



# Environmental factors involved in breaching behavior of manta rays in estuarine waters

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**ABSTRACT:** Improving our knowledge on the behavior of threatened species is essential for developing effective conservation actions. The Paranaguá Estuarine Complex (PEC), southern Brazil, is the only estuary in the world where manta rays have been observed performing breaches seasonally. The exact role of this breaching behavior and the environmental factors connected to it are unknown. Our goals were to determine the spatial distribution, and the temporal and environmental factors that influence the breaching behavior of this endangered group in a dynamic estuarine habitat for the first time. Manta rays were observed breaching in the PEC during austral summer and early autumn, when the sea surface temperature (SST) and precipitation were high. Generalized additive models revealed that the presence and frequency of the breaches were both influenced by the SST and hours of daily effort, while the breaching frequency was also influenced by the wind direction and speed, percentage of moon illumination, and year. The breaches were mainly concentrated near the mouth of a river. Likely these factors influenced not only the occurrence and behavior of manta rays, but also the distribution of their food source, potentially providing optimal conditions for foraging and reproduction. Based on the coloration pattern, it is possible that the observations were of *Mobula cf. birostris*. These results provide valuable insights into the breaching behavior of manta rays in estuarine waters that will assist future conservation initiatives and research on their behavioral ecology, to optimize fishery management and contribute to developing sustainable ecotourism in the PEC.

**KEY WORDS:** *Mobula birostris* · *Mobula cf. birostris* · Paranaguá Estuarine Complex · Brazil · Breaches · Generalized additive model · GAM · Elasmobranch

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## 1. INTRODUCTION

Pelagic elasmobranchs, unlike marine mammals, do not need to come to the surface to breathe (Notarbartolo-di-Sciara & Hillyer 1989), which makes investigating the distribution and behavior of these species challenging in turbid waters. However, some elasmobranchs are adapted to perform breaching behavior (jumping out of the water), enabling obser-

vation of these species as they propel themselves out of the water during such breaching events, e.g. sharks using their torpedo-shaped bodies, such as *Carcharhinus leucas*, *Carcharodon carcharias*, and *Cetorhinus maximus* (Pyle et al. 1996, Sims et al. 2000, Martin et al. 2005, Curtis & Macesic 2011), or rays using their well-developed pectoral fins, such as Aetobatidae, Rhinopteridae, and Mobulidae (Coles 1910, 1916, McEachran 1995).

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During the course of evolution, mobulid rays have moved from a benthic to a pelagic lifestyle, where they perform pectoral fin movements similar to the flapping movement of birds' wings, while their enlarged pectoral fins also allow them to propel their bodies out of the water (Bigelow & Schroeder 1953, Rayner 1986). Even with a disc width of up to 7 m and weighing up to 2 t (Marshall et al. 2009), manta rays, *Mobula birostris* (Walbaum 1792) and *M. alfredi* (Krefft 1868), can sometimes be observed performing breaching behavior (Marshall & Bennett 2010a, Medeiros et al. 2015, Stevens 2016).

According to the International Union for Conservation of Nature, the conservation status of *M. birostris* is Endangered (Marshall et al. 2020) and that of *M. alfredi* is Vulnerable (Marshall et al. 2019a), due to the natural characteristics that make these species highly susceptible to overfishing by the directed fisheries supplying the demand for gills from the Asian markets and for meat consumption, while pollution and accidental by-catch also contribute to their population decline (Couturier et al. 2012, Ward-Paige et al. 2013, Dulvy et al. 2014, Germanov et al. 2018).

A potentially separate manta ray species, *M. cf. birostris*, with distinct coloration and markings that seem to differ from the 2 currently described species has been mentioned in previous studies (Marshall et al. 2009, Hinojosa-Alvarez et al. 2016). They are currently reported to occur in the western Atlantic, southeastern Florida (Pate & Marshall 2020), Gulf of Mexico (Hinojosa-Alvarez et al. 2016, Hosegood et al. 2020), and northeast Brazil (Bucair et al. 2021), but no clear description is available to date in order to clearly distinguish between *M. birostris* and *M. cf. birostris*.

Manta rays have been observed to perform only 1 to 3 sequential breaches, landing in the water either on the dorsal or ventral side (Homma et al. 1999, Medeiros et al. 2015, Stevens 2016). The behavior is rarely observed and remains poorly studied, but the prevalence of breaches in Mobulidae indicates that it has an adaptive benefit (Rayner 1986, Homma et al. 1999). Various hypotheses have proposed that the breaches may be connected to courtship (Marshall & Bennett 2010, Stevens et al. 2018), parturition, removal of remoras and/or parasites, play, feeding, and/or communication (Rayner 1986, Homma et al. 1999, Deakos 2010, Medeiros et al. 2015, Stevens 2016), while it is possible that this behavior may have multiple functions.

The Paranaguá Estuary Complex (PEC) in southern Brazil is a large estuarine system (Noernberg et al. 2006) where it is impossible to see manta rays while SCUBA diving because the water is extremely

turbid due to the presence of suspended particulate matter carried into the area by several rivers. However, they can be seen breaching during austral summer and early autumn (Medeiros et al. 2015).

Although manta rays have been sighted sporadically in some estuaries on the west coast of the North and South Atlantic Ocean (Coles 1916, Adams & Amesbury 1998, Schwartz 2000, Milessi & Oddone 2003), the PEC is the only estuary in the world so far where they have been observed seasonally (Medeiros et al. 2015), and only those in the PEC (Medeiros et al. 2015) and in the Maldives (Stevens 2016) have been reported to demonstrate breaching behavior with high frequency, making this area particularly important to study and protect.

Estuaries are key habitats for many elasmobranchs that use these areas as nurseries, for parturition, and for feeding (Plumlee et al. 2018). However, estuaries have active hydrodynamics and are influenced by tides, freshwater input, and winds that generate fluctuations in the water parameters resulting from the convergence between fresh and saltwater (Able 2005). Consequently, the estuarine fauna is often exposed to sudden daily and seasonal variations in environmental factors (Collins et al. 2008, Plumlee et al. 2018). Changes in temperature, freshwater input, and ocean circulation have the most widespread effects on estuarine occurrence and behavior of sharks and rays, especially ectothermic elasmobranchs that feed on zooplankton that respond rapidly to changes in the environment (Chin et al. 2010, Jaine et al. 2012, Schlaff et al. 2014).

Understanding how environmental factors influence the temporal and spatial distribution of manta rays can provide important information to predict their occurrence and activity, and to identify the potential motivating factors for using estuarine waters and performing breaching behavior. During this study, we performed multiple-year observations, to analyze and identify key factors influencing temporal and spatial patterns of the breaching behavior of manta rays at the PEC, a dynamic estuarine area, for the first time. The results provide valuable insights into the behavioral ecology of manta rays and can help conservation initiatives in the area.

## 2. MATERIALS AND METHODS

### 2.1. Study area

The PEC is an interconnected subtropical estuarine system with an area of 551.8 km<sup>2</sup> composed of man-

groves, marshes, and beaches (Noernberg et al. 2006), located in southern Brazil (Fig. 1). The area has a large terrestrial and marine biodiversity that is located on the edge of the Atlantic Forest Biosphere Reserve, a UNESCO World Natural Heritage area (Lana et al. 2001, Medeiros et al. 2015). The area is also inhabited by the endangered mobulid species *Mobula hypostoma*, which reaches a maximum size of 120 cm disc width (DW) (Barleta et al. 1989, Marshall et al. 2019b).

The tidal regime is semi-diurnal (with diurnal inequalities), and during neap tides, strong non-linear interactions allow the formation of up to 6 high and low tides per day (Lana et al. 2001, Marone et al. 2005). The height of the tide can be up to 2.7 m in the interior and 1.5 m at the mouth of the estuary, and the average tide velocity measures  $0.85 \text{ m s}^{-1}$  at low tide and  $1.10 \text{ m s}^{-1}$  during flood tide (Mantovanelli et al. 2004, Marone et al. 2005). The mean residence time of the water is 3.5 d (Marone & Jamiyanaa 1997).

The average rainfall is higher in the summer and spring than in autumn and winter (Marone et al. 2005). The mean horizontal gradient of salinity varies from 12–29 psu (summer, rainy season) to 20–34 psu (winter, dry season) and the mean water temperature varies from 23 to  $30^\circ\text{C}$  during the summer and 18 to  $25^\circ\text{C}$  during the winter (Marone et al. 2005).

Local water circulation varies daily, as well as seasonally, and is mainly influenced by tide, wind, and precipitation that can generate distinct salinity conditions in the water column (i.e. well mixed or partially mixed water column), which in turn influences the distribution of the local fauna (Lana et al. 2001, Mantovanelli et al. 2004, Marone et al. 2005, Salvador & Bersano 2017).

## 2.2. Fixed-point observations

As the waters in the region are very turbid with low visibility, underwater observation is impossible (Medeiros et al. 2015). Therefore, in this study, we include only sightings of manta rays breaching, obtained using an ad libitum sampling method that consists of opportunistic observations during which all activities performed by the observed individual are

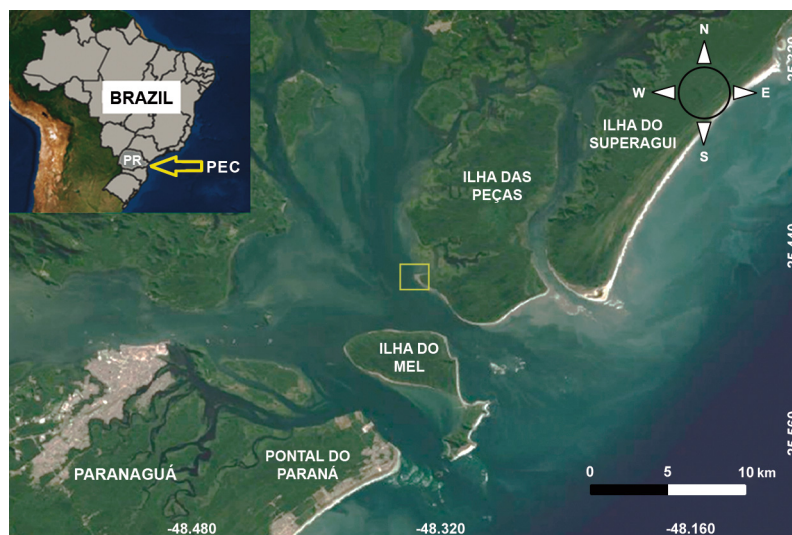


Fig. 1. Paranaguá Estuarine Complex (PEC), Paraná state (PR), southern Brazil. Yellow rectangle: observation area

recorded (Altmann 1974, Lehner 1996). Breaching events were recorded by a single observer (A. M. Medeiros) from the beach, observing towards the front at a  $180^\circ$  angle, at random times between 08:00 and 18:00 h.

Samplings were done from December 2011 to July 2015. The observations were performed from 2 fixed points: point 1 (P1;  $25^\circ 27' 38.4'' \text{ S}$ ,  $48^\circ 20' 7.2'' \text{ W}$ ) and point 2 (P2;  $25^\circ 27' 32.39'' \text{ S}$ ,  $48^\circ 20' 27.63'' \text{ W}$ ). The location of each sighting was estimated from the angle of the observer (with a protractor) and the distance from the sighting. Additionally, to reduce the errors of the estimates, the distance between known points in the area, such as buoys, islands, and points on the coastline, were measured to help identify the location of the breaches, by using rangefinder (Rioux 1984) and a nautical chart of the area with distance lines from the coast (Brazilian Navy Nautical chart no. 1820; [www.marinha.mil.br/chm](http://www.marinha.mil.br/chm)). Even so, the distance estimates are subject to errors, which increase with the distance from the coast, with a tendency to underestimate the number of sightings over long distances (Laake et al. 2012).

Every year, when breaching events were observed, data was collected for at least 20 field hours per month of effort. After more than 20 field hours without sightings, the monthly field hours were reduced to  $10 \text{ h mo}^{-1}$ . When breaching events were registered by the observer, the field observation routine was resumed to at least  $20 \text{ h mo}^{-1}$ . The daily hours of effort varied between 1 and  $12 \text{ h d}^{-1}$ .

### 2.3. Species identification

Identification of the manta rays was based on the body size, shape, and coloration of the animals. Their general coloration was black on the dorsal surface and white on the ventral side. The presence of cephalic fins and terminal mouth could often be observed, while the body size was estimated to be greater than 3 m DW (Marshall et al. 2009). Species identification followed the descriptions of Marshall et al. (2009) and Hinojosa-Alvarez et al. (2016). Due to the difficulties in detecting the presence of manta rays in the area (poor visibility underwater, no surface feeding recorded), we focused on documenting only the breaching events of manta rays during this study. When possible, species identification was based on photographs of the breaching behavior.

The shape and intensity of the white marks on the dorsal side and the coloration of the mouth and cephalic fin areas were recorded when possible. The breaching behavior was characterized according to Medeiros et al. (2015) as 'backward' or 'ventral' breaching, depending on whether the dorsal or ventral surface reached the water surface first, respectively, and as a single breaching (1 breaching only) or sequential breaching (the breaching behavior is repeated twice or more within less than 10 s by the same animal, less than approximately 3 m from the first breaching).

## 2.4. Data analysis

### 2.4.1. Environmental variables

In order to analyze the influence of environmental and temporal factors on the presence of breaching events and the number of breaches per day (samples unit), the following variables were considered for the models: 8 d sea surface temperature (SST), monthly precipitation, wind direction, wind velocity, percentage of moon illumination, tidal amplitude, hours of daily effort, day of year, and year (Table 1). The precipitation data was obtained from the Hydrological Information System of the Instituto de Águas do Paraná (www.aguasparana.pr.gov.br), for the 'Rio Guaíra' meteorological station. This is located in Guaíra, Paraná State, 15 km from the observation points, and is responsible for the largest continental drainage for the area (Falkenberg 2009).

Wind direction and speed data were obtained from the meteorological station at Instituto Nacional de Meteorologia (INMET), located 4 km from the observation points in Ilha do Mel, Paraná State. The 8 d

and monthly SST data were obtained from satellite images of the MODIS-AQUA sensor, with a resolution of 4 km, from the database available at <https://oceancolor.gsfc.nasa.gov> and analyzed with the SeaDAS software. The percentage of moon illumination was provided by the NASA Scientific Visualization Studio (<https://svs.gsfc.nasa.gov/>).

The tidal state was provided by the Paranaguá Port tide gauge ([www.portosdoparana.pr.gov.br/](http://www.portosdoparana.pr.gov.br/)) and was categorized as flood (from the ocean towards the interior of the estuary), ebb (from the interior of the estuary towards the ocean), high (moment after the flood tide, when the tide reaches its maximum height and loses speed until it stops and changes direction), and low (moment after the ebb tide, when the tide reaches its minimum height and loses speed until it stops and changes direction). Tidal amplitude was calculated by subtracting the height of the lowest tide from the highest tide of the day.

### 2.4.2. Spatial distribution

The location of the sightings was imported into GIS databases in Quantum GIS software 2.8.3, Wien, (QGIS Development Team 2020), from which a 95 % kernel density estimation analysis was performed to identify variation in the density of breaching events in the area with study period and tidal state. Depth data was obtained from the Brazilian Navy Nautical chart no. 1820 ([www.marinha.mil.br/chm](http://www.marinha.mil.br/chm)).

### 2.4.3. Statistical analysis

In order to evaluate the relationship between the presence and absence of breaching events and their

Table 1. The explanatory variables considered for the generalized additive models of manta ray breaching behavior related to environmental and temporal variables in the Paranaguá Estuarine Complex, from December 2011 to June 2015

Explanatory variable	Range and unit
Day	1–365
Year	2012–2015
Daily effort	1–12 h d <sup>-1</sup>
8 d sea surface temperature	19–30.4°C
Wind direction	0–360°
Wind velocity	0.3–6 m s <sup>-1</sup>
Tidal range	0.36–1.69 m
Moon illumination	0–100 %



daily frequency with environmental and temporal factors, generalized additive models (GAMs) were used (Hastie & Tibshirani 1990). A GAM is a non-parametric extension of the generalized linear model in which part of the linear predictor is specified in terms of a sum of smooth function covariates (Wood 2017). The general form of the GAM is as follows:

$$g[E(Y)] = \beta_0 + f_1(X_1) + \dots f_i(X_i) \quad (1)$$

where  $g$  is a link function,  $E(Y)$  is the expectation of the response variable  $Y$ ,  $\beta_0$  is the intercept, and  $f_i$  is the smoothing function of the predictor variable  $X_i$ .

Initially, an exploratory analysis of the data distribution was performed to select the family of models. Daily presence and absence data (1 for presence and 0 for absence of breaches) was used as a variable response to evaluate the presence of species based on breaching events, with binomial family distribution and logit link function. Daily number of breaches with zero-inflated family distribution (due to zero excess) and identity link function were used as a variable response to evaluate the breaching frequency.

The independent variables considered for the analysis were 8 d SST, monthly precipitation, percentage of moon illumination, wind direction, wind velocity, number of hours of daily effort, day of year, and year. Since the day of the year and 8 d SST variables are cyclical, these variables were adjusted as cyclical penalized cubic regression splines. To avoid multicollinearity, correlation analysis of the explanatory variables was performed (Zuur et al. 2009). When variables with high correlations were identified ( $r > 0.7$ ), the variable with the greatest ecological importance or easiest acquisition was maintained in the model. The models were performed in R (R Core Team 2020) version 3.6.1, using the 'mgcv' package. Estimation of additive functions was performed by the method of maximum restricted likelihood (REML), to reduce bias and balance model adjustment (Wood 2017).

We evaluated the concurvity of independent variables from the global models (including all inde-

pendent variables) with the function `concurvity`. Concurvity is the term used for the existence of non-linear dependencies between predictor variables that, when present, can lead to biased estimates of parameters and underestimation of standard errors (Amodio et al. 2014). This analysis results in an index bounded between 0 and 1, where a higher index correlates with higher concurvity. As performed with the correlations, when variables with high concurvity were identified ( $r > 0.7$ ), the variable with the greatest ecological importance or easiest acquisition was maintained in the model.

The best potential fitting models were evaluated from the global model using the dredge function of 'MuMIn' package (Barton 2017), which evaluates models with all possible combinations of variables using their respective corrected Akaike's information criterion ( $AIC_c$ ) and the AIC delta value ( $\Delta AIC$ ). We considered models with  $\Delta AIC$  less than 2 (Burnham & Anderson 2004) as potential best models. When more than 1 model was considered potentially best, the model with the lowest  $AIC_c$ , the highest explained deviance (Zuur et al. 2009), and the best fit in the standardized residual (Yee 2015) was selected as the best model.

For comparison of statistical differences between the tidal states, years, coastal distance, and depth of the occurrences, the Kruskal-Wallis non-parametric test and Wilcoxon rank-sum tests were used for differences between the groups (McKight & Najab 2010). Values were considered statistically significant when  $p < 0.05$ .

### 3. RESULTS

A total of 1002 h of field observation was performed in which, a total of 387 breaching events were recorded on 65 of the 129 observation days between 2011 and 2015, averaging 0.38 breaches per hour in the field (Table 2). Between June 2012 and

Table 2. Summary of data describing the effort of observations of manta ray breaching behavior in the Paranaguá Estuarine Complex

Field period	Field hours	Breaches (n)	Breaches per hour	Occurrence period	Peak of breaches
December 2011 to December 2012	337	99	0.29	January to May 2012	March
January to December 2013	261	147	0.56	January to May 2013	February
January to December 2014	227	64	0.28	January to April 2014	January
January to July 2015	177	77	0.43	January to June 2015	March and April
Total	1002	387	0.38 <sup>a</sup>		

<sup>a</sup>Total number of breaches / total hours

June 2015 observations were performed only at P1, the point with a greater number of observations in 2012 ( $n = 65$ ). There were 174 single breaching events (45%), 204 double sequential breaches (53%), and 9 triple sequential breaches (2%). In total, 385 breaches (99.5%) were 'backward' and only 2 breaches (0.5%) were 'ventral' (Fig. 2). On 15 occasions, more than 1 individual of the same or different sizes were observed breaching together at the same time.

The white marks on the dorsal side of the specimens were intense in 90% of the 125 breaches where it was possible to see the marks. When it was possible to clearly see the region of the mouth and cephalic fin areas (Fig. 2), those areas were white on most occasions ( $n = 34$ ); however, black cephalic fins were also observed twice. Based on the white coloration of the mouth and cephalic fins, the white triangular shoulder patterns, and the 'V'-shaped white shoulder patches observed in some individuals, it is possible that the observations were of *Mobula cf. birostris* (Fig. 2).



Fig. 2. A manta ray breaching in the Paranaguá Estuary Complex. Image credit: Andrielli Maryan Medeiros

Manta rays were observed breaching seasonally in the estuary during the austral summer and early autumn, when the highest monthly SST and precipitation occur (Fig. 3). The months of occurrence and the peak in the monthly number of breaches per field hour varied over the years, occurring in March in 2012, in February in 2013, in January in 2014, and in March and April in 2015. During late austral autumn, winter, and spring months, when the SST and precipitation go down, no breaching event was detected.

The 8 d SST presented a high inverse correlation with day of year ( $r_s = -0.75$ ,  $p < 0.01$ ) and monthly precipitation ( $r_s = 0.74$ ,  $p < 0.01$ ), which also showed high concavity ( $>0.7$ ). Tidal range was dropped due to high concavity with percentage of moon illumination ( $>0.8$ ) (Fig. 4). Monthly precipitation, day of the year, and tidal range were removed from the models and 8 d SST was maintained due to its ecological importance, while percentage of moon illumination was maintained due to the ease of data acquisition.

For the selection of the best model for the daily presence and absence of breaching events, 64 models were formulated (Table S1 in the Supplement at [www.int-res.com/articles/suppl/m674p203\\_supp.pdf](http://www.int-res.com/articles/suppl/m674p203_supp.pdf)). Fourteen potential models were selected ( $\Delta AIC < 2$ ), but 2 models demonstrated the best adjustments, with the lowest  $AIC_c$  (182.1) and  $\Delta AIC$  (0.00), and the same df (7) and level of deviance explained (19.5%). The first model used 8 d SST ( $p < 0.01$ ) and daily effort ( $p < 0.05$ ) as explanatory variables and the second model used 8 d SST ( $p < 0.01$ ), daily effort ( $p < 0.05$ ), and wind velocity ( $p > 0.05$ ).

The first model was selected as the best model, because even with the removal of the variable wind speed in the second model, the  $AIC_c$ ,  $\Delta AIC$ , and level of deviance explained did not change, demonstrating that this variable was not important for the model, since it was not significant. The selected model

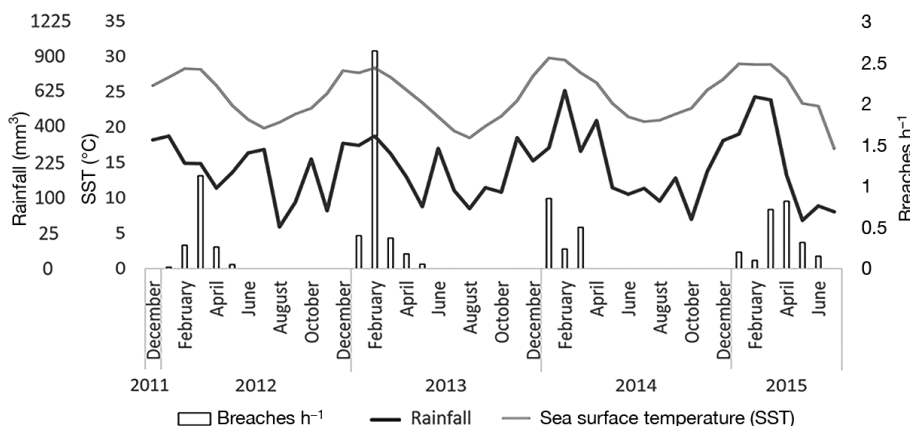


Fig. 3. Number of manta ray breaching events by hour of effort by month, monthly sea surface temperature variation, and monthly precipitation variation in the Paranaguá Estuary Complex between 2011 and 2015

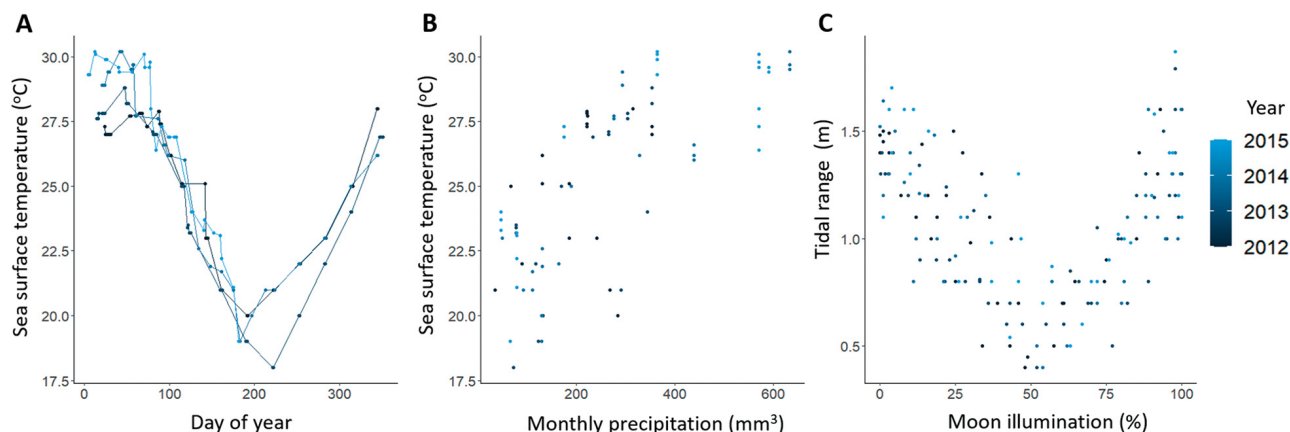


Fig. 4. Correlation between (A) sea surface temperature (SST) and day of year, (B) SST and monthly precipitation, and (C) tidal range and moon illumination between 2011 and 2015

showed that the highest probability of observing breaching behavior in the PEC occurred between 26 and 30°C SST, peaking at 29°C and decreasing toward 30°C, while increasing linearly with more hours of daily effort (Fig. 5A).

For the selection of the best model correlating the daily number of breaches, 64 models were formulated (Table S2 in the Supplement). The best model included all explanatory variables (8 d SST, percentage of moon illumination, wind direction, wind velocity, effort, and year), which was the only 1 with  $\Delta AIC < 2$ . The selected model obtained an  $AIC_c$  of 551.3,  $\Delta AIC$  of 0.00, and df of 28, and explained 61 % of the deviance. All variables showed high significance ( $p < 0.001$ ).

The number of breaches increased with increasing SST, peaking near 28.5°C, and decreased towards higher temperatures. It also increased during north-easterly wind (45°), peaked in easterly wind (80°), then decreased and showed 2 smaller peaks in the south (180°) and north-northwest quadrants (340°). The number of breaches also increased when the percentage of moon illumination was between 40 and 70 %, with a peak near 60 %, and at wind speeds between 2 and 5 m s<sup>-1</sup>, with a peak near 4 m s<sup>-1</sup>. These numbers were higher in 2013 and the breaching frequency increased linearly with increasing in hours of daily effort (Fig. 5B).

Spatially, in general, breaching events were observed between 20 and 3500 m distance from the observer, mainly less than 1500 m (92 %) with a higher concentration between 100 and 500 m (69 %), and between 4 and 20 m depth, mainly less than 10 m (69.5 %) with another peak at 14 m (24 %) (Fig. 6). Even from a long distance, the breaches could be observed due to the large size of the animals, the

black and white contrast, and the large ‘splashes’ generated by the impact on the water. In 2012, more breaches were documented in depths less than 4 m (40.4 %), in 2013 and 2015, the breaches were concentrated in depths less than 10 m (approximately 52 % in both years), and in 2014, they were concentrated in depths less than 6 m (64.1 %) (Fig. 7).

The mean ( $\pm$ SD) coastal distance varied between the years, with shorter average distances in 2012 ( $641 \pm 738$  m) and 2014 ( $537 \pm 467$  m) than in 2013 ( $895 \pm 630$  m) and 2015 ( $779 \pm 547$  m), showing significant differences (Kruskal-Wallis,  $\chi^2 = 33.664$ , df = 3,  $p < 0.001$ ; Wilcoxon rank sum test,  $p < 0.05$  between 2012–2013, 2012–2015, 2013–2014, and 2014–2015). Following the distance from the coastline, the mean depth varied between the years with shallower depths in 2012 ( $7.7 \pm 4.2$  m) and 2014 ( $7.7 \pm 3.8$  m) than in 2013 ( $9.2 \pm 4.0$  m) and 2015 ( $9.0 \pm 3.9$  m), showing significant differences (Kruskal-Wallis,  $\chi^2 = 15.139$ , df = 3,  $p < 0.01$ ; Wilcoxon rank sum test,  $p < 0.05$  between 2012–2013, 2012–2015, 2013–2014, and 2014–2015).

The pattern of breaches during tides were similar in all years with more breaches during the ebb tide, except in 2012 when similar numbers were observed during ebb and flood tide (Fig. 8). More breaching events were observed near the coast (less than 1000 m) in ebb (82.2 %,  $691 \pm 568$  m) and flood (81.2 %,  $678 \pm 533$  m) than in high (74.6 %,  $823 \pm 618$  m) and low tides (65.6 %,  $1158 \pm 1062$  m) (Fig. 9). However, these differences were not significant (Kruskal-Wallis,  $\chi^2 = 7.0864$ , df = 3,  $p = 0.06$ ). There were no significant differences between depth and tide, because in all years the occurrences were concentrated in depths of up to 10 m with another concentration at 14 m in all tidal states and at a mean depth of 8 m (Kruskal-

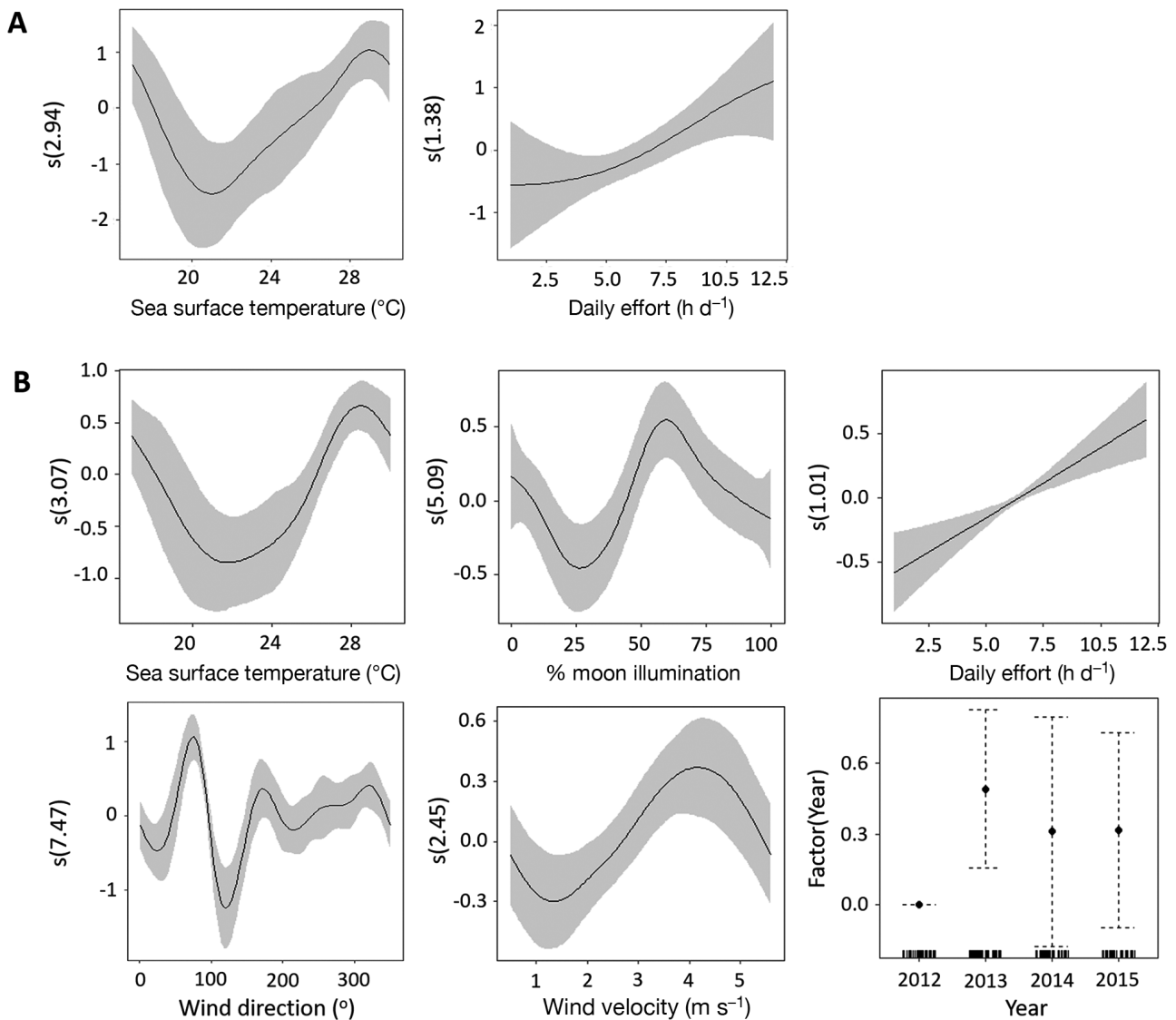


Fig. 5. Effects of the variables selected by the generalized additive model that analyze the influence of environmental variables on the (A) daily breaching frequency and (B) presence and absence of manta ray breaching events in the Paranaguá Estuary Complex. For numeric variables, the smooth functions (s) with their estimated degrees of freedom in parentheses are shown on the y-axis. Tick marks on the x-axis are observed points and shading indicates the 95% confidence interval

Wallis,  $\chi^2 = 0.46414$ ,  $df = 3$ ,  $p = 0.9267$ ). Details about the distance and depths of the observed breaching events are presented in Table S3 in the Supplement.

#### 4. DISCUSSION

This study is the first to describe the influence of spatial, temporal, and environmental factors on the breaching behavior of manta rays in an estuarine area. The GAMs revealed that the presence of breaching events and their frequency both increased with increasing SST and hours of daily effort, while the

breaching frequency also increased in northeast and east wind directions, at wind speeds near 4 m s<sup>-1</sup>, at a percentage of moon illumination near 60%, and in 2013. Spatially, the breaches were concentrated near the mouth of a river in depths less than 14 m.

Based on the general coloration pattern, it is possible that the observations were of a possible third species, previously referred to as *Mobula cf. birostris* (Fig. 2) (Marshall et al. 2009, Hinojosa-Alvarez et al. 2016, Hosegood et al. 2020, Pate & Marshall 2020). Additional images of individuals in the PEC with white mouths and cephalic fins were presented in a previous study by Medeiros et al. (2015). Misidentifi-



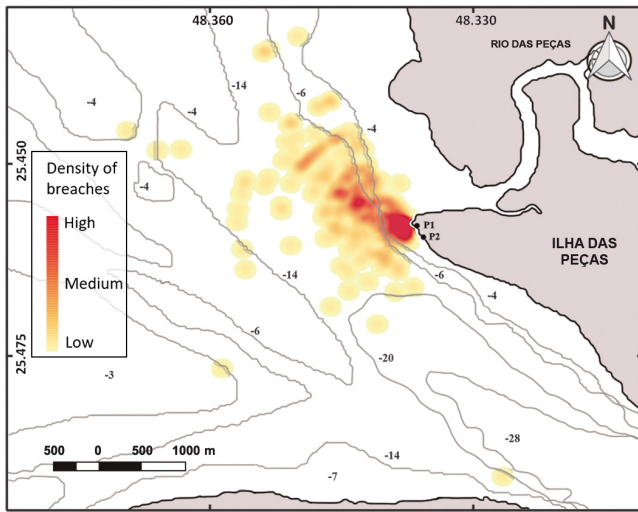


Fig. 6. Kernel density estimation of manta ray breaching events observed in the Paranaguá Estuary Complex over the total study period (2012 to 2015). P1: observation point 1; P2: observation point 2

cations are possible since manta rays maintain some level of overlap in their phenotypic descriptions (Hinojosa-Alvarez et al. 2016), and their coloration might not be stable over time, as they have been described to change their coloration within minutes during different behaviors (Ari 2014) and within months during their development (Ari 2015). Therefore, morphological and molecular analyses might be needed to identify the species.

#### 4.1. Environmental drivers of breaching events

Manta rays were observed breaching seasonally in the PEC during austral summer and early autumn, mainly between January and May, when the SST and, consequently, precipitation increase. The high correlation of day of the year and precipitation with SST suggests that this variation is linked mainly to the SST variation. Under these conditions, the daily

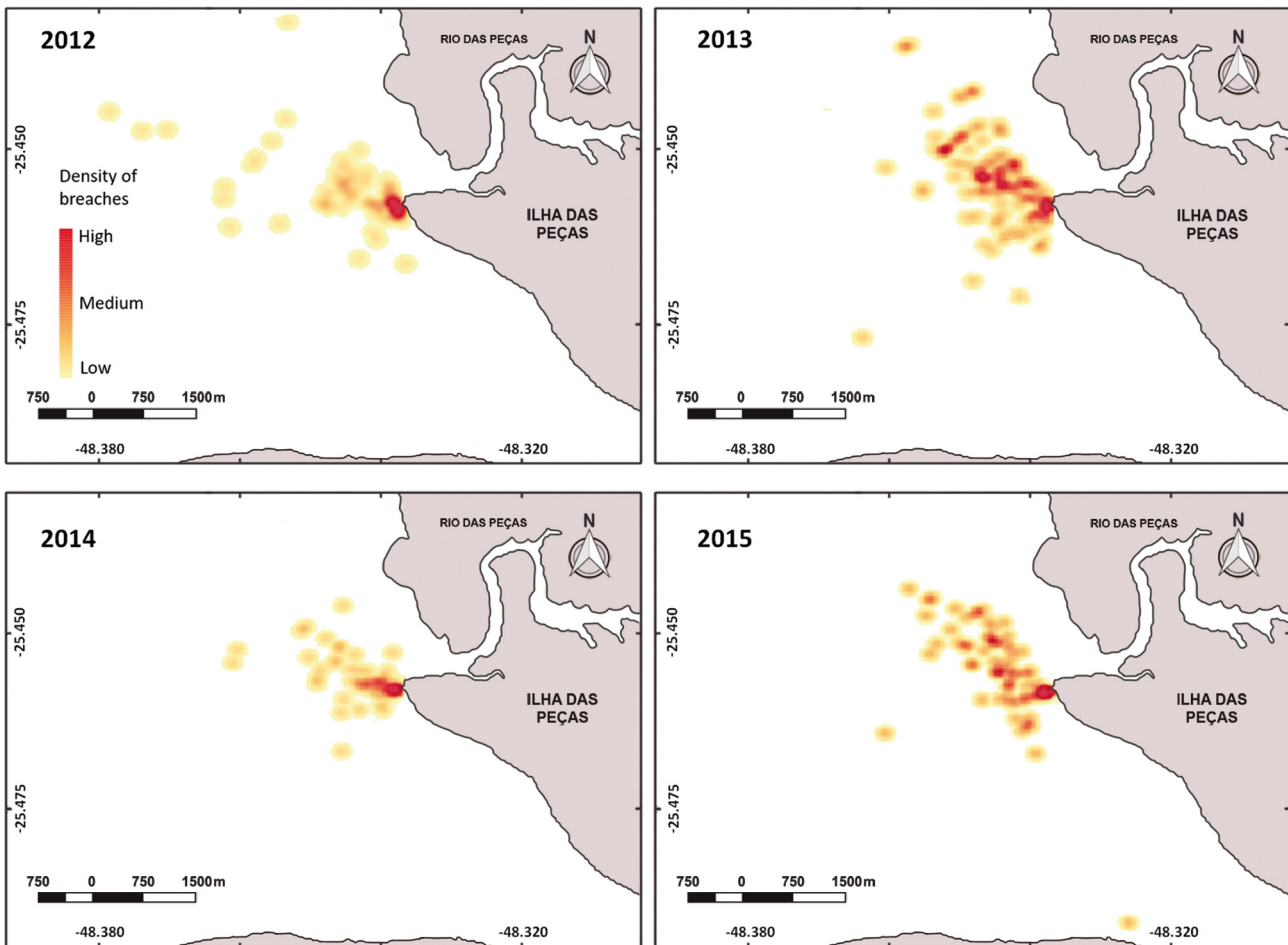


Fig. 7. Kernel density estimation of manta ray breaching events observed in the Paranaguá Estuary during the years of the study

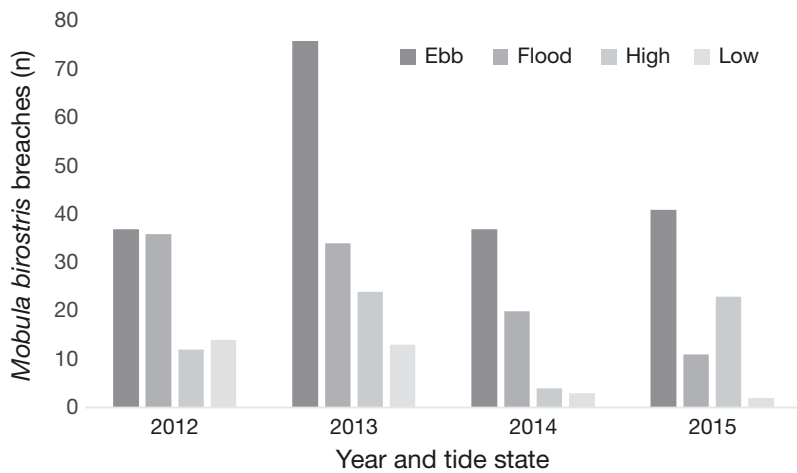


Fig. 8. Annual manta ray breaching event frequency in different tidal states

effort and SST influenced the presence of breaching events. With increasing numbers of hours of effort per day and increasing SST, the probability of observing the species in the estuary was higher, while it

decreased at temperatures greater than 29°C. A similar pattern was observed for *M. birostris* in Indonesia (Beale et al. 2019). SST seems to be an important driver influencing the presence in estuaries of other elasmobranch species that also prefer warm waters (Plumlee et al. 2018, Swift & Portnoy 2021).

It remains unknown whether the manta rays remain in the area without performing breaches or migrate elsewhere during the winter and spring months when SST is lower. However, no breaching event was detected during late autumn, winter, or spring months, suggesting the absence of the

species in the area or lack of breaching behavior during that period. Reports from fishermen support the hypothesis that the occurrence of manta rays inside the estuary is seasonal (Medeiros et al. 2015). Migra-

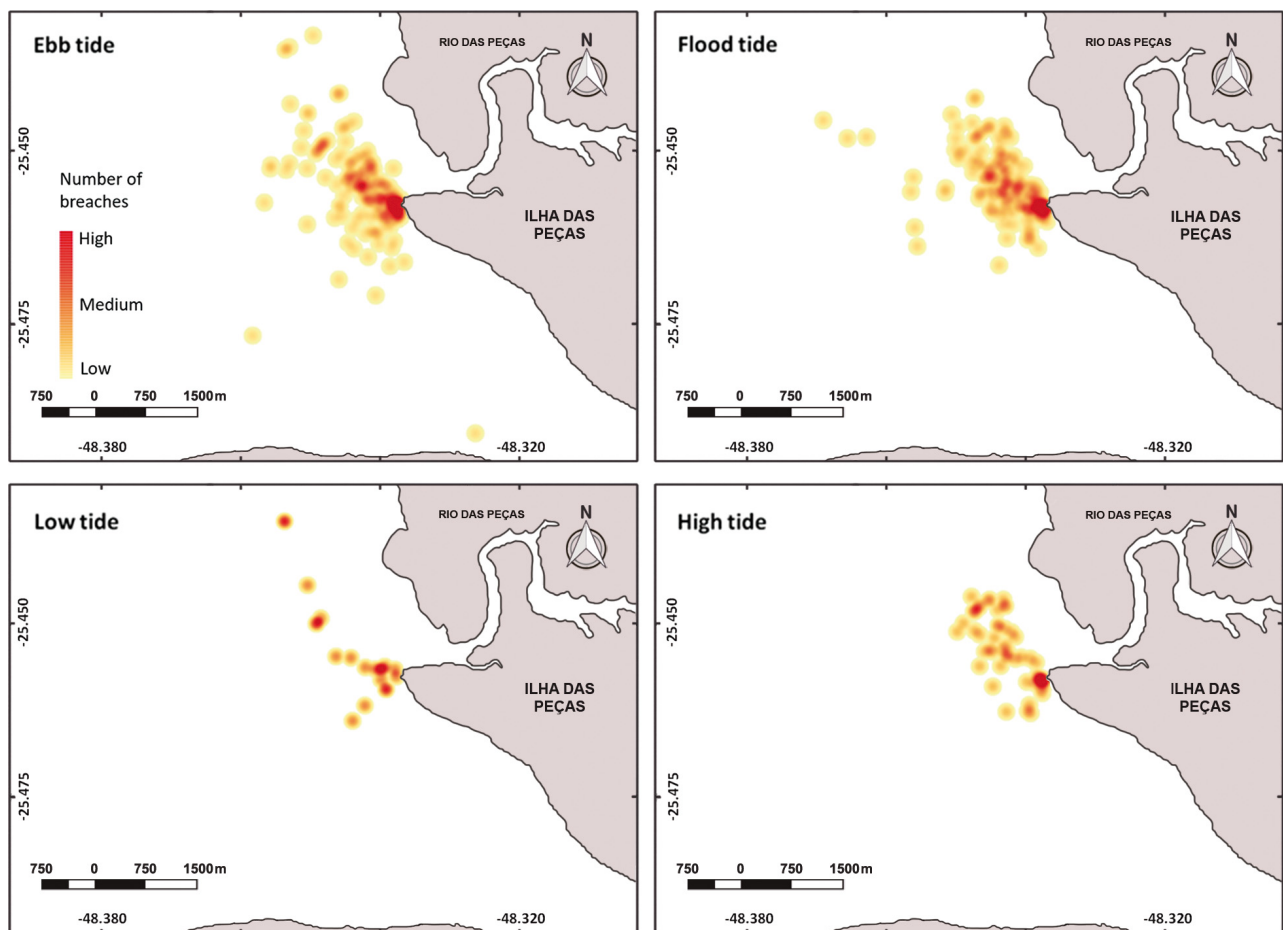


Fig. 9. Kernel density estimation of manta ray breaching events in the Paranaguá Estuary Complex during ebb, flood, high, and low tide states

tory behavior is common for manta rays that demonstrate seasonal aggregation and habitat fidelity, undertaking short- and long-distance migrations, visiting specific sites at specific times of the year to reproduce, to feed, or to be cleaned (Marshall et al. 2009, Kashiwagi et al. 2011, Jaine et al. 2014, Stewart et al. 2016a).

Manta rays are ectothermic and do not have the capability to accumulate fat; therefore, they need to constantly feed to maintain their large body mass (Alexander 1996). This requires them to migrate to areas with abundant zooplankton resources (Acuña-Marrero et al. 2014, Armstrong et al. 2016), whether these are cooler productive areas like oceanic islands, underwater mountains, and coastal upwellings (Marshall et al. 2009, Beale et al. 2019, Putra & Mustika 2020), or warm productive areas like coastal areas (Freedman & Sen Roy 2012, Graham et al. 2012, Girondot et al. 2015, Hacohen-Domené et al. 2017, Pate & Marshall 2020) and estuarine habitats, as documented in this study.

In the PEC, the SST influences the rainy season and consequently the input and productivity of the river, leading to a seasonal pattern in zooplankton abundance with higher density in the warm and rainy season, the same season that manta rays are seen (Montú & Cordeiro 1988, Lopes et al. 1998, Miyashita et al. 2012, Salvador & Bersano, 2017). A similar correlation can be observed in other estuaries of the world (Lopes 1994, Masundire 1994, Marques et al. 2007, Primo et al. 2009), as well as in coastal areas, such as in French Guiana, where the peak of *M. birostris* presence occurred on the coast at water temperatures between 27 and 29°C, in the season when the flow of the Amazon River is directed towards the region, generating an increase of nutrients, favoring an increase in local productivity (Girondot et al. 2015).

We did not collect data on prey availability during this study, but based on previous studies, high zooplankton density occurs in the PEC during summer (Lopes et al. 1999, Miyashita et al. 2012, Salvador & Bersano 2017). These studies also reported the occurrence of zooplanktonic species in the PEC that are considered prey items for manta rays, such as calanoid and cyclopoid copepods, fish and gastropod larvae, mysid shrimp, and chaetognaths (Armstrong et al. 2016, Stewart et al. 2016b, Rohner et al. 2017). Considering this information, it is possible that manta rays are seasonally migrating to the PEC to use the area for feeding, while Medeiros et al. (2015) suggested that the PEC might be also used for reproduction.

Some elasmobranchs use estuaries for fundamental processes, such as feeding, mating, gestation, and parturition, and as nursery habitat (Martins et al. 2018, Plumlee et al. 2018, Swift & Portnoy 2021). La Paz Bay in Mexico, an area with estuarine influence, has been described to serve as a nursery, mating, and feeding area for mobulids (Notarbartolo-di-Sciara 1988, Palacios et al. 2021) and a possible nursery area of *M. cf. birostris* has been identified in Florida, in a coastal region that is under the influence of several estuaries (Pate & Marshall 2020).

In the PEC, a *M. hypostoma* (the only other species of Mobulidae observed in the region) was collected pregnant (Barleta et al. 1989) and another one was captured pregnant by a local artisanal fisherman (A. M. Medeiros pers. obs.). A pregnant *M. birostris* was also observed in the Cape Lookout estuary, USA (Coles 1916), supporting the hypothesis that the PEC might serve as an area for parturition. It is also a possibility that some of the observed animals were juveniles, as the mouth coloration has been described to change from white to black after months of development, indicating that it may be linked to ontogenetic changes or related to maturation (Ari 2015).

#### 4.2. Environmental drivers of breaching frequency

The daily effort and SST both influenced the daily number of breaches of the manta rays. With increasing numbers of hours of effort per day and increasing SST (peaking at 28.5°C), the probability of observing the species in the estuary was higher. In addition, another 4 factors influenced the daily number of breaches observed: year, percentage of moon illumination, wind direction, and wind speed. There was a variation in the number of breaches observed per hour of effort per year, but no conclusions could be drawn due to the limited study period.

The percentage of moon illumination distinctly affected the behavior of manta rays at different locations, as described in previous studies (Dewar et al. 2008, Rohner et al. 2013, Couturier et al. 2018). In the PEC, more breaches occurred when the percentage of illumination was around 60% (waning and waxing gibbous, close to the first and last quarter phases), when neap tides with lower tidal ranges occur (0.5 m, Fig. 4), and consequently, the tidal force and flow speed are weaker (Noernberg 2001). The flow speed influenced manta ray behavior in the Philippines (Barr & Abelson 2019). According to Couturier et al. (2018), *M. alfredi* were less likely to be present dur-

ing high moon illumination (around full moon) at Lady Elliot Island (LEI), Australia.

Rohner et al. (2013) reported that *M. alfredi* was seen most often during the second quarter of the lunar cycle (~20 to 40 % of the moon illuminated) and observations of *M. birostris* were also more frequent during the first quarter of the lunar cycle (~0 to 25 % of the moon illuminated) in South Africa. These results are different from those of Dewar et al. (2008), who observed increased visitations of *M. alfredi* during the new and full moon in Indonesia, and of Jaine et al. (2012), who most often observed manta rays in LEI cruising at the new moon, at cleaning stations near the full moon, and foraging around the new moon and preceding the full moon, when tidal changes are greatest.

The number of breaches per day increased when wind speed was greater than 3 m s<sup>-1</sup> with a peak around 4.5 m s<sup>-1</sup>, and then decreased at higher wind speeds. It also increased during northeasterly wind, with a peak in easterly wind, and decreased in southeasterly wind with smaller peaks in wind from the south and northwest. The average easterly wind can increase water residence time in the estuary, allowing the planktonic community to grow and thrive successfully (Salvador & Bersano 2017). According to Jaine et al. (2012), *M. alfredi* foraging, cleaning, and cruising around LEI strongly decreased as wind speed increased and during northerly and northwesterly winds.

Couturier et al. (2018) reported that manta rays were less likely to be present when winds exceeded 20 knots, suggesting that this could be linked to decreased visibility, decreased prey concentration through dispersal, and/or increased predation risk, and during westerly wind, which probably creates higher surge and wave activity on the usually sheltered side of the island that may disturb the feeding and cleaning activities of *M. alfredi* in LEI.

The breaches were concentrated in the coastal region in depths less than 14 m, near to the mouth of Rio das Peças, which may indicate an area of higher productivity, as a result of the increase in nutrient-rich waters caused by the increased volume of continental drainage (Marone et al. 2005, 2007, Girondot et al. 2015). The manta rays breached more during ebb tide conditions, probably due to greater freshwater input and possibly consequent productivity (MacKenzie & Leggett 1991). Although a different ecosystem, the tide at the Great Barrier Reef in Australia significantly influences zooplankton biomass, which is higher during ebb tide, consequently influencing the foraging of *M. alfredi* (Armstrong et al. 2016).

The breaches may also present a signaling mechanism for manta rays to aggregate for courtship (Marshall & Bennett 2010, Stevens et al. 2018), feeding, or both (Stevens 2016), similar to the zooplanktivorous basking shark *Cetorhinus maximus* (Sims et al. 2000). On 15 occasions, more than 1 individual was observed breaching together in the PEC and the white markings on the backs of the specimens were intense during most observations when it was possible to see the marks in detail. This suggests that the breaching behavior in the PEC might be a part of social communication during group feeding, intense social interaction, or courtship, or indicate dominance hierarchy (Ari 2014). If the breaching behavior of the species is part of courtship, they may also perform parturition in the region, because parturition can be a stimulus for copulation in elasmobranchs (Carrier et al. 2004), and *M. alfredi* has been observed to mate after giving birth (Marshall & Bennett 2010).

#### 4.3. Study limitations and future directions

Due the difficulties in detecting the presence of manta rays in the area, we focused on documenting and discussing only breaching events during this study. As the visibility is very poor for SCUBA diving, and since the species has never been seen swimming on the water surface, tagging individuals in the region to follow their movements does not seem feasible. As manta rays can only be seen in the PEC during breaching events, we used this parameter as an indicator of the species' presence in the area. However, it is possible that the species was present in the region without performing breaches, and that there were occasions when breaches remained undetected by the observer. Therefore, we remain cautious about making conclusions about the presence of manta rays in the area, and rather focus on the role of factors affecting the occurrence and frequency of breaches. It is possible that the species migrates to nearby locations where the visibility is sufficient to detect the animals underwater, but more studies are needed to identify these locations.

The other limitation of this study was that it was not possible to determine the exact location of the breaches, as these events happen quickly, making it extremely difficult to identify the location using binoculars. Therefore, the reference points were demarcated in order to estimate the locations, which leads to some error. This error was reduced by using the same observer throughout the study. Even from a long distance, the breaches could be observed due to

the species' large size, and black and white contrast, and the large 'splashes' generated by the impact on the water, but even so, the locations of the breaches observed close to the coast probably have greater accuracy than long-distance observations.

Detailed surveys of zooplankton dynamics and abundance where breaches were concentrated in the PEC are needed to facilitate understanding of the ecology of the species, including the influence of the environment on habitat use and behavior. The study area should be expanded to include other regions of the estuary and the coastal region, and possible climatic and anthropologic impacts should also be monitored. The presence of elasmobranchs in estuarine waters has been described to be greatly influenced by salinity fluctuations (Plumlee et al. 2018, Swift & Portnoy 2021); therefore, research into how salinity influences the occurrence of manta rays in the PEC area is necessary.

The species that inhabit estuaries face an array of anthropogenic pressures, such as overfishing, pollution, and habitat destruction (Courrat et al. 2009, Plumlee et al. 2018). Fishing in the internal region of the PEC is artisanal and manta rays are not target species. The fishermen avoid placing nets during the period of occurrence of the species to avoid the risk of losing nets that can be dragged, broken, or tangled up by them (Medeiros et al. 2015). In addition, the capture and commercialization of mobulids are prohibited in Brazil (Governo do Brasil 2013), but even so, accidental catches can occur (Lessa et al. 1999, Cintra et al. 2015).

Our hope is that the results of this study can assist in fisheries management measures in the region and the presented information about the environmental factors that influence the frequency and the range of this behavior needs to be passed on to fishermen so they can avoid accidental bycatch, preventing negative impact on the population of manta rays and to the nets of fishermen in the PEC. Further research is needed to expand the study area to the coastal region, where artisanal and industrial fisheries occur.

The manta rays that occur in the PEC inhabit the surroundings of a conservation unit that prevents the destruction of habitat. Even so, they can suffer from the pollution coming from the city of Paranaguá that is within the estuary and has a high population density and a large port terminal. Despite the distance, the sewage pollution from Paranaguá reaches the region where the manta rays occur in Ilha das Peças (Cunha et al. 2011). High levels of metals, such as As, Cr, Pb, Se, and Zn, have also been found in fishes from the PEC (Trevizani et al. 2019).

Marine debris pollution also occurs in the region (Possatto et al. 2015). The port of Paranaguá imports and exports pellets, an activity in which there may be losses in the loading, transport, and unloading of this material (Pereira, 2014). Pellets and microplastics in general have a negative impact on marine animals, mainly filter feeders such as manta rays and whale sharks (Germanov et al. 2018). It is essential to understand how anthropogenic stressors can affect manta rays in the PEC.

Manta ray watching tourism is increasing in the world (O'Malley et al. 2013). Identifying the best conditions in which breaching events happen will assist in predicting and observing breaches, thereby enabling us to assist ecotourism, the local tourism industry built on sightings of manta ray breaches in the region. This activity could be developed working together with the PEC communities in a planned and conscious way, in order to prevent any potential negative impact.

Our study has identified environmental factors that influence manta ray breaching behavior in the PEC, which are essential for a better understanding of their behavioral ecology and for assisting future studies. Global warming may affect these environmental variables (Hewitt et al. 2016), consequently affecting the occurrence and distribution of manta rays in the PEC, as already observed in Indonesia (Beale et al. 2019). These changes can make seasonal movement less predictable over time, highlighting the importance of continuing to monitor their occurrence and the environmental conditions in the area (Robinson et al. 2009).

## 5. CONCLUSION

We have identified the spatial distribution, as well as environmental and temporal factors linked to manta ray breaching events for the first time in an estuarine habitat in southern Brazil. Data on the spatial ecology and behavior of these animals from this region is sparse. These results will assist future research on the breaching behavior and habitat use of manta rays, and will help to optimize fishery management, as well as contribute to developing sustainable ecotourism. It is possible that (at least some of) the observed animals were *Mobula cf. birostris*, which, if confirmed, will expand our knowledge of this species and their range of occurrence. The present study provides valuable insights into the ecology and behavior of the endangered manta rays to aid the future conservation efforts in the PEC and around the world.



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