http://dx.doi.org/10.32360/acmar.v56i1.93315
ISSN 0374-5686
e-ISSN 2526-7639

ORGINAL PAPER

Labomar
Arquivos de Ciências do Mar

BETWEEN BUCCAL PUMPING AND BITING - EVIDENCE OF INTRAUTERINE MOUTH MOVEMENTS AND REPRODUCTIVE ASPECTS OF NEOTROPICAL FRESHWATER STINGRAYS (ELASMOBRANCHII: POTAMOTRYGONINAE)

Entre bombeamento bucal e mordida - Evidências de movimentos bucais intrauterinos e aspectos reprodutivos de raias de água doce neotropicais (Elasmobranchii: Potamotryginae)

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ABSTRACT

Preliminary findings regarding the reproduction of three neotropical freshwater stingray species, *Potamotrygon henlei*, *P. motoro*, and *P. orbignyi*, are presented herein. These species are characterized by the presence of a singular functional left ovary and two operational uteri. Two distinct fertility models have been identified: fecundity dependent, and fecundity independent of maternal size. Furthermore, this study briefly outlines the process of capture-induced parturition and its implications for the fate of the litter. Additionally, it sheds light on novel embryonic behavioral patterns, particularly highlighting the active swimming exhibited by embryos within the uterine environment, notably during the latter stages of

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Received: 1 October 2021
Accepted for publication: 20 April 2023

development. Consequently, it is suggested that these movements, coupled with oral activities and active biting, facilitate branchial ventilation and potentially intrauterine foraging - a phenomenon previously documented only in sharks belonging to the order Lamniformes.

Keywords: Abortion, Histotroph, Capture stress, Embryonic nutrition.

RESUMO

Informações preliminares sobre a reprodução de três espécies de raias de água doce neotropicais, Potamotrygon henlei*,* P. motoro *e* P. orbignyi, *são aqui apresentadas. Estas espécies compartilham a presença de um único ovário funcional esquerdo e dois úteros funcionais. Foram detectados dois modelos de fecundidade distintos: fecundidade dependente, e fecundidade independente do tamanho materno. O processo de parto induzido por captura e o destino dos embriões são brevemente descritos, bem como novos aspectos comportamentais da prole, indicando que os embriões realizam natação ativa no ambiente uterino, especialmente nos estágios finais de desenvolvimento. Desta forma, é sugerido que esses movimentos, juntamente com a atividade oral e a capacidade de mordida ativa são usados como suporte para a ventilação branquial e de possível forrageamento intrauterino, até agora descrito apenas para tubarões da ordem Lamniformes.*

Palavras-chave: *Aborto, Histotrofo, Estresse de captura, Nutrição embrionária.*

INTRODUCTION

Reproduction in elasmobranchs has been extensively documented across various families (Wourms, 1977; Worms et al., 1988; Hamlett et al., 1996; Hamlett & Koob, 1999; Gilmore et al., 2005; Hamlett et al., 2005a; Hamlett et al., 2005b; Awruch, 2015). However, ongoing research continues to reveal new variations in reproductive modes and mother-embryo nutritional strategies (Yano, 1992; Sunyé & Vooren, 1997; Sato et al., 2016; Buddle et al., 2020; Nakaya et al., 2020), astonishing the scientific community with their uniqueness and evolutionary implications (Wourms & Lombardi, 1992; Dulvy & Reynolds, 1997; Musick & Ellis, 2005; Nakaya et al., 2020). To accurately assess populations of chondrichthyan species, a quantitative approach to reproductive studies is imperative (Walker, 2005). Comprehensive evaluations of age-structured fecundity and reproductive cycles, including the ovarian and uterine cycles alongside resting periods, are essential for estimating population growth. Thus, a thorough investigation of all facets of reproduction is necessary to establish the biological parameters crucial for reliable population assessments.

Several reproductive studies on elasmobranchs have recently been conducted., particularly focusing on categorizing the mode of birth (parity), which can occur either directly from the mother (viviparity) or through an egg (oviparity). Various mechanisms serve as the primary sources of embryonic nutrition, including the absorption of yolk reserves from the egg (lecitotrophy), the consumption of ova provided by the mother or smaller embryos (oophagy and intrauterine cannibalism), the absorption of mucoid or lipid secretions (limited histotrophy, lipidic histotrophy), or even the development of a yolk sac placenta (Awruch, 2015; Luer &Wyffels, 2022). Interestingly, some of these nutritional strategies can occur in combination. Moreover, among elasmobranchs, only lamniform sharks exhibit active intrauterine feeding — a behavior characterized by the embryo's ability to actively search for, bite, ingest, and swallow food (Gilmore et al., 1983; Gilmore, 1993; Sato et al., 2016). This behavioral trait likely plays a crucial role in facilitating rapid intrauterine growth and avoiding predation, as the embryo must actively locate and consume provided ova or potentially even siblings to mitigate the risk of predation throughout its intrauterine development.

Myliobatiform embryos are hypothesized to passively receive nourishment through epithelial absorption and inactive ingestion of lipid histotroph. This histotroph is delivered directly to the buccopharynx by trophonemata, entering through the spiracles and mouth of the developing embryo (Wourms et al., 1988; Hamlett & Koob, 1999; Charvet-Almeida, 2001; White et al., 2001; Colonello et al., 2013). Thus far, aside from yolk sac absorption, no additional embryonic nutritional strategy has been described for this group (Penfold & Wyffels, 2019; fig. 15.1). Consequently, the transfer of histotroph from mother to embryo is believed to occur entirely passively, without any direct action from the embryo. Recent studies in elasmobranch fishes have employed ultrasound methodologies, providing deeper insights into intrauterine life (Tomita et al., 2012; Tomita et al., 2018; Wosnick et al., 2018; Murakumo et al., 2020) and proposing a series of new and intricate embryonic behaviors.

Castex (1963) provided the initial modern description of the reproductive characteristics of freshwater stingrays, followed by Thorson et al. (1983), who made observations on both wild-caught and captive specimens. In recent years, there has been a growing interest in the reproduction of freshwater stingrays, resulting in morphological descriptions of their reproductive systems (Zaiden et al., 2011; Pedreros-Sierra et al., 2016; Anaya-López & Ramírez-Pinilla, 2017; Da Silva et al., 2017), studies on reproductive biology and ecology (Teshima & Takeshita, 1992; Lasso et al., 1996; Araújo, 1998; Charvet-Almeida, 2001; Charvet-Almeida et al., 2005; Rincon, 2006; Almeida, 2008; Garrone-Neto, 2010; Rosa et al., 2010; Rincon et al., 2019; Torres et al., 2021; Lizcano-Gutiérrez & Ramírez-Pinilla, 2022; Shibuya & Duncan, 2022), and a growing body of research on the physiology of reproduction (Morales-Gamba, 2018; Dzyuba et al., 2019a; Dzyuba et al., 2019b; Morales-Gamba et al., 2019; Rangel et al., 2020; Padilha et al., 2021). However, the number of reports based on field observations remains limited. Certain reproductive aspects, such as the size of the embryo in relation to the presence and volume of intrauterine fluid, absorption of the external yolk sac, and size at birth, can only be fully understood through effective field observations.

Commercial fisheries and scientific fishing expeditions present promising opportunities to investigate the reproductive aspects of elasmobranchs through both traditional methods (such as capture and dissection) and opportunistic observations. These approaches can shed light on behavioral aspects that remain poorly understood for sharks and rays in general. For instance, the documentation of presumed capture-induced parturition, defined as the premature expulsion of a litter due to capture stress – also identified as premature birth or abortion (Adams et al., 2018) – was made possible only through field observations (Rincon, 2007). Moreover, these circumstances allow for the collection of data on the uterine behavior of embryos, which can elucidate important adaptive aspects of viviparity within these taxonomic groups.

This study aimed to uncover novel insights into various aspects of reproduction through opportunistic field observations, with a specific focus on providing evidence of potential intrauterine active feeding in late-stage embryos of freshwater stingrays. Additionally, detailed descriptions of the females' reproductive system, ultrasonographic imaging of a pregnant female, and observations of embryo behavior during capture-induced parturition are included to corroborate the observed intrauterine activity.

MATERIAL AND METHODS

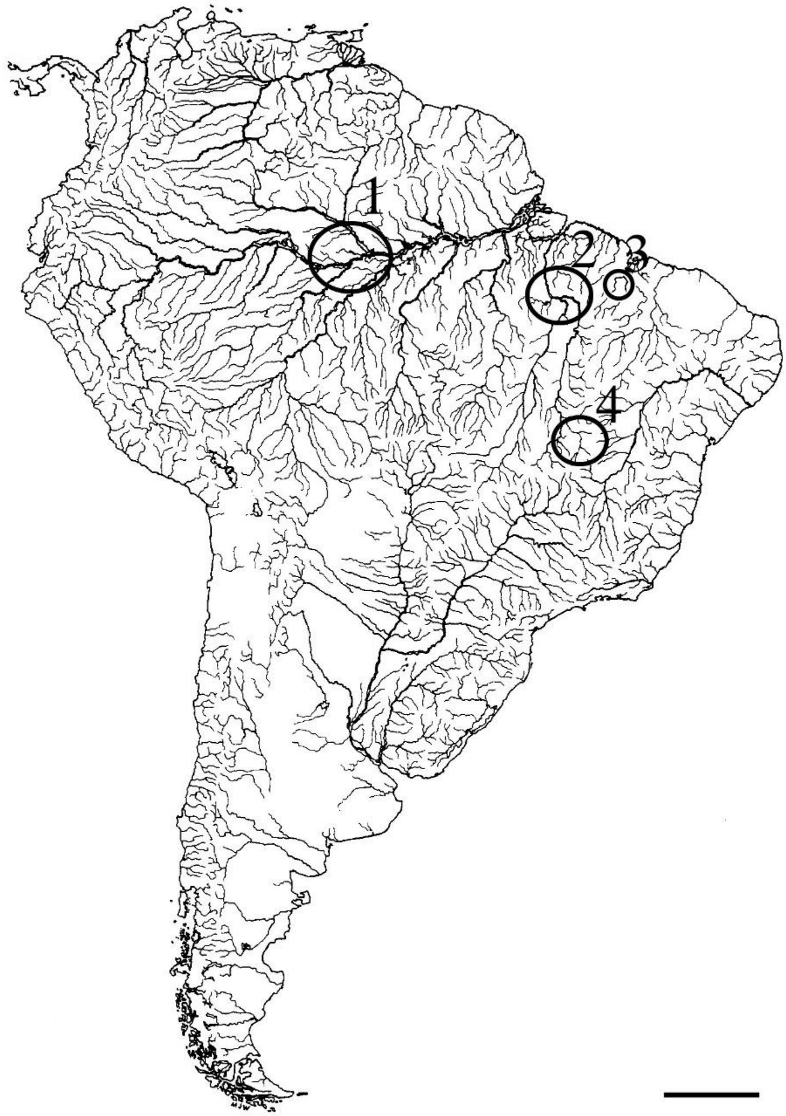
Specimens of freshwater stingrays belonging to the genus *Potamotrygon* were collected for purpose of this study. This comprised eight specimens of *P. henlei* (comprising five females and three males), 15 specimens of *P. motoro* (comprising 12 females and three males), and 18 specimens of *P. orbignyi* (comprising eight females and 10 males). These specimens were captured employing a combination of line and hook (Exori 3/0) alongside fishing nets spanning the temporal interval from July 2000 to November 2019. Preceding the expulsion of embryos in pregnant females, the specimens underwent thorough analysis to evaluate parameters such as intrauterine fecundity, embryonic positioning, and developmental stage. Specimens of *P. henlei* and *P. orbignyi* were captured in the Tocantins River (Marabá, State of Pará) and the Paranã River (Paranã, State of Tocantins), respectively. Two specimens of *P. motoro* were sourced from the Manacapuru Lake complex (specifically São Lourenço Lake, located at 03° 17’ 555’’ S and 60° 43’ 759’’ W) along the left margin of the Solimões River (Manacapuru, State of Amazonas), by local fishers. Furthermore, 13 specimens of *P. motoro* were captured in the Viana Lake Complex (Viana Lake, forming part of the Pindaré/Mearim Rivers drainage, located at 03° 12’ 57” S and 44° 58’ 59” W, in Viana, State of Maranhão) by a research fishing team (Figure 1).

In the field, female specimens and their embryos underwent dissection and subsequent analysis. Various parameters were recorded, encompassing the number of embryos per uterus, developmental stage, sex, total length (TL) measured in millimeters, presence of uterine milk (histotroph), and the size of the female (TL) (Table 1; Figure 2). Embryonic development stages were categorized as follows: stage I – eggs up to 50 mm TL; stage II – ranging from 51 to 99 mm TL; and stage III – exceeding 100 mm TL, following Fahy et al. (2007). Morphometric measurements adhered to the methodology delineated by Rosa (1985), with precise measurements recorded to the nearest millimeter using a digital caliper (Cescorf, 1-meter, digital Starret). All specimens underwent temporary preservation in ice for subsequent laboratory analysis, followed by permanent preservation in a 10% formalin solution. The terminology utilized for describing the reproductive system aligns with the established conventions outlined by Hamlett & Koob (1999).

A single large freshwater stingray (*P. henlei*, measuring 710 mm LD) was captured and subsequently deceased by a fisher. Upon the initiation of capture-induced parturition, steps were taken to prevent the mixing of embryos from different uteri by palpating live embryos located at the posterior portion of the uteri using a protocol adapted from Unkels (2012).

To augment our field observations, ultrasound imaging was conducted on a midterm to late-pregnant female *P. motoro* (specific information regarding her size was unavailable). The ultrasound video was generously provided by the clinical veterinarians at the John G. Shedd Aquarium in Chicago, Illinois (USA). Utilizing a Sonosite™ 180 Plus Veterinary unit and various transducers operating at frequencies of 2.0, 3.5, and 5.0 MHz, the imaging process entailed positioning the transducer carefully on the dorsal surface of the abdominal cavity in the

Figure 1. Schematic view of South American river basins. Sampling localities marked by circles and corresponding numbers: 1. Manacapuru Lake, Manacapuru, State of Amazonas, where *Potamotrygon motoro* was collected; 2. Tocantins River, Marabá. State of Pará, where *P. henlei* was collected; 3. Pindaré River, Viana Lake, and Aquiri Lake, where *P. motoro* was collected; 4. Paranã River, Paranã, State of Tocantins where *P. orbignyi* was collected. The scale bar corresponds to 500 km.



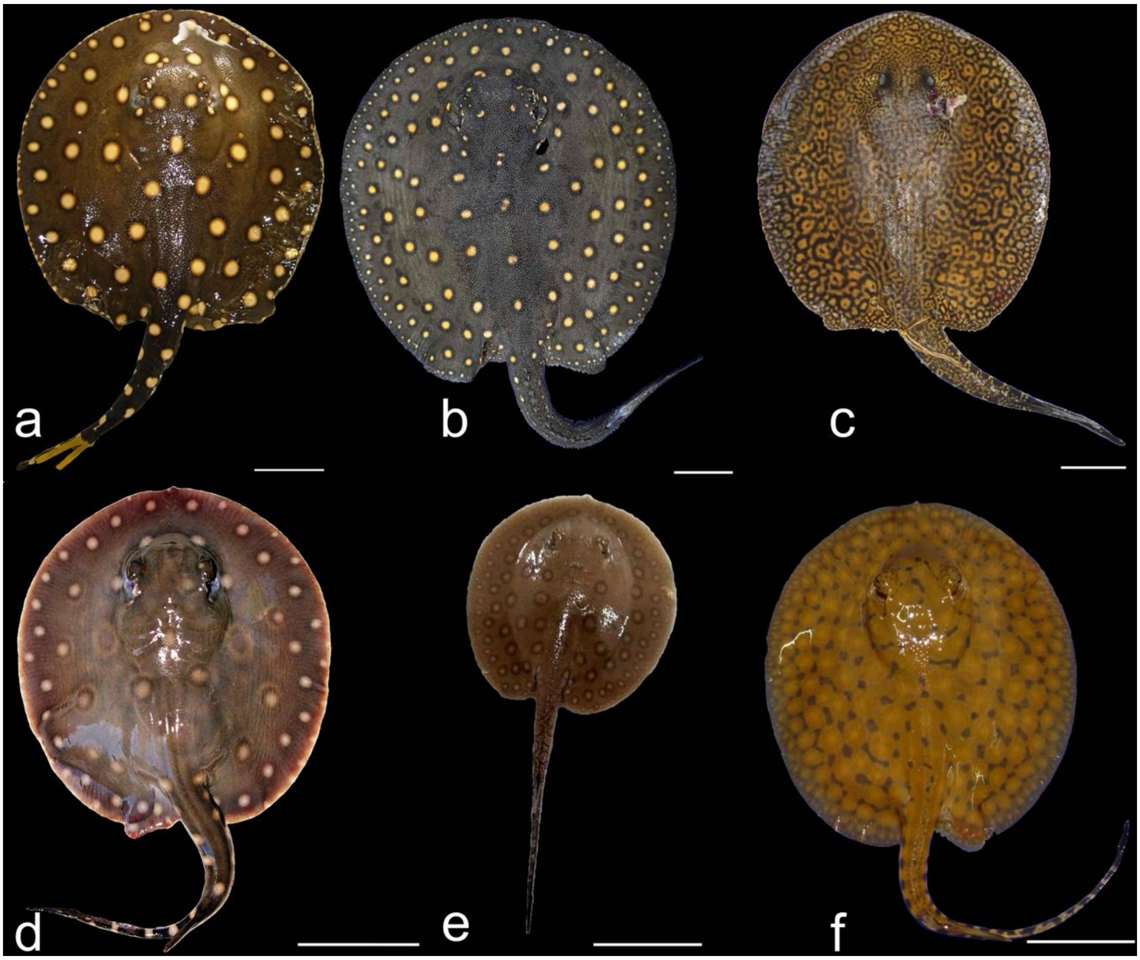
uterus. We have included the video herein and still images were extracted from the recording for illustrative purposes (accessible at: https://vimeo.com/913999066) to depict the mouth and intrauterine movements.

In this study, the term "capture-induced parturition" has been employed to characterize the expulsion of all embryos subsequent maternal capture and exposure to air, a phenomenon believed to be initiated by mechanisms that are still not fully understood (Rincon, 2007; Adams et al., 2018; Wosnick et al., 2018). Instances were classified as premature birth when embryos were born alive and as abortion when embryos were expelled deceased. Comprehensive descriptions of both maternal and litter behavior were recorded opportunistically and are presented below.

Table I – Average disk width (DW) and average uterus fecundity (UF) for Neotropical freshwater stingrays *Potamotrygon henlei*, *P. orbignyi*, and *P. motoro* from different river basins in Brazil analyzed in the present study. Each average DW and UF value is accompanied by its respective standard deviation. Data for each species is presented by sex, with indication about sample size.

	P. henlei		*P. orbigny*		*P. motoro* (Viana Lake)		*P. motoro*
	Female (n=5)	Male (n=3)	Female (n=8)	Male (n=10)	Female (n=10)	Male (n=3)	Female (n=2)
DW	587.2±90.8	328.3±41.5	325.5±47.4	236.8±73.8	497.7±94.2	336.6±65.5	460±7.0
UF	4.6±3.3	-	1±0	-	4.5±2.8	-	6±2.8

Figure 2. Adult female specimens of Neotropical freshwater stingrays sampled in different river basins in Brazil as part of the present study and one of its respective embryos: (a and d) *Potamotrygon henlei*; (b and e) *Potamotrygon motoro*; and (c and f) *Potamotrygon orbignyi*. Scale bars for adults correspond to 100 mm. Scale bars for embryos correspond to 50 mm.



All *P. motoro* specimens caught in the Manacapuru Lake by local fishers were retrieved alive and euthanized onboard by the fishers, who severed their spinal cords for subsequent human consumption. Conversely, all other specimens, including those of *P. motoro* from the Viana Lake of the Pindaré/Mearim Rivers drainage, as well as specimens of *P. henlei* and *P. orbignyi* from Tocantins River drainage, were exclusively captured for scientific purposes. These specimens were euthanized using a clove oil solution. Scientific captures were conducted under the following permits: (a) the Brazilian Environmental and Renewable Natural Resources Agency (IBAMA) permits no. 118 DIFAP/IBAMA and SISBio no. 58240-3; and (b) the Ethics Commission on the Use of Animals of the Federal University of Maranhão permit no. 23115.014176/2018-94.

RESULTS

Reproductive characteristics of females

The reproductive system of all examined specimens showcased a well-developed, irregularly shaped, and functional left ovary, occasionally accompanied by a smaller yet functional right ovary. Each anterior oviduct traversed dorsally through the visceral cavity and laterally alongside the vertebral column from the ostium to the olive-shaped oviducal gland. Subsequently, the uterus widened distinctly before tapering abruptly to the cervix. The internal lining of the uterus comprised highly vascularized trophonemata, appearing red and soft in both pregnant and post-parturition females, and paler in sexually resting females. Gravid females displayed a distended uterus with increasingly flexible walls as pregnancy progressed and embryos grew. Fecundity varied, with litter sizes ranging from a single embryo per litter in *P. orbignyi* to nine in *P. henlei* and eleven in *P. motoro*. Initial observations suggest that fecundity increases with female size in *P. motoro* and *P. henlei* (Figure 3). Both uteri exhibited equal development and functionality across all three species. Large litters with more than six embryos were less common and challenging to verify due to onboard birth. Embryos initiated development relying on yolk-sac reserves, with histotroph supply commencing at a later stage, although all three species still exhibited embryos relying on conspicuous yolk-sacs even in their final developmental stages. The correlation between pre-ovulatory ovarian vitellogenic follicle weight and term embryo weight unveiled a significant matrotrophic input of up to 1,579% per embryo, based on a wet weight gain from uterine eggs (3.8 g) to term embryos (60 g) (*P. orbignyi*, G. Rincon, unpublished data).

Figure 3. Uterine fecundity in relation to female size for three species of Neotropical freshwater stingrays collected from various river basins in Brazil. Species and or localities are represented by colored shapes: orange circle, *Potamotrygon orbignyi*; grey diamond, *Potamotrygon motoro* from Manacapuru Lake; yellow square, *Potamotrygon motoro* from Viana Lake; and blue triangle, *Potamotrygon henlei*. Ellipses encircle specimens that abide to one of two hypotheses for the relationship between uterine fecundity and female size: the blue ellipse represents the size-independent uterine fecundity group, including only *P. orbignyi*; the red ellipse represents the size-dependent uterine fecundity group, including *P. henlei* and *P. motoro* from both Manacapuru Lake and Viana Lake.

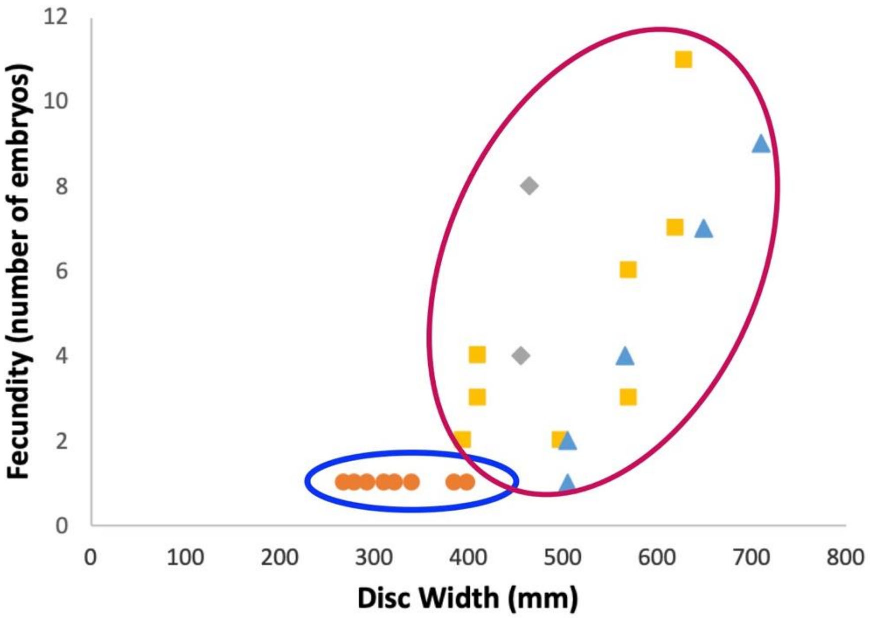


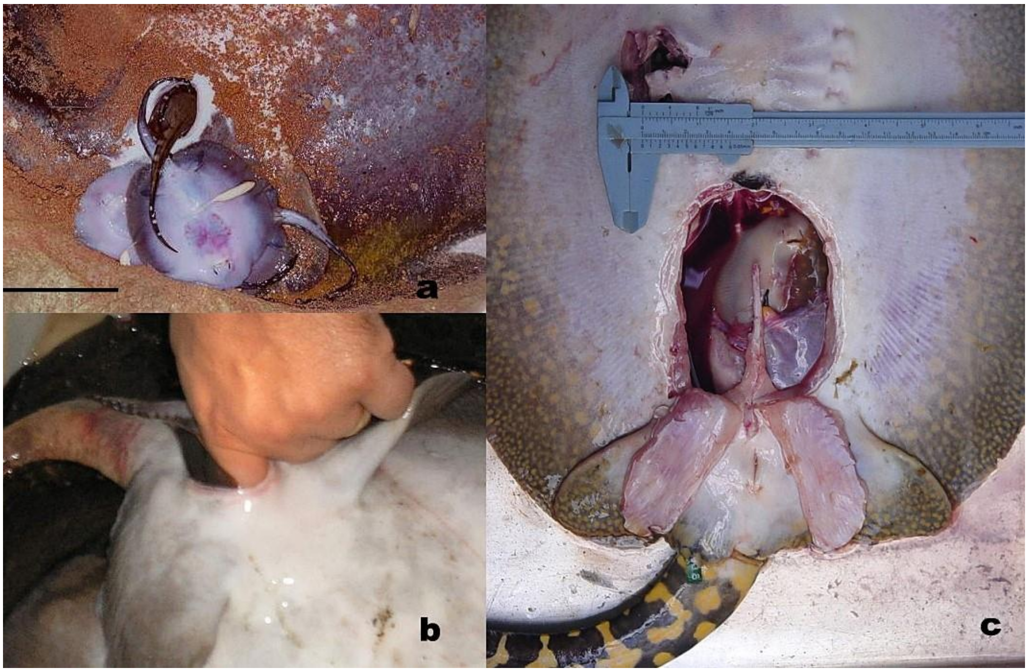
Table II – Estimated reproductive aspects for Neotropical freshwater stingrays collected from various river basins in Brazil: *Potamotrygon orbignyi*, *Potamotrygon henlei* and *Potamotrygon motoro* (Viana and Aquiri lakes).

Species	Size at birth (disc width; mm)	Maximum size (disc width; mm)	Estimated Size at Maturity (disc width; mm)		Maximum Uterine Fecundity
			Female	Male	
Potamotrygon orbignyi	115	460	251	260	1 (2?)
Potamotrygon henlei	140 (130 – 150)	710	350	400	9(14?)
Potamotrygon motoro (Viana and Aquiri lakes)	120-130	630	290	410	11

Capture-induced parturition

All three species of freshwater stingrays initiated parturition approximately five to 10 minutes after being captured and exposed to air (Figure 4a). Upon capture, females displayed resistance to the fishing gear, exhibiting vigorous behavior while submerged. Upon removal from the water, captured females exhibited conspicuous spiracular activity, expelling water from their gill slits and spiracles while emitting short, sequential oropharyngeal grunting sounds. Their tails exhibited lateral movement, often synchronized with the motion of their pelvic fins or motion across the disc in response to tactile stimulation. Parturition commenced following relaxation and distension of each cervix. The presence of a greenish intrauterine fluid was observed as embryos traversed the urogenital sinus. Although uterine wall contractions were not directly observed, increased embryo movement likely facilitated their expulsion. As embryos traversed the cloaca, their cranial-caudal orientation varied, although the majority assumed the typical birth position (i.e., caudal region first, aligning with the natural orientation of the female). Partial folding of pectoral fins was consistently noted, with one side elevated towards the dorsal surface and the other oriented towards the ventral surface.

Figure 4 – Capture-induced parturition in three species of Neotropical freshwater stingrays collected from various river basins in Brazil: a) *Potamotrygon henlei* (Scale bar corresponds to 150 mm); b) *Potamotrygon motoro* with embryo partially outside the cloacal aperture; c) partial ventral view of male embryo in the left uterus of *Potamotrygon orbignyi* (caliper main scale displayed reaches 172 mm).



Individual expulsion of embryos was observed, with occasional instances of uterine retention of one or two embryos. Expulsion of embryos occurred across all developmental stages, with increased uterine activity noted during the latter stages of development (stages II and III). While embryos at all stages were expelled alive, indicative of premature birth, larger embryos often retained intact external yolk sacs. Early-stage embryos were typically expelled alive but frequently exhibited damaged external yolk sacs. Notably, all live embryos (stages I and II) introduced into local water succumbed within hours, resulting in a 100% mortality rate post-capture-induced parturition. In one instance, two litters in stage III, from *P. motoro* (Viana Lake), were maintained in local water (3 and 4 embryos, respectively) demonstrating an 85% survival rate over a period exceeding 48 hours (detailed data to be published separately).

The deceased 710 mm LD *Potamotrygon henlei* that underwent capture-induced parturition had nine embryos, all at stage III of development but still possessing external yolk sacs. The palpation revealed that the embryos exhibited a significant movement activity, potentially linked to decreasing oxygen levels. Additionally, the palpation detected a biting behavior, though it remains uncertain whether this response was directly triggered by the palpation or merely coincidentally observed during the procedure.

Regarding the duration of parturition, it typically lasted for about 10 minutes in *P. motoro* and *P. orbignyi*. However, occasionally, this duration extended for longer periods or resulted in embryo retention for *P. orbignyi*, despite efforts to facilitate litter release (Figure 4b and 4c).

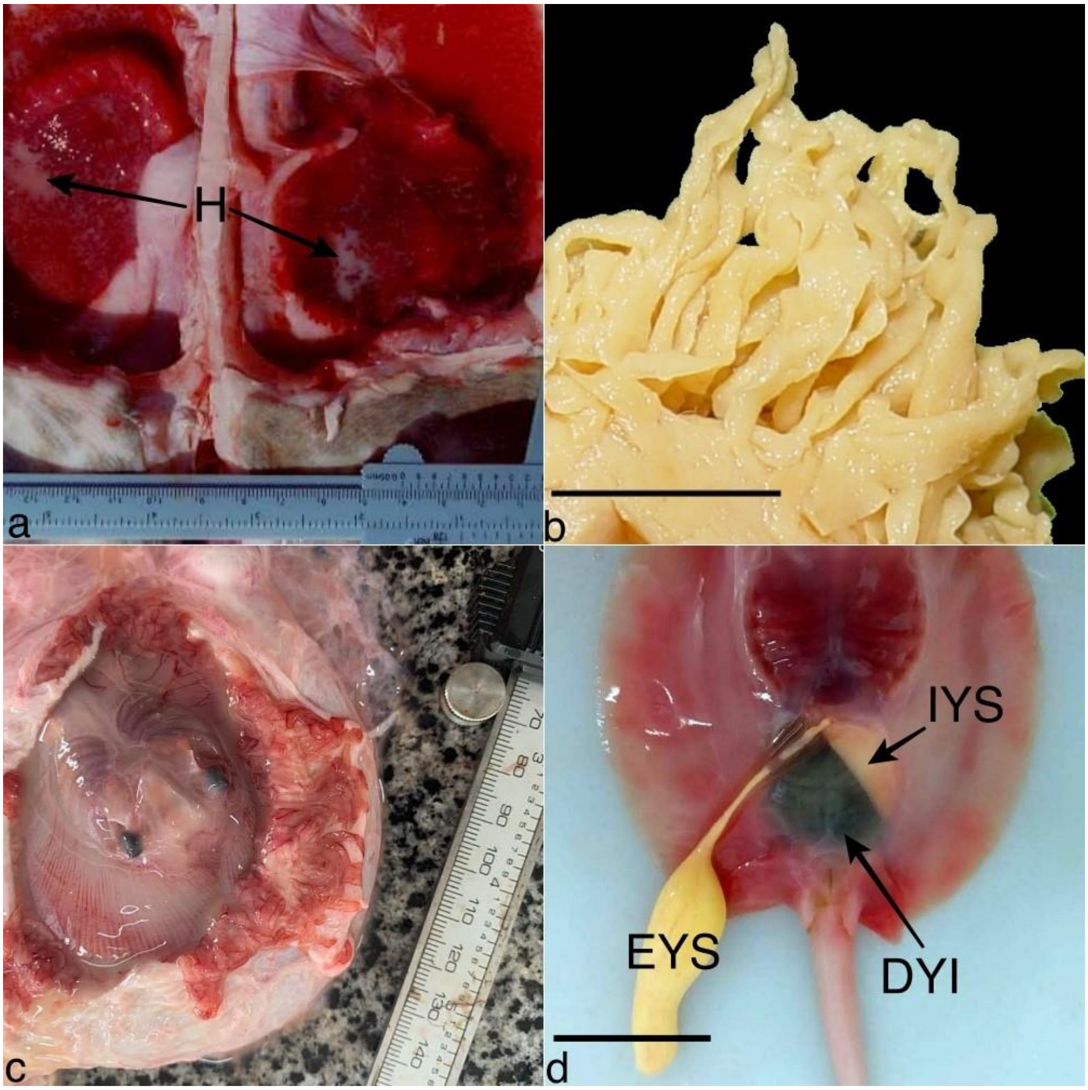
Uterine nutrition

The red, highly vascularized trophonemata were frequently observed entering the embryos' mouths and/or spiracles, directly accessing the buccopharyngeal cavity. The length of the trophonemata's villi ranged from a few millimeters in *P. orbignyi* to about 2.0 cm in *P. henlei* (Figure 5a and 5b) and *P. motoro* (Figure 5c). However, all villi exhibited a similar gross morphology, characterized by long, spatula-shaped structures. The volume of histotroph, excluding that adhered to the embryos and uterine eggs, ranged from 1.8 to 19 ml in stages I and II, respectively. However, in litters at stages II to III, an increase in intrauterine fluid was noted, with an estimated total volume of up to 500 ml, which drained after cervical distention. Embryos at stages II and III were immersed in intrauterine fluid (H) (Figure 5c) and exhibited signs of digestion in their stomachs, along with an internal yolk-sac (IYS), an external yolk-sac (EYS), and bluish digesting yolk in their intestines (DYI) (Figure 5d).

Ultrasound Images

All static images extracted from the ultrasonography video depict the contents of a single uterus, although it is not specified whether it is the left or right uterus. The trophonemata, positioned on the dorsal and ventral surfaces of the uterine lining, aggregate near the mouth, branchial (Figure 6d), and spiracular regions due to the embryo's buccal pumping process. These sequential movements of opening and closing the mouth create an inflow and outflow of intrauterine fluid, accompanied by the movement of trophonemata within the mouth (Figure 6 a, 6b, and 6c). These motions are closely linked to the breathing process. At least two embryos within the same uterus were identified, both demonstrating the ability to move (rolling and changing position) while immersed in the uterine fluid.

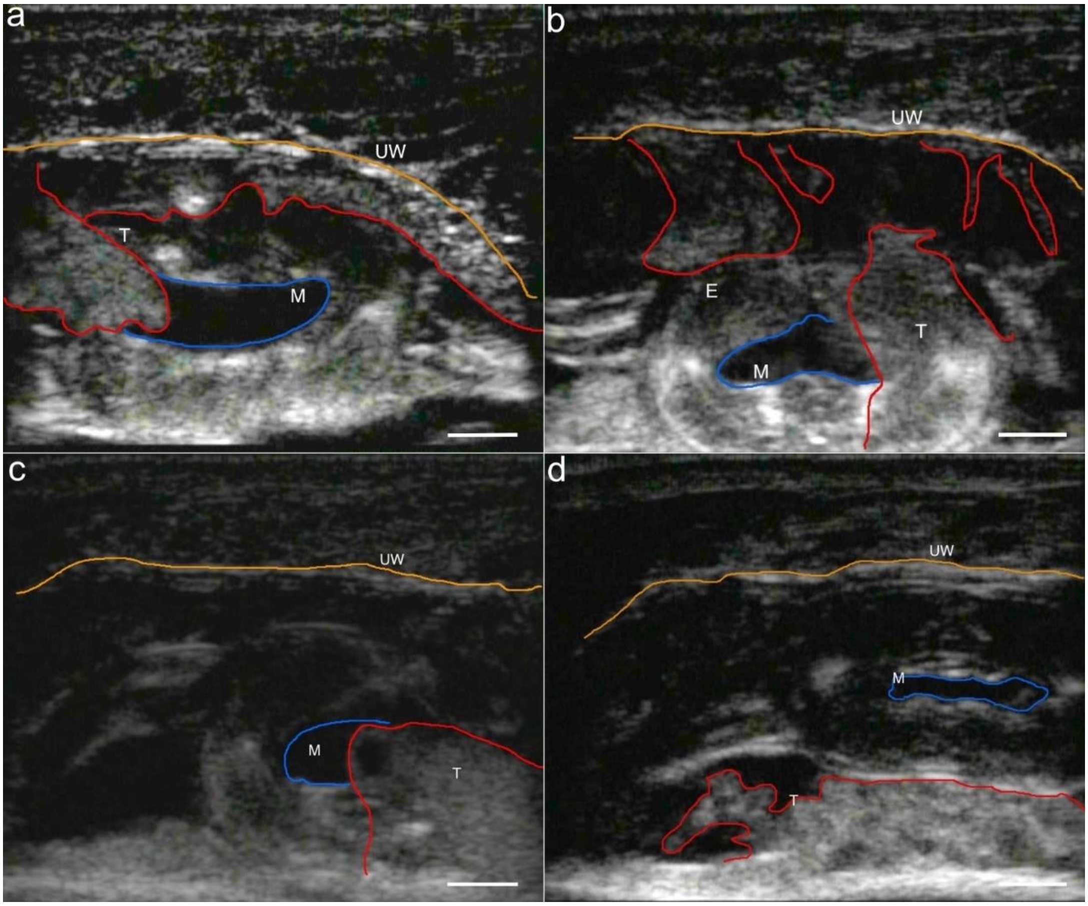
Figure 5. Uterine nutrition in two species of Neotropical freshwater stingrays: (a) uterine milk (histotroph; H) in uteri (*) of *Potamotrygon henlei* (caliper main scale displayed reaches 134 mm); (b) trophonemata of *P. henlei* with long villi (scale bar corresponds to 10 mm); (c) embryo of *Potamotrygon motoro* in uterine trophonemata (scale displayed reaches 85 mm); (d) embryo of *P. motoro* in ventral view with the external yolk sac (EYS) connected to the internal yolk sac (IYS) and the greenish digested yolk in the intestine (DYI) (scale bar corresponds to 30 mm).



DISCUSSION

The general structure of the female reproductive system in freshwater stingrays has been extensively outlined by various authors (Thorson et al., 1983; Teshima & Takeshita 1992; Lasso et al., 1996; Charvet-Almeida, 2001; Charvet-Almeida et al., 2005; Rincon, 2006; Pedreros-Sierra et al., 2016), aligning with the broader myliobatoid pattern (Hamlett & Koob, 1999; Fahy et al., 2007). Variations primarily revolve around the presence and functionality of the right ovary, noted as absent in species such as *P. motoro*, *P. brachyura*, *P. constellata*, *Potamotrygon wallacei*, and *Plesiotrygon iwamae* (Castex & Maciel, 1965; Achenbach & Achenbach, 1976; Thorson et al., 1983; Araújo, 1998; Charvet-Almeida, 2001), infrequent in *P. orbignyi* (Lasso et al., 1996; Rincon, 2006), and commonly found in *P. magdalenae* (Teshima & Takeshima, 1992). This pattern diverges from that of most stingrays, where the left ovary typically functions as the primary producer of oocytes (Hamlett & Koob, 1999). Thus, it is apparent that freshwater stingrays may exhibit functional right ovaries, with individual species sometimes displaying both functional ovaries within one river basin, while only the left ovary is functional in another basin. However, utilizing these characteristics as evidence of cryptic species should be approached cautiously and in conjunction with other morphological and genetic markers.

Figure 6. Static images obtained from ultrasonography conducted on a pregnant Neotropical freshwater stingray *Potamotrygon motoro* captive in the John G. Shedd Aquarium (Chicago, Illinois, USA): (a and b) trophonemata extending dorsally and reaching the mouth-buco-pharyngeal cavity (M; blue line) through spiracles or mouth opening; (c) trophonemata extending ventrally and reaching mouth-buco-pharyngeal cavity (M) through gill slits or mouth opening, with identification of embryo (E); and (d) trophonemata concentrated below the branchial region. (Scale bars corresponds to 25 mm). The video is available at https://vimeo.com/913999066.



Achenbach & Achenbach (1976) were the first to report capture-induced parturition in *P. motoro* and *P. brachyura*. They documented critical biological aspects of this phenomenon, including the relationship between maternal size and uterine fecundity, the position of embryos at birth, and the vigorous mobility and aggressiveness of more developed embryos in defending themselves. These findings, supported by our study, underscore the significance of field observations in elucidating aspects of reproduction in these animals. Similarly, Charvet-Almeida (2001) reported capture-induced parturition in *P. iwamae* immediately after capture, with embryos expelled at various positions, suggesting uterine movement before parturition. This observation aligns with reports on *P. orbignyi*, which typically has one or possibly two embryos per litter and shows no evidence of increasing fecundity (Winemiller & Taphorn, 1989; Lasso et al., 1996; Rincon, 2006). Consequently, it can be inferred that uterine fecundity in freshwater stingrays may follow different patterns, namely size-dependent and size-independent models (Rosa et al., 2010). Species that likely belong to the size-dependent group may include, *P. motoro*, *P. brachyura*, *P. henlei*, *P. leopoldi*, and *P. scobina* (Achenbach & Achenbach, 1976; Thorson et al., 1983; Almeida, 2003; Charvet-Almeida et al., 2005; Charvet-Almeida, 2006; Almeida, 2008). Conversely, species that likely belong to the size-independent group may include *P. orbignyi*, *P. wallacei*, *P. schroederi*, and *Plesiotrygon iwamae* (Araújo, 1998; Charvet-Almeida, 2001; Rincon 2006). This variation in uterine fecundity is believed to be linked to the relationship between the mother's maximum size and the size of term embryos, with intrauterine space and relatively large embryos acting as physical constraints on litter size (Martin & Cailliet, 1988). Nonetheless, factors such as mother's trophic considerations, prey nutritional value, and the efficiency of mother-embryo energy transference should also be considered potential contributors to the fecundity model in freshwater stingrays.

In this study, we noted an accelerated occurrence of capture-induced parturition, particularly evident in embryos at stages II and III, characterized by increased intrauterine movement. This observation parallels findings in various species such as *P. brachyurus* (Achenbach & Achenbach, 1976), *P. amandae* (Rangel et al., 2020), and the Tope shark, *Galeorhinus galeus* (Rincon, 2007), where embryo motility has been implicated as a potential mechanism or a significant contributing factor to premature birth. While numerous species demonstrate substantial intrauterine movement (Carrier et al., 2004; Jirik & Lowe, 2012; Tomita et al., 2018; Murakumo et al., 2020), further investigation is needed to elucidate the precise correlation between heightened uterine movement and capture-induced parturition. Additionally, comprehensive investigations are warranted to assess the physiological maternal aspects of induced parturition and to explore the hypothesis implicating uterine hypoxia as the principal driver of increased intrauterine movement in embryos (Rincon, 2007).

The histotroph, secreted by the trophonemata lining the uterus, serves to protect and nourish the embryos (Amoroso, 1960; Wourms et al., 1988; Charvet-Almeida, 2001; Carrier et al., 2004). This specialized tissue closely envelops the embryo, with earlier studies indicating its penetration into the spiracles, gill slits, and mouth, delivering uterine milk directly into the embryo's gut (Smith & Merriner, 1986; Hamlett & Koob, 1999; Charvet-Almeida, 2001; White et al., 2001). While this process represents passive nutrition, there may also be active behavior involved in seeking out histotroph-rich areas of the trophonemata, akin to the intrauterine egg-search observed in lamnoids (Gilmore et al., 1983a; Gilmore et al., 1983b), or ingestion of free histotroph present in the uterine milieu (Figure 5). This active feeding hypothesis is further supported by observed biting behavior, suggesting embryos are capable of biting the uterine lining to induce histotroph secretion or ingest free histotroph. Such behaviors entail significant intrauterine movement, indicative of intrauterine active feeding. Ultrasonography of a freshwater stingray in midterm to late pregnancy depicted at least two embryos loosely arranged, rolling in their axis immersed in uterine fluid. Although the mouth movement identified as buccal pumping appears to be part of the breathing process, similar to observations in other batoid species such as *Mobula alfredi* (Marakumo et al., 2020), the intense embryonic mobility and buccal pumping behavior, coupled with intrauterine biting behavior facilitating the ingestion of nutritive and oxygenated intrauterine fluid (Tomita et al., 2012), support the hypothesis of intrauterine foraging, identified here as an intrauterine active feeding hypothesis.

Essentially, the presence of free histotroph within the intrauterine fluid, combined with the embryo's buccal pumping and active movement before and during parturition, along with its biting behavior, suggests intrauterine activity likely associated with both breathing and feeding functions. To gain deeper insights into the significance of active foraging and to identify the gestational phase when it becomes relevant for embryo nourishment, targeted reproductive studies focusing on trophonemata secretion and detailed intrauterine behavioral analyses are crucial. Furthermore, additional investigation into the microanatomy of trophonemata in gravid term females may reveal evidence of embryos actively seeking a richer supply of histotrophe, potentially indicated by bite marks.

Acknowledgements – This issue of *Arquivos de Ciências do Mar* pays tribute to Dr. Carolus Maria Vooren, an esteemed founding member of the Sociedade Brasileira para o Estudo de Elasmobrânquios (SBEEL; the Brazilian Elasmobranch Society). His significant contributions to the study and conservation of Brazilian elasmobranchs, along with his cherished friendship, are commemorated here. Dr. Carolus M. Vooren, who supervised the Master's theses of two authors of this research (G. Rincon and M.C. Oddone), was renowned for his meticulous approach to measuring ovarian follicles and embryos at various developmental stages. He dedicated himself to comparing uterine trophonematas among different species of myliobatiform rays, drawing inspiration from the pioneering work of Silvio Ranzi in the 1930s (Ranzi, 1934a, b). Additionally, we fondly remember Dr. Jack Musick, whose recent passing deeply saddened us. Following a presentation conducted by G. Rincon at the VI meeting of SBEEL, in Itajaí-SC, Brazil, in 2006, on uterolactation, capture-induced parturition, and intrauterine embryo movements in freshwater stingrays, Dr. Musick enthusiastically encouraged the publication of the findings presented in this article. Through this article, we pay homage to these exceptional elasmobranch researchers whose profound knowledge, unique perspectives on biology, guidance, and friendship continue to inspire us all. We thank Dr. Erica Clayton and Dr. William Van Bonn, at the time at the John G. Shedd Aquarium (USA), for generously providing the ultrasonography video of the *Potamotrygon* specimen. We are grateful to the three anonymous reviewers for their comments and recommendations on the manuscript. We would also like to express our gratitude to Dr. Vicente Faria, Associate Editor of *Arquivos de Ciências do Mar*, for his hard work in helping us improve this manuscript. Financial support for this research was provided by FAPEMA Universal 002/18 (01350/18). The CAPES Foundation (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil) provided a Ph.D. fellowship to G. Rincon.

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