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Effects of biological traits on capture-induced parturition in a freshwater stingray and perspectives for species management

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Abstract

Elasmobranchs are particularly vulnerable to overexploitation and population depletion, especially due to their life-history traits, such as low reproductive output and slow growth. Given that capture-induced parturition (abortion or premature birth) is a common consequence of fisheries in elasmobranchs, but still little studied, we investigated how the abortion/premature birth process varies in response to reproductive traits in a freshwater stingray, *Potamotrygon amandae*. Our results revealed that capture-induced parturition was affected by reproductive traits, such as litter size (one to seven) and gestation stage. The event occurred faster in pregnant females with high litter size during late pregnancy. Also, as found in other elasmobranchs, litter size was positively correlated with maternal size. These findings indicate that larger pregnant females in late pregnancy are more vulnerable to capture-induced parturition. This study improves our understanding of the capture-induced parturition process in stingrays, and provides useful information for management strategies and future recommendations for elasmobranch conservation.

KEYWORDS
conservation, fisheries management, fishing stress, premature birth, upper rio Paraná, viviparity

1 | INTRODUCTION

Elasmobranchs (sharks, rays and skates) share a number of life-history traits, such as low reproductive output and slow growth, which make them particularly sensitive to overexploitation and population depletion/collapse (Dulvy *et al*., 2014; Stevens *et al*., 2000). As a result, numerous studies have explored how fisheries interactions negatively affect the survival and fitness of elasmobranchs (*e.g*., Lambert *et al*., 2018; Skomal & Mandelman, 2012). For example, studies have been performed to assess species that are more vulnerable to capture stress and mortality (Gallagher *et al*., 2014; Mandelman & Skomal, 2009), to understand the variation in the stress response over ontogeny (Prohaska *et al*., 2018) and to unravel the occurrence of sublethal effects on important biological processes such as growth and reproduction (Guida *et al*., 2017). Although capture-induced parturition is a common consequence of fisheries interactions, the occurrence and cause of abortion and premature birth remain unknown, making specific regulations difficult to incorporate into fisheries management (reviewed by Adams *et al*., 2018).

Viviparous elasmobranchs are particularly sensitive to capture-induced parturition due to high maternal investment that enables females to gestate multiple and large offspring through a long gestation period (Adams *et al*., 2018; Hamlett *et al*., 2005). Given the wide diversity of reproductive modes of elasmobranchs, understanding the mechanisms that trigger the abortion/premature birth process may be challenging, considering that this mechanism can be driven by multiple factors (*e.g*., physical injury, air exposure, physical pressure and/or handling) (Adams *et al*., 2018). As capture-induced parturition was reported more frequently in stingrays than other elasmobranch species (order Myliobatiformes, Adams *et al*., 2018), in this study we used a Neotropical freshwater stingray, *Potamotrygon amandae* (Loboda & Carvalho, 2013), sampled in a non-native environment (upper rio Paraná, Brazil) as a model species to assess biological traits that may influence abortion/premature birth events. Previous reports have observed frequent abortions in potamotrygonid pregnant females and subsequently mortality of embryos regardless of their developmental stage (Charvet-Almeida *et al*., 2005; Garrone-Neto, 2010), yet several important questions remain unanswered.

To contribute to knowledge about the vulnerability to abortion in elasmobranchs, we integrated capture-induced parturition data of two consecutive reproductive seasons to address two primary questions about what factors might influence abortion/premature birth events. First, is the abortion/premature birth response associated with litter size? If so, are females with a higher litter size more susceptible to capture-induced parturition events? Second, is the gestation stage related to time to abortion? This study improves our understanding of how the abortion process varies in response to reproductive traits. Such information can be useful for management strategies and future recommendations for elasmobranch conservation.

2 | MATERIALS AND METHODS

Pregnant female *P. amandae* were collected from November 2017 to January 2018 and November 2018 to January 2019 during the gestation period (Table 1), on the Paraná River (reservoir of HPP Engenheiro Souza Dias) between the states of São Paulo and Mato Grosso do Sul, Brazil (20°23′–20°46′S, 51°40–51°22′W). The average air temperature ranged from 26.4 to 28.7°C on the sampling days. The specimens were captured during the day using casting nets (Figure 1a) by adopting the region's most common commercial fishing practice model. In the Paraná-Paraguay basin, *Potamotrygon* species of the “ocellate group” (*e.g.*, *P. motoro*, *P. pantanensis* and *P. amandae*) have high phenotypic plasticity with discrete and generally overlapping specific diagnostic characters. Therefore, all collected specimens were carefully analysed and their identification checked (see diagnosis in Loboda & Carvalho, 2013). *P. amandae* voucher (DZSJRP-21426) was deposited in the DZSJRP fish collection (Department of Zoology and Botany of the Institute of Biosciences, Letters and Exact Sciences) of the São Paulo State University ‘Júlio de Mesquita Filho’, São José do Rio Preto, SP, Brazil. The care and use of experimental animals complied with Ethical Conduct Committee on Animal Use (CEUA, 15/2018) animal welfare laws, guidelines and policies as approved by the Universidade Estadual Paulista and the National Council for Animal Experimentation Control. Permission for collecting was provided by IBAMA/ICMBio (SISBIO authorization number 50019-1 and SISGEN register number A001CBE).

Following the Adams *et al*. (2018) terminology, abortion is the expulsion of embryos before they can survive outside the uterus and premature birth is the parturition of preterm offspring that may survive. In this work, since we do not aim to analyse the survival of the embryos, we considered premature birth as developed embryos/foetuses with well-defined species coloration, with almost all yolk sac consumed (Figure 2d–i), which were alive when they were expelled, whereas abortion was considered to be when the embryo was underdeveloped, with a large yolk sac (Figure 2a–c), and born dead.

The stingrays were individually kept in plastic boxes and had water level and time monitored until parturition. Once in the box, the females were kept without any intervention apart from water renewal every 30 min so that temperature and oxygen levels were kept similar to their natural environment, and methodological standardization was ensured for all specimens. After 330 min without observing any abortion, the females were anaesthetised with 0.5% benzocaine (CEUA/FEIS authorization number 15/2017) and euthanized for verification of the presence of embryos/foetus retained in the uterus, which was not observed. All aborted and nonaborted embryos/foetuses were also euthanized following the same protocol. They will be used in future studies on maternal investment. The gestation stages were classified based on embryo size (disc width, DW) and month of capture (November to January; Table 1). The number and size of embryos

TABLE 1 Number of pregnant females and aborted embryos, embryo size (disc width, mm), gestation stage (from 1 to 3) and classification of specimens from which abortion/premature birth was observed in the freshwater stingray *P. amandae* from the Paraná River, Brazil (mean values ± S.D.)

Month	Pregnant female (*N*)	Aborted embryos (*N*)	Embryo size (mm)	Gestation stage	Classification
November	20	69	45.2 ± 9.21	Stage 1	Abortion
December	14	40	66.4 ± 10.95	Stage 2	Abortion
January	18	69	103.9 ± 10.27	Stage 3	Premature birth

Note. For gestation stage description please refer to Figure 2

FIGURE 1 (a) Capture of a freshwater stingray (*P. amandae*) from the Paraná River, Brazil, using casting nets. (b), (c) Pregnant females, easily identified by the presence of a distended abdomen (arrow). (d) A female post abortion, with the abdomen visibly empty (arrow), compared to a nonpregnant female (e)

and their respective mothers (DW also measured), and time to parturition by each female were recorded to test if the parturition process response is related to litter size and gestation stage. The time to parturition was also compared between gestation stages (1, 2 and 3; Table 1) using one-way ANOVA and the *post hoc* test of Holm–Sidak. The correlations between maternal disc width and number of embryos, and between time to parturition and number of embryos were determined using Pearson correlation analysis. Statistical significance was stated at $P < 0.05$, and all analyses were conducted in SigmaPlot for Windows 10.0 (Systat Software Inc., San Jose, CA, USA).

3 | RESULTS

Capture-induced parturition events were recorded in all stingrays on capture (n = 52; Figure 2), from early stage (presence of egg only) to late gestation stage (November to January; Table 1). Pregnant females were easily identified by the presence of a distended abdomen (Figure 1b,c). After parturition, the abdomen was visibly empty (Figure 1d) when compared to a pregnant (Figure 1c) and non-pregnant female (Figure 1e). Litter size ranged from one to seven embryos (mean ± S.D. = 3.4 ± 1.6; Figure 3) and was positively correlated with maternal size (Pearson r = 0.46; Figure 3a). Uterine fecundity corroborated the findings for other species of the genus *Potamotrygon* (Charvet-Almeida *et al*., 2005), but not reported in *P. amandae*. A total of 109 embryos were aborted and there were 69 premature births between November 2017 and January 2019 (Table 1). The abortions were recorded in the early and middle gestation stages (*i.e*., stages 1 and 2), when undeveloped embryos still had a large yolk sac (Figure 2a–c). Premature birth was observed in late gestation (*i.e*., stage 3).

Time to parturition ranged from 5 to 300 min (n = 20; mean S.D. = 126 ± 99.1) and was negatively correlated with the number of aborted embryos (Pearson r = −0.55; Figure 3b). The time to parturition decreased progressively as the gestation stage progressed, and there were differences between stages 1 (n = 6), 2 (n = 12) (ANOVA, t = 2.49; P = 0.02) and 3 (n = 7) (ANOVA, t = 2.49; P = 0.0003), and between stages 2 and 3 (ANOVA, t = 2.28; P = 0.03) (Figure 3c).

4 | DISCUSSION

Our results demonstrate for the first time how the abortion/premature birth process in stingrays can be affected by important reproductive traits such as litter and maternal size, and how gestation stage modulates such relationships. We found that parturition occurs faster in pregnant females with higher litter size during late pregnancy (Figure 4). As all pregnant stingrays were collected as done in commercial fishing, our study presents the first report that evaluates with strict methodological criteria the effects of commercial capture in this species, considering the importance of the data for management and conservation. These results may therefore be used to understand the capture-induced parturition process in elasmobranchs, as well as to determine the most susceptible stages for designing effective conservation measures.

The high abortion/premature birth rate observed (100%) shows that all pregnant stingrays, from early stage (presence of egg only) to late stage pregnancy, are susceptible to capture-induced parturition in a wide range of time (5–300 min), despite the rapid capture methods

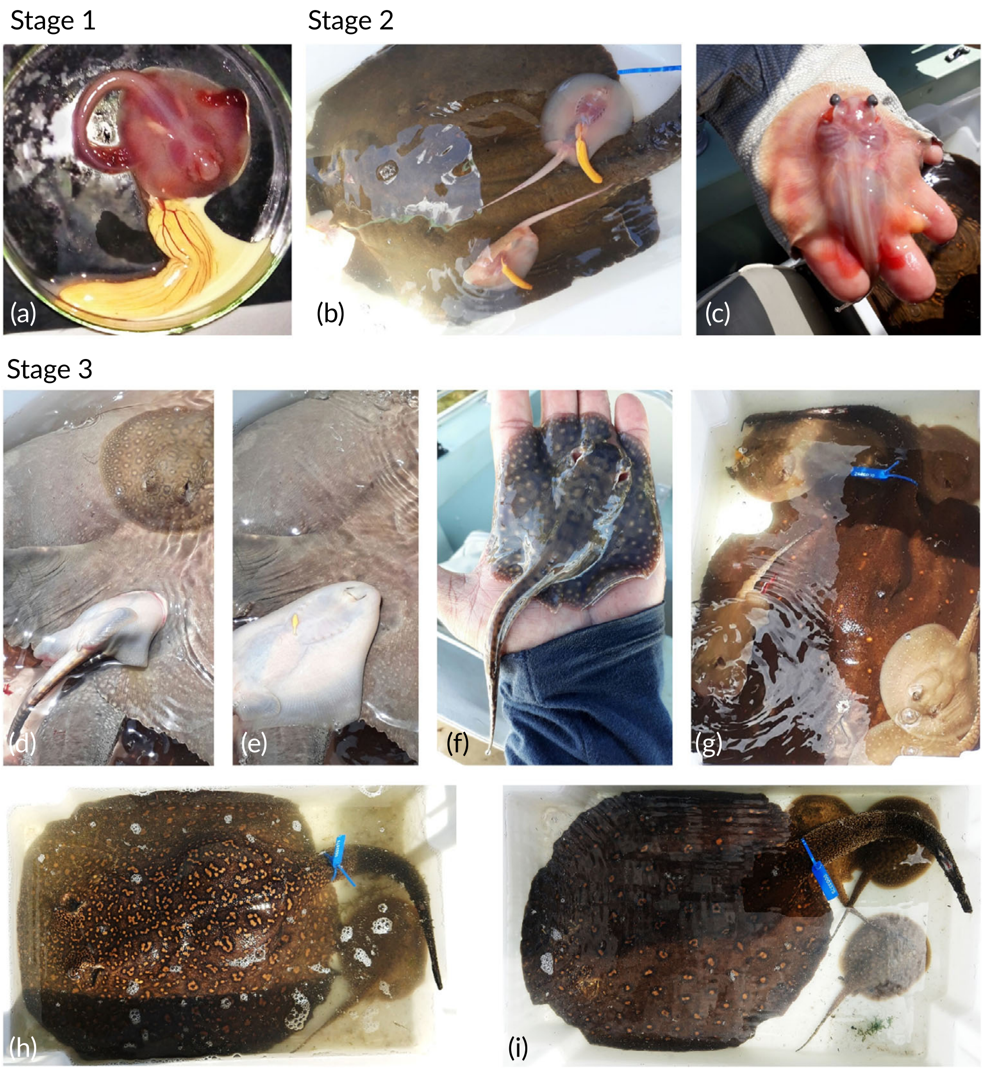

FIGURE 2 Embryos of gestation stages 1 to 3, *P. amandae*. (a) Stage 1, November: undeveloped embryo with a large yolk sac. (b), (c) Stage 2, December: more developed embryo with the presence of the yolk sac and without colouring. (d)–(i) Stage 3, January: developed embryos with well-defined coloration and almost all yolk sac consumed. (d), (e) Premature birth of pups

used in the present study (approximately 2 min from the moment the stingray was trapped in the net until the moment of its removal). Similar findings were observed in the pelagic stingray *Pteroplatytrygon violacea* (85%; Mollet, 2002) and in the shortnose guitarfish *Zapteryx brevirostris*, in which 93% of abortions occurred in the first 10h (Wosnick *et al*., 2019). Together, these results suggest the occurrence of continued physiological disruptions, regardless of time to parturition, caused by capture and potentiated or not by handling during monitoring. New methodologies, such as "birthing tags" inserted into the uterus of pregnant females (Holt *et al*., 2014), could help to investigate whether parturition is induced by handling during monitoring or just by acute stress of capture, and also if the immediate release may avoid the event.

Our results show an increase in reproductive output with maternal size, a pattern commonly observed in elasmobranchs (*e.g*., Fahy *et al*., 2007; Hussey *et al*., 2010). Such a reproductive trait, however, directly affected the time to parturition in the stingrays, since larger-bodied females had more pups and therefore faster parturition (Figure 3a,b). The recommendation for specific conservation measures for females during critical reproductive periods, for example temporary fishing bans, has been the advice of several studies (*e.g*., Guida *et al*., 2017; Adams *et al*., 2018; Wosnick *et al*., 2018). Although this work was performed in a non-native environment for the species (some locations in the Paraná-Paraguay basin), the results obtained are representative for places where the species is native and threatened by commercial fishing. However, our results indicate that specific sizes can be even more prone to early parturition, which should be integrated into current management recommendations based on the degree of vulnerability to abortion of these stingrays due to capture stress.

The time to abortion/premature birth was also significantly affected by gestation stage. Females in late gestation stages (with stage 3 embryos) aborted faster than those in earlier stages (stages 1 and 2), indicating that the size or movement of the embryos may facilitate this process (*e.g*., Rincon, 2007), especially if they are in larger numbers in uteri. Our findings demonstrate for the first time that females in late gestation are more susceptible to premature birth events. Because capture-induced premature birth occurred in a short time (mean 40 min, stage 3), our results further suggest that females at this stage may also be sensitive to other stressors (*e.g*., urbanization, pollution, climate changes; *e.g.*, Chin *et al.*, 2010; Lyons and Wynne-Edwars, 2019) that may cause physiological disruption and premature birth. Despite the high vulnerability to premature birth in females during late gestation, pups at this stage appear to have a higher survival capacity. Although we did not report the survival time of pups, we observed that most of them were born very active. However, timing for parturition seems to be crucial in embryo survival, since in later abortion the pups were born dead.

Since all pregnant rays are susceptible to capture-induced parturition, an efficient management strategy would be to apply seasonal closures to key areas such as maternities and nurseries during the entire reproductive period (Adams *et al.*, 2018). However, as such decision-making depends on other issues, our study shows that closure during more critical periods (*i.e.*, late gestation, ~1 month), when females are most vulnerable, could increase the chances of maternal and offspring survival. Additionally, given that capture-induced parturition occurs in a relatively short time, other complementary management actions could be implemented, such as (a) immediate release after capture, ensuring shorter time of exposure to additional stressors (*e.g.*, air exposure, handling, temperature change); (b) assisted fishing in regions with high capture of pregnant rays, that is, verification of animals caught in the fishing gear during short time periods, based on time to abortion at each gestation stage/month (*e.g.*, each 1 h); and (c) encouragement of the use of more selective and less invasive fishing gear. We understand that the recommendations presented here will have species-specific variations, but this approach may be particularly useful to assist decision makers aiming to conserve elasmobranchs.

Our study revealed a close relationship of the abortion/premature birth process with the gestation period, litter size and size of pregnant freshwater stingrays. Our findings suggest that pregnant females with high litter sizes are more susceptible to capture-induced parturition and that the time to parturition is dependent on the gestation stage, helping to elucidate some of the reproductive and evolutionary traits related to capture-induced parturition in elasmobranchs and to propose specific recommendations for the management of these animals. Future studies on the survival and degree of vulnerability of postpartum females (natural or stress-induced) are recommended, as the high mortality of postabortion females has already been observed in another elasmobranch species, the shortnose guitarfish (Wosnick *et al.*, 2019). In addition, it is recommended that the effect of different fishing gear and duration, as well as species-specific physiological responses on the abortion/premature birth process in species with differing reproductive strategies is examined.

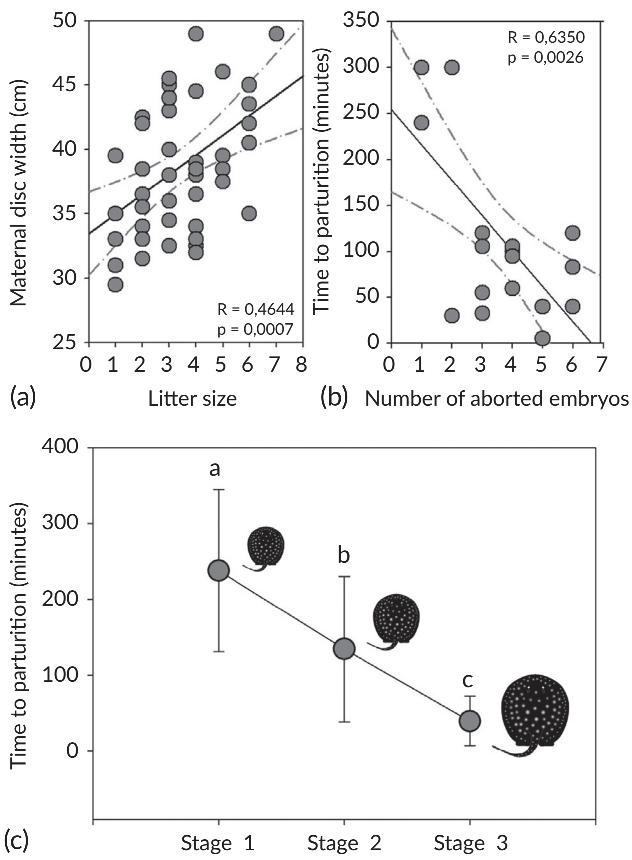

FIGURE 3 Linear correlations of the relationship between (a) maternal disc width and litter size (n = 22) and (b) time to parturition and number of embryos/foetuses (n = 20). (c) Time to parturition in each gestation stage (1, n = 6; 2, n = 12; 3, n = 7) of pregnant females of *P. amandae*. [a,b,c]Different letters indicate significantly different means through ANOVA + Holm-Sidak (P < 0.05)

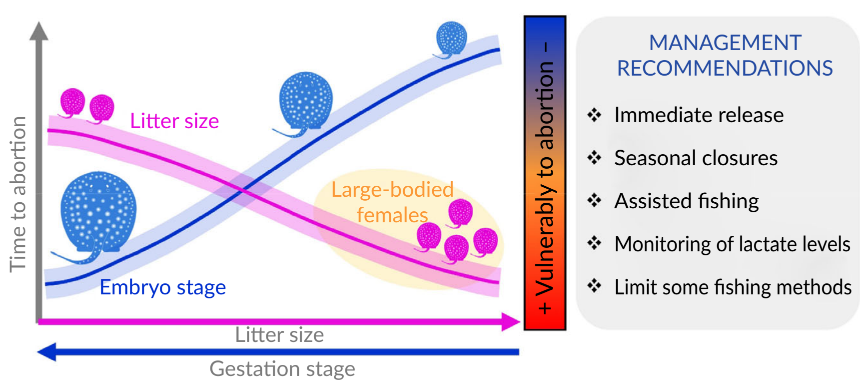

FIGURE 4 Conceptual figure of the degree of vulnerability to abortion of pregnant female freshwater stingrays (*P. amandae*), integrating all the responses observed in the present study

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