for the fish to recover from the capture event (Cooke et al. 2001; Brownscombe et al. 2017). Guindon (2011) suggested limiting air exposure of tarpon to 2 minutes or less and reported no significant physiological difference in blood chemistry response with 1 minute air exposure. Whereas evidence suggests longer air exposure has adverse effects on the survivability of some species (Arlinghaus and Hallermann 2007), it might be advantageous to minimize air exposure, as suggested by Edwards (1998) for tarpon.

Anglers like to share their experience with others, especially through photography. Clients were encouraged to hold fish horizontally supported (58%) when taking photographs; only a few fish (13%) were supported vertically or on the deck, particularly for larger fish due to their heavy weight. All fish were gently revived and released back into the water after capture. Horizontally supporting and gently releasing the fish minimizes injury and stress (Frawley 2015). Practicing these efforts are now considered etiquette and part of safe handling practices of catch-and-release angling. Some researchers recommend leaving the fish in the water for hook

removal and photography, minimizing air exposure and possible injury (Cooke and Sneddon 2007).

It is common for tarpon to show "partial migration", that is intraspecific variation in migration distance and the species commonly resides in estuarine habitat for part of the year (Luo et al 2020). The uniqueness of the San Juan Lagoon network is that tarpon are contained within a semi-closed system and can only enter and exit via a shallow opening (Boca de Cangrejos, Figure 1C) that is about 35 m wide. It is unclear whether tarpon in the San Juan lagoon network are year-round residents or migrate out of the system during some part of the year. In this study, only 3 fish approached the exit to the Atlantic Ocean but did not leave for any significant duration within the 9-week observation period. If fish are year-round residents, it raises concern about how continuous angling pressure impacts individuals confined within a semi-closed system. Although angling effort was not measured, it is substantial, with many charter outfits with client anglers and private recreational anglers using their personal watercraft daily. It was common during this study to be targeting tarpon within a small area with 5 or more other boats nearby. Further, telemetered fish from this study were caught multiple times by different angling charters, as researchers received multiple reports of tagged fish being caught. While this indicates that some fish recover quickly and return to normal feeding behavior (Cooke et al. 2013), it also suggests that fish in this lagoon fishery are repeatedly exposed to the risk of injury, air exposure, and post-release mortality.

Caution is warranted when using the upper mortality rate estimates due to the classification process. Immobile fish were classified as mortalities without verification. There were 2 reports of fish captured that looked like the tag was pulled out and another that was disconnected from the anchor line. This indicates that tag shedding occurred, but the timing and effect is currently unknown. Tag loss soon after tagging would lead to potential false classifications if those fish survived after shedding the tag. Further, this research occurred only during the summer. Although environmental conditions are relatively stable in Puerto Rico, some seasonal variation occurs and could influence seasonal results. Summer water temperatures are greater and therefore may represent a period of greater thermal stress. Finally, this study assessed specific practices of a single angling charter operator. The angling and handling techniques of other charter companies, freelancing captains, and independent anglers may differ (Brownscombe et al. 2017).

With optimal angling and handling practices, catch-and-release angling can be successful with minimal mortality (Brownscombe et al. 2017). Angler techniques can be altered to minimize catch-and-release angling from having negative impacts (Bartholomew and Bohnsack 2005). Hook type, gear action, landing procedures, and air exposure were discussed as areas where modifications could yield a significant improvement in post-release survival. The use of heavier action rods and greater line test could reduce fight time, shortening the period of angling stress (Mohan et al. 2020). This may not be the best option to maximize the angling experience, as anglers pursue tarpon for the fight. Circle hooks are well-researched to reduce deep hooking,

and a circle hook requirement when using natural bait could increase post-release survival (see reviews by Cooke and Suski 2004; Keller et al. 2020). Maintaining fish in the water during landing and photography would eliminate air exposure and injuries related to lifting fish from the support water provides (Edwards 1998). If the fish must be removed from the water, it is necessary to provide support to the body and reduce potential for injury. Finally, when tarpon are exposed to air, the exposure time should be kept at 2 minutes or less (Guindon 2011). Although most released tarpon survived, angling pressure in the San Juan lagoon system is intense and these recommendations could help reduce the risk to fish that are subjected to multiple capture events.

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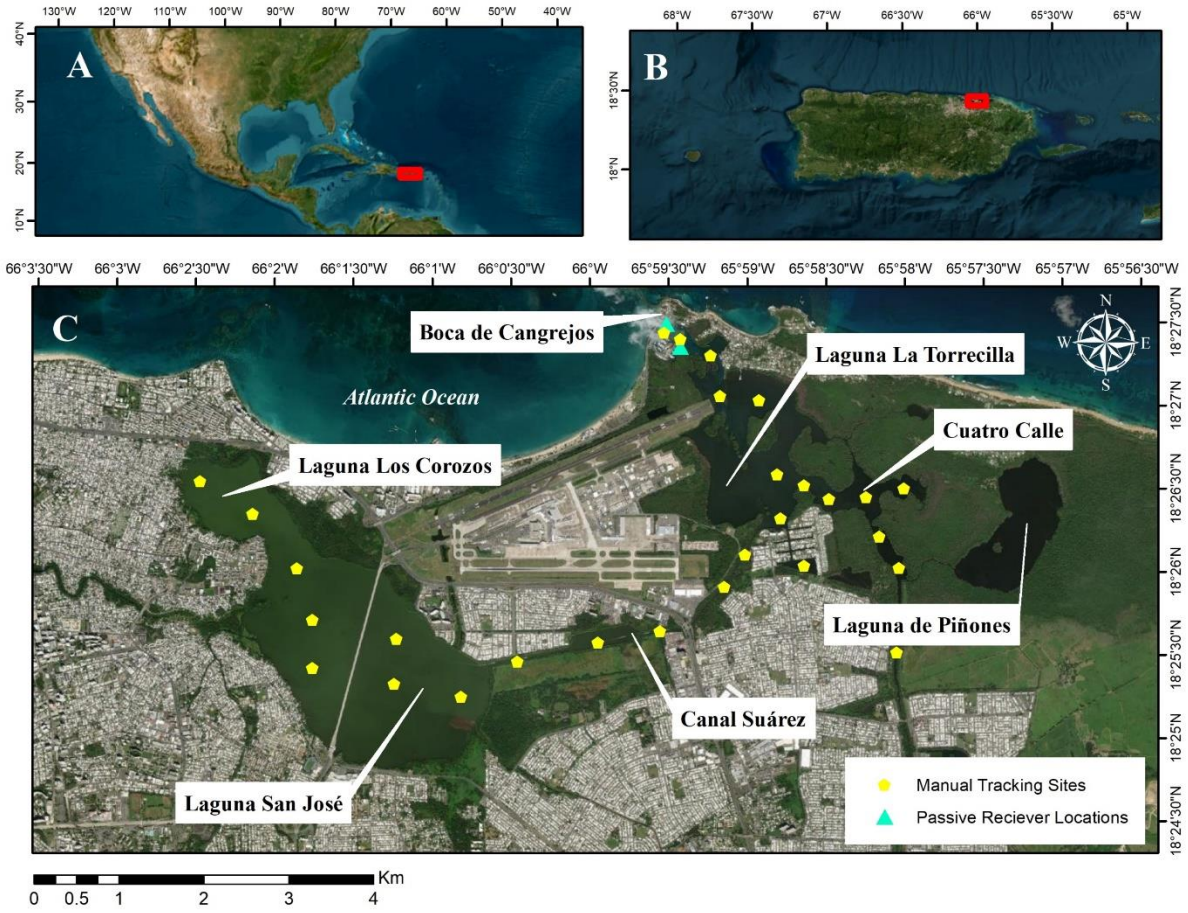

Figure 1. Map of A) the location of Puerto Rico in the Caribbean Sea, B) the location of the San Juan Lagoon System in Puerto Rico, and C) a closeup of the San Juan lagoon system and surrounding area with the location of tracking sites and passive receivers; Boca de Cangrejos connects the system to the Atlantic Ocean. Maps were produced by Karold Coronado-Franco.

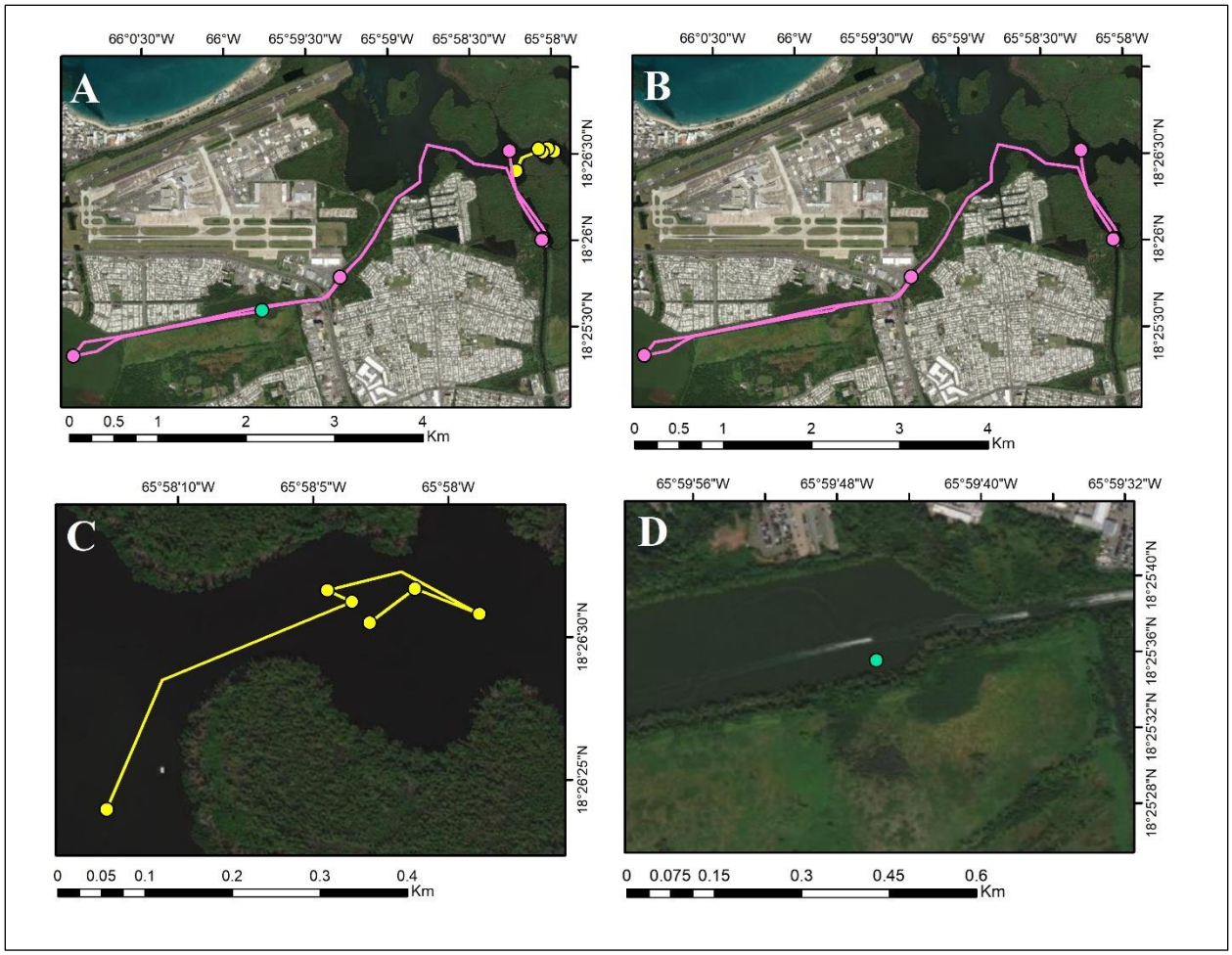

Figure 2. Examples of fish movement and status classification as alive, dead, or censored based on rules. A) Comparative map of all 3 classifications represented by panels B-D. B) Fish considered alive because of significantly movement after 5 days. C) Fish considered dead, as it moved from the initial site of capture, but did not move significantly following the 5-day evaluation period. D) Fish unable to be classified and censored from analyses due to insufficient relocations. Maps were produced by Karold Coronado-Franco.

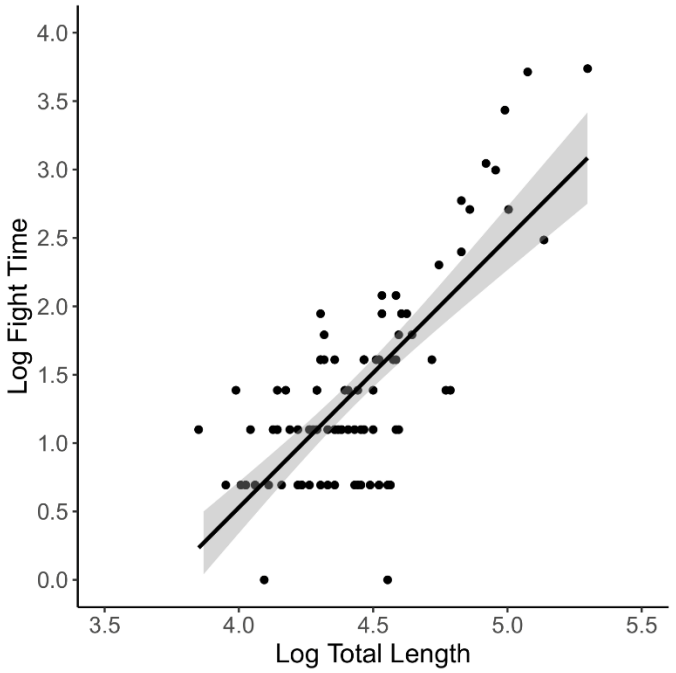

Figure 3. Linear regression showing the relationship of fish total length (cm) with fight time (min).

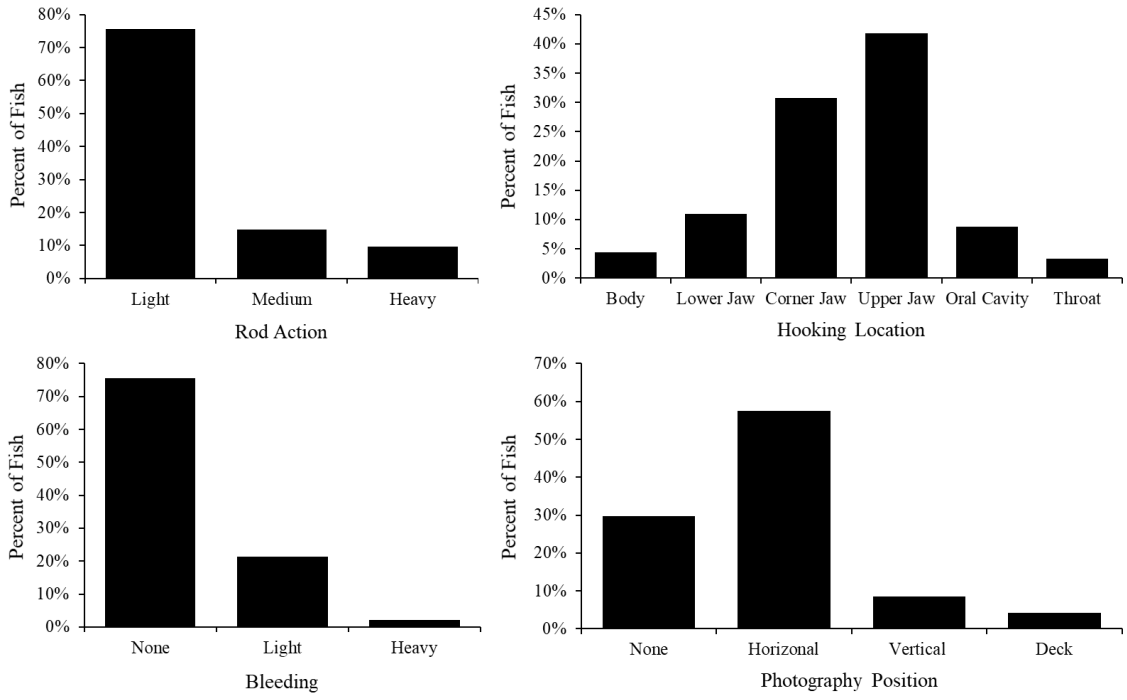

Figure 4. Factors during the angling process that could influence mortality. Rod action (Top Left), hooking location (Top Right), observed bleeding (Bottom Left), and handling position during photography (Bottom Right) are presented.

Chapter 2: Physiological effects of catch-and-release fishing on Atlantic Tarpon: simulated and wild studies

Harvest-oriented fisheries result in 100% mortality of harvested fish; thus, fishing mortality must be restricted using size limits, bag/trip limits, quotas, and seasonal closures to promote the sustainability of the fisheries (Bartholomew and Bohnsack 2005). In contrast, catch-and-release fishing is designed to allow a fish to be angled, released, and possibly caught again in the future. As a management tool, catch-and-release fishing is particularly beneficial for maintaining spawning stock (Cooke et al. 2002), protecting long-lived species (Wegner et al. 2021), and conserving species that receive intense fishing pressure (e.g., Hutt et al. 2008). This approach is intended to impart sustainability to a fishery (Pollock and Pine 2007) but requires that fish are as physiologically fit after release compared to pre-capture. In other words, released fish must survive the angling experience and be able to defend and support themselves and reproduce successfully (Orr 2009).

The process of angling is not without injurious effect. There are numerous potential stressors that may act individually or synergistically to affect fish health. These repercussions vary by duration (Meka and McCormick 2005) and magnitude of the stressors and are influenced by gear type (Sass et al. 2018), environmental conditions (Suski et al. 2006), and post-capture handling and release practices (Guindon 2011). Physical wounds from catch-and-release fishing may involve direct tissue damage in the mouth, digestive tract, and body, which in turn may decrease mobility, therefore impacting physiological performance (Cooke et al. 2002). Wounds to the body, organs, and spine can occur from the fish having to support its weight out of the water when held by anglers (Gould and Grace 2009). Physiological impacts may include disturbances in cardiac output and functionality, respiratory challenges, and diminished short- and long-term immune responses (Cooke et al. 2002), all of which may lead to behavioral changes. Reduced physiological performance can affect individual health through diminished stressor response, predator avoidance, and immune capacity, potentially leading to delayed mortality. These factors may impact individual and correspondingly population-level fitness (Cooke et al. 2002; Brownscombe et al. 2017).

The physiological stress response of fish is similar to many other vertebrates (Wendelaar Bonga 1997; Davis 2006). In teleost fishes, stressors induce primary responses such as cortisol release. In turn, primary responses produce secondary responses, including increased heart rate, permeability of gill epithelia, and increased circulating glucose levels, mobilizing energy to respond to a stressor but leading to greater hydromineral fluxes and depletion of stored energy substrates (Wendelaar Bonga 1997). These variables can be used to quantify intensity and duration of the stressor (Davis 2006), which are important for understanding implications to health and survival. While acute responses to stressors can be beneficial and extend normal adaptive ability, chronic exposure may result in decreased performance or survival (Davis 2006). Identifying this stressor threshold is key to guiding fisheries management.

Controlled experiments in a laboratory setting are commonly used to determine the physiological impact of fishing, stress response of the fish, and delayed mortality rate. Experiments can range from catch-and-release studies (Campbell et al. 2010) to simulated commercial net trawls (Davis 2007) designed to measure stress responses and recovery at discrete time intervals (Wedemeyer and Wydoski 2008). For example, marine fishes, including Walleye Pollock *Gadus chalcogrammus*, Coho Salmon *Oncorhynchus kisutch*, Rock Sole *Lepidopsetta bilineata* and Pacific Halibut *Hippoglossus stenolepis* were chased in tanks with motorized nets to simulate the fishing experience and measure fishing mortality (Wedemeyer and Wydoski 2008). Wild trout species and Arctic Grayling *Thymallus arcticus* were angled at select study sites for set durations, representing average times of an angling experience, and held in holding pens to extract blood to measure stress parameters over 72 hours (Wedemeyer and Wydoski 2008). Hatchery reared Senegalese Sole *Solea senegalensis* were chased with dip nets for 5 minutes at various timepoints throughout 1 day and/or for multiple days, and then blood was drawn to test for physiological stress (Conde-Sierira et al. 2018). Paddlefish *Polyodon spathula* were chased for 1 hour with some treatments having air exposure (in dip nets for 30 seconds) to simulate a fishing experience and determine fishing stressors (Barton et al. 1998).

Yet in any study, researchers must be cautious not to induce procedural stress unrelated to the angling experience. For example, drawing blood from fish can be a stressful process, and researchers must minimize induced stress from handling during blood sampling (Acerete et al. 2004). Likewise, wild fish can be studied; but moving wild fish to aquaculture settings can increase stress due to confinement (Woodward and Strange 1987). When fish husbandry and experiments are conducted with care and consideration to potential confounding effects, controlled environments allow for the use of standardized stressors such as chasing methods and aerial exposure times (Barton et al. 1998).

Physiological responses to stressors associated with catch-and-release angling have been examined in a wide variety of species. For example, Largemouth Bass *Micropterus salmoides* and Smallmouth Bass *M. dolomieu* are two of the most targeted freshwater fishes in the United States (White et al. 2008). In these species, maladaptive responses to catch-and-release fishing have been correlated to the degree of exhaustion (Kieffer et al. 1995), air exposure (Cooke et al. 2002), water temperature, and dissolved oxygen (Suski et al. 2006; Keretz et al. 2018). Survival decreases as water reaches maximum temperature tolerance and minimum dissolved oxygen thresholds (Keretz et al. 2018). Conversely, rapid capture, minimal handling, and rapid release can minimize immediate and latent mortality (Keretz et al. 2018).

In large migratory and pelagic fishes, blood chemistry stress-response variables may not be as effective at quantifying physiological impacts of stressors. This is due to the homeostatic disruptions caused by high anaerobic muscular activity (Skomal 2007). Understanding the physiology of marine fish, especially larger species, is often challenged by the difficulty of obtaining unstressed specimens that can be handled adequately in captivity (Skomal 2007). For this reason, an acclimation period for wild fish should be implemented to allow confinement

stressors to equilibrate. Further, stress response variables are often species-specific (Skomal 2007; Fanouraki et al. 2011). For example, blood glucose, lactate and osmolality increased following capture in Atlantic Sharpnose Shark *Rhizoprionodon terraenovae* (Hoffmayer and Parsons 2001), whereas blood lactate and glycogen were more responsive in Skipjack Tuna (*Katsuwonus pelamis*) and Yellowfin Tuna *Thunnus albacares* (Barrett and Connor 1962; Skomal 2007). There have been relatively few studies conducted on large marine species despite their popularity and importance as sport fishes.

Atlantic Tarpon are a popular marine inshore sport fish species distributed in the Atlantic Ocean (Virginia to Florida coast), Bermuda, the Gulf of Mexico, the Caribbean Sea to Brazil, and across to Africa (Wade 1962). In the United States, the tarpon fishery generates millions of dollars in revenue (Anyanwu et al. 2009). For example, in Florida's Caloosahatchee River and Charlotte Harbor region, tarpon fishing generates > $65 million annually (Felder 2011). Within these fisheries, varying regulations control the legal harvest of tarpon. In Mississippi, anglers can keep 1 legal-size tarpon per angler per day (Graham et al. 2021). In Alabama, there is a harvest tag system which requires the purchase of a tag to allow for harvesting a tarpon, yet there is no limit to the tags an angler can purchase (Graham et al. 2021). In Louisiana, there are no management regulations for tarpon (Graham et al. 2021). In Puerto Rico, past fishing pressure and harvest caused a population decline of tarpon (Zerbi et al. 1999); with up to 10,189 kg harvested annually from 2000 to 2003 (Guerrero Pérez et al. 2013). As a result, in 2004, the Puerto Rico Department of Natural and Environmental Resources (DNER) imposed harvest prohibition on tarpon to protect stocks for local and tourist sport fishing to ensure the sustainability of the resource (Guerrero Pérez et al. 2013; Luo et al. 2020).

Tarpon have been studied for tagging efficiency (Zerbi et al. 1999), movement (Griffin et al. 2018), conservation genetics (Seyoum et al. 2008, Blandon et al. 2003) and ageing (Elmo 2020). However, few studies have examined physiological effects of angling on tarpon. In a simulated angling study on Atlantic Tarpon, blood lactate, glucose, hematocrit, and electrolytes increased following a stressor; however, duration of angling and intensity of angling stressors were not examined (Guindon 2011). Therefore, the purpose of the present study is to 1) characterize the physiological response of tarpon to simulated angling stressors, 2) evaluate responses to different stressor intensities, and 3) determine the time duration of post-angling recovery over a 24-hour period. Results are compared to physiological responses of wild tarpon from catch-and-release fishing in Puerto Rico.

Methods

Fish collection and laboratory setting

Wild tarpon (n=50) were collected from the northern Gulf of Mexico at Ocean Springs, Mississippi, from August-November 2020. Tarpon were captured using cast nets deployed into

estuarine inlets and coastal marshes. Fish were held in holding tanks at the Gulf Coast Research Laboratory until November 2020, and then transferred to the South Farm Aquaculture Facility at Mississippi State University. Smaller tarpon (< 205 mm total length (TL); n = 25) were placed into an 800-L tank recirculating system, whereas larger tarpon (> 205 mm TL; n = 25) were placed into a 3,500-L tank; separation of size classes was used to minimize competition and enhance acclimation of tarpon. The recirculating systems were composed of a holding tank, sump tank, biofilter, ultraviolet sterilizer, aeration via forced air and water circulation pump.

Fish were transitioned from a diet of live fish to prepared fish. Fish immediately consumed live feed consisting of Guppy *Poecilia reticulata* and required approximately 5 weeks to fully transition to prepared fish (mix of cut pieces of catfish *Ictalurus* spp. and sunfish *Lepomis* spp.). Fish were fed to satiation daily throughout the study. After 4.5 months, small and large fish were placed together in the larger holding tank. Acclimation conditions consisted of mean ± standard error (SE) water temperature of 22.7°C ± 0.2°C, dissolved oxygen of 82.5 ± 1.1% saturation or 10.8 ± 0.9 mg/L, and salinity of 19.4 ppt ± 0.1 ppt. Dissolved oxygen and salinity measurements were conducted using a YSI 85 (YSI, Inc., Yellow Springs, Ohio), pH using a pH10A (YSI, Inc.) and ammonia/nitrite using colorimetric assays (DR/850, Hach Company, Loveland, Colorado). Experiments began after 5 months of acclimation to tank systems.

Two standardized stressor treatments were applied: 1) low fishing intensity (2-min chase, 1-min air exposure), and 2) high fishing intensity (6-min chase, 2-min air exposure). Each trial consisted of 3 tanks with 6 fish per tank per treatment. The experiment was repeated 1 month later for a total of 6 tanks per treatment (36 fish; 6 time-series replicates per treatment). Tanks were 800-L with a diameter of 127 cm and a water height of 61 cm. Tanks used in experimental trials were partially submerged in 2 flow-through raceways to maintain homogenous temperature.

Fish were randomly selected from the holding tank and assigned to treatments using a complete randomized design. Total length ranged 191-395 mm (mean ± SE = 292 ± 9 mm) in the first trial and 209-399 mm (302 ± 9 mm) in the second trial. Feeding was suspended 48 hours prior to experiments to minimize stress. Following transfer to the treatment tanks, fish were allowed to acclimate for 24 hours. The top of each tank was continuously covered with foam insulation to prevent escape and avoid visual stimuli by researchers. Oxygen was supplied by forced air via air stones. Water temperature (mean ± SE) averaged 22.8 ± 0.2 °C, dissolved oxygen averaged 7.11 ± 0.07 mg/L, and salinity averaged 18.5 ± 0.4 ppt (Table 1). Water quality was monitored daily to ensure minimal variation among tanks.

Covers were removed during each experimental procedure and immediately replaced between sampling periods to reduce external stimuli. Chasing and air exposure were accomplished using 1 dip net (0.6-cm-mesh, 40.6 x 40.6 cm mouth frame) per tank. The net was moved vigorously in each tank to chase fish and produce the simulated angling effect for each allotted treatment time. Immediately following chasing, all fish were captured in the net and held

out of the water for the allotted treatment air exposure time. Fish were then immediately returned to the water. Specific protocols for fish sampling are presented below. Signs of fish impairment, short-term mortality, and delayed mortality were monitored daily for 30 days following the experimental trial, with no signs of significant injury or mortality detected.

Data collection

Blood was collected from one experimental fish per tank per sampling period. The time series sampled was 0 (before simulated angling), 0.25, 1, 4, 8, and 24 hours (after simulated angling). To collect blood, each fish was removed from the tank and placed in an anesthetic bath (200 mg/L tricaine methanesulfonate (MS-222) at treatment conditions; Popovic et al. 2012). Blood (~1-mL) was collected from the caudal vasculature or via cardiac puncture (Clark et al. 2011) using sodium heparin-coated 1- or 3-mL syringes (20-gauge needle). After blood collection, the fish was transferred to a recovery tank until visible signs of recovery occurred (i.e., movement of caudal fin and resumed swimming) and subsequently transferred to the original holding tank.

Once a blood sample was collected, it was immediately placed on ice in a 1-mL snap cap vial. Whole blood pH was measured with a pH meter (Accumet Basic AB15 pH Meter, Fisher Scientific) and microelectrode (#13620850, Fisher Scientific) using a temperature-controlled water bath (WB10, Polyscience) set to the same temperature as the experimental tanks. Immediately afterwards, hematocrit (Hct) was measured in Hct tubes (heparinized capillary tube) after centrifugation for 5 minutes at 6,000 x *g*. Remaining blood was centrifuged for 3 minutes at 5,000 x *g* and plasma was aspirated into a new vial, flash-frozen in liquid nitrogen and subsequently stored at -80°C.

To determine if fish exhibited stress when moved into experimental tanks, six fish from the original holding tank were collected 1 month after the 2nd trial (April 2021) and processed identically to experimental fish. These fish were collected in April to avoid any impact on experimental fish. Furthermore, these fish were used as a baseline control to compare with Time 0 fish in each treatment to assure experimental fish did not experience elevated stress levels due to being in different tanks. The size range of fish in the control group was 229- 352 mm (TL; mean ± SE = 348 ± 21 mm).

The frequency of air-breathing was quantified by visual observation over a 5-min period in each experimental tank. The number of air breaths was divided by the number of fish in the tank at the time of the count to provide a per fish rate of breathing. Air-breathing was measured prior to 0 minutes (immediately before chasing stressor), and at 0.08 (immediately after chasing to 5 min), 2-, 5-, and 23-hours post-angling stressor.

Analyses

When thawing blood plasma, it was noted that a high frequency of plasma samples had presumably fibrinogen clots which were removed prior to analytical tests. Commercial kits were

used in blood plasma analyses to measure cortisol (EA65; Oxford Biomedical Research, Oxford, Michigan), corticosterone (EA66; Oxford Biomedical Research), glucose (DIGL-100; BioAssay Systems), and lactate (A-108L; Biomedical Research Service Center, Buffalo, New York). Osmolality was measured using a vapor pressure osmometer (Vapro 5520; Wescor, Logan, Utah). Methods were similar to those used by Dinken et al. (2020).

The stress response curves of lactate, cortisol, glucose, and osmolality from the simulated angling treatments were compared to values from wild fish caught from chartered sport fishing trips in Puerto Rico. Caribbean Fishing Adventures, located in San Juan, Puerto Rico, hosted the researchers on fishing charters with clients to observe their fishing experience and collect blood from angled fish. Trips ranged from 4 to 6 hours and were available 3 times per day (morning, afternoon, and night). Fishing was conducted between May 2021 to July 2021. Tarpon were angled by guides or clients, lifted from the water via a handhold on the jaw, and handled briefly in the air for pictures and blood collection. Blood was collected from the caudal artery or gill arch without anesthesia. Syringes (5-mL) were used, and plasma was centrifuged immediately after blood collection. Once blood draw was completed, fish were immediately released. Blood was immediately placed on ice in a cooler, and subsequently stored at -20 °C until transportation to Mississippi State University, where it was stored at -80 °C until processing. In total, blood samples were collected from 43 fish in the San Juan lagoon system, where fish ranged between 43-125 cm in total length (Mean ± SE: 78 ± 3 cm). Due to limited equipment, pH and Hct were not measured in fish collected in Puerto Rico. In addition, air breathing frequency was not able to be measured for Puerto Rico fish due to release after capture.

All statistical analyses were conducted using R statistical software 64-bit version 3.6.2. Data from laboratory studies were analyzed for outliers using Dixon's Q tests with a maximum of 1 outlier removed from a treatment group. Comparisons between control (fish from the 3,500-L holding tank) and experimental fish at Time 0 (low and high angling intensity groups) were conducted for each blood parameter with a one-way analysis of variance (ANOVA). To evaluate if there was any effect from using the same fish for both trials (i.e., February and March), differences in blood parameters between trial runs were tested via a three-way ANOVA to determine the effects of treatment group (i.e., low and high fishing intensity) and time (i.e., 0, 0.25, 1, 4, 8, and 24 hours after simulated angling). No differences in any of the measurement variables were detected, therefore samples from separate months were pooled into a two-way ANOVA with factors of stressor intensity and time. Before implementing ANOVA models, residuals were assessed by quantile comparison plots, histograms, and Shapiro Wilk’s test of normality and Levene’s test for homogeneity of variance. If any assumptions were violated, data were logarithmically, square root, or inversely transformed. If the transformation was ineffective, the ANOVA proceeded with caution as it is a robust analysis (Oliver-Rodríguez and Wang 2015). If an ANOVA was significant, differences among groups were tested using a Tukey’s *post hoc* test. Normality was assessed similarly for wild fish sampled in Puerto Rico, and linear regressions were conducted to assess relationships between physiological parameters and body

size or duration of angling (i.e., fight time and handling time). For all statistical tests, a significance level of $\alpha = 0.05$ was used.

Results

Simulated angling consisted of rapid chasing, impact with the net and tank walls, and rapid changes in direction. Experimental fish exhibited evasive jumping behavior in response to angling stressors as observed during angling of wild fish. Further, some evidence of light abrasions and scale loss was observed. However, no mortality occurred during simulated angling or during the 30-day post-stressor monitoring period. In two instances, blood was unable to be collected from a fish.

Control fish were statistically indistinguishable from Time 0 low and high stressor intensity treatments for all blood parameters except glucose, which was lower in control fish (control average of 66.1 mg/dL compared to the experimental fish of 119.2 mg/dL in low treatment group and 106.3 mg/dL in high treatment group; $p < 0.05$). There was no interaction between stressor intensity and time except for plasma osmolality ($F_5 = 2.497$, $p < 0.041$). There were no main effects of stressor intensity ($p > 0.05$); however, all variables measured had a main effect of time ($p < 0.05$).

The cortisol assay had an intra-assay CV of 7.6%. Cortisol spiked pre-extraction into tarpon plasma at 12.5, 25, 50 and 100 ng/mL yielded a mean recovery of 99.3% (range: 75.5 - 114.7%). Tarpon plasma diluted 1:5 to 1:160 showed parallelism to the standard curve following methods by Sink et al. (2008). Cortisol concentration was greater at Time 0 and 0.25 hours compared to 24 hours post stressor (Figure 1). In wild caught fish from Puerto Rico, there was not a linear relationship between cortisol and total length or time duration of angling.

The corticosterone assay had an intra-assay CV of 20.0%, with relatively high variability presumably due to low concentrations in samples. Corticosterone spiked pre-extraction into tarpon plasma at 5, 10, and 20 ng/mL yielded a mean recovery of 95.6% (range: 87.7 - 101.6%) with a CV of 14.9%. Lower concentration spiked samples (1.25, 2.5 ng/mL) yielded a mean recovery of 67.8%. High and low stress pooled tarpon plasma samples diluted 1:5 to 1:40, showed parallelism to the standard curve. Corticosterone concentration was greater at Time 0 and 0.25 hours compared to 24 hours post stressor (Figure 1).

Glucose concentration peaked at 4 hours and returned to baseline by 8 hours post-stressor (Figure 2). In wild caught fish from Puerto Rico, fish total length or time duration of angling did not impact glucose concentration.

Lactate increased at Time 0.25, 1 and 4 hours and returned to baseline by 8 hours post stressor (Figure 3). In wild caught fish from Puerto Rico, lactate increased with fish total length ($p < 0.001$) and time duration of angling ($p = 0.004$).

There was an interactive effect of angling intensity*time and an effect of time on plasma osmolality (Figure 4). The high angling intensity resulted in a greater, but non-significant, stress response compared to the low angling intensity. In the low angling intensity treatment, plasma osmolality was elevated at 0.25 hours post-stressor but was indistinguishable from Time 0 by 1 hour post-stressor. In the high angling intensity treatment, plasma osmolality was elevated at 0.25 hours post-stressor but was indistinguishable from Time 0 hour by 4 hours post-stressor. In wild caught fish from Puerto Rico, osmolality increased with time duration of angling ($p = 0.012$) but not fish total length ($p = 0.855$).

Blood pH decreased at Time 0.25 and 1 hour compared to Time 0 and returned to baseline 4 hours post-stressor (Figure 5). There was a decline in Hct with time, with decreased Hct at Time 8 and 24 hours compared to Times 0, 0.25, and 1 hours post-stressor. Air breathing frequency increased approximately 4-fold and 7-fold immediately following the low and high intensity treatments (Time 0.08 hours) compared to Time 0 and returned to baseline by 2 hours post stressor.

Discussion

This is the first study to characterize the time course of angling stress response in tarpon, investigate the response to differing magnitudes of angling stressors, and investigate stress responses of wild tarpon angled from Puerto Rico. The physiological response of fish to an acute stressor typically consists of an increase in primary stress responses, which mobilize secondary stress responses to provide energy substrates to respond to the stressor, followed by a return to homeostatic initial or baseline conditions. Blood plasma cortisol is a well-established primary indicator of stress in fish, as large differences in the corticosteroid responses to stress are dependent on magnitude of stress and are species-specific (Barton 2000). Corticosterone is important as a primary stress response in some fishes with ancestral characteristics. Blood glucose, lactate, osmolality, pH and Hct are all secondary indicators of stress; these parameters occur over a slower timescale and allow for a greater understanding of how the organism is responding to a stressor (Sopinka et al. 2016). Based on this established relationship, blood was analyzed for physiological indicators of stress, including cortisol, corticosterone, glucose, lactate, osmolality, pH, and Hct (Wendelaar Bonga 1997).

Although cortisol is the primary stress hormone in teleosts (Vijayan et al. 2010; Ellis et al. 2012), less is known in primitive fishes (Youson 2007). In this study cortisol did not respond in a classical manner, with no apparent response to the stressor. Guindon (2011) also found cortisol did not respond to an acute stressor in tarpon. A related Elopiformes species, Bonefish (*Albula* spp.), showed no cortisol response following strenuous exercise and varying levels of dissolved oxygen (Shultz et al. 2011). Because tarpon are descendants of a primitive lineage of teleost fish (Ault and Luo 2013), it is possible that cortisol is not the primary stress hormone in this group, or that these fish have a minimal cortisol response to a stressor, such as with some

other groups of primitive fishes. For example, sturgeons *Acipenseridae* (Haukenes et al. 2008), gars *Lepisosteidae,* and bowfin *Amia calva* (Davis and Parker 1986) have reduced or no detectable cortisol responses. Therefore, it is quite possible that tarpon do not have a predictable cortisol response as a primary indicator of stress. Guindon (2011) reported that larger tarpon had significantly lower cortisol responses compared to smaller tarpon. It is possible that fish size is not a significant proxy for cortisol concentration whereas fish age is. In the Silver Catfish, *Rhamdia quelen*, there were large differences in the stress response of fish that were of different ages even though they were the same size (Barcellos et al. 2012). Age could, in this case, be more influential in stress response than fish size as tarpon can live a maximum age of at least 55 years (Crabtree et al. 1995).

Secondary stress response indicators displayed a time-series effect that followed the classical pattern. Plasma glucose increased in response to the simulated angling stressor, peaking at 4 hours and then returning to baseline concentrations by 8 hours. These findings are supported by Guindon (2011), who reported angling tarpon for 15 minutes increased glucose concentrations from 87 mg/dL to 94 mg/dL. In this study, glucose did not respond differentially to the intensity of the angling experience. Increases in glucose may suggest utilization of metabolic energy reserves (Hemre et al. 2002), facilitating greater energy expenditure needed to respond to a stressor and return the body to homeostasis. As tarpon were noted to have rapid jumping behavior in both laboratory and field settings, the need for increased energy to be drawn to the muscles would support glucose increases within the body (Driedzic and Hochachka 1976).

Control fish displayed significantly lower glucose than experimental fish at Time 0; therefore, some level of pre-treatment stress may have been present in experimental fish. Further, average glucose concentration at Time 0 was 113 mg/dL in the controlled experiment compared to 81 mg/dL for angled Puerto Rico fish, which was closer to the value of 87 mg/dL in tarpon reported by Wells et al. (2003). Many factors could account for this observed variability. One possible explanation is that tarpon have a varying glucose response. Cortisol activates glucocorticoid receptors which can alter glucose levels (Vijayan et al. 2010). Glucose responses in salmonids differ to some extent depending on the stressor and species, with concentrations of glucose potentially remaining elevated for extended periods of time (> 72 hours) (Wedemeyer and Wydoski 2008). In the present study, experimental fish were stressed for a maximum of 8 minutes in total (chasing and air exposure), so with longer stress periods, results might change. Lastly, increased concentration of glucose could be related to fish size as this has been determined to be an influential factor in species such as wild Rainbow Trout *Oncorhynchus mykiss* (Meka and McCormick 2005). In the current laboratory study, glucose averaged 112.7 mg/dL compared to 81.0 mg/dL in wild-caught fish; wild-caught fish were larger in size compared to laboratory fish indicating that these trends may be opposite for species such as tarpon.

Lactate also displayed a predictable temporal response to a stressor, peaking at 1 hour and exhibiting return to baseline by 8 hours after simulated angling. No influence of angling

intensity was detected. Lactate is produced in conditions of anerobic respiration to provide energy to the individual (Allen and Holm 2008). Many studies have reported that lactate continues to build after exercise ceases (Wood et al. 1983; Wells et al. 2007). Furthermore, lactic acid in fish species using burst swimming is rapidly accumulated and then is released in excess after 5-10 minutes (Wood 1991). Since tarpon use burst swimming to leap out of water when stressed, it is intuitive that lactate would increase rapidly with exertion and then decrease rapidly to a base level following cessation of exertion. Guindon (2011) reported that lactate was the physiological characteristic with the greatest response to stress in Atlantic Tarpon. In the congener Pacific Tarpon *Megalops cyprinoides*, lactate levels followed a similar pattern following exertion, with recovery within 4 hours (Wells et al. 2007). Although no effect of intensity was found in this study, the duration of chase and air exposure has been shown to affect lactate in other species (e.g., Australasian Snapper *Pagrus auratus*; McArley and Herbert 2014).

Blood plasma osmolality was the only stress response variable measured displaying both a temporal response and an intensity response. Osmolality peaked at 0.25 hours before returning to baseline (statistically similar at 4 hours), with a greater response to the high intensity treatment. Osmolality had not previously been studied in tarpon or other bonefish in a simulated angling scenario. Results from this study are in line with those found for many species, including White Sturgeon *Acipenser transmontanus* and Elephant Fish *Callorhinchus milii*, which, when exposed to increasing intensities of simulated fishing and air exposure, the response of osmolality increased (McLean et al. 2016; Martins et al. 2018).

Blood pH declined immediately following simulated angling but recovered by 4 hours post-stressor; no influence of angling intensity was detected. Blood pH is an important metabolic indicator with increasing lactate causing a decrease in blood pH (Aoi and Marunaka 2014). In addition, other factors such as respiratory acidosis can cause a reduction in blood pH (Lopez et al. 2002). Low blood pH in fishes can increase disease susceptibility, impair osmo- and ionic regulation, inhibit hormone production/activity, create genetic damage to future generations of brood, and increase susceptibility to toxic substances (Fritz 1980). Blood pH has not been previously examined in Atlantic Tarpon, but Pacific Tarpon have shown a similar response (Wells et al. 2007). In that study, angling intensity did influence pH, which also recovered following a 4 hour recovery period (Wells et al. 2007). For Bonefish, blood pH was negatively correlated with fight time and fish length, but lactate was not the sole cause of pH decline (Brownscombe et al. 2015). In Rainbow Trout, exercising fish for 10 minutes with the addition of air exposure for 1 minute significantly altered acid-base relationships when comparing stressed fish to those that were only exercised (Ferguson and Tufts 1992). This suggests that the additional stress of air exposure may be detrimental.

Hematocrit (Hct) exhibited a steady decline following the angling stressor with no effect of stressor intensity but had not recovered to baseline level at the end of the 24-hour assessment period. Hct measures the proportion of red blood cells in the blood which influences oxygen carrying capacity (Gallaugher et al. 1995). Aerobic stress due to exercise is linked to increases in

Hct percentages (Gallaugher et al. 1995), yet this was not the case in the current study. In previous tarpon studies, Hct was measured but did not have any significant effects in terms of fight time, handling time, size, or angling treatment (Guindon 2011). The mean Hct percentage for tarpon previously studied was 46.9% (Guindon 2011), whereas this study's angled tarpon had a mean value of 35.1%. Tarpon naturally have a high resting Hct percentage of 37.6% (Wells et al. 2003), yet our results are lower compared to previous studies in tarpon. Hct has been shown to decrease due to factors such as confinement, induced stress, and lack of food (Affonso et al. 2002), which might explain the observed results since tarpon were not fed 48 hours before experimental simulations. Decreased Hct percentages have been a factor of starvation (Larsson and Lewander 1973), but the specific timeframe where this occurs is currently unknown. As the laboratory fish were well-fed throughout their holding period, determining whether the starvation factor impacts the Hct following the angling stressor in the current study will have to be further investigated.

Although angling intensity did not influence tarpon Hct, exertion level did affect Bonefish Hct, with a response only detected for the greater level of exertion (4 minutes stressed) compared to low level exertion (1 minute stressed), and no return to base levels during the 4-hour post stress observation period (Suski et al. 2007). Under harsh water quality conditions, Tambaqui (*Colossoma macropomum)* exhibited a decreased Hct percentage at 96 h post stressor (Affonso et al. 2002). It is currently unknown how long it takes tarpon to fully recover to a resting Hct percentage.

Air-breathing increased 3- to 7-fold immediately following both low and high angling intensity stressors, respectively, and returned baseline within 2 hours. Tarpon are facultative air breathers (Geiger et al. 2000; Seymour et al. 2008), and air breathing can aid in recovery from oxygen debt by restoring physiological parameters back to baseline levels (Wells et al. 2003). Tarpon increase air breathing frequency in non-optimal environments and due to exercise (Wells et al. 2003). In this study, the increase in air breathing was presumably also beneficial for recovery of other physiological parameters such as lactate (Wells et al. 2003, 2007), pH (Gonzalez et al. 2001) and hematocrit (Wells et al. 2003). In Pacific Tarpon, the air breathing organ has been shown to help prolong aerobic activity in events that require a high energy demand, but it is gill breathing that is used to recover from oxygen debt (Wells et al. 2007). Although not documented in Puerto Rico fish, air breathing frequency may differ in adult tarpon (Clark et al. 2007).

Due to the nature of charter angling and field research, time series analysis of wild angler-caught tarpon in Puerto Rico was not feasible. Physiological stress variables were not related to fish size or time of fishing experience. This may be due to the lack of significant variability in size range or fight and handling time (O'Toole et al. 2010), as most angled tarpon were smaller juveniles and were captured quickly (average ~ 5 minutes; maximum of 24 minutes). Further, sampling was conducted immediately upon landing, which may not have allowed sufficient time for physiological effects to manifest. Bower et al. (2016) found that Blue-

finned Mahseer *Tor putitora* physiological factors had pronounced effects the longer the fight time continued.

Following an acute induced stressor (angling and air exposure), the stress response in tarpon was characterized by temporary changes in the secondary stress indicators of osmolality, glucose, lactate, and pH. These physiological variables returned to baseline by the end of the 24-hour observation period. Osmolality was the only physiological parameter to respond to stressor intensity, but other parameters displayed patterns suggestive of an intensity effect and it is possible that greater sample sizes or longer stress times might yield more conclusive differences. Air breathing also proved to be a useful tool in visually determining stress of tarpon exposed to fishing experiences. This study provides insight into how wild tarpon react to and recover from the fishing experience. This information can be used to refine catch-and-release protocols to minimize the response and recovery times of osmolality, glucose, lactate and pH.

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Table 1. Mean and standard error water quality variables for the laboratory experiment conducted during February (n=24) and March (n=23) of 2021 at the Mississippi State University South Farm Aquaculture Facility.

Water Quality Variable	Month	Mean	Standard Error
Temperature (°C)	February	21.90	0.30
	March	23.70	0.10
Salinity (ppt)	February	18.40	0.00
	March	18.60	0.10
Dissolved Oxygen (mg/L)	February	7.42	0.07
	March	6.79	0.06

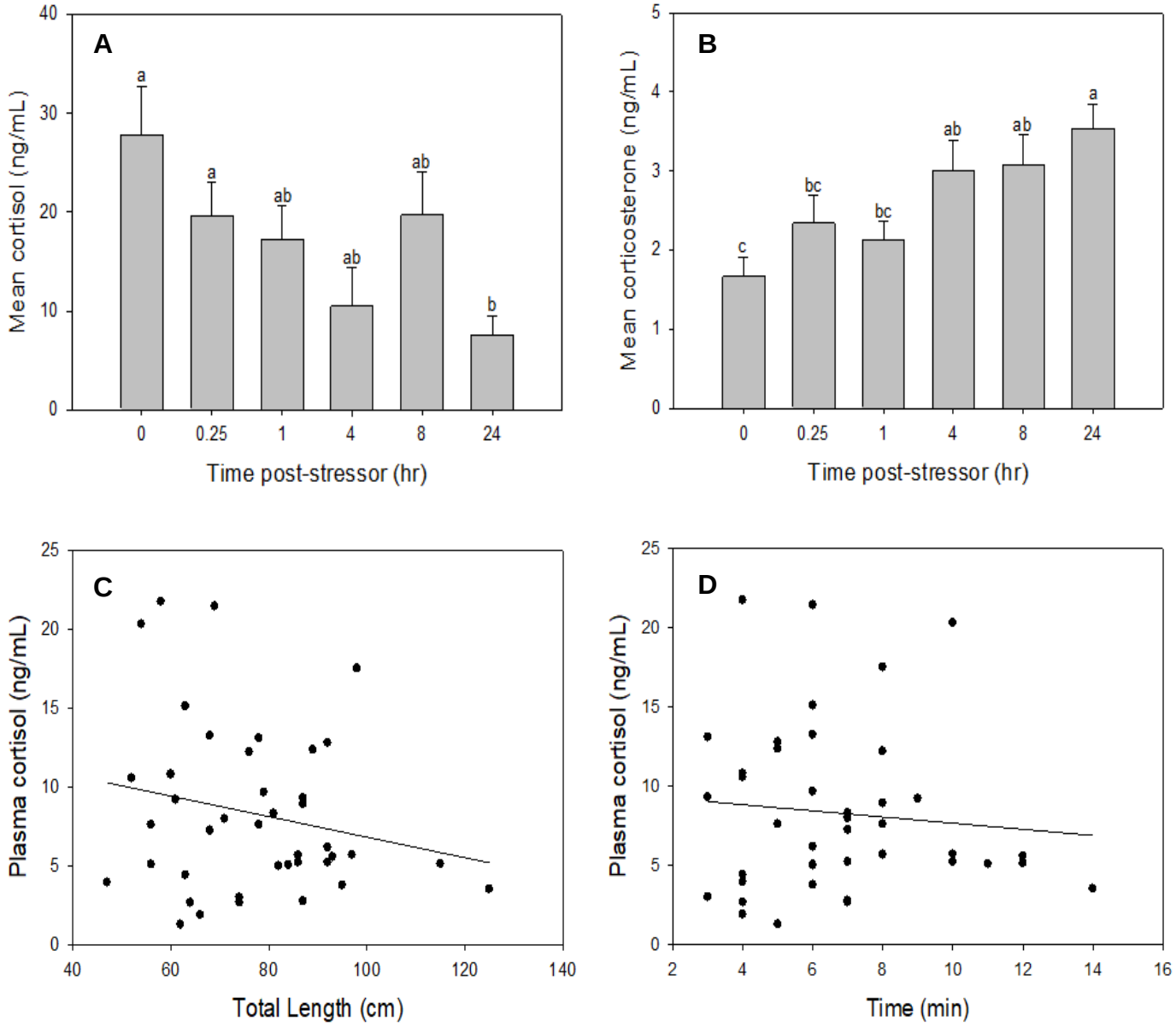

Figure 1. Atlantic tarpon (*Megalops atlanticus*) mean + standard error (A) plasma cortisol (ng/mL) and (B) corticosterone at sequential times following a low- or high-intensity simulated angling stressor (n = 67). Different letters indicate statistical differences. Plasma cortisol of individual wild-angled fish are presented as a function of (C) total length (cm) and (D) angling time (min).

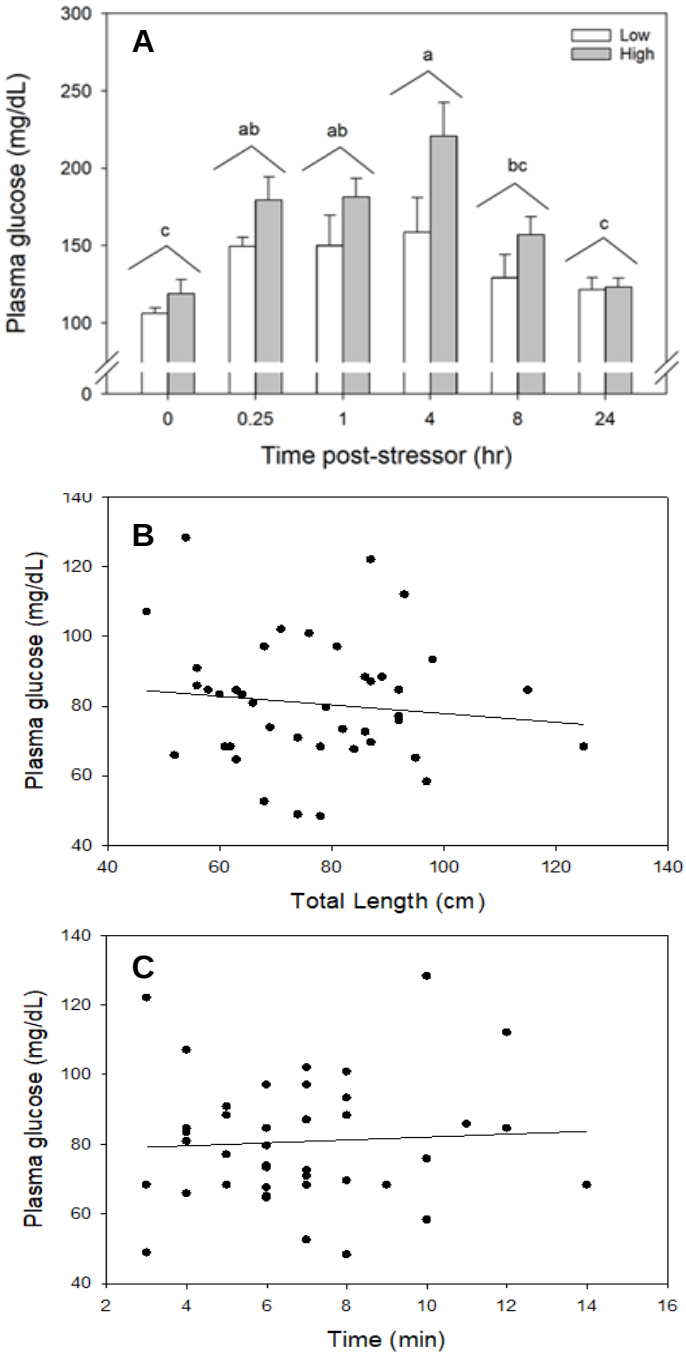

Figure 2. Atlantic tarpon (*Megalops atlanticus*) mean + standard error (A) plasma glucose before and at sequential times following a low- or high-intensity simulated angling stressor (n = 67). Different letters indicate statistical differences. Plasma glucose (mg/dL) of wild-caught fish (n = 42) as a function of (B) total length (cm) and (C) fishing time of the total fishing experience (i.e., caught and handled). Dots represent individual fish with a linear regression line presented.

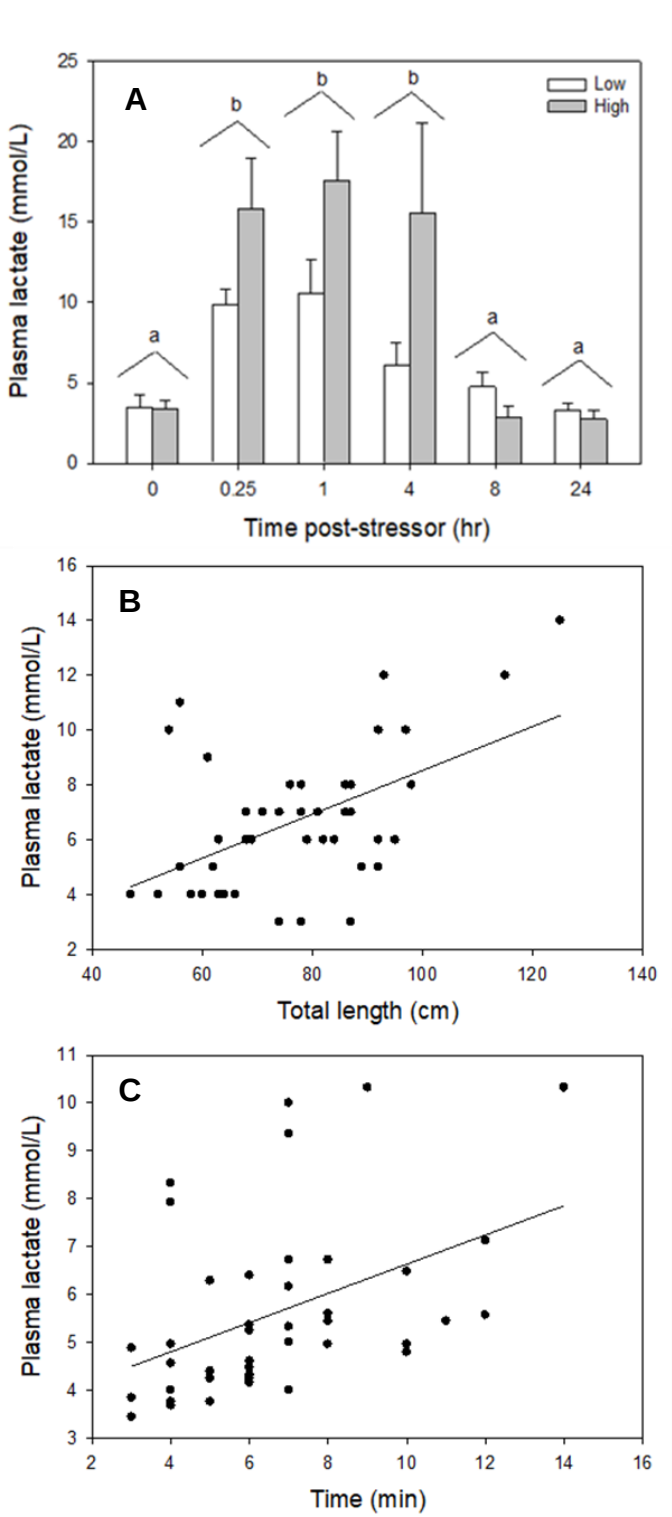

Figure 3. Atlantic tarpon (*Megalops atlanticus*) mean + standard error (A) plasma lactate before and at sequential times following a low- or high-intensity simulated angling stressor (n = 67). Different letters indicate statistical differences. Plasma lactate (mmol/L) of wild-caught fish (n = 42) as a function of (B) total length (cm) and (C) angling time (min). Dots represent individual fish with a linear regression line presented.

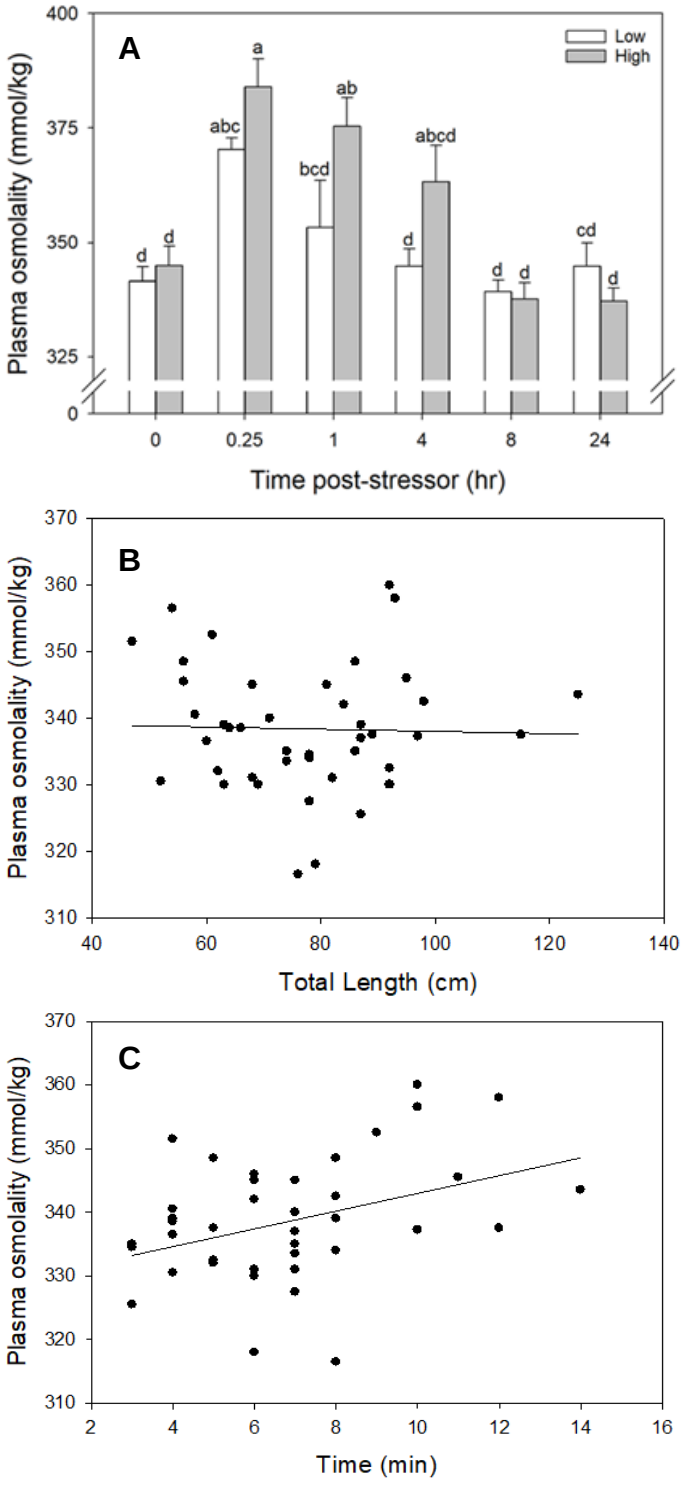

Figure 4. Atlantic tarpon (*Megalops atlanticus*) mean + standard error (A) plasma osmolality (mmol/kg) at sequential times following a low- or high-intensity simulated angling stressor (n=67). Different letters indicate statistical differences. Plasma osmolality (mmol/kg) of wild-caught fish (n=42) as a function of (B) total length (cm) and (C) angling time (min). Dots represent individual fish with a linear regression line presented.

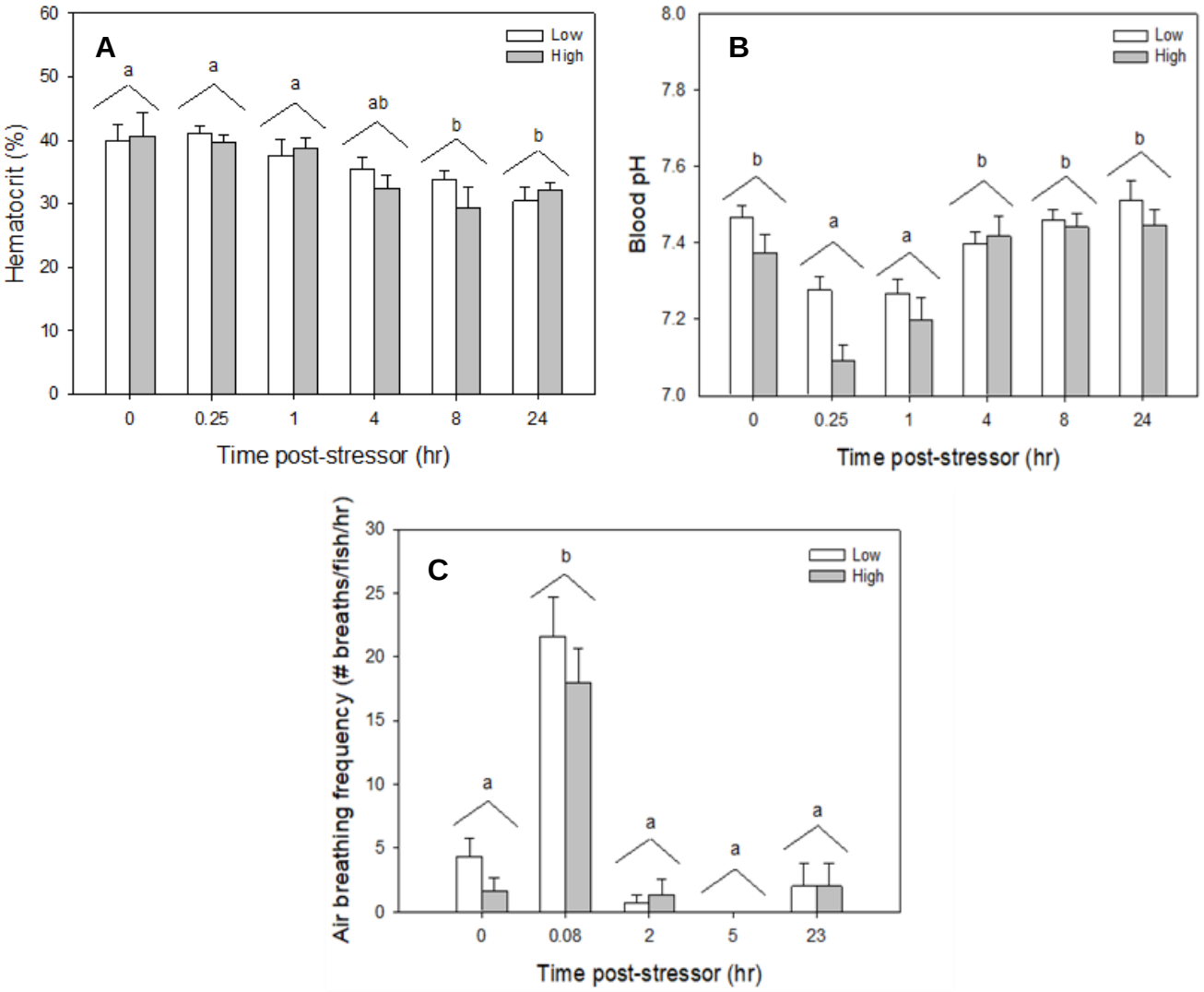

Figure 5. Atlantic tarpon (*Megalops atlanticus*) mean + standard error (A) blood hematocrit (%), (B) blood pH, and (C) air breathing frequency before and at sequential times following a low- or high-intensity simulated angling stressor (n = 67). Different letters indicate statistical differences.

Chapter 3: Effects of temperature and salinity on blood chemistry and survival of juvenile Atlantic tarpon

Introduction

Tarpon are large migratory, elopiform fish (Family Megalopidae) of ecological importance in tropical, subtropical, and temperate waters of the Atlantic Ocean where they support valuable recreational fisheries (Wade, 1962; Crabtree *et al.*, 1995, 1997). Tarpon demonstrate a dependence on upper estuarine habitats during their early life history. These habitats include brackish lagoons, tidal creeks, stagnant pools, backwaters, ephemeral coastal ponds, hurricane and storm over washes, swales, mangrove swamps, marshes, man-made mosquito impoundments, and artificial wetlands (Wade, 1962; Rickards, 1968; Jud *et al.*, 2011; Graham *et al*., 2017; Mace *et al.*, 2018; Wilson *et al.*, 2019) which have a wide range of temperature, salinity, pH, and dissolved oxygen concentrations (Geiger *et al.*, 2000). Salinity is one of the most influential abiotic factors determining the composition of estuarine fish communities (Cyrus and Blaber, 1992; Barletta *et al.*, 2005). Estuarine ecosystems are subject to seasonal fluctuations in salinity, primarily due to freshwater discharge (Conroy *et al.*, 2020), and vertical and horizontal salinity stratification is a common feature of these systems (Schroeder *et al.*, 1990). However, tarpon are facultative air breathers and possess euryhaline osmoregulatory capabilities that facilitate occupancy of these habitats (Wade, 1962; Adams *et al.*, 2013). Among juvenile tarpon habitats are those that provide access for later emigration as sub-adults to deeper coastal waters (Ault, 2007), such as sounds, bays, and bayous. Juvenile and sub-adult tarpon are thought to depend on these estuarine habitats for approximately seven to ten years (Kurth *et al.*, 2019), followed by an ontogenetic habitat shift to coastal waters as they approach maturity, where they presumably join the adult stock. Coastal waters are relatively well oxygenated, yet adult tarpon exhibit behaviors associated with gas bladder ventilation, such as surfacing, rolling, and bubble release, presumably for orientation and social and reproductive interactions (Seymour *et al.*, 2008).

The northern latitudinal range of Atlantic Tarpon is primarily limited by their thermophilic physiology (Overstreet, 1974; Howells and Garrett, 1992). Estuaries in U.S. waters that are utilized by tarpon as nursery areas extend into the northern Gulf of Mexico (GOM) and along the South Carolina and North Carolina coasts (Wade, 1962; Franks *et al.*, 2013; Graham *et al.*, 2017; Mace *et al.*, 2020), where juveniles can be exposed to lethally low water temperatures during winter months (Rickards, 1968; Franks *et al*., 2013; Graham *et al.*, 2017, Mace *et al.*, 2020). Mace *et al.* (2017) investigated thermal threshold requirements for juvenile tarpon in South Carolina estuaries and reported that their findings, combined with all published data on the cold tolerance of juvenile tarpon, resulted in an overall mean ± SD minimum lethal temperature of 12.0 ± 2.8 °C. However, there is limited information on the synergistic effects that salinity and temperature can have on haematology, osmoregulation, and survival of tarpon. Blood

chemistry can be used to measure physiological well-being under different environmental conditions (Kirk, 1974; Fajt and Grizzle, 1998; Dinken *et al.,* 2020), and physiological adaptive capacity plays a central role in responses to environmental changes (Young *et al.,* 2006). The objective of this study was to examine the combined effects of water temperature and salinity on blood physiochemical variables, osmoregulation, and survival of juvenile tarpon. Temperatures and salinities selected for the study (15 and 25°C; ≤2 and ≥30 ppt) were based on environmental metrics associated with reported juvenile tarpon collections in U.S. GOM and South Atlantic coastal habitats (Stein *et al.,* 2016, Graham *et al.,* 2017, Mace *et al.,* 2020).

Methods

Fish Source and Acclimation

Wild juvenile tarpon (n=50) were collected from Mississippi Sound estuarine salt marsh tidal sloughs in September and October 2020 using monofilament cast nets (0.9–3.0 m radius, 0.9–1.6 cm mesh). The specimens were immediately placed in a 45-L holding container with aerated water from the collection sites, transferred to an aquaculture facility located at the University of Southern Mississippi, Gulf Coast Research Laboratory (USM-GCRL), then transported to Mississippi State University's South Farm Aquaculture Facility for experimentation. Fish were reared over a period of 10 months in large (3,600-L) recirculating aquaculture systems (RAS) where they were fed to satiation initially daily and after several months every other day using small pieces of fresh catfish *Ictalurus* spp. During this period, temperature was maintained at 23°C and salinity at 19 ppt. Following this period and two months prior to experiments, conditions were held at 28°C and 16 ppt for a one-month period, then conditions were gradually adjusted to 25°C at a rate of 1°C/day and to either ≥30 ppt or ≤2 ppt (n=2 tanks/treatment) at a rate of 1 ppt/day.

All fish were implanted with a passive integrated transponder (PIT) tag (8 mm long x 1.4 mm in diameter, 0.03 g, 134.2-kHz ISO FDXB, Biomark Inc., Boise, ID, USA) in the left dorsal musculature anterior to the dorsal fin using a MK165 implanter (Biomark Inc.) with a 16-gauge, 50-mm stainless steel injector needle (N165, Biomark Inc.). Before implantation, food was withheld for 48 hours and the fish were anaesthetized in a buffered anaesthetic bath (150 mg/L tricaine methanesulfonate [MS-222], 9 g/L NaCl, and 400 mg/L $NaHCO_3$), then measured for total length (nearest mm) and weighed (wet, nearest 0.01 g).

Fish were held in four 3,600-L RAS and acclimated to 25°C, with two tanks maintained at ≤2 ppt and two tanks maintained at ≥30 ppt for >2 weeks. Following acclimation, blood was collected from fish in the treatment tanks (n=7/tank). Afterwards, temperatures were decreased at a rate of 1°C/day in all tanks until 15°C was reached, and blood was collected (n=7/tank) after fish were acclimated for >2 weeks. This methodology provided duplicate tanks for each

treatment combination. Water quality parameters (temperature, dissolved oxygen, and salinity) were monitored approximately every other day (Pro2030, YSI Inc., Yellow Springs, OH, USA).

Blood Collection

Fish were anesthetized in a buffered bath (same as stated above), measured for fork length and total length (nearest mm), and wet weight (nearest 0.01 g). Fish were placed into the supine position onto a wet surface and blood samples (~0.5 mL) were collected from the caudal vein using a heparinized syringe or vacutainer and a 22 to 25-gauge hypodermic needle. Blood was then transferred to a 1.5-mL microcentrifuge tube and placed onto ice. Immediately following collection, pH was measured in whole blood using a microelectrode (Accumet AB15, Fisher Scientific, Hampton, NH, USA) in a water bath set at the treatment temperature. Hematocrit (Hct), haemoglobin (Hb), and red blood cell counts (RBC) were also measured from whole blood. Hematocrit was measured by filling blood into a 75-mm heparinized capillary tube (Drummond Scientific Company, Broomall, PA, USA), centrifuging at 6,000 rcf for five minutes, and read using a microcapillary tube reader as the ratio of red blood cells to the total volume of blood. Haemoglobin was analyzed with Drabkin's reagent (Sigma-Aldrich, St. Louis, MO, USA) and RBC concentration was determined at a 1:200 dilution with saline using a hemocytometer and an automated cell counter (Cytosmart, Corning Cell Counter, Glendale, AZ). From the whole blood samples, 50-200 μL were transferred into a 0.6-mL microcentrifuge tube and centrifuged for 3 minutes at 5,000 x *g*. Plasma was collected, stored at -80°C, and later used to measure osmolality via a vapor pressure osmometer (Vapro 5520, Wescor Inc., Logan, UT, USA).

Loss of Equilibrium (LOE)

Following blood collection at 15°C, temperatures were maintained at 15°C for an additional 3 weeks before loss of equilibrium (LOE) experimentation. After 2 weeks, or approximately 1 week before experimentation, most fish stopped feeding, presumably due to exposure to the prolonged cool temperature. During this period, mortality occurred in both treatments, with approximately 20% mortality at ≥30 ppt and approximately 70% mortality at ≤2 ppt. Mortality events during this time affected sample size availability for LOE experiments.

For LOE experiments, 4-5 fish were transferred into two 950-L, 1.5-m diameter tanks per trial. Fish were placed in the tanks at the same salinity and temperature as the acclimation tanks the night prior to experiments (17:00-21:00). During the night, water was recirculated to the two tanks, which drained into a 340-L sump tank where a 1,700-W heater maintained water temperature at 15°C. A magnetic-drive pump moved water from the sump tank to each of the two test tanks. Tanks were covered by a net and blue 1.3-cm foam board insulation, which reduced light, external stimuli, and helped to maintain water temperature. Each tank had a ½-HP, 115-Volt chiller with a programmable thermostat (Cyclone Drop-In, Aqualogic Inc., San Diego, CA, USA), and air stones supplied with forced air. Water temperature in the tanks was decreased at 0.9 ± 0.1°C/hr starting at 08:00-09:00 on the following day.

For both high salinity (n=20 fish) and low salinity (n=8 fish) treatments, temperature and dissolved oxygen were measured every 30 minutes. Fish were monitored initially every 30 minutes and at approximately 10-minute intervals after the first fish lost equilibrium. Loss of equilibrium was determined by an inability to maintain an upright position in the water column. When a fish lost equilibrium, water temperature in the tank was measured, and the fish was quickly removed, scanned for PIT tag number, and released into the acclimation tank. Dissolved oxygen concentration during the LOE experiments was maintained above 7 mg/L and above 80% saturation for both salinity treatments.

Statistical Analyses

Statistical analyses were performed using RStudio 4.0.3 (R Core Team, 2022) at a significance level of $\alpha = 0.05$. Normality and homogeneity of variance were tested on residuals with Shapiro-Wilk and Levene's tests, respectively. Data were $\log_{10}$ transformed to meet these parametric assumptions, as needed. A two-way analysis of variance (ANOVA), with factors of temperature (15 and 25°C) and salinity (≤2 and ≥30ppt), was used to analyze fork length, total length, weight, and blood physiochemical variables. Following a significant ANOVA, group means were compared using Tukey's Honestly Significant Difference (HSD) test. A student's *t*-test was used to compare LOE between different salinities (≤2 and ≥30 ppt). Data are reported as mean ± standard error (SE).

Results

Water quality variables in rearing tanks during the acclimation period are shown in Table 1. There were no differences in total length ($F_{1,52}$=0.132, P=0.7179) or wet weight ($F_{1,52}$=0.438, P=0.5110) among temperature and salinity treatments at the beginning of experimentation (Table 2).

For blood physiochemical variables, the interaction between temperature and salinity was significant for pH ($F_{1,52}$=24.433, P<0.001) (Figure 1A). The pH varied between salinity concentrations at cold temperature (15°C) but not at high temperature (25°C). The interaction between temperature and salinity was not significant for Hb ($F_{1,42}$=0.441, P=0.5100), Hct ($F_{1,52}$=0.176, P=0.6770), RBC concentration ($F_{1,24}$=0.081, P=0.7785), MCV ($F_{1,24}$=2.213, P=0.1499), MCH ($F_{1,24}$=1.4982, P=0.2328), MCHC ($F_{1,42}$=0.2931, P=0.5911), or plasma osmolality ($F_{1,52}$=1.451, P=0.2339). However, a main effect of temperature was observed for Hct ($F_{1,52}$=28.622, P<0.001) and RBC concentration ($F_{1,24}$=8.349, P=0.0081), and a main effect of both temperature ($F_{1,52}$=11.661, P<0.001) and salinity ($F_{1,52}$=49.211, P<0.001) was observed for plasma osmolality (Figure 1B). A significantly lower Hct was observed at both 15°C treatments, and elevated plasma osmolality was observed for both temperature treatments at ≥30 ppt compared to the same temperature at ≤2 ppt. Loss of equilibrium occurred at a lower temperature

for fish acclimated to 30 ppt salinity and at a higher temperature for fish acclimated to 1 ppt salinity (Figure 2).

Discussion

Temperature and salinity are essential physical components forming abiotic environmental conditions for aquatic fauna. Each fish species has a range of optimal conditions (e.g., fundamental niche) typically defining the habitats they occupy (Craig and Crowder, 2002; Bacheler *et al.*, 2009; Stevens *et al.*, 2022), although conditions outside of optimal ranges may be occupied due to energetic benefits of increased forage opportunities or refugia (e.g., realized niche) (Freitas *et al.*, 2016; Brownscombe *et al.*, 2022). Temperatures and salinities above and below the optimum range can affect metabolic and immune mechanism performance, hormonal control, blood composition, osmoregulatory energy expenditure, gill Na^+/K^+-ATPase activity, feed consumption rate and efficiency, digestion times, and survival (Stuenkel and Hillyard, 1981; Kolok and Sharkey, 1997; Imsland *et al.*, 2001; Sampaio and Bianchini, 2002; Cuesta *et al.*, 2005; Resley *et al.*, 2006; Saoud *et al.*, 2007; Soegianto *et al.*, 2017; Galkanda-Arachchige *et al.*, 2021).

For euryhaline fishes, the capacity to regulate internal solutes in the face of daily and seasonally changing environments is vital for function and survival, particularly for those species inhabiting estuarine environments. In this context, blood plasma osmolality is an excellent measure of overall regulatory ability and well-being (Kolok and Sharkey, 1997; Sampaio and Bianchini, 2002). In this study, plasma osmolality of juvenile tarpon was elevated at low temperature (15°C compared to 25°C) and at high salinity (≥30 ppt compared to ≤2 ppt). Although moderately increased osmolality in hyperosmotic environments is typical in fishes (Holmes and Donaldson, 1969; Marshall, 2012; Edwards and Marshall, 2013), high blood osmolality can indicate an osmoregulatory imbalance, which can relate directly to limitations in habitat occupancy (McCormick *et al.*, 1987; Kammerer *et al.*, 2010; Tsui *et al.*, 2012). Low temperature has been shown to be problematic for osmoregulation in fishes (Thomsen *et al.*, 2007; Anderson and Scharf, 2014; Allen *et al.*, 2017), due in part to effects on cellular membrane fluidity (Hochachka and Somero, 2002) and gill Na^+/K^+-ATPase activity (Sardella *et al.*, 2007; Hansen *et al.*, 2022). The effects of cold stress in warm-water euryhaline species may be reduced by acclimation to seawater, in part through reduced effects on gill Na^+/K^+-ATPase activity (Kang *et al.*, 2015). The relative level of low temperature where effects are incurred are species-specific and may be exacerbated by short-term exposure to rapid decreases in temperature (Beitinger *et al.*, 2000; Donaldson *et al.*, 2008; Anderson and Scharf, 2014). For juvenile tarpon, survival has been shown to diminish at low temperatures under laboratory conditions (Mace *et al.*, 2017) and during rapidly dropping temperatures from cold fronts in the wild (Storey and Gudger, 1936; Overstreet, 1974; Franks *et al.*, 2013; Graham *et al.*, 2017).

Haematological and blood physiochemical variables are helpful for understanding osmoregulatory ability and adaptive capacity. Perhaps the most telling blood variable in our study was pH, which was highest at low temperature and low salinity but also elevated in low temperature and high salinity. Acid-base balance, which is indicated by blood pH, is extremely important to physiological processes, and extreme pH has been related to temperature effects and survival in other species (Smit *et al.*, 1981; Stewart *et al.*, 2019; Dinken *et al.*, 2022). In air-breathing fishes, blood pH is typically related to using the air-breathing organ with decreased pH resulting from greater reliance on air-breathing (Graham, 1997). This is due to a reduction of blood flow to the gills, the primary site of CO_2 excretion, with increased reliance on air breathing, therefore increasing retention of CO_2 and reducing blood pH (Shartau and Brauner, 2014). Thus, the observed increase in blood pH at low temperatures in tarpon could be partly due to the lack of use of the air-breathing organ due to cold temperatures, as evidenced in other fishes with bimodal respiration (Rahn *et al.*, 1971; Damsgaard *et al.*, 2018). Although not quantified, our observations indicated that air-breathing frequency in tarpon was greatly diminished at 15°C compared to 25°C, a similar trend to reported quantifications (Geiger *et al.*, 2000). Additionally, the highest blood pH at low temperature and low salinity may be due partly to impaired osmoregulation, possibly manifested in part by reduced internal HCO_3^- exchange for external Cl^- (Cameron, 1978). Several other blood variables also had diminished values at low temperature, with decreases in RBC concentration, Hct, and Hb, with the lowest value of Hb in the low temperature and low salinity treatment. These measures all relate to blood oxygen capacity, although lower temperatures are typically associated with higher oxygen availability and a decreased metabolic oxygen demand in ectothermic species. Changes in blood composition due to temperature, salinity, or a combination of both, have been reported for various fishes (Toneys and Coble, 1980; Adeyemo *et al.*, 2003; Ziegeweid and Black, 2010; Soegianto *et al.*, 2017). Interestingly, salinity had no effect on haematological variables at the higher temperature treatment (25°C), likely because *Megalops* spp. are broadly adapted to tropical and sub-tropical temperatures (>20°C) (Chen *et al.*, 2008; Luo and Ault, 2012; Luo *et al.*, 2020).

Results from the LOE experiment indicate tarpon can tolerate lower temperatures under a hyper-osmotic environment (≥30 ppt) than in a hypo-osmotic environment (≤2 ppt), as evidenced in other fishes (Zale and Gregory, 1989; Atwood *et al.*, 2001; Anweiler *et al.*, 2014). In predominantly marine species, this may be partially due to the increased difficulty to osmoregulate in a hypo-osmotic environment, which leads to a passive osmotic influx of water and diffusive loss of ions, which can lead to mortality (e.g., red drum *Sciaenops ocellatus,* Crocker *et al.*, 1981; cobia *Rachycentron canadum,* Resley *et al.*, 2006; rabbitfish *Siganus rivulatus,* Saoud *et al.*, 2007; red snapper *Lutjanus campechanus,* Galkanda-Arachchige *et al.*, 2021). Our results are similar to prior lower thermal tolerance studies conducted on smaller (length range: 83-239 mm SL) juvenile tarpon (mean ± SD: 12.0 ± 2.8°C; Mace *et al.*, 2017).

Euryhaline capabilities allow juvenile tarpon to inhabit many estuarine habitats regardless of ambient salinity. Juveniles are able to maximize prey availability and seek refuge from

predators, cool temperatures, and natural events (e.g., storms and plankton blooms) by moving across salinity gradients. Osmoregulation in fishes can have high energy demands, which may account for >50% of the total energy budget (Bœuf and Payan, 2001), therefore, the ability to regulate plasma osmolality is an important physiological mechanism that allows fish to perform routine activities under varying environmental salinities.

We conclude that low salinity has a synergistic effect with low temperature on the survival of juvenile tarpon. Juveniles can tolerate lower temperatures when inhabiting hyper-osmotic environments, suggesting they can increase overwintering survival by utilizing these habitats during winter months when temperatures drop <20°C in the latitudinal range of their nursery distribution. Alternatively, mortality may be high if juvenile tarpon are trapped in low salinity habitats during the occurrence of extreme cold weather events.

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Table 1: Mean (± SE) water quality variables of tarpon rearing tanks during acclimation.

Acclimation period	Trt-Temp	Trt-Sal	DO (mg/L)	DO (%)	Temp (°C)	Salinity (ppt)
week-1	High	High	6.36 ±0.06	88.8 ±1.0	23.6 ±0.6	30.6 ±0.9
week-2			6.08 ±0.09	89.3 ±1.5	25.0 ±0.1	30.7 ±0.9
sample day			6.04 ±0.15	89.9 ±0.4	25.3 ±0.2	30.0 ±0.3
week-1	High	Low	7.18 ±0.04	85.8 ±0.8	23.8 ±0.1	2.0 ±0.2
week-2			7.04 ±0.02	87.5 ±0.1	25.3 ±0.3	1.0 ±0.0
sample day			7.08 ±0.19	89.7 ±1.8	25.7 ±0.3	1.0 ±0.0
week-1	Low	High	7.63 ±0.14	94.5 ±2.2	16.2 ±0.4	31.2 ±0.3
week-2			8.25 ±0.11	99.0 ±1.2	14.8 ±0.0	31.3 ±0.2
sample day			8.93 ±0.33	107.3 ±4.3	14.5 ±0.0	31.4 ±0.0
week-1	Low	Low	8.96 ±0.00	92.7 ±0.4	16.5 ±0.1	1.1 ±0.0
week-2			9.93 ±0.05	99.2 ±0.2	14.9 ±0.1	1.2 ±0.0
sample day			10.57 ±0.03	105.1 ±0.0	14.3 ±0.2	1.1 ±0.0

Trt = treatment, Temp = temperature, Sal = salinity, DO = dissolved oxygen. Water samples were collected from two tanks for each treatment 7-12 times during acclimation.

Table 2. Mean (± SE) blood characteristics of tarpon acclimated to different temperatures (15 and 25°C) and salinities (≤2 and ≥30 ppt).

Trt Temp (°C)	Trt Salinity (ppt)	Fork Length (mm)	Total Length (mm)	Wet weight (g)	Hb (g/dL)	Hct (%)	RBC (x 10^9 cells/L)	MCH (pg/cell)	MCV (fL)	MCHC (g/dL)
15	≤2	404 ± 11	462 ± 12	888 ± 82	11.17 ± 1.77^a	29 ± 1^a	2.52 ± 0.15^a	45.3 ± 9.5	106 ± 11	39.5 ± 6.5
15	≥30	372 ± 16	425 ± 17	694 ± 91	12.52 ± 0.73^b	28 ± 2^a	2.32 ± 0.10^a	59.6 ± 1.1	125 ± 2	45.5 ± 2.4
25	≤2	390 ± 12	446 ± 14	743 ± 80	12.80 ± 0.54^c	36 ± 1^b	2.91 ± 0.12^b	45.4 ± 2.9	130 ± 4	35.4 ± 1.1
25	≥30	367 ± 18	420 ± 21	663 ± 94	12.96 ± 0.55^c	34 ± 1^b	2.79 ± 0.13^b	47.8 ± 3.4	124 ± 9	38.2 ± 1.2

Trt = treatment, Temp = temperature. Due to partial clotting of blood prior to quantification of red blood cell (RBC) concentration in some samples, the number of samples for that particular assay, and calculations directly related (MCH and MCV) were reduced to N = 4-10/treatment. Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$).

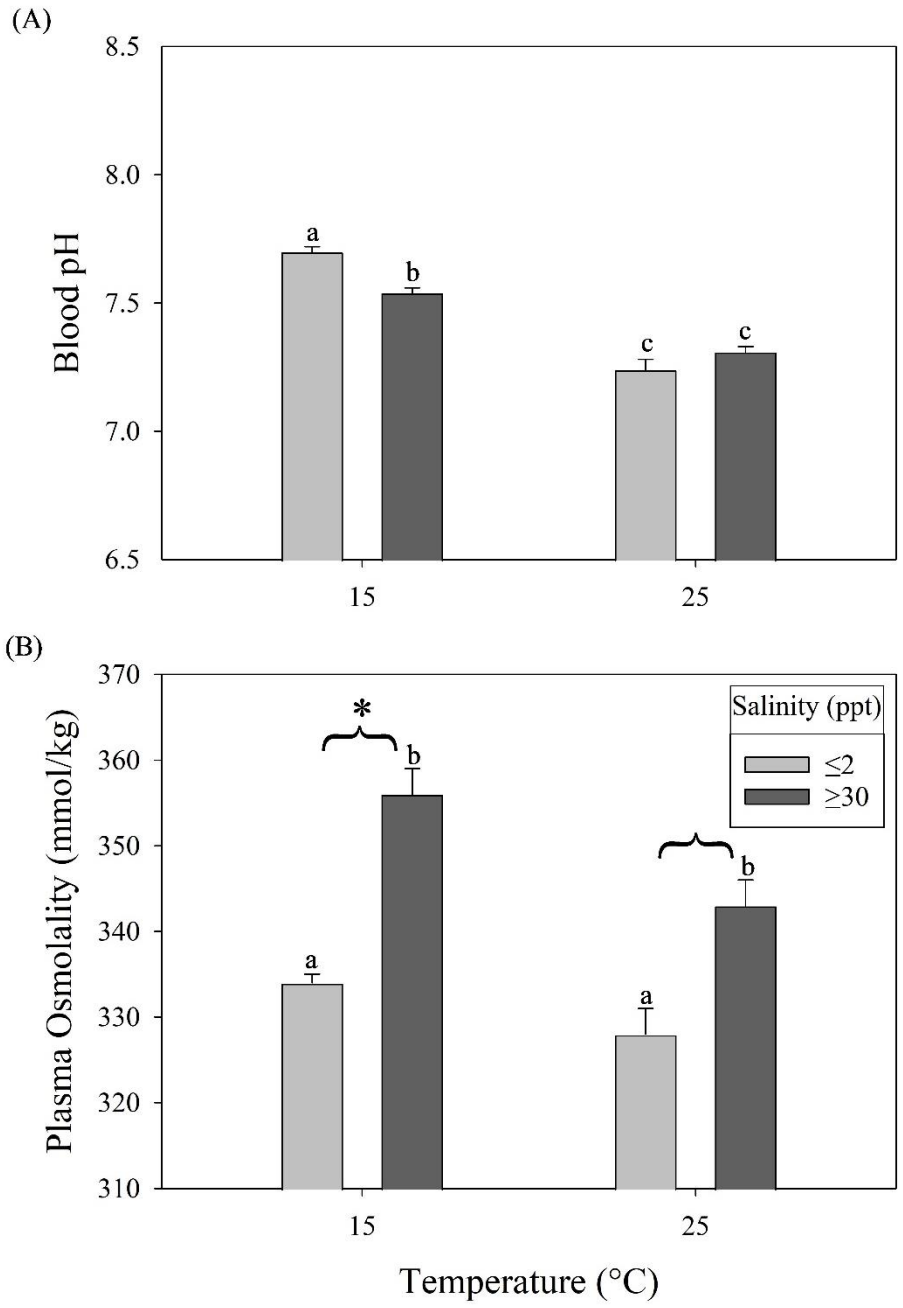

Figure 1. Mean (± SE) blood pH (A) and plasma osmolality (B) of tarpon acclimated to different temperatures and salinities. In (A) different letters indicate differences among individual treatment groups. In (B) different letters indicate statistical differences between salinity treatments, while asterisk denotes statistical differences between temperatures (ANOVA; $P<0.05$, n=14/treatment).

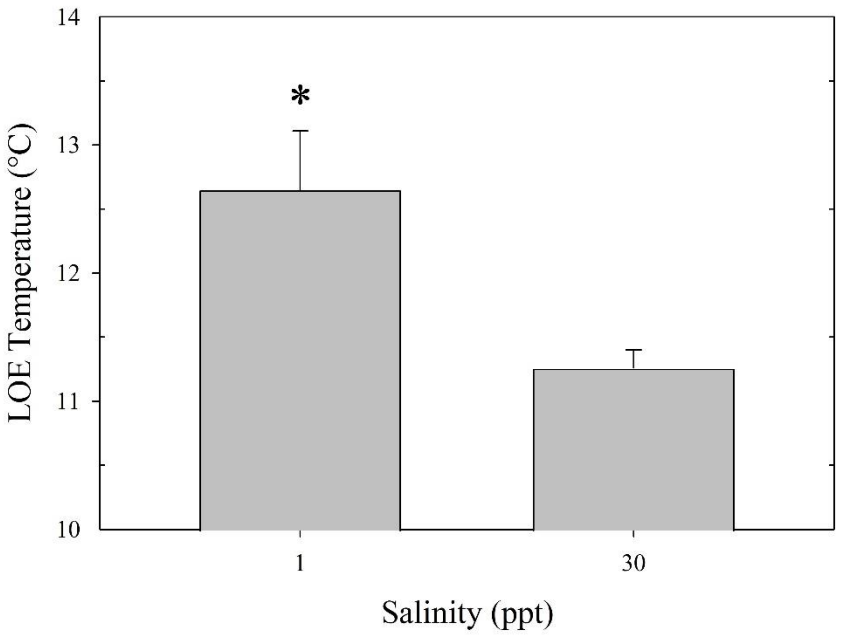

Figure 2. Mean (± SE) loss of equilibrium of tarpon acclimated to different salinities. Asterisk indicates statistical differences between salinity treatments (*t*-test; $P<0.05$, n=8 for 1ppt and n=20 for 30ppt).

Products and Publications

Publications

Horowitz, L. B., P. J. Allen, J. W. Neal, and S. B. Correa. 2023. Post-release mortality of angled Atlantic Tarpon *Megalops atlanticus* in Puerto Rico. Marine and Coastal Fisheries 15:e10238 (online).

Coffill-Rivera, M. E., Y. Paez Mendez, L. Little, P. M. Graham, J. S. Franks, S. B. Correa, J. W. Neal, and P. J. Allen. (*In press*). Effects of temperature and salinity on blood chemistry and survival of juvenile Atlantic tarpon *Megalops atlanticus*. Journal of Fish Biology. (Submitted 1 March 2023).

Horowitz, L. B., P. J. Allen, J. W. Neal, and S. B. Correa. (*In preparation*). Physiological impact of simulated and wild catch-and-release fishing on Atlantic Tarpon.

Presentations

Neal, J. W., L. B. Horowitz, S. B. Correa, and Peter J. Allen. 2023. Effects of catch-and-release fishing on Atlantic tarpon (*Megalops atlanticus*) in Puerto Rico. Annual Meeting of the Mississippi Chapter of the American Fisheries Society. Natchez, Mississippi.

Neal, J. W., L. B. Horowitz, S. B. Correa, and Peter J. Allen. 2022. Remediating release mortality of Atlantic Tarpon in Puerto Rico. Annual Meeting of the Puerto Rico Chapter of the American Fisheries Society. Río Piedras, Puerto Rico.

Paez Mendez, Y., P. M. Graham, J. S. Franks, Jr., J. W. Neal, and P. A. Allen. 2022. The effects of temperature and salinity on juvenile Atlantic Tarpon *Megalops atlanticus*. Annual Meeting of the Mississippi Chapter of the American Fisheries Society. Hattiesburg, Mississippi.

Horowitz, L. B., P. J. Allen, J. W. Neal, J. S. Franks, P. M. Graham, and S. B. Correa. 2022. Physiological response of Atlantic Tarpon to catch-and-release angling. Annual Meeting of the Southern Division of the American Fisheries Society. Charleston, South Carolina.

Neal, J. W., L. B. Horowitz, S. B. Correa, and Peter J. Allen. 2022. Effects of catch-and-release fishing on Atlantic tarpon (*Megalops atlanticus*) in Puerto Rico. Annual Meeting of the American Fisheries Society. Spokane, Washington.

Horowitz, L. B., P. J. Allen, S. B. Correa, J. S. Franks, P. M. Graham, and J. W. Neal. 2021. Stress response and recovery of tarpon to catch-and-release angling. Mississippi Chapter of the American Fisheries Society, Virtual.

Outreach Materials

Neal, J. W., L. B. Horowitz, S. B. Correa, and Peter J. Allen. 2022. How to improve release survival of tarpon – Cómo mejorar la sobrevivencia después de soltar un sábalo. Poster distributed to boat ramps, tackle shops, and marinas. (See next page).

How to Improve Release Survival of Tarpon

Cómo Mejorar la Sobrevivencia Después de Soltar un Sábalo

1. Gear Up! *¡Prepárese!*

Use heavy action rod/reel/line combos when possible to reduce fight time. Always use circle hooks with natural bait to prevent deep hooking.

Use una combinación de caña/carrete/línea de acción intensa cuando sea posible para reducir el tiempo de pelea. Utilice siempre anzuelos circulares con carnada natural para evitar enganches profundos.

2. Keep Wet! *¡Mantengalo húmedo!*

Air exposure is harmful to fish. When possible, keep tarpon in the water to support their weight while removing hooks and taking photos.

La exposición al aire es dañina para los peces. Cuando sea posible, mantenga el sábalo en el agua para soportar su peso y retire el anzuelo y tome fotos en el agua.

3. Support! *¡Sosténgalo!*

If you must lift fish from the water for hook removal or photography, carefully support the head and abdomen with both hands, especially with big fish to prevent internal damage and bone fractures.

Si debe sacar el pez del agua para quitarle el anzuelo o fotografiarlo, sujete con cuidado la cabeza y el abdomen con ambas manos; particularmente con los peces grandes para evitar daños internos.

MISSISSIPPI STATE UNIVERSITY EXTENSION

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