Maternal Investment and Gestational Indicators in the Atlantic Stingray

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Abstract

Reproduction in elasmobranchs (sharks, skates, rays, and sawfish) is not widely known. Understanding their reproduction is vital for their future conservation and management. This study aims to develop new methods capable of determining species-specific reproductive patterns and life history of elasmobranchs vulnerable to fisheries bycatch.

Many threatened and endangered sharks and rays are caught as bycatch during fishing operations, resulting in their death. This research will allow us to determine reproductive patterns and gestational stages from post-mortem elasmobranchs. Reproductive macroscopic assessments can be conducted in the field or in markets by anyone to contribute to our worldwide knowledge of elasmobranch reproduction.

This study collected *Hypanus sabinus* as a model species to assess these methodologies. Intrauterine macroscopic morphometrics were collected from post-mortem *H. sabinus;* including uterine length, uterine width and uterine villi length (mm) at each gestational stage. Gestational stages were divided into early, late, and postpartum stages based on their 4-month gestation period and averages were calculated for each stage (**N = 36**). A standard T-test was used to test for significant differences in macroscopic measurements between gestational stages. Analyses comparing the descriptive changes of the uterus, such as color, will further develop these techniques to determine reproductive patterns macroscopically.

Introduction:

Elasmobranchii reproduce through internal fertilization nourishing their embryos in ten different modes of embryonic nourishment. The level of nourishment ranges from lecithotrophic to matrotrophic maternal investment. Stingrays provide embryos with Histotrophic milk secretion for nourishment.

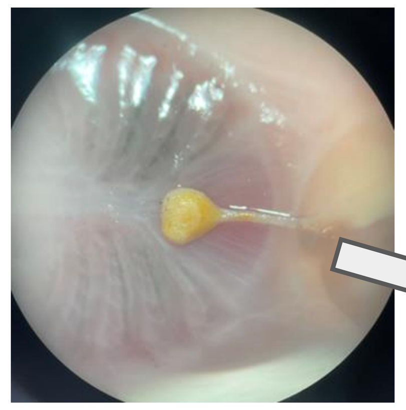


Image 1

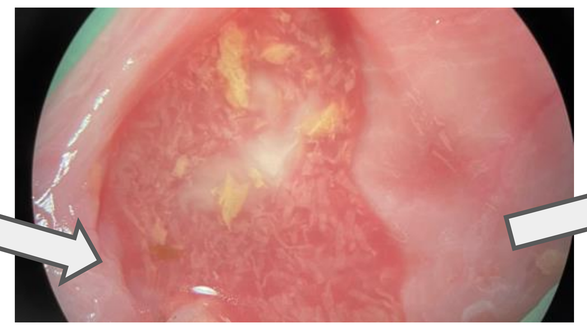


Image 2

Image 3

Goal: Determine intrauterine macroscopic morphological characteristics of matrotrophic elasmobranch species to predict the reproductive state of post-mortem specimen.

Predictions: There will be significant differences in uterine morphology between gestational stages that will allow gestational staging of post-mortem specimen collected in the field and artisanal fishing markets.

Methodology:

- Mature female *H. sabinus* were collected from April and August of 2023 (with a gestation period of 4 to 4.5 months) using seines with Fish and Wildlife Research Institute and through independent collections along the coast of FSU's Coastal and Marine Laboratory. Mature female *H. sabinus* (220 - 300 cm) were collected and euthanized following approved IACUC protocols (PROTO20230000004).
- Uterus length, width, and villi (mm) were measured with calipers. Photographs were taken using Olympus Model CHTSZ4045 Dissecting Microscope and further analyzed through ImageJ software.
- T-test was used to test for statistical significance.

Results:

Image 4

This displays a microscopic image of a mid-term gestational uterus with distinct and elongated villi.

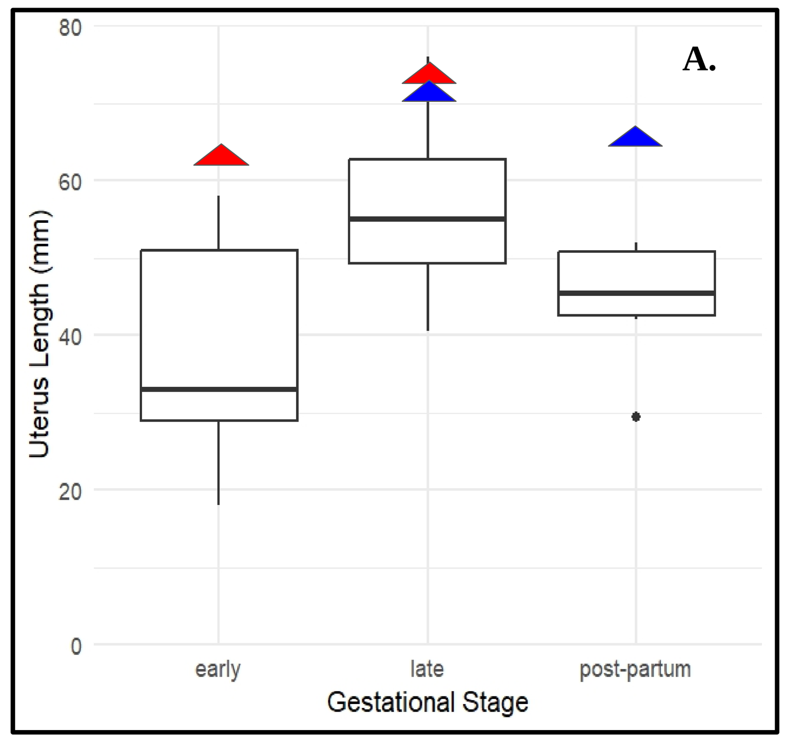
Image 5

This displays a microscopic image of postpartum uterus with no distinct villi.

Table I. This table shows the average of uterine morphology throughout all stages of gestation including overall uterus length, uterus width, and villi length.

	Mean	Sample Size
Uterus Width	18.367561 (+-7.458385)	41
Uterus Length	46.88122 (+- 15.348175)	41
Villi	5.729167 (+- 3.493553)	36

Figure A. Uterine length increases throughout gestation, reaching its peak in late gestation. There is a statistically significant difference in uterine length between 1) late and post-partum stages and 2) early and late stages. However, there is no statistically significant differences between the early and postpartum stages in uterine length. These results suggest we can determine gestational stage using uterine length morphology.



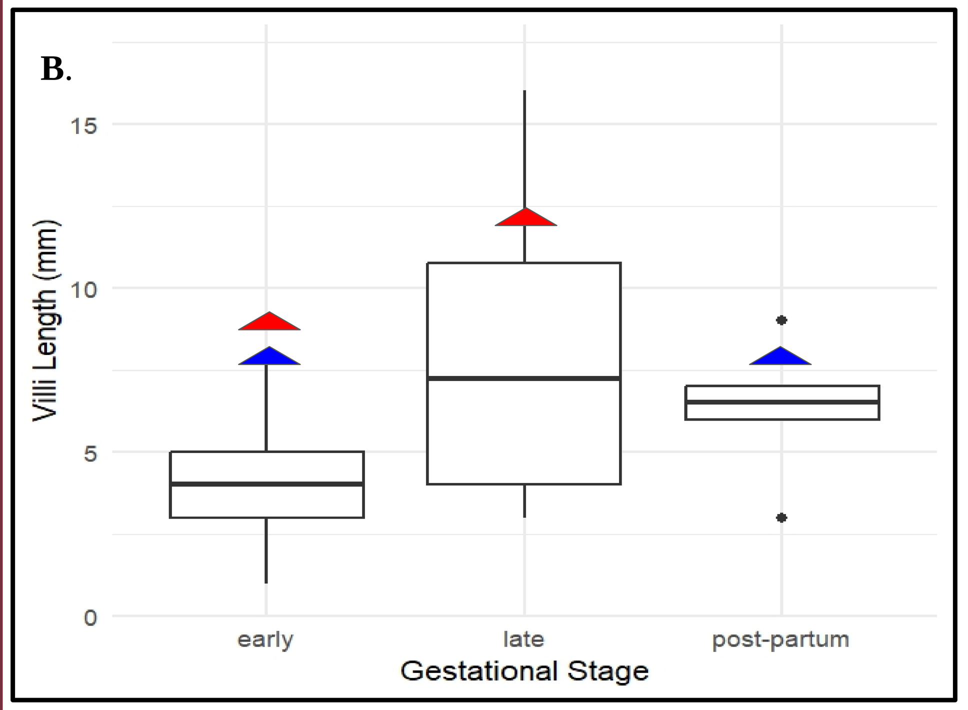


Figure B. Villi length increases throughout gestation, peaking in late gestation. There is statistically significant difference in villi length between early and late gestational stages. There is a statistically significant difference in villi length between early gestation and postpartum. These results suggest we can determine gestational stage using uterine villi morphology.

Conclusion:

This study investigated the intrauterine morphological development of *Hypanus sabinus* throughout gestation. We have found that villi length can be used to differentiate between early and late stages along with early and post-partum stages, but not late and post-partum stages. Meanwhile, the uterus length can identify differences in early and late stages along with late and post-partum stages, but not differences in early and post-partum stages. Overall, villi and uterus length generally decrease from early to post-partum stages, but will not shrink back to their original pre-pregnancy size, showing differences are visible between gestational stages. These differences in villi length and uterus length will allow us to successfully determine gestational stage from uterine and villi measurements, providing a valuable tool for assessing reproductive status in various species.

This data provides insight into methodology which can be used to determine differences in gestational stages of *H. sabinus*, and contributes to understanding reproductive patterns in species affected by bycatch mortality, which is essential for conservation efforts.

Future Plans:

We are currently analyzing oxygen consumption data to determine the energetic cost to physical and physiological changes that occur throughout gestation. We plan to further analyze the uterine width data to retest for significant differences between early and late gestation

References:

Abel, D. C. & Grubbs, R. D. The Lives of Sharks: A Natural History of Shark Life. (2023).

Rueden, C., Dietz, C., Horn, M., Schindelin, J., Northan, B., Berthold, M. & Eliceiri, K. (2021). ImageJ Ops [Software].

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

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