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Physiological stress in the smalltooth sawfish: effects of ontogeny, capture method, and habitat quality

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ABSTRACT: Similar to other elasmobranchs, the smalltooth sawfish *Pristis pectinata* is slow growing, matures late in life, and produces relatively few young, all factors that have contributed to its sensitivity to dramatic population declines from overfishing and habitat loss. Currently, the physiological stress response of these fish to capture or to other physiological challenges such as habitat loss, climatic changes, or pollution is unknown. In the absence of these data, conservation plans may be less effective, making populations susceptible to further declines. We examined basic stress physiology over ontogeny and as a function of capture using different fishing gears. We also examined stress parameters to test whether degraded habitat and water quality from altered habitats may have resulted in chronic stress in juveniles. Results suggested that the stress response to capture by all methods was low, particularly for blood lactate, compared to other elasmobranchs examined to date. Metabolic stress was found to change over ontogeny, with young of the year (YOY) eliciting the highest responses. Glucose, pCO_2, bicarbonate, potassium, and hematocrit indicated that gillnet capture induced greater stress responses than longline capture. Significantly higher metabolic stress was observed in YOY and juveniles captured in the 2 nurseries most influenced by anthropogenic activities, the Peace and Caloosahatchee rivers, than in the 2 relatively pristine nurseries in Everglades National Park. Overall, the low physiological stress responses to all capture methods investigated in this study suggest that this species is resilient, which should promote optimism for recovery of the population.

KEY WORDS: *Pristis pectinata* · Stress physiology · Metabolic stress · Chronic stress · Anthropogenic effects · Nursery · Habitat loss · Blood chemistry

INTRODUCTION

The Pristidae, a small family (5 species) of batoids, is considered the most imperiled of all shark and ray families (Dulvy et al. 2014), and sawfishes are perhaps the most endangered marine fishes in the world (Wueringer et al. 2009, Dulvy et al. 2016). The smalltooth sawfish *Pristis pectinata* (Latham 1794) occurs in tropical and subtropical waters within the Atlantic basin (Faria et al. 2013). *Pristis pectinata* are born at a stretch total length (STL) of approximately 70 cm, and reach maturity at around 340 cm for males and 380 cm STL for females, although this species is thought to grow to over 520 cm STL (Poulakis et al. 2011, R. D. Grubbs & J. Gelsleichter unpubl. data).

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Young of the year (YOY) and smaller juvenile *P. pectinata* inhabit shallow estuaries and coastal bays for the first few years of life until reaching around 220 cm STL (Seitz & Poulakis 2002, Poulakis & Seitz 2004, Simpfendorfer et al. 2008, 2010). Adults occupy habitats ranging from shallow coastal estuaries to depths of approximately 100 m on the continental shelf (Seitz & Poulakis 2002, Poulakis & Seitz 2004, Carlson et al. 2014, Waters et al. 2014). The historic range of *P. pectinata* in the United States was from Texas to New York (Poulakis & Seitz 2004), though the core of the range is southwest Florida. The overall range was reduced dramatically in the last half of the 20th century, and by 2000, reports of sightings outside southwest Florida were uncommon (Seitz & Poulakis 2002, Poulakis & Seitz 2004, Wiley & Simpfendorfer 2010, Waters et al. 2014). Two major nursery regions exist in Florida and were federally designated as Critical Habitat for juveniles: the Charlotte Harbor Estuary Unit and the Ten Thousand Islands National Wildlife Refuge Everglades National Park (TTI/ENP) Unit (Norton et al. 2012). The Charlotte Harbor Estuary Unit is a highly anthropogenically influenced nursery, whereas the TTI/ENP Unit is relatively pristine. In the Charlotte Harbor Estuary Unit, the Caloosahatchee River has been highly altered by the creation of extensive canal systems, and its freshwater flow is regulated through a lock system (Barnes 2005). In contrast, the Peace River is less developed with more natural shorelines, and has unaltered freshwater flow (Poulakis et al. 2011). Within the TTI/ENP Unit, Goodland, Chokoloskee, and Everglades City have some anthropogenic development, and lower Everglades National Park has almost no development (Hollensead et al. 2016).

In the United States, *P. pectinata* experienced population declines because of bycatch in gillnet and trawl fisheries, direct harvest for the rostra, and habitat loss. Overfishing and habitat loss are believed to be responsible for up to a 95% decline in the *P. pectinata* population in the United States over the 20th century (Simpfendorfer 2000, Norton et al. 2012). *Pristis pectinata* were commonly captured in net fisheries in the 19th and 20th centuries, because their toothed rostra easily became entangled in fishing gear (Seitz & Poulakis 2006). If the fish were still alive when captured, they were likely killed because they were a nuisance species and because their rostrum could be sold in the curio trade (Simpfendorfer 2000, 2005, Wiley & Simpfendorfer 2007, 2010). Because bycatch was not well recorded in fishery statistics, population declines went unnoticed for many years (Seitz & Poulakis 2006). Population declines were also likely a result of habitat loss to urban development of mangrove shorelines and adjacent seagrass habitats that *P. pectinata* relied upon (Simpfendorfer 2000, Seitz & Poulakis 2002, Poulakis & Seitz 2004, Simpfendorfer 2005, Seitz & Poulakis 2006, Wiley & Simpfendorfer 2010). *Pristis pectinata* are now listed by the IUCN as Critically Endangered, endangered under the US Endangered Species Act (ESA), and under CITES Appendix I, which bans international trade (NMFS 2003, 2009, Carlson et al. 2013).

Despite being listed as endangered, *P. pectinata* are still negatively affected by anthropogenic influences. For example, *P. pectinata* are often encountered by recreational rod and reel fishers that target other species (Poulakis & Seitz 2004), and in bottom longline fisheries that target sharks (Enzenauer et al. 2016). While gillnet fisheries were eliminated in Florida waters in 1994 (FWC 1999, NMFS 2003), there has been one incident of a *P. pectinata* captured in shark gillnets in federal waters (Carlson & Baremore 2003), and as the population of *P. pectinata* begins to recover, there will likely be more interactions with this fishery. Furthermore, *P. pectinata* are still captured as bycatch in the shrimp trawl fishery, which is likely the largest source of direct fishing mortality (Simpfendorfer 2000, NMFS 2009, Carlson & Scott-Denton 2011). In addition to continued fisheries interactions, *P. pectinata* are also entangled in marine debris (Seitz & Poulakis 2006), and mortalities and purposeful injuries, such as rostrum removal, still occur in the United States (Seitz & Poulakis 2006). Additionally, habitat loss within ESA-designated critical habitat, particularly in the Charlotte Harbor Estuary Unit, continues.

Anthropogenic stressors such as habitat degradation and fisheries interactions can cause acute and chronic stress, which may surpass stress that occurs from natural stressors such as seasonal changes in the environment, capturing prey, and avoiding predators (Skomal & Bernal 2010). Physiological stress responses are of interest because the physiology of an animal can determine its life history, behavior, and fitness (Ricklefs & Wikelski 2002, Wikelski & Cooke 2006). Much of the stress physiology research that has been conducted on teleosts has focused on the primary stress hormone, cortisol, which is readily quantifiable in teleosts; however, the primary stress hormone in elasmobranchs, 1α-hydroxycorticosterone, has yet to be validated (Anderson 2012). In fishes, including elasmobranchs, stressors are known to cause a series of other quantifiable physiological changes to the blood chemistry (Skomal & Bernal 2010). Blood-gases, acid-base status, and blood lac-

tate are most often used in studies of stress physiology to determine the condition of the fish following a stressor such as capture (Cliff & Thurman 1984, Wells et al. 1986, Harrenstien et al. 2005, Skomal 2007). These parameters can also be indicators of present or imminent mortality (Hoffmayer & Parsons 2001, Young et al. 2006, Arlinghaus et al. 2009). Furthermore, in teleosts, the stress response is known to change over ontogeny (Skomal & Mandelman 2012); however, variation in the stress response over ontogeny has only been investigated in a few species of elasmobranchs, and not over their entire size ranges (Mandelman & Skomal 2009).

The stress physiology parameters that are typically examined in elasmobranchs include glucose, partial pressure of CO_2 (pCO_2), lactate, bicarbonate, hematocrit, pH, and potassium. Blood glucose is measured as a proxy of the glucocorticoid stress response (Cliff & Thurman 1984, Hoffmayer & Parsons 2001, Skomal 2006, Frick et al. 2010a), during which gluconeogenesis occurs and stored hepatic glycogen is mobilized into the blood to serve muscle tissue (Barton & Iwama 1991, Hoffmayer & Parsons 2001). Respiratory stress results in elevated blood concentrations of pCO_2, and occurs as a result of decreased ventilation, which would otherwise expel excess carbon dioxide. This often occurs for elasmobranchs that are entangled in a gillnet and cannot properly ventilate, or for ram-ventilating elasmobranchs that have limited mobility when caught on a line (Manire et al. 2001, Mandelman & Farrington 2007). Increases in blood lactate, termed metabolic acidosis, result from an animal increasing its energetic demands (e.g. when evading predators, pursuing prey, or seeking suitable habitat) (Murdaugh et al. 1965, Rasmussen & Rasmussen 1967, Piiper & Baumgarten 1969, Piiper et al. 1972, Martini 1974, Cliff & Thurman 1984). This causes a switch from aerobic to anaerobic respiration in white muscle tissue, which results in the movement of lactate and H^+ ions from the muscle to the blood (Black 1958, Schmidt-Nielsen 1997, Skomal 2007). Previous work has identified bicarbonate as having the capacity to potentially buffer pH alterations caused by lactate (Holeton & Heisler 1983), indicated by decreasing levels of bicarbonate with increasing metabolic stress. High lactate in muscle tissue may also result in a compensatory mechanism called hemoconcentration (Piiper et al. 1972), which is the movement of fluid from the blood to the muscle to dilute these high concentrations, and can be quantified by observing increases in hematocrit. As a result of increases in pCO_2 and lactate, an overall blood acidosis can occur (Cliff & Thurman 1984, Hoffmayer & Parsons 2001, Spargo 2001, Lindinger et al. 2005, Robergs et al. 2004, Mandelman & Skomal 2009, Brooks et al. 2012). Elevated concentrations of potassium in the blood have been observed in stressed elasmobranchs (Cliff & Thurman, 1984, Wells et al. 1986, Manire et al. 2001), and occur from intracellular acidosis and the resulting efflux of potassium from muscle cells (Cliff & Thurman 1984, Moyes et al. 2006). The change in the potassium gradient can alter the excitability of muscle cell membranes (Adams & Galvan 1986), which has been shown to result in myocardial malfunction in spiny dogfish *Squalus acanthias* (Martini 1974), and has also been associated with neuromuscular interference (Cliff & Thurman 1984, Frick et al. 2010a).

Stress physiology can inform researchers of intraspecific or interspecific differences in the response and the major factors contributing to stress (Mandelman & Skomal 2009, Gallagher et al. 2010). With this knowledge, the stress response can be an indicator of population health (Creel et al. 1997, Wasser et al. 1997). Understanding the physiological response to stress in individual organisms can inform species-specific management for conservation (Ferguson & Tufts 1992, Wikelski & Cooke 2006, Young et al. 2006), but this has not been studied in any sawfish species. Because data regarding the physiological stress response of the critically endangered *P. pectinata* could be useful to species recovery, we investigated 3 main questions: (1) does the stress response change over ontogeny; (2) does capture method affect the stress response; and (3) does habitat quality affect stress physiology in YOY and juveniles?

MATERIALS AND METHODS

Smalltooth sawfish *Pristis pectinata* were sampled from the 3 research surveys that currently exist for the species in the United States, allowing us to sample the broadest geographic area possible, and the full size range of the species. These include the Florida State University (FSU) survey, the National Marine Fisheries Service (NMFS) survey, and the Florida Fish and Wildlife Conservation Commission (FWC) survey.

FSU survey

The ongoing FSU survey targeted large juvenile and adult *P. pectinata* using fishery-independent longlines consisting of a 4.0 mm monofilament main-

line that was anchored on each end and marked with a surface buoy bearing the permit numbers. Each mainline set was approximately 750 m long. A standard set included 50 gangions consisting of a stainless steel tuna clip with an 8/0 stainless steel swivel attached to 2.5 m of 300 kg monofilament that was doubled in the terminal 25 cm and attached to 16/0 non-offset circle hook. Hooks were baited with ladyfish *Elops saurus* or Spanish mackerel *Scomberomorus maculatus*. Depth (m), turbidity (cm), water temperature (°C), salinity, and dissolved oxygen (mg l^{-1}) were recorded from the surface to the bottom for all sets made in depths of less than 10 m, and bottom water temperature (°C) was recorded for those greater than 10 m deep. Soak times were 1 h to minimize mortality, and all lines were set during daylight hours. The line was hauled in the order and direction it was set and *P. pectinata* were sampled as they were caught during retrieval. Areas sampled included the Atlantic side of the Florida Keys from Key West to Islamorada and inside ENP from Florida Bay north to Ponce de Leon Bay.

Opportunistically, YOY and juvenile *P. pectinata* were also captured during this survey using rod and reel as well as a dip net. Hook size for rod and reel ranged from 10/0 to 16/0 circle hooks, and were baited with *E. saurus* or *S. maculatus*. Fight time for all rod and reel captures was less than 1 min. The dip-net-captured YOY *P. pectinata* was visually spotted in the shallows at Eagle Key in Florida Bay. The YOY swam into the large dip net, and was immediately restrained and sampled within 1 min.

NMFS survey

During NMFS surveys, gillnets were used to capture YOY and juveniles. Gillnets were 1.5 m deep and either 30.5 or 61.0 m long with stretched mesh sizes of either 7.6 or 10.2 cm, respectively. Nets had continuous float and lead lines, were anchored at each end with a 3.6 kg mushroom anchor, and marked with large surface buoys at each end. Depth (m), turbidity (cm), water temperature (°C), salinity, and dissolved oxygen (mg l^{-1}) were recorded at the beginning of each set. All sets were made during daylight hours. One net was fished at a time, monitored continuously, soaked for 1 h, and checked for catch every 0.5 h or immediately if any animal was observed in the gear. *Pristis pectinata* were untangled and sampled as soon as possible. Areas surveyed were in southwest Florida from Marco Island to Florida Bay, and were divided into 2 distinct geographic areas for analyses. Upper Everglades (UE) was denoted as the area from Marco Island southeast through the Ten Thousand Island National Wildlife Refuge and northern ENP. Florida Bay and the lower Everglades (FLBLE) was denoted as the area encompassing Whitewater and Coot bays, Flamingo, and Florida Bay.

FWC survey

Pristis pectinata were captured from the Peace and Caloosahatchee rivers using the methods described by Poulakis et al. (2011). Briefly, *P. pectinata* were captured in gillnets soaked for 1 h in areas where *P. pectinata* had recently been reported by the public or sites where they were previously caught. Depth (m), water temperature (°C), salinity, and dissolved oxygen (mg l^{-1}) were recorded at the beginning of each set. Nets were constantly monitored and checked when fishes of any type were seen in them (e.g. when splashing was observed) or every 0.5 h, whichever came first. When water clarity was favorable, *P. pectinata* were actively searched for and gillnets were used to catch any animals seen. After being untangled, captured *P. pectinata* were placed in the net well of the vessel or in tubs filled with ambient water and sampled as soon as possible. Dissolved oxygen concentrations were monitored and water changes occurred as necessary to maintain water quality.

Sampling and sample analyses

As soon as a *P. pectinata* was removed from the gear, it was restrained and a 1–5 ml blood sample was immediately collected, in 30 s or less, by caudal venipuncture using a 16–22 gauge needle attached to a heparinized syringe. To assess pCO_2, lactate, bicarbonate, and pH, a small aliquot of blood was immediately loaded into a CG4+ cartridge and then inserted into a VetScan i-STAT 1 point of care device (Abaxis), which has been validated for use in elasmobranchs (Mandelman & Farrington 2007, Mandelman & Skomal 2009, Gallagher et al. 2010). Glucose was then measured using an Accu-Chek glucose meter (Roche Diagnostics), which has been validated for use in fishes (Cooke et al. 2008). Blood samples were placed on ice in a cooler (4°C) for up to 12 h.

After blood sampling, all captured *P. pectinata* were measured (STL) and assessed for life stage, and sex was determined (YOY were <150 cm STL, juve-

niles were immature animals ≥150 cm STL, adult males had calcified claspers and were ≥340 cm STL, and adult females were ≥380 cm STL) (Simpfendorfer et al. 2008, R. D. Grubbs & J. Gelsleichter, unpubl. data). Rostral teeth were counted (left and right, independently). All animals were then externally tagged and released.

Upon returning to land, hematocrit was measured in duplicate by filling a capillary tube with the homogenized blood sample, capping one end with clay, and spinning the tube in a hematocrit centrifuge at 15 000 × *g* for 5 min. Hematocrit was determined by calculating the red blood cell percentage of the whole blood volume. The remaining whole blood was then centrifuged at 1800 × *g* for 5 min (Unico). The separated plasma was stored at −20°C. Plasma potassium (K^+) concentrations were measured using a Single-Channel Digital Flame Photometer (Model 02655-00, Cole-Parmer). Each sample was prepared using a 1:100 dilution of plasma to Cole-Parmer diluent. Potassium standards (K^+: 0.5, 1, 2, and 5 ppm) were prepared with a 1000 ppm stock solution. Potassium ions were measured by running a standard curve (in triplicate) before the samples, which were then measured in triplicate and in groups of 5. This process was repeated to ensure proper calibration. Measurement of each standard and sample dilution followed protocol developed by the manufacturer (Cole-Parmer), wherein the standard or sample was aspirated for 20 s prior to recording the concentration. Between each measurement, air was aspirated for 10 s, followed by Cole-Palmer diluent for 20 s and air again for 10 s.

Statistical analyses

Stress physiology data for pCO_2, bicarbonate, and pH were temperature-corrected to water temperature measurements at the time of capture (Mandelman & Skomal 2009, Gallagher et al. 2010). Hematocrit data were arcsine transformed prior to analyses.

To investigate physiological differences over ontogeny, one-way ANOVA or Kruskal-Wallis tests, depending on whether data were normally distributed, were conducted between the YOY, juvenile, and adult blood parameters: glucose, pCO_2, lactate, bicarbonate, pH, potassium, and hematocrit. For these analyses, *P. pectinata* were assessed by ontogenetic stage only and the capture method was not taken into account. If the results indicated a significant difference, a Tukey post hoc test was conducted to determine significant pairwise differences between ontogenetic stages. When a significant difference was identified between YOY and juvenile life stages, an additional independent *t*-test or Welch's *t*-test, depending on whether data were normally distributed, was conducted only on gillnet captured YOY and juveniles to control for any effects of capture method.

To assess differences in the physiological stress response of *P. pectinata* to different methods of capture, one-way ANOVA or Kruskal-Wallis tests were conducted on the following blood parameters between shallow longline (<5 m), deep longline (>50 m), and gillnet captured individuals, regardless of life stage: glucose, pCO_2, lactate, bicarbonate, pH, potassium, and hematocrit. Dip net and rod and reel data were omitted from these analyses because of low sample size. When the results of the ANOVA were significant, a Tukey post hoc test was conducted to determine significant pairwise differences between the different capture methods. If a significant difference was identified between shallow and deep longline captured *P. pectinata*, an additional independent *t*-test or Welch's *t*-test was conducted only on data from adults captured by shallow longline and deep longline to control for any influence of juveniles that were also captured by longline. While juveniles were omitted from these analyses, juveniles and adults captured by shallow longline were analyzed using independent *t*-tests, and no significant differences were noted for any of the parameters.

To investigate the potential effects of habitat quality on YOY and larger juvenile stress physiology, one-way ANOVA or Kruskal-Wallis tests were conducted on glucose, pCO_2, lactate, bicarbonate, pH, and hematocrit data of gillnet captured *P. pectinata* comparing FLBLE, UE, the Caloosahatchee River, and the Peace River. If the results were significant, a Tukey post hoc test was conducted to identify significant pairwise differences between nurseries. Because the sample sizes for potassium ion results were smaller than those of the other stress parameters, the more pristine nurseries, FLBLE and UE, were pooled (FBEV), and the more anthropogenically influenced nurseries, Caloosahatchee River and Peace River, were pooled (PC) and a *t*-test was conducted. If a significant difference between YOY and juvenile stages was identified for a particular parameter in the previous ontogenetic analyses, only YOY data were used for this analysis; however, if no significant difference was noted for a particular parameter, then YOY and juvenile data were pooled.

All statistical analyses were conducted, and figures were made using R version 3.0.3 (R Development Core Team 2014). All tests were considered significant at $\alpha = 0.05$.

RESULTS

In total, blood samples were collected from 83 smalltooth sawfish *Pristis pectinata*, 42 YOY, 13 juveniles, and 28 adults (Table 1). A total of 22 individuals were captured by shallow longline (5 juveniles, 17 adults), 11 adults by deep longline, 46 by gillnet (39 YOY, 7 juveniles), 3 by rod and reel (2 YOY, 1 juvenile), and 1 YOY by dip net (Table 2). From the YOY and juvenile surveys, 7 were captured in FLBLE, 11 in UE, 9 in the Caloosahatchee River, and 22 in the Peace River (Table 3).

Ontogeny and capture method

With respect to ontogeny, no significant difference was found in *P. pectinata* blood glucose concentrations (ANOVA: $F_{2,65} = 0.37$, p = 0.696). Blood glucose was significantly higher in *P. pectinata* captured in gillnets than in those captured by shallow longlines, although there were no significant differences between those captured in gillnets and deep longlines, or between those captured by deep longline and shallow longline (ANOVA: $F_{2,60} = 6.02$, p = 0.004; Fig. 1A). However, adults captured by deep longlines had significantly higher blood glucose than those caught by shallow longlines (*t*-test: $t = 3.78$, df = 12, p = 0.003). Though not included in the analysis, the YOY *P. pectinata* captured by dip net had 2- to 3-fold higher blood glucose levels than YOY caught using the other capture methods.

Table 1. Concentrations of stress physiology parameters (mean ± SE) in smalltooth sawfish *Pristis pectinata* young of the year (YOY), juveniles, and adults. Sample sizes in parentheses

	Glucose (mmol l^{-1})	pCO_2 (torr)	Lactate (mmol l^{-1})	Bicarbonate (mmol l^{-1})	pH	Potassium (mmol l^{-1})	Hematocrit (%)
YOY	2.30 ± 0.14 (42)	10.13 ± 0.38 (37)	3.56 ± 0.46 (37)	7.05 ± 0.33 (37)	7.17 ± 0.02 (37)	12.58 ± 1.29 (12)	27.81 ± 0.83 (42)
Juvenile	2.31 ± 0.36 (11)	7.97 ± 0.83 (11)	2.00 ± 0.38 (11)	6.63 ± 0.63 (11)	7.25 ± 0.02 (11)	7.70 ± 1.26 (8)	22.54 ± 0.95 (13)
Adult	2.04 ± 0.36 (15)	5.52 ± 0.50 (24)	2.38 ± 0.14 (21)	4.58 ± 0.39 (24)	7.24 ± 0.01 (24)	6.64 ± 0.76 (16)	23.27 ± 0.81 (26)

Table 2. Concentrations of stress physiology parameters (mean ± SE) in smalltooth sawfish *Pristis pectinata* captured by shallow longline (SLL), deep longline (DLL), gillnet (GN), rod and reel (RR), and dip net (DN). Sample sizes in parentheses

	Glucose (mmol l^{-1})	pCO_2 (torr)	Lactate (mmol l^{-1})	Bicarbonate (mmol l^{-1})	pH	Potassium (mmol l^{-1})	Hematocrit (%)
SLL	1.63 ± 0.14 (13)	5.43 ± 0.52 (17)	2.54 ± 0.28 (16)	4.18 ± 0.31 (17)	7.22 ± 0.02 (17)	6.41 ± 0.55 (11)	23.13 ± 0.67 (21)
DLL	3.12 ± 0.90 (5)	6.10 ± 0.33 (9)	2.53 ± 0.26 (7)	4.82 ± 0.52 (9)	7.22 ± 0.04 (7)	6.72 ± 1.40 (8)	23.50 ± 1.76 (10)
GN	2.16 ± 0.07 (46)	10.01 ± 0.38 (41)	3.40 ± 0.43 (41)	6.96 ± 0.28 (41)	7.17 ± 0.02 (41)	12.26 ± 1.11 (15)	27.44 ± 0.78 (46)
RR	2.98 ± 1.12 (3)	9.80 ± 0.43 (3)	1.16 ± 0.68 (3)	8.76 ± 1.75 (3)	7.26 ± 0.08 (3)	4.58 (1)	23.08 ± 2.81 (3)
DN	7,44 (1)	10.80 (1)	0.93 (1)	9.67 (1)	7.40 (1)	5.84 (1)	17.75 (1)

Table 3. Concentrations of stress physiology parameters (mean ± SE) in smalltooth sawfish *Pristis pectinata* young of the year and juveniles captured in Florida Bay/lower Everglades (FLBLE), upper Everglades (UE), the Caloosahatchee River (CAL), and the Peace River (Peace). Sample sizes in parentheses

	Glucose (mmol l^{-1})	pCO_2 (torr)	Lactate (mmol l^{-1})	Bicarbonate (mmol l^{-1})	pH	Potassium (mmol l^{-1})	Hematocrit (%)
FLBLE	2.87 ± 0.81 (7)	8.00 ± 0.51 (7)	0.97 ± 0.25 (7)	7.72 ± 0.74 (7)	7.30 ± 0.03 (7)	10.75 ± 1.54 (7)[a]	20.89 ± 1.36 (7)
UE	1.97 ± 0.17 (11)	9.94 ± 0.66 (8)	1.11 ± 0.35 (8)	8.55 ± 0.62 (8)	7.26 ± 0.03 (8)		24.23 ± 1.45 (11)
CAL	1.90 ± 0.09 (9)	10.76 ± 0.89 (8)	2.30 ± 0.58 (8)	7.58 ± 0.61 (8)	7.18 ± 0.03 (8)	13.58 ± 1.51 (8)[a]	28.11 ± 1.30 (9)
Peace	2.35 ± 0.08 (22)	10.33 ± 0.54 (21)	5.09 ± 0.58 (21)	6.32 ± 0.37 (21)	7.12 ± 0.03 (21)		30.26 ± 0.87 (22)

[a]Because of low sample size, FLBLE and UE data were pooled, and CAL and Peace data were pooled

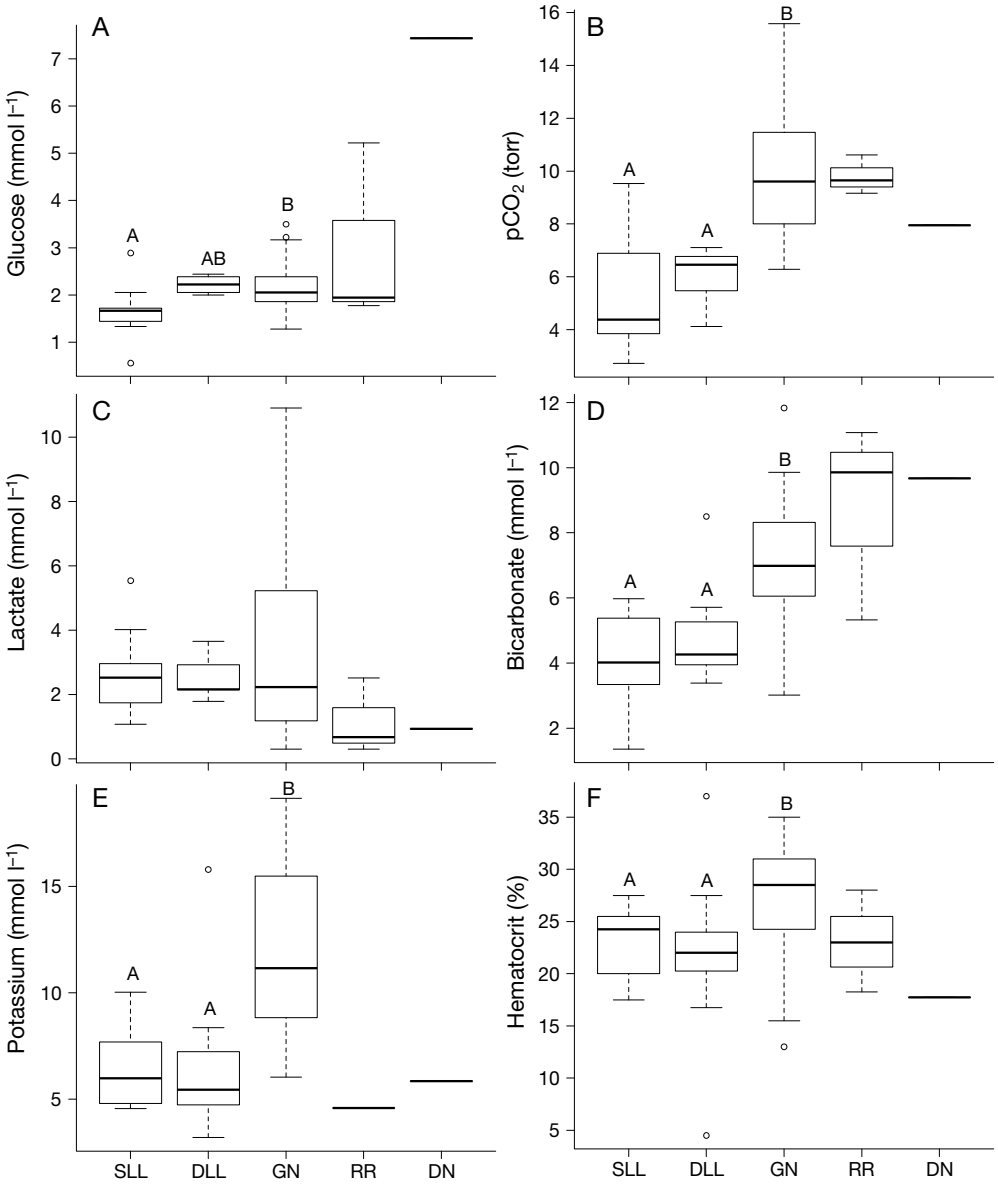

Fig. 1. Boxplots of (A) glucose concentration (mmol l^{-1}), (B) pCO_2 (torr), (C) lactate concentration (mmol l^{-1}), (D) bicarbonate concentration (mmol l^{-1}), (E) potassium concentration (mmol l^{-1}), and (F) hematocrit (%) in smalltooth sawfish *Pristis pectinata* captured via 5 different methods: shallow longline (SLL), deep longline (DLL), gillnet (GN), rod and reel (RR), and dip net (DN). Different letters indicate significant pairwise differences. RR and DN were not included in any statistical analyses. The top and bottom of the boxes indicate the 75 and 25% quartiles, respectively, the bold lines are the medians, the upper and lower whiskers indicate the maximum and minimum values within 1.5 times the inter-quartile range, and the points outside of the whiskers indicate outliers

Blood pCO_2 concentrations were significantly higher in YOY than in juveniles and adults, and concentrations of pCO_2 were significantly higher in juveniles than in adults (ANOVA: $F_{2,69} = 25.91$, $p < 0.001$; Fig. 2A). There was no significant difference in pCO_2 concentrations between gillnet captured YOY and juveniles (*t*-test: $t = -1.14$, df = 40, $p = 0.263$). Gillnet captured *P. pectinata* had significantly elevated pCO_2 compared to both shallow and deep longline captured individuals (ANOVA: $F_{2,64} = 31.67$, $p < 0.001$; Fig. 1B). While not included in the ANOVA, rod and reel captured *P. pectinata* also had elevated pCO_2 levels, comparable to those of gillnet captured individuals.

There was a significant difference in lactate over ontogeny; however, no significant pairwise differences between life stages were found (ANOVA: $F_{2,66} = 3.14$, $p = 0.0497$; Fig. 2B). In gillnet captured YOY and juveniles, YOY lactate levels were significantly

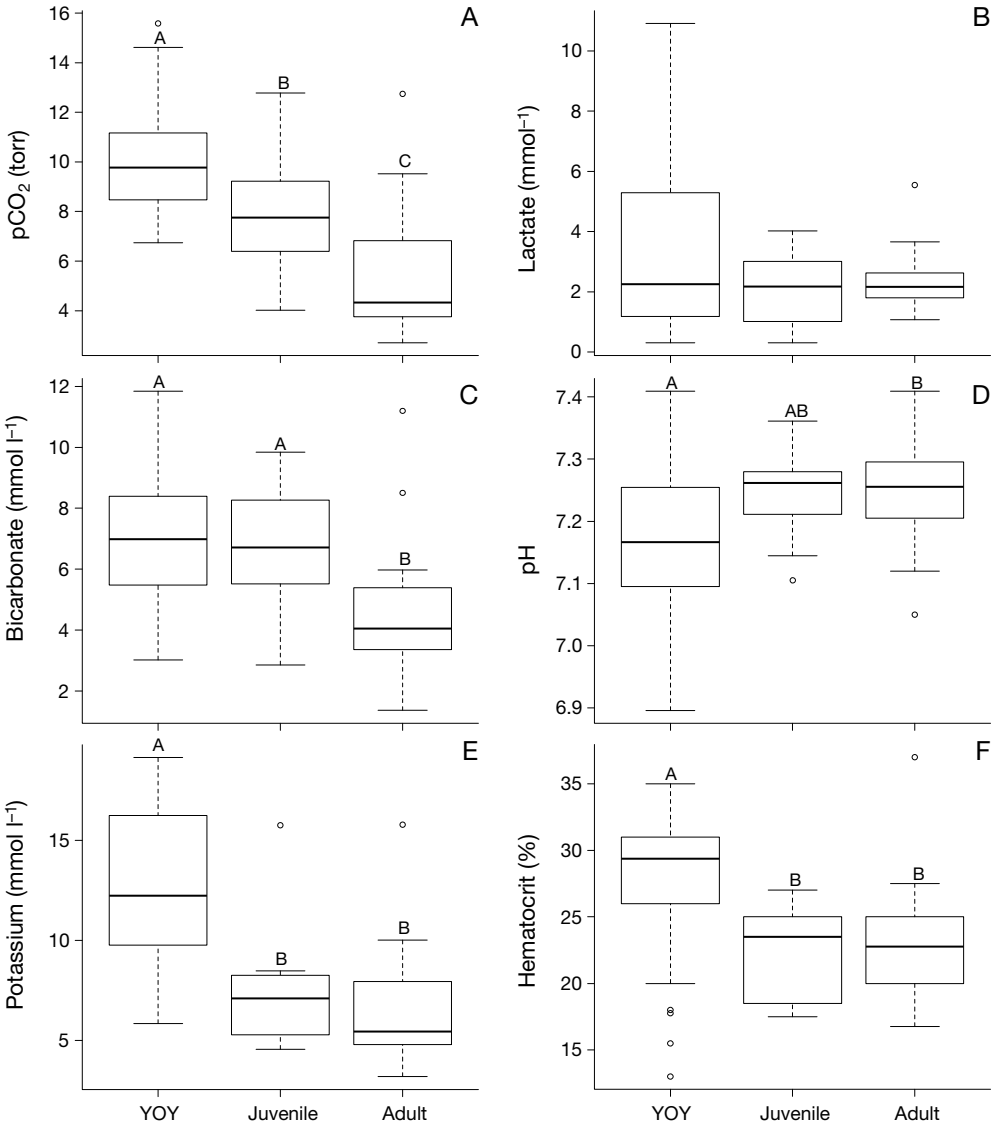

Fig. 2. Boxplots of (A) pCO_2 (torr), (B) lactate concentration (mmol l^{-1}), (C) bicarbonate concentration (mmol l^{-1}), (D) pH, (E) potassium concentration (mmol l^{-1}), and (F) hematocrit (%) in the 3 smalltooth sawfish *Pristis pectinata* ontogenetic stages: young of the year (YOY), juvenile, and adult. Different letters indicate significant pairwise differences. For an explanation of the box plots see Fig. 1

higher than those of juveniles (Welch's *t*-test: $t = -4.00$, df = 28.19; $p < 0.001$). No significant differences in lactate were found between shallow longline, deep longline, and gillnet captured *P. pectinata* (ANOVA: $F_{2,61} = 0.86$, $p = 0.427$). While not statistically compared, rod and reel and dip net captured *P. pectinata* displayed lactate levels about half those of shallow longline, deep longline, and gillnet captured individuals (Fig. 1C).

YOY and juvenile *P. pectinata* blood contained significantly higher concentrations of bicarbonate than adults (ANOVA: $F_{2,69} = 11.14$, $p < 0.001$; Fig. 2C). Bicarbonate was significantly depressed in *P. pectinata* captured by shallow and deep longlines compared with that of those captured in gillnets (ANOVA: $F_{2,64} = 20.21$, $p < 0.001$; Fig. 1D). While not statistically compared, rod and reel and dip net captured *P. pectinata* had higher concentrations of bicarbonate, similar to those of gillnet captures.

Blood pH of YOY was significantly lower than that of adults; however, no significant difference in blood pH was identified between YOY and juveniles or juveniles and adults (ANOVA: $F_{2,69} = 4.66$, $p = 0.013$; Fig. 2D). When analyzed separately, YOY blood pH was significantly lower than juveniles for *P. pectinata* captured in gillnets only (*t*-test: $t = 2.41$, df = 40, p =

0.021). No significant differences were found in *P. pectinata* blood pH between the 3 capture methods (ANOVA: $F_{2,64}$ = 1.30, p = 0.279). While not statistically compared, the 1 *P. pectinata* captured by dip net had a pH that was higher than that of the mean pH of other capture methods (Table 2).

Concentrations of potassium in the plasma were significantly higher in YOY than in juvenile and adult *P. pectinata* (ANOVA: $F_{2,33}$ = 9.43, p <0.001; Fig. 2E). When analyzing gillnet captured YOY and juveniles, no significant difference was found in plasma potassium (*t*-test: *t* = 1.44, df = 13, p = 0.173). Plasma potassium was significantly higher in *P. pectinata* captured in gillnets compared to individuals captured by both shallow and deep longlines (ANOVA: $F_{2,31}$ = 10.64, p < 0.001; Fig. 1E). While not statistically compared, the concentrations of plasma potassium in the rod and reel and dip net captured *P. pectinata* were lower compared to individuals caught using the other methods.

A significantly higher percent hematocrit was observed in YOY compared with juveniles and adults (ANOVA: $F_{2,78}$ = 10.45, p <0.001; Fig. 2F). When comparing only gillnet captured YOY and juveniles, YOY percent hematocrit was still observed to be significantly higher than that of juveniles (*t*-test: *t* = –3.41, df = 45, p = 0.001). A significant hemoconcentration was observed in *P. pectinata* captured in gillnets compared to those caught by shallow and deep longlines (ANOVA: $F_{2,75}$ = 7.92, p <0.001; Fig. 1F). While not statistically compared, rod and reel and dip net captured *P. pectinata* percent hematocrit was lower than that of those captured in gillnets, and more comparable to that of those captured by shallow and deep longlines (Fig. 1F).

Habitat quality

No significant difference in YOY or juvenile *P. pectinata* blood glucose or pCO_2 was observed between the 4 nurseries sampled (glucose ANOVA: $F_{3,45}$ = 2.13, p = 0.11; pCO_2 ANOVA: $F_{3,40}$ = 2.31, p = 0.091).

Significantly elevated blood lactate was identified in YOY *P. pectinata* sampled from the Peace River when compared to the other nurseries (ANOVA: $F_{3,33}$ = 8.19, p < 0.001; Fig. 3A). While not significant,

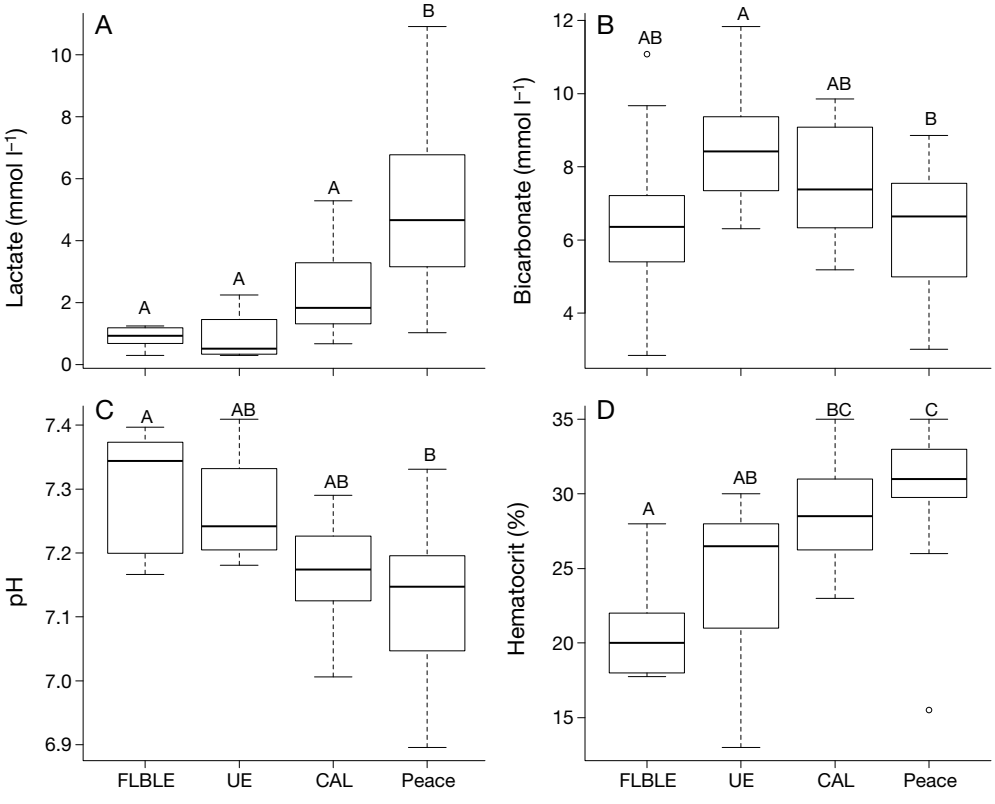

Fig. 3. Boxplots of (A) lactate concentration (mmol l^{-1}), (B) bicarbonate concentration (mmol l^{-1}), (C) pH, and (D) hematocrit (%) in young of the year smalltooth sawfish *Pristis pectinata* captured in the more pristine Florida Bay and lower Everglades (FLBLE) and upper Everglades (UE), and in the anthropogenically influenced Caloosahatchee River (CAL), and the Peace River (Peace). Plot B includes juveniles. Different letters indicate significant pairwise differences. For an explanation of the box plots see Fig. 1

the median blood lactate concentration in YOY from the Caloosahatchee River was nearly double that from the FLBLE and UE nurseries.

YOY and juveniles in the UE had significantly higher bicarbonate than those from the Peace River (ANOVA: $F_{3,40} = 3.63$, p = 0.021; Fig. 3B). When solely investigating YOY, there was no significant difference in blood bicarbonate between the 4 nurseries (ANOVA: $F_{3,33} = 2.86$, p = 0.052).

YOY blood pH was significantly lower in the Peace River than in the FLBLE, although it was not significantly lower than that of UE or the Caloosahatchee River (ANOVA: $F_{3,33} = 4.57$, p = 0.009; Fig. 3C).

No significant difference in YOY and juvenile plasma potassium was identified when results from FLBLE and UE were pooled, or when those from the Peace River and Caloosahatchee River results were pooled (*t*-test: $t = -1.31$, df = 13, p = 0.214).

A significant hemoconcentration was observed in the blood of YOY sampled in the Peace River, when compared with individuals sampled in FLBLE and UE. The percent hematocrit was not significantly higher in the Peace River than in the Caloosahatchee River, and the Caloosahatchee River YOY did not have significantly higher percent hematocrit than that of the UE (ANOVA: $F_{3,38} = 8.02$, p < 0.001; Fig. 3D).

DISCUSSION

This study is the first to document stress physiology parameters in the smalltooth sawfish *Pristis pectinata* by ontogeny, capture method, and habitat quality. When comparing all of the parameters investigated between *P. pectinata* and previously examined elasmobranchs, *P. pectinata* appear to have a similar or less pronounced stress response, suggesting physiological resiliency in the species. In particular, glucose and lactate were relatively low when compared to other elasmobranchs. Glucose in *P. pectinata* was most similar to demersal species such as the southern stingray *Hypanus americana*, which had a median glucose concentration of 1.7 mmol l^{-1} after trawling (Cain et al. 2004), and the Port Jackson shark *Heterodontus portusjacksoni*, which had average glucose concentrations between 1.61 and 2.28 mmol l^{-1}, depending on the treatment and duration, before and after gillnet and longline capture simulations (Frick et al. 2010a). In contrast, in the 11 species of ram-ventilating sharks examined by Marshall et al. (2012), the lowest average glucose concentration was ~5 mmol l^{-1}, and in the gummy shark *Mustelus antarcticus*, glucose ranged from 3.98 to 6.14 mmol l^{-1} before and after gillnet and longline capture simulations (Frick et al. 2010a). While behavior was not directly recorded in the present study, sawfish are known to rest on the substrate and live a more sedentary lifestyle. During this study, *P. pectinata* were observed to rest on the substrate after capture, similar to *H. portusjacksoni* during simulated capture (Frick et al. 2010a), and as inferred from temperature depth recorder behavior data in longline captured *M. antarcticus* (Guida et al. 2016). This behavior could contribute to lower glucose concentrations, as observed in teleosts (Vijayan & Moon 1994, Waring et al. 1996), by either stopping glucose levels from rising higher or allowing time for clearance.

Average concentrations of lactate, one of the most commonly used indicators of stress, in *P. pectinata* ranged from 0.9 to 3.5 mmol l^{-1} over ontogeny and different capture methods and were very low relative to those of other elasmobranchs studied to date (Fig. 4). For example, Marshall et al. (2012) examined blood lactate in 11 shark species captured by longline, and only 1 species, the oceanic whitetip shark *Carcharhinus longimanus*, displayed a mean lactate concentration less than 4 mmol l^{-1}. Moyes et al. (2006) found that lactate was one of the best predictors of post-release mortality, with moribund blue

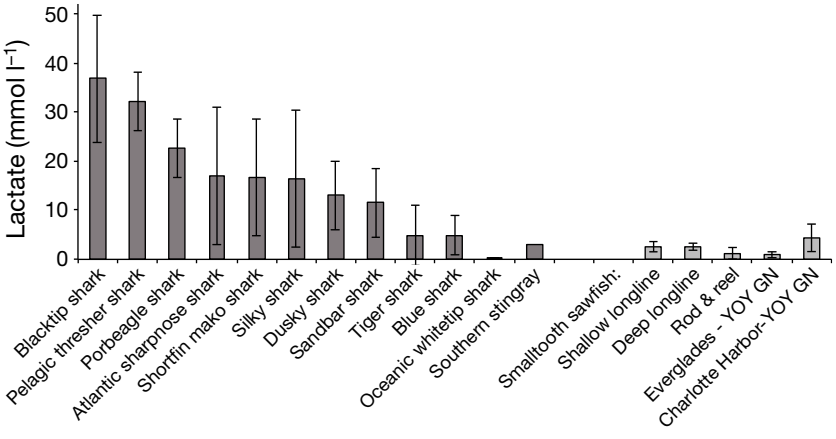

Fig. 4. Bar plot comparing smalltooth sawfish *Pristis pectinata* lactate concentrations (mmol l^{-1}) measured in the present study to concentrations from 11 species of longline captured sharks (from Marshall et al. 2012) and trawl captured southern stingray *Dasyatis americana* (from Cain et al. 2004). *Pristis pectinata* are subdivided into shallow longline, deep longline, rod and reel, Everglades young of the year (YOY) gillnet (GN) capture, and Charlotte Harbor YOY GN

shark *Prionace glauca* having blood lactate values around 20 mmol l^{-1}. Hight et al. (2007) reported mortality occurred with lactate ~16 mmol l^{-1} in *P. glauca*, 19 mmol l^{-1} in common thresher sharks *Alopias vulpinus*, and 20 mmol l^{-1} in shortfin mako sharks *Isurus oxyrinchus*; however, they also reported survival based on tag recaptures of *I. oxyrhinchus* with lactate values at tagging ranging from 10.3 to 14.7 mmol l^{-1} as well as for 1 *A. vulpinus* with a lactate value at tagging of 23 mmol l^{-1}. In Cain et al. (2004), bottom-trawled *H. americana* displayed a median lactate of 3.1 mmol l^{-1}, similar to that of *P. pectinata* in the present study (see Fig. 4).

Lactate concentrations have been found to be significantly higher in gillnet captured bonnethead sharks *Sphyrna tiburo*, bull sharks *C. leucas*, lemon sharks *Negaprion brevirostris*, and *M. antarcticus* than those captured by longline (Frick et al. 2010a, Hyatt et al. 2012). While lactate concentrations in *P. pectinata* had the same trend, the average concentration was lower than that for all the aforementioned elasmobranchs (Frick et al. 2010a, Hyatt et al. 2012). Additionally, the handling time prior to obtaining the blood sample is likely to increase the stress response cascade. Although this handling time is greatest for longline captured adults, the lactate level was comparable, if not higher, in gillnet captured *P. pectinata* which experienced less handling time prior to sampling. This reinforces the US research permit requirement for constant monitoring of all gillnets targeting *P. pectinata*.

Plasma potassium concentration of gillnet captured *P. pectinata* was the only parameter examined in the present study that was neither comparable to nor indicative of lower stress than that of other elasmobranchs (Frick et al. 2010a, Marshall et al. 2012). Despite relatively elevated concentrations of potassium, tag-recapture and tracking data suggested that no *P. pectinata* captured during this study died post-release. This may indicate that *P. pectinata* has a higher threshold for the damaging effects of hyperkalemia, that this species has the ability to recover from acutely elevated potassium, as has been observed in spiny dogfish *Squalus acanthias* (Mandelman & Farrington 2007), or that potassium may not be a reliable indicator of physiological stress in this species. Additionally, despite following identical protocols to other elasmobranch blood collections, *P. pectinata* blood samples all showed high levels of hemolysis, which may have resulted in greater concentrations of plasma potassium, similar to what was found in *S. acanthias* by Martini (1974). This could limit the use of this parameter for comparative purposes.

Ontogeny

Of the 7 stress physiology parameters that were investigated, 6 were found to vary significantly over ontogeny. However, when capture method was held constant to elucidate stage-specific differences, only bicarbonate, pH, and hematocrit varied significantly, suggesting that our results investigating physiological stress as functions of ontogeny and capture method were confounded.

Both YOY and juvenile *P. pectinata* had significantly higher concentrations of bicarbonate than adults. Because bicarbonate can act as a buffer for lactate increases, it is possible that this physiological functioning is more efficient, or only occurs in the adult stage, since YOY had the highest lactate as well as the highest bicarbonate concentrations. However, it is also likely that gillnet captured *P. pectinata* were removed from the net and sampled too quickly to observe a decline in bicarbonate. Significantly lower blood pH and a much larger range in pH were observed in YOY and gillnet captured juveniles than in adults. This is likely driven by life stage, since this was observed when capture method was held constant, and is likely a result of higher lactate, higher pCO_2, and higher bicarbonate concentrations, all of which can contribute to blood acidosis. Dissimilar to the results here, when investigating the pH response over total length in 5 shark species, Gallagher et al. (2014) did not find a significant interaction; however, that study had a smaller sample size over a smaller size range. A significant hemoconcentration of the blood was observed in the present study in YOY *P. pectinata* when compared with juveniles and adults, and this significant trend was still observed when only gillnet captured YOY and juveniles were analyzed. The significant hemoconcentration observed in YOY *P. pectinata* was likely because of their higher range of lactate concentrations. Bicarbonate, pH, and hematocrit are directly linked to metabolic and respiratory stress, which is typically first indicated by pCO_2 and lactate. Overall, when investigating all of the stress physiology parameters, there was likely little change in the response as a result of age, but rather from capture method. Similarly, Mandelman & Skomal (2009) compared stress parameters over fork length of 4 shark species, and did not find any differences as a result of length; however, they noted their low intraspecific variability in size and variation in capture duration as potential reasons for not detecting differences over ontogeny.

Capture method

Gillnet captured *P. pectinata* had significantly higher glucose, pCO_2, bicarbonate, potassium, and hematocrit compared to longline captured individuals; however, lactate and blood pH were not significantly different between these capture methods. Gillnet capture may induce a larger stress response relative to that of a longline because of the limitation in mobility, and potential impediment of ventilation (Manire et al. 2001), whereas longline capture allows a fish to continue swimming or lie on the bottom with unrestricted ventilation (Manire et al. 2001, Hyatt et al. 2012, Guida et al. 2016). However, field observations suggest that juvenile *P. pectinata* react by settling onto the substrate with the rostrum entangled in the mesh. Dissimilar to the results of the present study, Hyatt et al. (2012) found that lactate concentrations were significantly higher and pH was significantly lower in gillnet captured *S. tiburo*, *C. leucas*, and *N. brevirostris*, although they found no significant difference in blood pCO_2 and bicarbonate. In elasmobranchs, because bicarbonate concentrations can be directly related to lactate concentrations (Holeton & Heisler 1983), it would be expected that all of the capture methods in the present study should have comparable depletions of bicarbonate; however, bicarbonate was only found to be depleted in shallow and deep longline captured *P. pectinata*. The differences in blood bicarbonate across capture methods may be a result of the greater time lag between capture and sampling for longline, during which bicarbonate buffering may occur. Mean potassium in longline captured *P. pectinata* was similar to that observed in sharks (Frick et al. 2010a, Marshall et al. 2012); however, the significantly higher potassium concentrations observed in gillnet captured *P. pectinata* were likely related to greater instances of hemolysis in the blood of YOY. Similarly, the significant hemoconcentration observed in gillnet captured *P. pectinata* when compared with longline captured individuals could also be related to the predominance of YOY captured by gillnet. This is further supported by the significant hemoconcentration found in gillnet captured YOY compared to juveniles.

The only parameter that indicated any significant difference in the stress response between shallow and deep longline was blood glucose, which indicated greater stress in deep longline caught individuals. This may be because of the time required to pull the *P. pectinata* 40–80 m from the substrate to the surface.

While not statistically analyzed, lactate, bicarbonate, pH, potassium, and hematocrit indicated less stress with rod and reel and dip net captures. Although blood samples were collected as soon as the fish was restrained, some handling may have induced greater stress in longline and gillnet captured *P. pectinata*, because rod and reel and dip net captured individuals were sampled within 1 min of gear contact. The rod and reel gear used in this study was heavier than that commonly used by recreational fishers, reducing the fight time to less than 1 min. Baseline or near baseline levels of lactate in some species of sharks have been reported between 0 and 1 mmol l^{-1} (Cliff & Thurman 1984, Spargo 2001, Skomal 2006, Brooks et al. 2012); therefore, these similar concentrations in rod and reel and dip net captured *P. pectinata* may represent near-baseline levels of lactate for this species.

Overall, the results indicate that gillnet capture may induce both greater relative metabolic and respiratory stress in this species, although it is important to acknowledge that ontogeny may be confounding these results, particularly regarding potassium and hematocrit. Regardless, comparison to previous work on other elasmobranchs suggests that stress was relatively low for *P. pectinata* across all capture methods.

Habitat quality

Of the 7 stress physiology parameters assessed, lactate, bicarbonate, pH, and hematocrit significantly differed in YOY and juveniles between nurseries. These parameters suggest that there may be chronic metabolic stress occurring in the more anthropogenically altered nurseries, the Peace and Caloosahatchee rivers, than in the relatively pristine nurseries in the TTI/ENP Unit. Changes in these 4 stress physiology parameters are likely the result of habitat degradation in the form of less refuge from human and predator interactions, and poorer abiotic conditions, which could elicit behavioral and physiological compensations (Skomal & Mandelman 2012), potentially leading to increased metabolic activity and, subsequently, higher lactate and hematocrit, as well as lower bicarbonate and pH. Chronic stress related to habitat loss can have negative population-level effects through reproductive, growth, and immune system impairments (Sapolsky 1992, Wingfield & Romero 2001). These observations are especially relevant from conservation and management perspectives in light of recent evidence that *P. pectinata* exhibit interannual site fidelity to the Peace and Caloosahatchee rivers (Feldheim et al. 2017).

Conclusions

Lactate is a commonly used indicator of stress and capture survival in elasmobranchs, and *P. pectinata* blood lactate concentration was among the lowest studied in elasmobranchs to date. Lactate was similar among capture methods. The lower concentrations of lactate observed in *P. pectinata* are likely in part because this species is a benthic, sedentary fish, and thus less sensitive physiologically to capture than most other previously examined elasmobranchs.

Our results indicate there may be chronic metabolic stress associated with the more anthropogenically influenced nurseries than in the relatively pristine nurseries. *P. pectinata* in the Peace and Caloosahatchee rivers had significantly higher lactate and hematocrit, and decreased pH and bicarbonate in comparison to individuals in the TTI/ENP Unit.

Future directions of *P. pectinata* stress physiology research should focus on sampling more individuals using the rod and reel capture method. This is likely the most common capture method that *P. pectinata* are exposed to today, and understanding how *P. pectinata* respond physiologically to this capture method is of great importance for understanding how they may recover once released. Additionally, similar to studies by Frick et al. (2010a,b) in which stress responses were compared between simulated longline, gillnet, and trawl captured *H. portusjacksoni* and *M. antarcticus*, *P. pectinata* that are captured via shrimp trawl should also be sampled to determine if these fish have the potential to survive once released, based on comparing their biochemical parameters to those measured in other moribund elasmobranchs. Overall, the results of our study indicate that *P. pectinata* is a species resilient to capture using the methods studied here. Ongoing tagging and telemetry studies of animals captured in these surveys indicate that post-release survival is very high. These results suggest that if harvest restrictions are enforced and suitable habitats are protected, the recovery outlook is positive for this imperiled species.

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