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Sex and Length as Predictors of Vertical Movements in Smalltooh Sawfish(Pristis pectinata)

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SEX AND LENGTH AS PREDICTORS OF VERTICAL MOVEMENTS IN SMALLTOOTH SAWFISH (*Pristis pectinata*)

By

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ABSTRACT

Smalltooth sawfish (*Pristis pectinata*) are listed as 'critically endangered' species by the IUCN and listed as *Endangered* under the U.S. Endangered Species Act (ESA). Smalltooth sawfish are extremely vulnerable to being caught as bycatch due to their long, toothed rostrum that can easily become entangled in fishing nets, especially in shrimp trawling fisheries. Several studies have been conducted on smalltooth sawfish since being listed under the ESA in 2003, but their vertical movements and use of depth have still not been thoroughly studied. Until recently sawfish were thought to stay at a depth of 10 m or less, but studies have now shown that they occupy much deeper. Due to this, data on their depth use is limited and is the reason why depth was chosen to be the focus of this study. The purpose of this study was to assess whether sex or length has an influence over the percentage of time a sawfish spends at a particular depth. Satellite telemetry was used to track the movements of the tagged sawfish. The data collected from the pop-off satellite tags showed smalltooth sawfish spend most of their time in shallow water, but do frequently occupy deeper depths. Sex was found to be a significant factor, while length was not. Females were found to spend more time at deeper depths than males. Females frequently occured at deeper depths while males mostly remained in shallow waters. Understanding how sawfish use depth is important in order to predict population processes and dynamics. Knowing the factors that affect a sawfish's depth use would be beneficial to efforts to manage and conserve this species. With the decline of smalltooth sawfish and being vulnerable to population loss, more research needs to be conducted in order to create more effective management and recovery plans.

INTRODUCTION

Sawfish are large rays in the Class Chondrichthyes and are known for their long, toothed "saw" or rostrum (Harrison and Dulvy 2014). Their rostrum is an elongated snout that has horizontal rostral teeth and is used for feeding and defense (Poulakis and Seitz 2004; Whitty et al. 2009). About 20-28% of a sawfish's body length comes from its rostrum and it serves a vital role for capturing and detecting prey. The rostrum helps find and catch prey by having extensive sensory organs that detect minute electrical signals sent off by other animals. Their rostrum can also be used as a way to stunt or kill fish by slashing its toothed side into its prey (Harrison and Dulvy 2014). They live in coastal tropical and subtropical waters in both estuaries and freshwater. Sawfishes can be found across the world, but the only sawfish species currently found in the U.S. is the smalltooth sawfish (*Pristis pectinata*).

The average size at birth for a smalltooth sawfish is 80 cm Total Length (TL). The size at maturity is 370 cm TL for females and 340 cm TL for males (Brame *et al.* 2019). Their age of maturity is estimated to be 7-11 years for males and females (Carlson and Simpfendorfer 2015). Their lifespan is around 30 years. Smalltooth sawfish are yolk-sac viviparous, meaning young are nourished in utero by an external yolk sac and are then born live.. It is presumed that their reproduction occurs biennially and their average litter size is 7-14 pups (Feldheim *et al*. 2017). Due to their long lifespans and late maturity, sawfish have a slow population growth rate, therefore increasing their vulnerability to and difficulty recovering from population loss.

Many of the areas where sawfish reside are highly threatened habitats such as mangroves or seagrasses, two habitats that have seen a great decline in range over the years. Their coastal preference also tends to overlap with large cities and areas of high human population density where more activities like fishing occur (Dulvy *et al.* 2016). The current geographical distribution of *P. pectinata* is mainly in the western Atlantic, but they have been found in the eastern Atlantic (Harrison *et al.* 2014), and the largest population is found along the southwest coast of Florida (Poulakis and Seitz 2004). Smalltooth sawfish were historically found along the coast of the United States as far north as the Carolinas, all in the Gulf of Mexico, the Caribbean Sea, and along the coast as far south as Uruguay (Dulvy *et al.* 2016; Carlson *et al.* 2014; Feldheim *et al.* 2017; Wiley and Simpfendorfer 2010; Simpfendorfer 2001). The smalltooth sawfish is currently found in less than 20% of its historic range (Dulvy *et al.* 2016). Now smalltooth sawfish are only consistently found in the coastal waters of southern Florida with a slow increase or stable population growth, and an estimated population size of only a few thousand (Carlson *et al.* 2014; Feldheim *et al.* 2017; Wiley and Simpfendorfer 2010). Exact population reduction rates are hard to calculate due to limited scientific data, but it has been estimated that the population may have declined as much as 95% from the historic stock size (Wiley and Simpfendorfer 2010).

Smalltooth sawfish tend to stay near or within mangroves and seagrass beds as juveniles (Dulvy *et al.* 2016; Wiley and Simpfendorfer 2010). Sawfish are known to occupy shallow coastal waters typically 10 m deep or less, but they can occupy depths deeper than 10 m and can be found at depths up to 122 m (Poulakis and Seitz 2004; Carlson *et al.* 2014; Wiley and Simpfendorfer 2010; Simpfendorfer 2001). Smaller and immature sawfish are more commonly found in shallow water (Poulakis and Seitz 2004; Wiley and Simpfendorfer 2010; Whitty *et al.* 2009; Simpfendorfer 2001). They also prefer warm water temperatures, mainly 22–28°C, and their lower thermal tolerance is predicted to be around 20°C (Carlson *et al.* 2014). Smalltooth sawfish often feed on schooling fish such as clupeids, carangids, mugilids, elopids, sparids, and belonids, as well as dasyatids stingrays (Poulakis *et al.* 2017). They obtain their prey by slashing their rostrum sideways through a school and impaling the fish on their rostral teeth. After caught, they ingest their prey whole.

There has been evidence for sexual segregation in elasmobranchs and sawfish may be included among those. Sexual segregation is the separation of males and females of the same species; this separation may be spatial as well as temporal in nature, for example, occurring only during the non-breeding season (Wearmouth and Sims 2010). It is important to understand the habitat use of different sexes in order to predict population processes and dynamics. This would provide data that may be useful to the successful management and conservation of species since these spatial dynamics often overlap with area-focused human activities like fishing.

There has never been a large scale fishery that directly targeted smalltooth sawfish, but it is very common for a sawfish to get entangled in fishing nets, due to their long toothed rostrum, and therefore they are often caught as bycatch. The main threat responsible for the decline in smalltooth sawfish has been and remains commercial and recreational fisheries. Shrimp trawl fisheries present a major concern due to the high bycatch mortality of large, mature sawfish, which could reduce the population's reproductive potential. Fortunately, sawfish are expected to suffer less and recover quicker when caught and released on longline as opposed to trawls and gill nets (Brame *et al.* 2019).

Smalltooth sawfish are said to be one of the world's most vulnerable marine fishes (Dulvy *et al.* 2016; Feldheim *et al.* 2017). The United States population of smalltooth sawfish was listed as *Endangered* under the U.S. Endangered Species Act on 01 April 2003 (Poulakis and Seitz 2004). Penalties such as imprisonment or steep fines could be given to anyone who harasses, harms, or kills any animal listed on the U.S. Endangered Species Act. Smalltooth sawfish are classified as 'critically endangered' by the International Union for Conservation of Nature (IUCN). This is the highest level of alert (the closest to extinction) set by the IUCN and is defined by them as "a species facing an *extremely* high risk of extinction in the wild." The IUCN Red List states their population trend is still decreasing (Carlson *et al.* 2013).

Pop-off archiving satellite tags (PSATs) are a relatively new electronic tagging technology. PSATs detach from the tagged animal after a predetermined time, float to the surface, and transmit the archived data to satellites which provide the data to the researcher. This technology allows tracking the movements of pelagic fish in their natural environment to be much more accessible and economical. PSATs can sample temperature, depth and light levels at user-defined time intervals, then store and process these data (Luo *et al.* 2006). It has become more common to use PSATs to gather data on horizontal and vertical movements of pelagic fishes, especially since the vertical movement of PSAT-tagged fishes in sea water has a high degree of accuracy and precision (depth and temperature resolution are claimed to be 0.5 m and 0.05°C, respectively) (Luo *et al.* 2006).

Why we need to study and protect sawfish

The U.S. Endangered Species Act requires by law that critical habitat must be designated for any listed species. To date, critical habitat has only been designated for small juvenile smalltooth sawfish. The reasons satellite tagging of sawfish adults was done was to: 1) aid in defining critical habitat for adults and vertical space use, 2) determine potential aggregation sites for mating and areas where males and females overlap in depth, and 3) determine if the U.S.

population is distinct from adjacent populations (e.g. Bahamas, Cuba) by determining if sawfish frequently traverse deep water (up to 800 m depths). With the decline of sawfish, more research needs to be conducted in order to create more effective status assessments, management measures, and recovery plans (Dulvy *et al.* 2016). Fishery management, where it does occur, focuses mostly on commercially valuable fish populations, so populations like sawfish have rarely been the main concern. It is difficult to develop recovery strategies for species that do not have a sufficient amount of scientific data concerning their distribution and habitat use, especially if they are widely dispersed (Wiley and Simpfendorfer 2010). More research must be conducted about sawfish so adequate recovery plans can be developed. The purpose of this study is to access whether sex or length has an influence over the percent time a smalltooth sawfish spends at a particular depth. Conducting research on how sawfish use depth could help predict population processes and dynamics. Knowing the factors that affect a sawfish's depth would allow for the successful management and conservation of this species, so it is important that these factors are studied and considered.

METHODS

I analyzed pop-off archival satellite tagging data collected from 2011 to 2017. The sawfish were caught using a bottom longline of 50 or 100 16/0 hooks baited with ladyfish, *Elops saurus*. Longline stations were selected based on being potential habitats suitable for smalltooth sawfish and historic encounter records. Satellite telemetry was used to track the movements of the tagged sawfish. Pop-off archiving satellite tags (PSATs), which record depth, temperature, time, and light levels, were attached externally to the sawfish first dorsal fin. Three different models of PSATs (all manufactured by Wildlife Computers, Inc.) were used: Mini-PAT, MK10, and PATF. The tags were programmed to detach from the animals after a certain number of days and transmit the archived data to a satellite. The PSATs use light-based geolocation where the spatial track is based on the timing of local noon (used to estimate longitude) and day length (used to estimate latitude) and corrected for temperature (Luo *et al.* 2006). Depth data were collected every four hours. Each satellite tag or PTT (Platform Transmitting Terminal) was assigned a PTT number.

I analyzed data from fourteen satellite tags (Wildlife Computers, Inc.) deployed from March 2011 to March 2017. Six of the tags were MK10 tags (programmed for 150 days), five were PATF tags (track for 60 days), and three were Mini-PAT tags (programmed for 105 days).

Choosing viable data

The GPE2 program from Wildlife Computers was opened using iGOR Pro 6.37 software. The graphs that displayed viable data (daily depths measurements for at least a duration of two weeks) were chosen to examine further. Further examination was done by looking at the specific depth measures in the Excel sheets for each of those tags. Only depths with an error of 4 m or less were considered, since that was the most accurate reading recorded by any tag. Anything with a higher error than 4 m was considered to be inaccurate.

Used data

In total, forty-three satellite tags were inspected for data use. Only fourteen of those tags were analyzed due to some tags failing to report or record enough data. Statistics for each analyzed sawfish can be seen in Table 1. Only data recorded from “PDT” stations were used. Any negative minimum depths were regarded as 0m (at the surface).

Creating histograms of percentage time-at-depth

All of the recorded depth measurements, with an error of 4 m or less, for an individual sawfish were considered. Depth bins were created in 8 m intervals (0-8, 9-16, 17-24, etc.), since the recorded data were expressed in increments of 8 m (0, 8, 16, 24, etc.). The percent of time each sawfish spent in each depth bin was calculated and made into a histogram in Excel.

Average maximum depth

Excel was used to calculate the average maximum depth of each sawfish. The average maximum depth of each day was found, then all of these calculations were averaged together.

Linear mixed-effects model

R Statistical Computing (R Core Team, 2019) and lme4 (Bates, Maechler, Bolker & Walker, 2015) were used to perform a linear mixed effects analysis of the relationship between percent time and depth. A mixed-effects model was chosen because the data had both random and fixed effects due to temporal pseudoreplication resulting from repeated measurements on the same individuals. As the random effect, sawfish PTT number was entered (without interaction term) into the model. Intercepts for depth and sex were fixed effects, as well as by-subject and by-item random slopes for the effect of depth. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality for sex. The function "lmer()" was used since it allows for non-normal errors and non-constant variance with the same error as a generalized linear model.

Model choice

Akaike's Information Criteria (AICc) is a log-likelihood that penalizes any superfluous parameters in model. AIC estimates the quality of each entered model, relative to each of the other models. AICc has small-sample correction. The lower the value indicates better fit of one model over another to that data. If the delta AIC is lower than 2 it can be said to have substantial support. Various linear mixed-effects models were tested. The entered model that was the most simple was chosen by comparing AICc values to determine which model has a better fit. Due to the principle of parsimony, all the factors that did not cause a significant increase in deviance were removed from the model to form a minimal adequate model.

RESULTS

Histograms of percentage time-at-depth

The results of the histograms can be seen in Figure 1. These histograms showed the percentage of time a sawfish spent at a specific depth by analyzing all of the data recorded on that sawfish's tag. The majority of time for every sawfish was seen at a depth of 0-8 m, however a few sawfish were recorded to go as deep as 88 m. Most tagged sawfish spent over 60% of their time at 0-8 m, but two sawfish (one male and one female) spent over 50% of their recorded time at depths deeper than the 0-8 m bin. When the sawfish were not at a depth of 0-8 m, there were no patterns of depth use. After visual inspection, time spent at depth did not consistently decrease with increasing depth and there were no favored depths below 0-8 m. Sex-dependent trend were apparent, however. All of the females spent time in at least six other depth bins, while half of the males solely spent time in 0-8 m. This reveals female's preference for deeper depths than males.

Which sex has a greater maximum depth?

This t-test compared the average maximum depth each sawfish occupied each day, which shows the deepest depth a sawfish occupies on average. The average maximum depth occupied by males was 5.736 m (standard error = 2.475) and 22.61 m (standard error = 5.118) for females. An unpaired student t-test was performed and the two-tailed p-value equaled 0.0068, suggesting there was a significant difference between the means (t = 3.3985 ; df = 10 ; standard error of difference = 4.965).

Model choice

The model with a delta AICc value between 0-2 was chosen to be the best fit. The minimal adequate model found depth and sex to be siginifant factors. Length was not found to be significant factor. The AICc results can be seen in Table 2. Best model: *lmer(Percent.Time ~ Depth + Sex + (1|Sawfish))*. This means the interaction between sex and depth is not significantly important, so both sexes can be estimated to have the same slope.

Linear mixed-effects model

The variables that were tested were depth, length and sex to address the question: "does sawfish length and/or sex have an effect on the percent-of-time a sawfish spent at a particular depth?". Since length fell out of the model, it is not considered a significant factor. The percent time each sex stays at a specific depth is shown in Figure 2. In this figure, it is clear that sawfish spend more time at shallow depths, but definitely spend a considerable amount of time in deeper water. Due to the model choice conducted with AICc, both the male and female isoclines were given the same slope. Males spend a much greater time at shallower depths than females. This is consistent with the average maximum depth t-test results that show females have a significantly deeper average depth than males. This shows that females are expected to be found at deeper depths than males.

DISCUSSION

The goal of this study was to assess whether sex or length had an influence over the percent of time a sawtooth sawfish spent at a specific depth. Knowing what factors determine the depth of sawfish will help further understand the habits of sawfish and could help with conservation management for this critically endangered species. According to the t-test performed, females occupy greater maximum depths than males. This means we can expect to find females at greater depths than males and could potentially affect the way female, especially pregnant, sawfish are protected. Females are very important for population dynamics since they give birth to pups. In order to ensure the sawfish population grows, females must be protected. If females have different depth preferences than males, this could change the way the conservation of female sawfish is managed. It may be more important to ensure female survival, so this information can help tailor sawfish conservation more towards females. Further research on whether being pregnant affects the depth a sawfish occupies could be useful data and could help ensure these individuals are protected, therefore promoting the growth of the sawfish population.

The lowest AICc value indicated the most parsimonious model, relative to the other model fits with a higher AICc value. The best model to explain the influence of percent of time at depth indicated sex was relevant, while length was not. The minimal adequate model showed a common slope for percent time against depth with two intercepts, one for each sex. The delta AICc of the best linear mixed-effects model was between 0-2 and therefore can be said to have substantial support. Sex was shown to affect percent time at a particular depth significantly. This implies that males and females have a variation in the depths they occupy, which could change the way the conservation of female versus male sawfish are managed. Improvements to this model could be adding factors such as seasonality. Month was considered when sorting the data, but seasonality was not analyzed. Upon visual inspection of the data, it appears sawfish mostly occupied deeper depths during the late summer months. This makes sense since the surface water temperatures would be greater and would allow a sawfish to go deeper into the water column. Further analysis regarding month and the breeding season would need to be calculated to see if sex is only segregating at certain times of the year. Knowing what specific time of the day (e.g. day or night) could be another factor to consider and may be significant. Length was not a significant factor when considering what depth a sawfish occupies, but modeling length and depth could still be useful to see if any visual trends are spotted.

Trends in the histograms (Figure 1) reveal that a sawfish is found at 0-8 m a majority of the time. Every sawfish that was analyzed spent more time in 0-8 m than any other depth, but most of the sawfish did occupy deeper depths. This data confirms that sawfish can and do frequently occupy at deep depths. It also reveals that even if there is a difference between sex and depth, sawfish are still spend most of their time in shallow depths. This means that sawfish are still expected to spend most of their time in shallow depths and so shallow depths should still be the main concern for conservation and management practices. However, it is important to know that they do frequently occupy deeper depths. While shallow waters may be largely where adult sawfish occur, deeper waters still need to be considered for management measures to promote the increase of the population size of this critically endangered species. The entire sawfish population must be considered and conserved in order to encourage the growth of this species.

The results concluded that both male and female sawfish spend more time in shallow depths over deeper depths, as expected from previous sawfish literature (Poulakis and Seitz 2004; Carlson *et al.* 2014; Wiley and Simpfendorfer 2010; Simpfendorfer 2001). This knowledge is important to comprehend in order to understand their habitats and apply them to conservation practices. Expecting shallower depths to be favored, the main focus of this study was to see which sex spent more time occupying deeper depths. The results showed females tend to spend more time at deeper depths than males, and males spend more time at shallower depths than females. This was seen in the histograms of percentage time-at-depth (Figure 1), t-test conducted for greatest maximum depth and linear mixed-effects model (Figure 2). This may be due to females being larger than males on average (Poulakis *et al.* 2011) and smaller sawfish tend to stay in shallower depths (Poulakis and Seitz 2004; Wiley and Simpfendorfer 2010; Whitty *et al.* 2009; Simpfendorfer 2001). However, length fell out of the minimal adequate model, so length does not have a significant effect on the percent of time a sawfish spends at a particular depth. Therefore this should not be the case for the difference in depth between males and females. The fact that the analyzed sawfish did not have much size variety must also be considered. Length may be a significant factor, but did not have enough statistical power in this study to show any variation. Further testing with sawfish of greater size variety could be done to see if increasing the statistical power of length creates a difference.

It still can not be said for certain whether smalltooth sawfish exhibit sexual segregation in depth. Differences in depth between males and females could also be due to the reproductive cycle of females (Carlson *et. al* 2014). This could be counterintuitive since females are more often found at nurseries, which occur in shallow water areas like estuaries, and would not support the data showing females spend more time at deeper depths than males (Feldheim *et. al* 2017). However, this could potentially be evidence of sexual segregation, where females are actively avoiding males during non-reproductive seasons. Further studies that track the exact seasons the female sawfish are mating would have to be conducted in order to see if this could be a factor. Other factors that may affect the distribution of sexes could be due to feeding behavior or thermoregulation (Carlson *et al.* 2014). There has been evidence of sex-specific dietary requirements, such as females eating more than males of the same size. Sex-specific temperature preferences may also occur if a female is pregnant since warm waters can help an embryo develop (Wearmouth and Sims 2010). Feeding behavior and temperature preference could be different among sexes, but more data collection and analysis would need to be done to see if these factors have an effect on percent of time at a particular depth. Depth could also depend on the time of day. Sawfish have been recorded to move into shallower waters at night and deeper waters during the day, so diurnal movements could be a factor (Carlson *et al.* 2014). Overall, there are several potentially confounding variables that could have influenced the results of this study. Additional data and analyses would need to be collected and completed in order to see if these variables cause a significant difference.

Understanding how sawfish use depth is important in order to predict population processes and dynamics. Knowing the factors that affect a sawfish's depth preferences would allow for the successful management and conservation of this species, since these spatial dynamics often overlap with area-focused human activities like fishing. Until recently sawfish were thought to stay at a depth of 10 m or less (Simpfendorfer 2001), so data on their depth use is limited, but important to know in order to fully understand their full vertical range. Understanding their depth range could help with conservation effects and recover this critically endangered species. With the decline of sawfish, more research needs to be conducted in order to create more effective status assessments, management measures, and recovery plans (Dulvy *et al.* 2016). With the knowledge from this study, hopefully more effective recovery plans can be developed and key areas of research that still needs to be conducted can be identified.

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FIGURES AND TABLES

Table 1. Statistics for each sawfish that was used in the analysis. Sawfish numbers are their PTT numbers. STL means stretched total length. The location with the asterisk was adrift for 55 days before reporting. data. Females are highlighted in red.

Sawfish number	Tag type	Days Analyzed	Sex	STL	Tagging date	Tagging location	Pop up date	Pop up location
103429	MK10	151	M	427	4/24/13	25.098N 80.958W	9/22/13	25.307N 81.795W
103430	MK10	141	M	371	7/21/11	24.440N 82.083W	12/16/11	25.078N 80.423W
103432	MK10	55	M	409	5/1/11	25.130N 81.810W	6/23/11	25.012N 81.453W
103434	PATF	140	M	381	7/18/11	24.458N 82.087W	10/5/11	24.872N 82.242W
103435	PATF	46	M	399	3/13/12	25.035N 80.977W	4/28/12	25.112N 80.890W
103438	PATF	84	F	368	7/19/11	24.439N 82.087W	10/11/11	24.422N 81.749W
119906	MK10	150	M	412	8/11/13	25.105N 81.041W	1/9/14	N/A
127594	Mini-PAT	121	F	428	1/30/15	24.782N 80.657W	5/31/15	25.326N 80.267W
60745	PATF	62	M	395	4/24/13	25.098N 80.960W	6/24/13	28.293N 83.286W
60746	PATF	61	M	395	4/25/13	24.839N 80.610W	6/24/13	26.464N 82.064W
60749	MK10	138	F	323	7/17/11	24.455N 81.810W	1/27/12	24.013N 81.497W
60750	MK10	156	F	352	7/17/11	24.455N 81.810W	12/20/11	32.599N* 73.797W
136409	Mini-PAT	141	F	279	3/17/14	25.129N 81.066W	8/5/14	25.337N 81.240W
136410	Mini-PAT	133	F	283	9/6/14	25.124N 81.067W	1/17/15	25.110N 81.065W

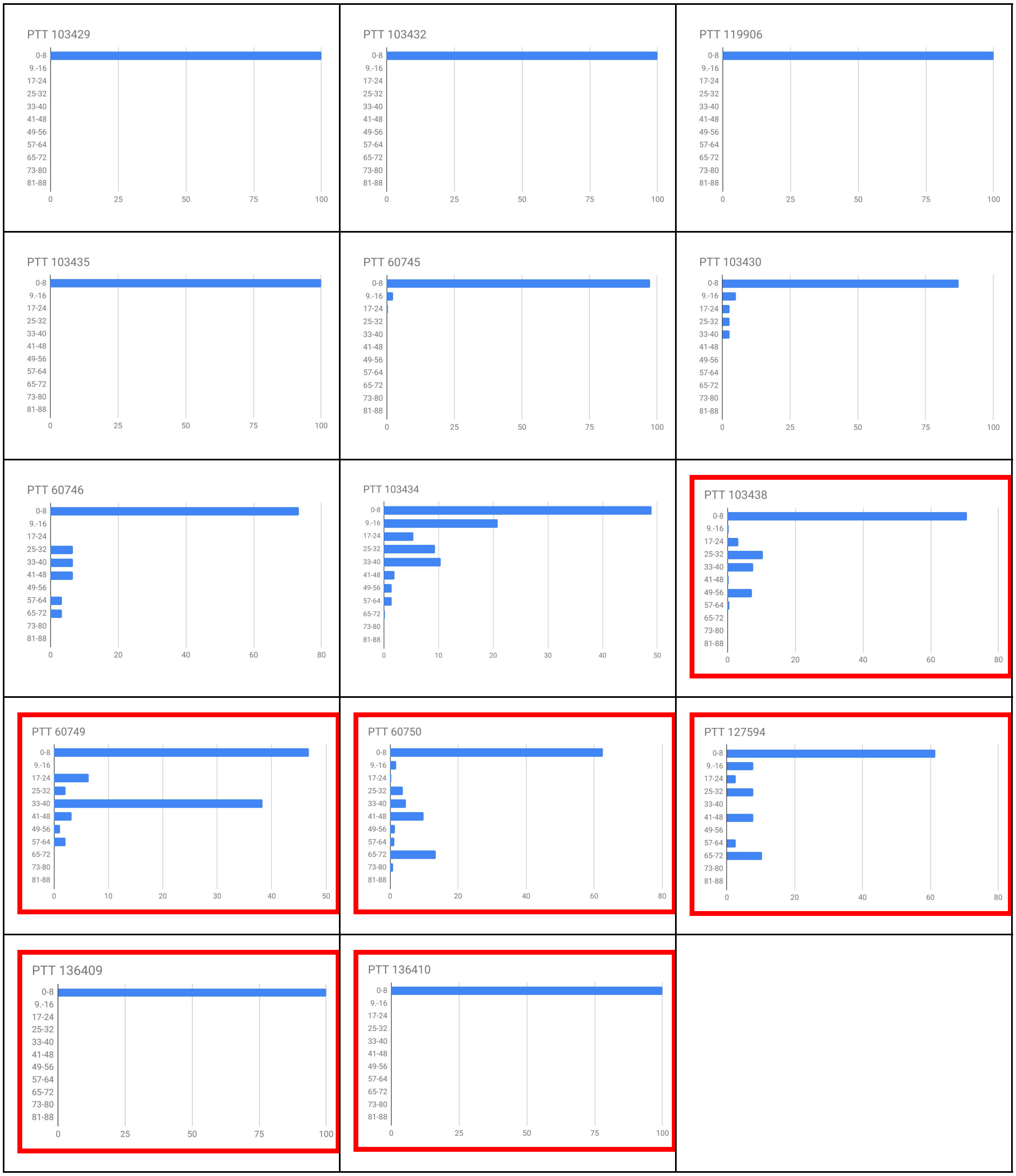

Figure 1. Histograms of percentage time-at-depth all of the analyzed sawfish. X-axis is "Percent of Time" and Y-axis is "Depth (m)". Females are highlighted in red.

Table 2. Table of the tested linear mixed-effects models and corresponding AICc and Delta AICc values. The lowest AICc value indicated the most parsimonious model, relative to the other

model fits with a higher AICc value. The delta AICc of the best linear mixed-effects model between 0-2 can be said to have substantial support.

Model- function:lmer()	AICc	Delta AICc
Percent.Time ~ Sex + Depth + (1|Sawfish)	58.25	0.00
Percent.Time ~ Length + Depth + (1|Sawfish)	66.93	8.67
Percent.Time ~ Depth * Sex + (1|Sawfish)	69.82	11.56
Percent.Time ~ Length + Depth + Sex + (1|Sawfish)	72.76	14.51
Percent.Time ~ Length * Depth + (1|Sawfish)	87.28	29.03
Percent.Time ~ 1 + (1|Sawfish)	115.48	57.23
Percent.Time ~ 1	115.48	57.23
Percent.Time ~ Length * Depth * Sex + (1|Sawfish)	123.87	65.62
Percent.Time ~ Length + Sex + (1|Sawfish)	132.52	74.27
Percent.Time ~ Length * Sex + (1|Sawfish)	139.54	81.29

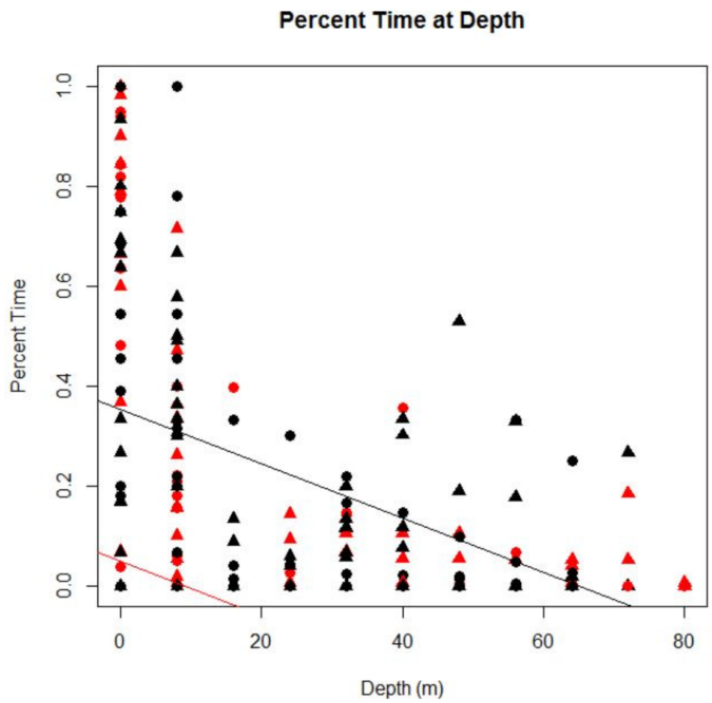

Figure 2. The model shows the percent time a sawfish stays at a specific depth. Black = females, red = males.