

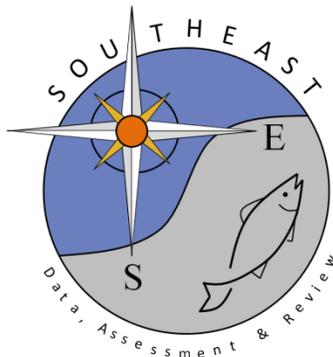
Identification, Life History and Distribution of *Mustelus canis*, *M. norrisi*  
and *M. sinusmexicanus* in the northern Gulf of Mexico

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Identification, Life History and Distribution of *Mustelus canis*, *M. norrisi* and *M. sinusmexicanus* in the northern Gulf of Mexico.

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## Summary

### Introduction

The life histories of triakid sharks inhabiting the northern Gulf of Mexico remain largely unknown. Data pertaining to the age, growth and reproduction of the three species of hound sharks inhabiting the northern Gulf of Mexico were obtained from numerous sources, including National Marine Fisheries Service (NMFS) Mississippi Laboratories Longline Surveys, Florida State University Coastal and Marine Lab Shark Survey (FSUCML), Texas A&M Department of Biology, Texas Parks and Wildlife, University of North Florida, primary literature and museum accessed specimens. The purpose of this document is to provide, when possible, sex-specific von Bertalanffy growth function (VBGF) parameter estimates, maturity ogives and a summary of the reproductive biology of *Mustelus canis*, *M. norrisi* and *M. sinusmexicanus*. Additionally, information on the spatial distribution of the three species in the northern Gulf of Mexico is provided.

### Material and Methods

Hound shark specimens were collected during surveys, covering waters from approximately the Dry Tortugas to Brownsville, Texas at depths from 2 to 408 meters. Due to the difficulty associated with differentiating among the three species occurring in the northern Gulf of Mexico, during NMFS surveys, sharks were identified to the genus level unless microscopic examinations of the denticle morphology could be conducted. To provide species-specific length-weight relationships, morphometric data from all *Mustelus* spp. collected during NMFS longline surveys, regardless if they were retained for life history examinations, were analyzed. Length-length (i.e., precaudal length (PCL), fork length (FL), natural total length (TL) and stretch total length (STL)) and length-weight relationships were compared among species and between sexes using an analysis of co-variance (ANCOVA). Body weight and FL data were log-transformed for this analysis.

Specimens used for life history studies were collected opportunistically. For NMFS collected sharks, specimens were frozen at sea and returned to the laboratory. After thawing, PCL, FL, TL and STL were measured and specimens were dissected. Sharks collected during FSUCML surveys were dissected in the field. Vertebrae used for age analyses were limited to those removed from sharks collected during NMFS surveys. Vertebrae were prepared for age analysis using standard techniques.

The reproductive systems of females and males were examined and pertinent measures were taken. Males were considered mature if they possessed calcified claspers that could freely rotate 180° and had freely opening rhipidions. Females were considered mature if they were gravid or possessed fully developed uteri and oviducal glands, and exhibited signs of advanced ovarian activity, such as the presence of vitellogenic or atretic follicles. Females that had vitellogenic follicles but did not show complete development of other reproductive organs were considered pubescent. When gravid, brood size was enumerated and the sex and length of each embryo was recorded. A chi-square test with Yates' correction was used to determine if the ratio of female to male embryos differed from the expected (1:1). When adequate data were available, maturity ogives were generated for both sexes of each species and ages (years) and length (cm) at 50% maturity estimated.

Capture locations of the three *Mustelus* species collected during NMFS surveys were plotted using QGIS (2.0.1 DuFour) to visually compare their distributions within the northern Gulf of Mexico. Multiple logistic regressions were conducted using a backwards stepwise procedure to identify factors that could influence distribution patterns. Factors in the logistic model included depth, bottom fluorescence, turbidity (% transmittance), dissolved oxygen, and salinity. In addition, analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed to determine if species-specific differences exist for any of these factors. These statistical tests were considered significant at a  $p < 0.05$ . Additionally, NMFS distribution data were compared to distribution data collected during FSUCML surveys, which occurred from the West Florida shelf to Louisiana.

## Results and Discussion

### *Identification*

Identifying *Mustelus* specimens based on external, macroscopically visible characters has been widely acknowledged to be problematic (e.g. Compagno, 1984; Castro, 2011). Jones et al. (in review) conducted morphometric analyses on genetically verified specimens of *M. canis* and *M. sinusmexicanus* and determined that no externally visible characters were capable of differentiating between the two species, due to overlap in measures of characters assessed. The authors considered the only reliable features to distinguish among species were denticle and vertebral column morphology. However, it should be noted that Jones et al. (in review) also reported variability in denticle morphology in both species examined. These two characters used during NMFS surveys to assign species designations. These species designations were utilized for morphometric relationships, distribution, age, growth, and some reproductive results reported below. While vertebral morphology was verified for some of the specimens, results should be considered preliminary until genetic verifications can be completed. Genetic analyses of *Mustelus* spp. specimens identified during NMFS surveys based on labial furrow lengths and denticle morphology indicated that 68% of *M. canis*, 40% of *M. norrisi* and 97% of *M. sinusmexicanus* specimens were correctly identified.

Based on genetically verified specimens, Giresi et al. (2012) identified several characters that the authors demonstrated are effective in differentiating among triakids in the northern Gulf of Mexico, including ampullary pore patterns and fin shapes. Therefore when available, genetically identified specimens were used to generate species-specific life history parameter estimates.

Length-length and length-weight relationships:

Species and sex-specific length-length and length-weight relationships are listed in Table 1. There were no significant differences in the length-length relationships among the three *Mustelus* species (all  $p > 0.150$ ) or between sexes (all  $p > 0.264$ ), resulting in the reporting of combined *Mustelus* spp. length-length relationships (Table 1). However, significant differences were observed in length-weight relationships among species ( $p < 0.001$ ) and between sexes ( $p < 0.001$ ). As a result, length-weight relationships are provided for each species and sex (Table 1).

*Age and Growth:*

Ages were assigned to 518 hound sharks. A summary of species and sex-specific VBGF parameter estimates is presented in Table 2. For all species combined, there was a significant difference in the VBGF models for females and males ( $\chi^2 = 54.31$ ,  $p < 0.01$ ). There was significant variability in size-at-age among the growth models, suggesting VBGF models combining the three species are not appropriate and that the combined-species model was likely driven by the relatively large number of nominal *M. canis* samples. A total of 369 nominal *M. canis* (166 female; 203 male) samples were aged. There was a significant difference in VBGF models for females and males ( $\chi^2 = 24.58$ ,  $p < 0.01$ ). Like the combined models, there was a significant amount of variability in size-at-age among models. Unlike the combined models, variability in size-at-age estimates was narrower in earlier age classes, suggesting that as *M. canis* grows, accurate ageing becomes more difficult. For *M. norrisi*, there were not adequate data to generate a reliable growth model for females; however, VBGF parameter estimates for the combined sexes and males are reported in Table 2. Highly variable size-at-age data suggest that vertebrae might not be well suited to age *M. norrisi*. Few *M. sinusmexicanus* were aged ( $n = 54$ ), therefore, only a combined model was generated (Table 2, Figure 9). Similar to the other two species aged, there was a significant amount of variability in the size-at-age. The oldest observed ages for female *M. canis*, *M. norrisi* and *M. sinusmexicanus* were 13, 6 and 14 years, respectively. The oldest observed ages for male *M. canis*, *M. norrisi* and *M. sinusmexicanus* were 11, 9 and 13 years, respectively.

Because of the identification problems for *M. norrisi* and *M. canis* associated with the NMFS samples used for vertebral analyses and the limited sample sizes for the reliably identified *M. sinusmexicanus*, it was not possible to present a reliable growth model for any of the species. Therefore, we felt the growth of the three species would be best described by the lowest and highest biologically realistic values of VBGF parameter estimates found for any of the three species. The growth models selected to represent these low and high values were associated with combined sexes models for *M. canis* and *M. norrisi*, respectively (Table 2; Figures 1 and 2).

## **Reproduction:**

*Age and Size at maturity:*

Based on identification issues and biological similarities (e.g. maximum observed age and length), size at maturity data for *M. canis* and *M. sinusmexicanus* were combined. However,

no nominal *M. sinusmexicanus* specimens had both maturity and age data. Therefore, age at maturity for *M. canis* was used as a surrogate for *M. sinusmexicanus* as maximum observed ages. Sex-specific size and age at 50% maturity for the *M. canis*/*M. sinusmexicanus* group are presented in Table 3 and Figures 3-6. Size and age at maturity schedules are presented in Tables 4 and 5.

As only 40% of *M. norrisi* were correctly identified within NMFS samples and the maximum size of *M. norrisi* is smaller than *M. canis* and *M. sinusmexicanus*. Length and maturity data from genetically verified *M. norrisi* specimens collected during FSUCML surveys were used for this analysis. Unfortunately, all specimens collected during FSUCML surveys that were assigned a maturity state were males, therefore, size at maturity had to be estimated for female *M. norrisi*. For *M. canis* and *M. sinusmexicanus* female size at maturity was 109% of male size at maturity. An estimate of female size at maturity for *M. norrisi* was derived using this relationship between male and female size at maturity for *M. canis* and *M. sinusmexicanus*. Age data also were not available for the *M. norrisi* specimens utilized for maturity analyses, so we chose to use age at maturity data from the combined *M. canis* / *M. sinusmexicanus* estimates as a conservative surrogate for *M. norrisi*. Size and age at maturity for *M. norrisi* are summarized in Table 3 and Figure 7. The size at maturity schedule for male *M. norrisi* is listed in Table 6.

#### *Brood size and Reproductive Cycle:*

Brood size ranged 11-20 in 10 genetically verified *M. canis* (mean = 15.5, S.D. = 2.8, n = 61). The ratio of female to male embryos was not different from the expected 1:1 ( $\chi^2 = 0.99$ ,  $p > 0.25$ ). There was no significant relationship between maternal length and brood size. Ovaries of all gravid females examined had vitellogenic follicles indicating that female *M. canis* reproduce annually. According to Baughman and Springer (1950) and Grubbs (personal observation), the pupping season for *M. canis* occurs in approximately July.

Brood size for *M. norrisi* was determined based on examination of three genetically verified specimens (Giresi, unpublished data) and three reports from the primary literature (Springer, 1939; Heemstra, 1997). The mean brood size for *M. norrisi* was 11.3 (S.D. = 2.1) and ranged from 8-14. This range was similar to the range of 6 -14 reported by other authors (e.g. Springer, 1939, 1961; Clark and von Schmidt, 1965; Heemstra, 1973). The female to male ratio of embryos was not different from 1:1 ( $\chi^2 = 0.04$ ,  $p > 0.50$ ). There was no significant relationship between maternal length and brood size for *M. norrisi* ( $F = 0.16$ ,  $p = 0.71$ ,  $r^2 = 0.18$ ). Based on three gravid females with vitellogenic follicles, *M. norrisi* appears to reproduce annually. Clark and von Schmidt (1965) proposed an annual cycle for this species as well based on a single gravid specimen with vitellogenic follicles, an observation corroborated by Giresi (personal observation). We did not observe any near term embryos or neonate specimens; however, according to Castro (2011), the pupping season for *M. norrisi* is approximately April.

Only five gravid female *M. sinusmexicanus* were collected. Brood size ranged from 3-10 (mean = 5.00, S.D. = 2.83) and the sex ratio of female to male embryos was not different from the expected ratio of 1:1 ( $\chi^2 = 0.04$ ,  $p > 0.50$ ). All five gravid females were collected during winter months and three had small vitellogenic follicles. It is likely that these females were at the onset of vitellogenesis. Therefore, we hypothesize that female *M. sinusmexicanus* reproduce annually. The smallest free swimming *M. sinusmexicanus* we observed was 48 cm STL; slightly larger than the size at birth of 35-43 cm STL reported by Heemstra (1973). This shark was captured in October, suggesting summer parturition. This was in agreement with Grubbs

(personal observation), who observed a gravid female with near term pups of similar size to the smallest free swimming individuals in May.

*Distribution:*

All *Mustelus* species collected during NMFS surveys occurred in depths ranging from 50 to 200 m, and there did not appear to be any distribution differences between the sexes (Figure 15). *Mustelus canis* ranged from the Florida Keys to the Texas-Mexico border, with their highest concentration occurring in the north central Gulf of Mexico (Figure 15a). Similar to *M. canis*, *M. sinusmexicanus* ranged from south Florida to southern Texas; however, their highest concentration occurred in the north western Gulf of Mexico (Figure 15b). *Mustelus norrisi* was not as abundant as its two congeners, but its highest abundance occurred in the eastern Gulf of Mexico, east of the Mississippi River delta (Figure 15c).

The only two variables that exhibited co-linearity were bottom temperature and depth, so bottom temperature was excluded from the analysis. Multiple logistic regression analysis revealed that depth (Wald = 4.86,  $p = 0.027$ ), bottom fluorescence (Wald = 8.04,  $p = 0.005$ ), turbidity (Wald = 3.82,  $p = 0.051$ ), dissolved oxygen (Wald = 7.06,  $p = 0.008$ ) and salinity (Wald = 6.28,  $p = 0.012$ ) all significantly influenced the distribution of *M. canis*. The overall model had a good fit (log-likelihood ratio = 73.0,  $p < 0.001$ ). Bottom fluorescence (Wald = 5.98,  $p = 0.015$ ), dissolved oxygen (Wald = 4.90,  $p = 0.027$ ), and salinity (Wald = 10.77,  $p = 0.001$ ) significantly influenced the distribution of *M. sinusmexicanus*. The overall model had a good fit (LR = 37.0,  $p < 0.001$ ). Bottom fluorescence (Wald = 10.55,  $p = 0.001$ ) was the only factor that significantly influenced the distribution of the *M. norrisi*. The overall model had a good fit (LR = 24.0,  $p < 0.001$ ).

Several species-specific differences in environmental factors were evident using NMFS collected specimens, but it is important to note that the species IDs were not genetically verified. From this analysis, there was a significant effect of depth ( $F_{2,343} = 13.8$ ,  $p < 0.001$ ) on the distribution of the three species, with *M. norrisi* occurring in deeper water (mean:  $160.3 \pm 12.7$ m) than *M. canis* (mean:  $122.3 \pm 4.1$ m) and *M. sinusmexicanus* (mean:  $100.1 \pm 3.8$ m). Similarly, all three species occurred at different bottom temperatures ( $F_{2,315} = 20.0$ ,  $p < 0.001$ ), with *M. norrisi* occurring in cooler waters (mean:  $15.9 \pm 0.5^\circ\text{C}$ ) compared to *M. canis* (mean:  $18.1 \pm 0.2^\circ\text{C}$ ), and *M. sinusmexicanus* (mean:  $19.5 \pm 0.2^\circ\text{C}$ ). No species-specific differences were evident related to bottom salinity, dissolved oxygen and fluorescence (all  $F < 0.360$ , all  $p > 0.085$ ).

Analysis by Giresi et al. (unpublished) compared genetically verified specimens to provide a spatial comparison among species as well. This study is in agreement with Jones et al. (2014), showing that there is an effect of depth on the distribution of species. However, the depth ranges differ from Jones et al. in that the depth ranges of *M. canis* and *M. sinusmexicanus* were generally caught in deeper water than *M. norrisi*. *Mustelus canis* was caught from 64-408m (mean  $210.68 \pm 106.05$ ), *M. sinusmexicanus* were caught from 51-233m (mean  $101.78 \pm 39.35$ ), and *M. norrisi* were caught at depths from 1-92m (mean  $13.94 \pm 26.6$ ).

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Table 1: Length-length and length-weight relationships for *Mustelus* spp. specimens collected in the northern Gulf of Mexico. All lengths are measured in cm. FL = fork length, PCL = precaudal length, TL = natural total length, and STL = stretch total length. Results of the ANCOVA revealed that there was no significant differences in the length-length measurements between species (all  $p > 0.150$ ) or sex (all  $p > 0.264$ ); however, there were significant differences observed in length-weight relationships between species ( $p < 0.01$ ) and sex ( $p < 0.01$ ).

Conversion	N	Equation	$r^2$
Combined: FL to PCL	97	$(0.9416 * FL) - 1.1133$	0.99
Combined: FL to TL	915	$(1.0914 * FL) + 3.5978$	0.98
Combined: FL to STL	41	$(1.1367 * FL) + 2.15$	0.99

Conversion	n	Equation	$r^2$
<i>M. canis</i> Female: FL to Wt	398	$2 \times 10^{-6} (FL^{3.258})$	0.95
<i>M. canis</i> Male: FL to Wt	148	$3 \times 10^{-6} (FL^{3.108})$	0.90
<i>M. sinusmexicanus</i> Female: FL to Wt	250	$3 \times 10^{-6} (FL^{3.135})$	0.88
<i>M. sinusmexicanus</i> Male: FL to Wt	76	$2 \times 10^{-6} (FL^{3.213})$	0.90
<i>M. norrisi</i> Female: FL to Wt	34	$2 \times 10^{-6} (FL^{3.287})$	0.92
<i>M. norrisi</i> Male: FL to Wt	26	$2 \times 10^{-5} (FL^{2.735})$	0.89

Table 2: von Bertalanffy Growth Function parameter estimates for *Mustelus canis*, *M. norrisi* and *M. sinusmexicanus* from the northern Gulf of Mexico.  $L_\infty$  and  $t_0$  are reported in cm FL and years, respectively. Models considered to represent low and high values for *Mustelus* spp. are highlighted in gray.

Species	Sex	$L_\infty$	$k$	$t_0$	$N$	$r^2$
Combined	Combined	109.62	0.15	-3.42	518	0.74
Combined	Female	130.32	0.12	-3.50	224	0.78
Combined	Male	93.89	0.23	-2.70	293	0.75
<i>M. canis</i>	Combined	113.78	0.13	-3.87	369	0.75
<i>M. canis</i>	Female	128.95	0.12	-3.65	166	0.80
<i>M. canis</i>	Male	96.88	0.19	-3.23	203	0.76
<i>M. norrisi</i>	Combined	95.05	0.25	-2.03	94	0.64
<i>M. norrisi</i>	Male	85.86	0.4	-1.40	57	0.74
<i>M. sinusmexicanus</i>	Combined	104.58	0.16	-3.99	54	0.58

Table 3. Summary of age and size at maturity for *Mustelus canis* / *M. sinusmexicanus* group and *M. norrisi* in the northern Gulf of Mexico.

Species	Sex	Age (years) at 50% maturity (a, b, n)	Size (cm FL) at 50% maturity (a, b, n)
<i>M. canis</i> / <i>sinusmexicanus</i>	Combined	3.61 (-5.67, 1.57, 346)	71.54 (-23.62, 0.33, 656)
	Female	4.11 (-6.31, 1.54, 145)	75.09 (-55.54, 0.74, 303)
	Male	3.28 (-5.36, 1.63, 201)	69.20 (-21.98, 0.32, 353)
<i>M. norrisi</i>	Combined	3.61 (-5.67, 1.57, 346)	-
	Female	4.11 (-6.31, 1.54, 145)	58.50
	Male	3.28 (-5.36, 1.63, 201)	53.86 (-74.15, 1.38, 39)

Table 4. Sex-specific size at maturity schedules for the *Mustelus canis* / *M. sinusmexicanus* group in the northern Gulf of Mexico.

Percent mature	Female (cm FL)	Male (cm FL)
10	72.12	62.29
20	73.22	64.84
30	73.95	66.54
40	74.55	67.93
50	75.10	69.20
60	75.64	70.48
70	76.24	71.87
80	76.97	73.57
90	78.07	76.12
100	84.43	90.94

Table 5. Sex-specific age at maturity schedules for the *Mustelus canis* / *M. sinusmexicanus* group in the northern Gulf of Mexico.

Percent mature	Female age (years)	Male age (years)
10	2.68	1.94
20	3.20	2.44
30	3.55	2.77
40	3.84	3.04
50	4.11	3.28
60	4.37	3.53
70	4.66	3.80
80	5.01	4.13
90	5.54	4.63
100	8.60	7.51

Table 6. Size at maturity schedule for male *Mustelus norrisi* in the northern Gulf of Mexico.

Percent	Male (cm FL)
10	52.2605
20	52.8495
30	53.2409
40	53.5618
50	53.8563
60	54.1508
70	54.4717
80	54.8632
90	55.4521
100	58.8726

Figure 1. von Bertalanffy Growth Function (VBGF) for combined *Mustelus canis* in the northern Gulf of Mexico. See Table 2 for a summary of VBGF parameter estimates.

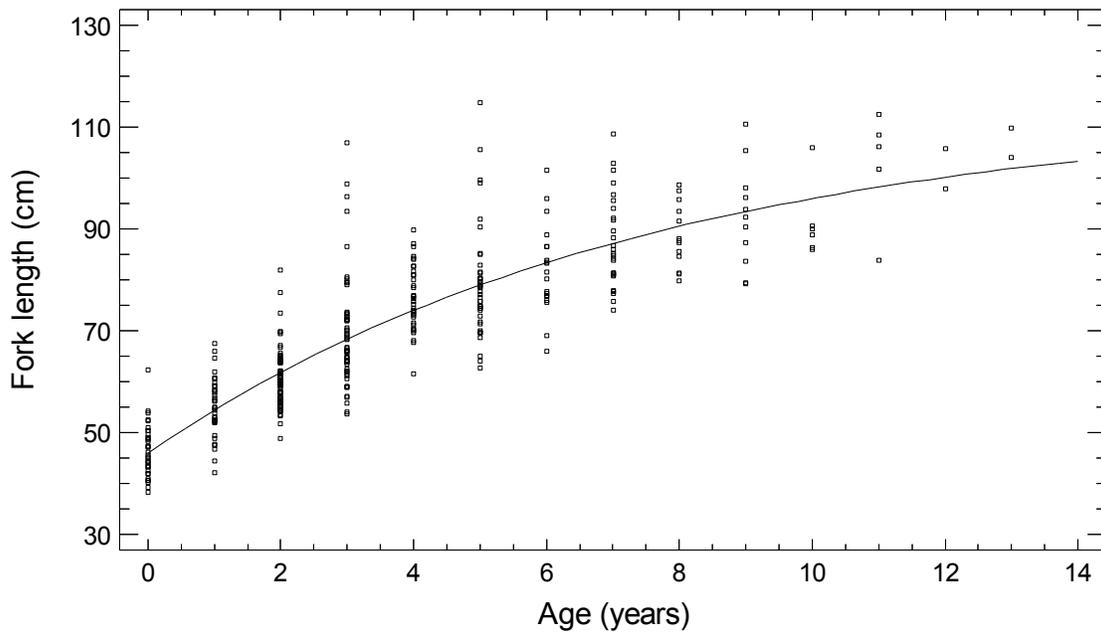


Figure 2. von Bertalanffy Growth Function (VBGF) for combined *Mustelus norrisi* in the northern Gulf of Mexico. See Table 2 for a summary of VBGF parameter estimates.

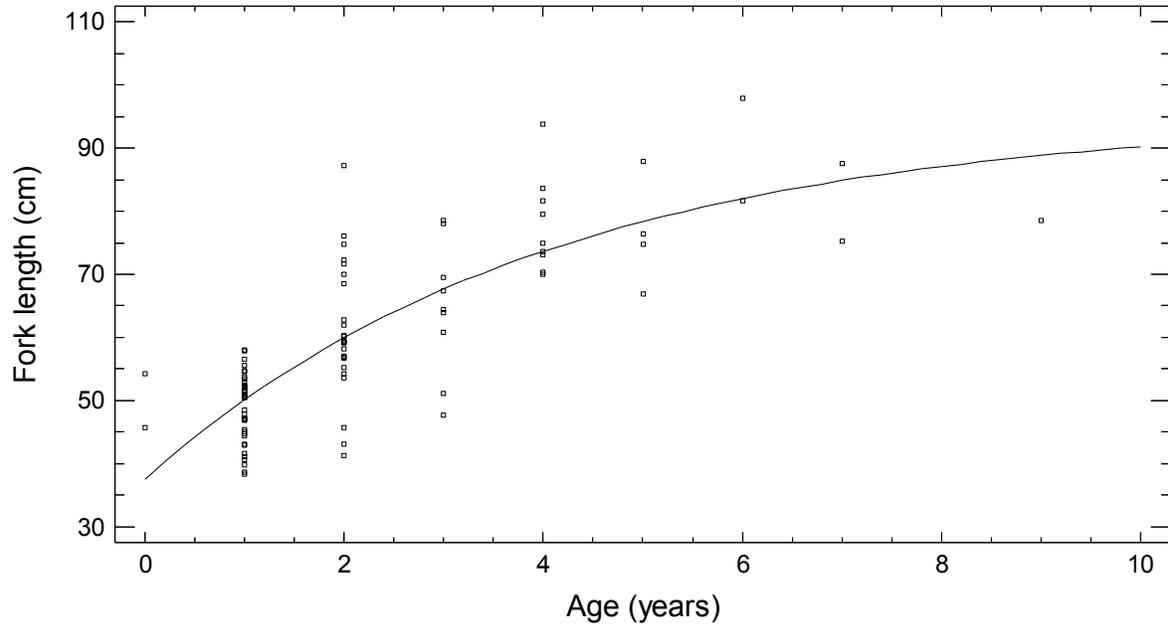


Figure 3. Size at maturity ogive for females within the *Mustelus canis* / *M. sinusmexicanus* group in the northern Gulf of Mexico. 95% confidence intervals are indicated by red lines.

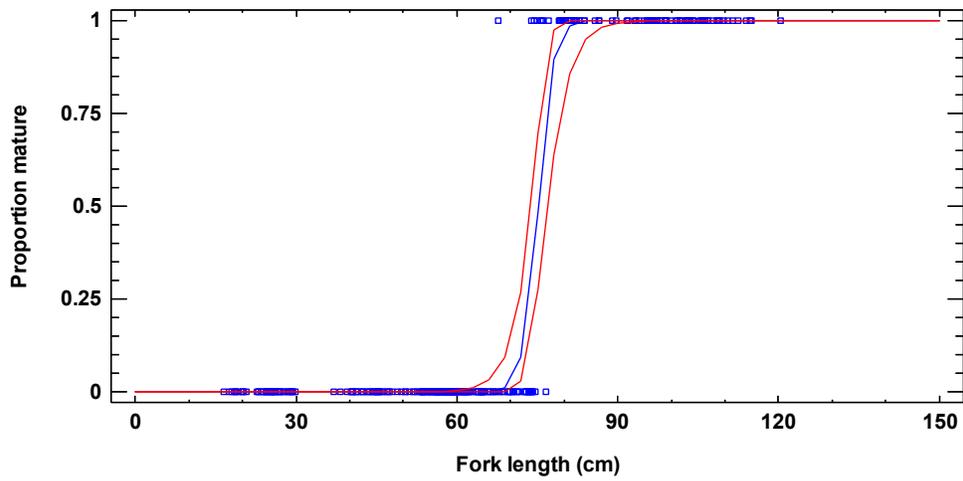


Figure 4. Size at maturity ogive for males within the *Mustelus canis* / *M. sinusmexicanus* group in the northern Gulf of Mexico. 95% confidence intervals are indicated by red lines.

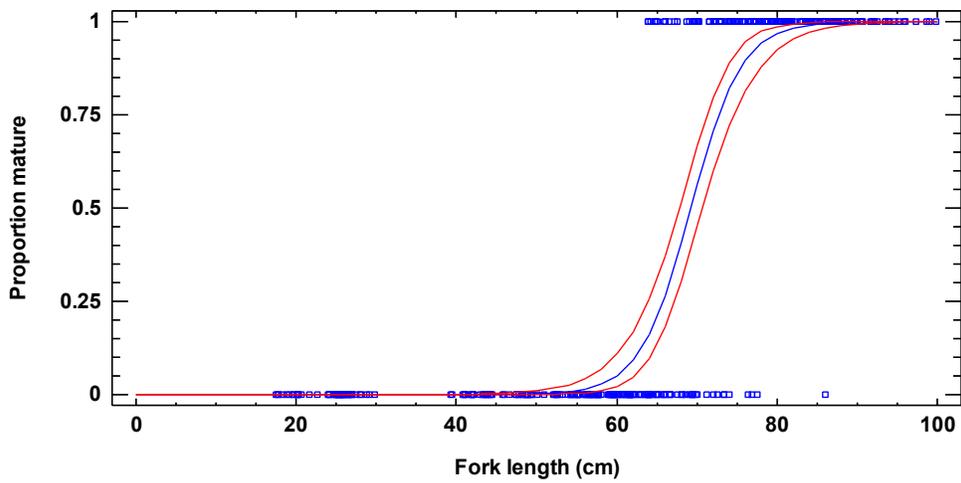


Figure 5. Age at maturity ogive for females within the *Mustelus canis* / *M. sinusmexicanus* group in the northern Gulf of Mexico. 95% confidence intervals are indicated by red lines.

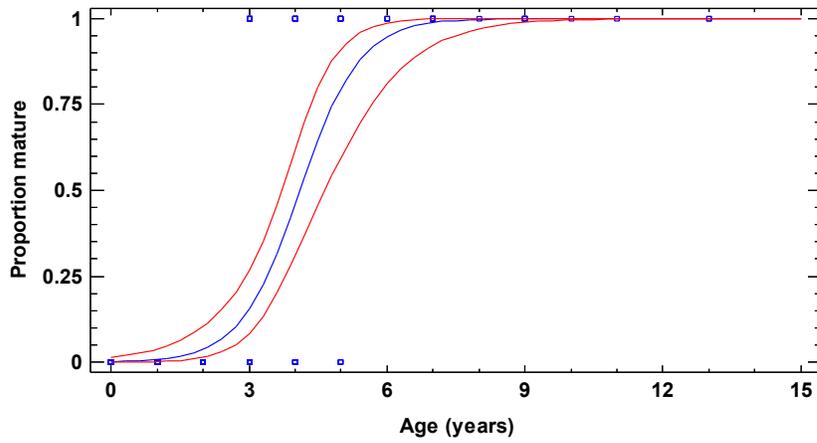


Figure 6. Age at maturity ogive for males within the *Mustelus canis* / *M. sinusmexicanus* group in the northern Gulf of Mexico. 95% confidence intervals are indicated by red lines.

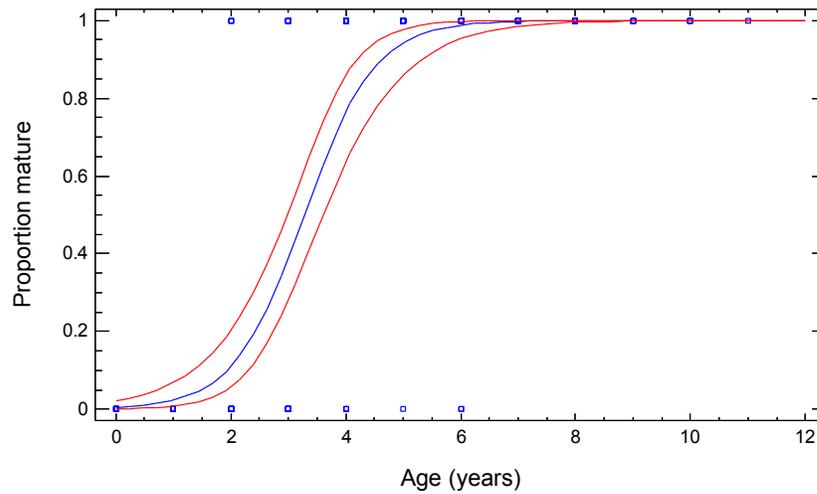


Figure 7. Size at maturity ogive for male *Mustelus norrisi* group in the northern Gulf of Mexico. 95% confidence intervals are indicated by red lines.

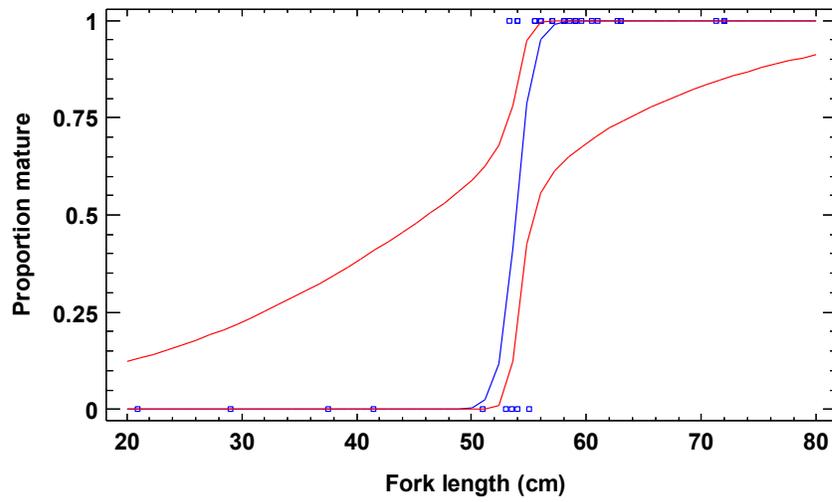


Figure 15. Station locations where a) *Mustelus canis*, b) *M. sinusmexicanus*, and c) *M. norrisi* were captured during the NOAA/NMFS offshore bottom longline survey from 2000-2013. Gray and white circles indicate sex.

