Maternal Investment and Gestational Indicators in the Atlantic Stingray

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Abstract

Reproduction in Elasmobranchii (cartilaginous fish) is not widely known. Understanding their reproduction is important for their conservation and particularly important for species vulnerable to bycatch mortality. This study aims to investigate reproductive patterns and gestational development of *Hypanus sabinus*.

To investigate these patterns, embryos of *Hypanus sabinus* collected during the 2023 gestation season were analyzed across developmental stages. Samples were weighed, dried to determine water content, and combusted to quantify inorganic and organic matter. Organic matter increased from an average of 0.3 g in recently fertilized ova to 2.86 g in near term embryos, representing an average increase of 862.7%. Segmented regression analysis revealed a biphasic pattern, with little change in organic matter early in development followed by a rapid increase after mid gestation, indicating that most maternal nutrient contribution occurs during later embryonic stages.

Elasmobranch species are increasingly threatened and require both in-vito and ex-situ reproductive conservation and population management. Our understanding of their reproductive patterns and required level of provisioning will assist ongoing projects aimed at the conservation of elasmobranchs.

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.

Histrophy is a reproductive strategy of nourishment that allows embryos to receive additional nutrients from secretions produced by its mother.

Goals:

- Determine the patterns, quantity, and distribution of maternal investment throughout the gestation in a matrotrophic elasmobranch, the Atlantic Stingray.

Predictions:

- *Hypanus sabinus* will contribute over a 800% increase in organic matter from recently ovulated ovum to full-term embryos.
- The contribution of organic matter will be bi-phastic, with no increases in organic matter in early embryonic stages, with a exponential growth following and predicting when the secretion of histotroph occurs.

Embryonic Development:

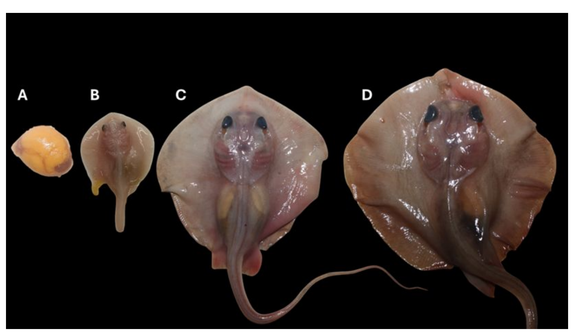

Figure A. Determination of embryonic stages throughout the collection period. A) early, B) mid, C) late, and D) full-term samples were collected between 24 April and 6 July 2023.

Methodology:

- Mature female *H. sabinus* (220 - 300 cm) were collected and euthanized following approved IACUC protocols (PROTO20230000004).
- Embryonic developmental stage was determined for each specimen (Figure A) based on external morphological features at the time of collection. Embryos were classified into one of four stages: early, mid, late, or full term, following a staging scheme adapted for *Hypanus sabinus* (Kina et al., 2021; Furumitsu et al., 2019).
- Embryos and their internal and external yolk sacs were weighed to obtain initial wet weights (g) across various developmental stages. Samples were dried in an oven at 60°C until weights stabilized, dry weights (g) were recorded.Dried samples were transferred to a muffle furnace, where temperatures were gradually increased from 90°C to 550°C and maintained at 550°C for 24 hours to ensure complete combustion. Water, inorganic, and organic content in each sample was calculated using the equations below:

$$\text{Water content (g)} = \text{wet } M - \text{dry } M$$

$$\text{inorganic content (g)} = \text{ash } M$$

$$\text{organic content (g)} = \text{dry } M - \text{ash } M$$

- The percent change in mass for water, inorganic, and organic components across embryonic stages was calculated using the percent change formula for average early stage ovum and average late stage embryos:

$$\text{Change} = \frac{M_{\text{near term}} - M_{\text{early}}}{M_{\text{early}}} \times 100\%.$$

Sample type	Stage	N (samples)	N (females)
Ovum	Early[a]	7	7
Embryo	Mid	2	1
Embryo	Late	12	9
Embryo	Full	8[b]	6[b]
Total		29	23

Table I. Sample size and sample type summary for collections and analysis. This shows the number of samples (ovum or embryos) collected from mature *H. sabinus* between 24 April 2023 and 6 July 2023. Each sample was staged as early, mid, late or full-term, and include the number of samples collected and number of females those samples were collected from.

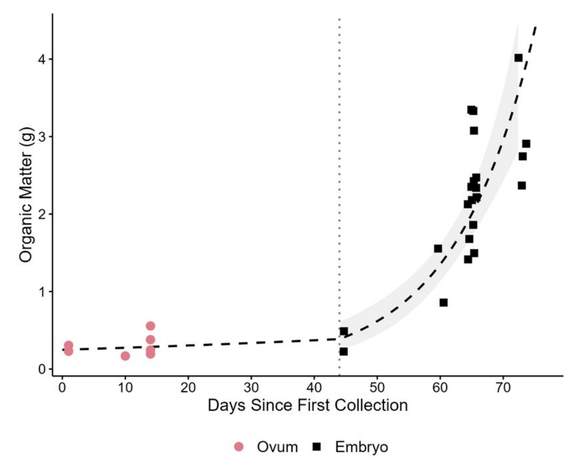

Figure B. Organic matter (g) plotted against days since first collection for ova (pink circles, n = 7) and embryos (black squares, n = 22) of *Hypanus sabinus*. The dashed line represents a segmented regression fit to log-transformed organic matter back-transformed to the raw scale, with a statistically determined breakpoint at day 44 (dotted vertical line). The shaded ribbon represents the 95% confidence interval for the post-breakpoint phase. Prior to day 44, organic matter showed no significant change (slope 1.0% per day, $p = 0.670$). After day 44, organic matter increased exponentially (8.14% per day, $p < 0.001$), reflecting rapid contribution of organic matter between days 44 and 73 of the sampling period ($R^2 = 0.898$, $p < 0.001$).

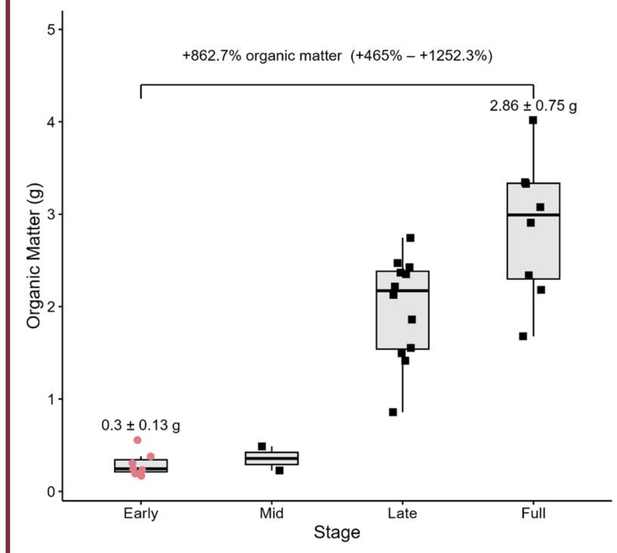

Figure C. Percent Change in Organic Matter. Organic matter (g) per embryo across developmental stages in *Hypanus sabinus*. Boxes represent the interquartile range with the median line shown; individual data points are overlaid (embryos: black squares; ova: pink circles). Mean ± SD are indicated for early-stage ova (0.30 ± 0.13 g, n = 7) and full-term embryos (2.86 ± 0.75 g, n = 8). Percent change was calculated from mean early-stage ova to individual full-term embryos, yielding a mean increase of 862.7% (range: +465.0% to +1,252.3%; Welch's t-test: $t = -9.45$, $df = 7.51$, $p < 0.001$).

Conclusion:

Recently fertilized zygotes maintained an average organic matter mass of 0.3 g, which increased to 2.86 g in near-term embryos. This represents a substantial 862.7% mean increase in organic matter throughout the developmental process, with some individuals capable of contributing over 1252% increases. Segmented regression confirmed this bi phasic pattern, showing minimal change in organic matter prior to day 44 (1.0% per day, $p = 0.670$) followed by rapid exponential increase after (8.14% per day, $p < 0.001$). Only 10% of the total maternal contribution occurred prior to mid-stage development, with 89.5% of total maternal contribution of organic matter was a result of histotrophic provisioning during the last 40% of measured gestational period.

This gives us insight into the reproductive patterns of histotroph elasmobranchs and will contribute valuable insight for both in-vito and ex-situ reproductive conservation and population management plans.

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