

SHORT COMMUNICATION

Pre-copulatory bite wounds as evidence of aggressive competition for mating in the neotropical freshwater stingray *Potamotrygon motoro*

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Reproductive aspects of Amazonian freshwater stingrays are still poorly studied, however, it is known that the copulatory behavior in some species is related to the hydrometric seasons (high and low water). In order to assess the reproductive activity prior to copulation, we examined bite wounds in *Potamotrygon motoro* of Catalão Lake, in Amazonas state, Brazil. Specimens of both sexes showed wounds on the pectoral fins. Males presented a large amount of bite wounds, distributed randomly throughout the body, while females had more bite wounds on the posterior region. The higher number of wounds in males is assumed to indicate aggressive interactions among males in competition for females, caused by attempts to prevent other males from mating with the target female. The behavior is likely owed to the confinement of the stingrays in the lake during the low-water period.

KEYWORDS: dermal injuries; male-male aggressiveness; Potamotrygoninae; reproductive aspects

Lesões por mordidas pré-copulatórias como evidência de competição agressiva para acasalar na raia de água doce neotropical *Potamotrygon motoro*

RESUMO

Os aspectos reprodutivos das raias de água doce ainda são pouco estudados, no entanto, sabe-se que algumas espécies apresentam comportamento de cópula relacionado com as estações hidrométricas (águas altas e baixas). Com o intuito de avaliar as atividades reprodutivas que antecedem a cópula, nós examinamos as lesões por mordidas em *Potamotrygon motoro* do Lago Catalão, Amazonas. Espécimes de ambos os sexos apresentaram lesões nas nadadeiras peitorais. Os machos apresentaram uma maior quantidade de mordidas distribuídas aleatoriamente por todo o corpo, enquanto as fêmeas apresentaram mais lesões de mordidas na região posterior. Acreditamos que o maior número de lesões nos machos indica interações agressivas entre os machos em competição por fêmeas, causada por tentativas de impedir outros machos de acasalar com a fêmea alvo. Tal comportamento provavelmente deve-se ao confinamento das raias no lago durante o período de águas baixas.

PALAVRAS-CHAVE: lesões dermais; Potamotrygoninae; agressividade entre machos; aspectos reprodutivos

Elasmobranchs present a variety of reproductive modes and their complex courtships mostly involve pre-copulatory-biting (Pratt and Carrier 2005). The copulation initiates with males grasping the margin of the female's pectoral fins, followed by the insertion of the clasper into the female's cloaca (Conrath and Musick 2012).

Bite scars on the male's pectoral fins are not uncommon and have been reported for some elasmobranchs. Bite wounds on the body of a male sand-tiger shark, *Carcharias taurus* Rafinesque, 1810 (Carchariidae) were assumed to

be a potential result of copulatory activities (Gilmore *et al.* 1983). Biting behavior was also observed during agonistic interactions, such as expressing dominance hierarchy or group feeding (Gordon 1993; Pratt and Carrier 2005; Brunnschweiler and Pratt 2008). The mating behavior of batoid species is still poorly known, although the biting of the female's pectoral fins during copulation has been observed in marine Myliobatiform rays (Chapman *et al.* 2003; Arnés-Urgellés *et al.* 2018; McAllister *et al.* 2020). Mating bite wounds in both sexes were observed in Atlantic stingray,

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Hypanus sabinus (Lesueur, 1824) (Dasyatidae), indicating that biting behavior among males may be relatively common for this species (Kajiura *et al.* 2000).

Despite the paucity of data on reproductive aspects of neotropical freshwater stingrays, there is evidence that the mating of Potamotrygoninae species is driven by the hydrological pulse (Charvet-Almeida *et al.* 2005). Physical-chemical changes in water characteristics probably act as a trigger for the onset of the reproductive cycle in Potamotrygonin species during the rainy season, when river levels are high, so that copulatory and associated biting behaviors should also occur seasonally (Thorson *et al.* 1983). In the ocellate stingray, *Potamotrygon motoro* (Müller & Henle, 1841), however, the onset of mating occurs in the low water period (Charvet-Almeida *et al.* 2005). Here, we report the occurrence of bite wounds in *P. motoro* during the low-water season in an Amazonian floodplain lake and discuss their role as a pre-copulatory activity.

Two field collections were carried out in October 2014 and November 2015 (five days per month), in Catalão Lake (3°09'42.3"S, 59°54'55.4"W), a floodplain lake located at the confluence of the Solimões and Negro rivers, in the municipality of Iranduba, Amazonas state, Brazil. During fieldwork, the water in the lake was turbid and sediment-rich. The level of the Negro River measured at the port of Manaus (Porto de Manaus 2021), was 19.9 and 16.6 m (means values of the two five-day collection periods), respectively, in October 2014 and November 2015. The typical water level in the lake during the low-water season is between 17 and 19 m, while levels under 17 m occur in abnormally dry years (Bittencourt and Amadio 2007). In moderately or strongly dry years, Catalão Lake can maintain a narrow and shallow channel connecting to the Negro River or can become completely disconnected from the river (Röpke *et al.* 2018). During the low-water season of 2015, we observed that the connection between the lake and the main channel of the river was completely severed, trapping fishes inside the lake.

Stingrays were captured using bottom longlines over the muddy substrate. Species identification followed Loboda (2010). All specimens were euthanized with clove oil and the sex was determined macroscopically by direct observation of the claspers in males. Both dorsal and ventral sides of the disc were photographed for posterior identification of bite marks, but only the ventral surface was examined in detail, due to the lightly pigmented epidermis on the edge, which facilitates the visualization of wounds. Marks on the dorsal surface were not counted, to avoid overestimation. The ventral surface of the disc was divided into four quadrants to quantify the bite wounds: left and right anterior quadrants (from the rostral edge to the fifth branchial slits) and left and right posterior quadrants (from the fifth branchial slits to the posterior edge) (modified from Kajiura *et al.* 2000) (Figure 1). The wounds

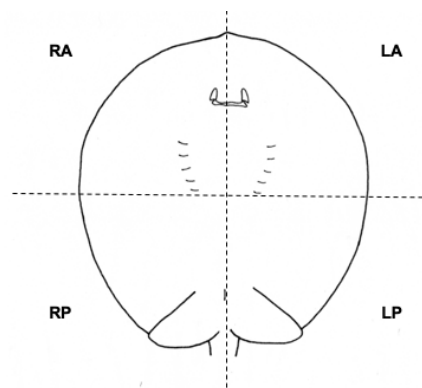


Figure 1. Division of the ventral surface of the disc of *Potamotrygon motoro* for bite wound quantification. RA = right anterior; LA = left anterior; RP = right posterior; LP = left posterior.

were carefully examined to avoid mistaking with other marks, probably caused by litter and rocks present on the substrate.

We considered the following marks as evidence of bite wounds: (a) bite (the superficial triangular pattern of the stingray's teeth); (b) abrasion (epidermis wear with pigmentation loss caused by a deep dental mark); and (c) excision wound (loss of tissue of the disc edge due to extremely strong biting). The categorizations followed Kajiura *et al.* (2000). Only recent bite wounds were considered, i.e., those which could be clearly identified as bites and abrasion marks. We considered only excision wounds that were not completely healed. According to Kajiura *et al.* (2000), dermal wounds on *H. sabinus* in captivity were completely healed within two weeks. Small and dubious excisions were not considered, as fishermen from a nearby riverside community frequently capture stingrays as bycatch and return most of them to the water. In addition, typical excisions caused by bites of piranhas (*Serrasalmus* spp.) or trairas (*Hoplias malabaricus* (Bloch, 1794)) were not counted. A chi-square test was applied to test the association between number and type of wounds with sex ($P \leq 0.05$).

The specimens were collected with permission of Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio (SISBIO license # 22055/2012) and in accordance with the Ethical Committee of Animal Experimentation (CEUA-UFAM authorization protocol # 070/2012).

We collected 20 individuals of *P. motoro* (nine females, disc width between 25.5 and 50.0 cm; 11 males, disk width 25.0 – 48.0 cm). Ten specimens (four females and six males) had bite wounds on the surface of the pectoral fins (Figure 2; Table 1). Bite, abrasion and excision wounds were observed in both sexes, with higher numbers in males (Table 1), independently of the body quadrant (Figure 3). Despite the higher numbers in males, there was no significant difference between the sexes in the number of wounds per body quadrant ($\chi^2 = 4.29$; $p = 0.23$) or types of wounds ($\chi^2 = 1.08$; $p = 0.58$). Bite overlaps were observed in most male specimens, especially those with

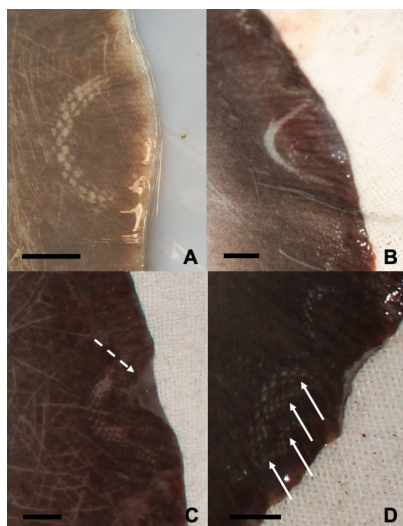


Figure 2. Bite wounds observed on the ventral surface of *Potamotrygon motoro*. A – bite; B – abrasion; C – excision, indicated by the dashed arrow; D – bite overlap indicated by arrows. Scale bar = 1 cm. This figure is in color in the electronic version.

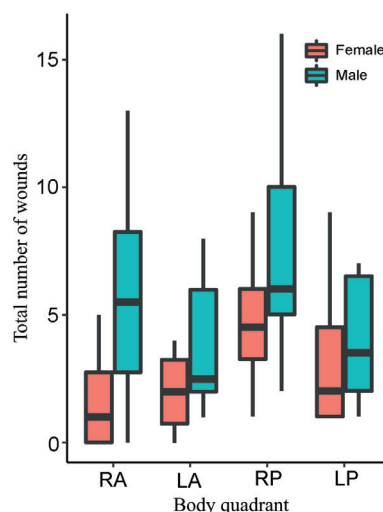


Figure 3. Total number of wounds (mean and standard deviation of bites, abrasions and excisions) on four females and six males of *Potamotrygon motoro* per body quadrant. RA: right anterior; LA: left anterior; RP: right posterior and; LP: left posterior. This figure is in color in the electronic version.

Table 1. Single bites, multiple (overlapping) bites, abrasions and excisions observed on the pectoral fins of females and males of *Potamotrygon motoro* in Catalão Lake (Amazonas, Brazil). Values are the mean \pm standard deviation, followed by the range. N = number of individuals.

Sex	N	Single bites	Multiple (overlapping) bites	Abrasions	Excisions	Total number of wounds
Females	4	8.5 \pm 11.8 (2 - 22)	3	2.4 \pm 2.1 (1 - 6)	2.0 \pm 1.3 (1 - 2)	48
Males	6	21.3 \pm 2.9 (4 - 24)	4.0 \pm 7.7 (1 - 5)	2.5 \pm 1.7 (1 - 2)	4.2 \pm 1.9 (2 - 5)	128

multiple dental marks (Figure 2d). Only two males had bites on the pelvic fins (one wound each).

The high number of bite wounds on the posterior quadrants compared to the anterior quadrants of females agrees with previous findings that males grasp the females near the cloaca during pre-copulatory behavior (Chapman *et al.* 2003; Arnés-Urgellés *et al.* 2018; McAllister *et al.* 2020). The large number of wounds on the posterior disc edge might be related to the possible release of pheromones through the females' cloaca during the reproductive season, as males are not able to visually discriminate females (Kajiura *et al.* 2000). The copulation probably also is mediated by other sensory systems, such as the electrosensory ampullae of Lorenzini, which needs to be further investigated (Tricas *et al.* 1995).

The high number of bite wounds in males of *P. motoro* in Catalão Lake may have a different explanation from those presented in previous studies on agonistic interactions in elasmobranchs (Gordon 1993; Pratt and Carrier 2005). During the transition from the period of receding to low water, stingrays remain in the lake, probably due to the increase in prey abundance (A. Shibuya, unpubl. data). During the low-water period, when the connection to the river is restricted or severed, the confinement likely leads to competition for females during the reproductive season. Although other agonistic male-male interactions have been

previously described for elasmobranchs, such as dominance hierarchy and feeding behavior in group (*e.g.* Brunnschweiler and Pratt 2008), our observations are probably related to copulatory behavior, as *P. motoro* reproduces in the low-water period (Charvet-Almeida *et al.* 2005). A pregnant female was collected in 2014, which also indicates that they were in reproduction. The random distribution of bite wounds on the anterior and posterior quadrants of males indicates aggressive male-male interactions in competition to copulate with females. This behavior was observed in *Hypanus sabinus* (Dasyatidae) by Kajiura *et al.* (2000), who proposed that ovulation may influence the high occurrence of wounds related to competition during the mating season.

The lack of connection between the lake and the river in 2015 confined individuals of *P. motoro* in a limited space during the low-water period, trapping them in the lake until rising water. Thus, this temporary limitation likely led to aggressive interactions during the reproductive period. Furthermore, it is likely that the turbidity of the water in Catalão Lake prevents the visual identification of sexual partners in *P. motoro*, reinforcing the possibility that the release of pheromones by females during ovulation could trigger the copulatory behavior.

Though non-significant, the observed tendency of overall higher number of bite wounds, and of more excision

wounds in males than in females suggests that the aggressive interactions among males of *P. motoro* occur with certain frequency. In addition, multiple overlapping bites that were observed mostly in males are probably related to male-male “grasping” behavior as an insistent attempt to avoid any other male mating with the target female.

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