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**Amazonian river dolphin density estimates in the
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Miriam Marmontel^{1,*}, Jessica Fernandes de Melo¹, Mariana Paschoalini Frias^{1,2,3}, André Giovanni
de Almeida Coelho¹, Hilda Isabel Chávez-Pérez¹, Heloíse Pavanato Julião^{1,4}

¹ Research Group on Amazonian Aquatic Mammals, Mamirauá Institute for Sustainable
Development, Estrada do Bexiga 2584 69553-225 Tefé AM Brazil *marmontel@mamiraua.org.br

² Instituto Aqualie, Avenida Doutor Paulo Japiassu Coelho 714 – Salas 201 e 202 36033-310 Juiz
de Fora MG Brazil

³ Laboratory of Ecological Behavior and Bioacoustics at Federal University of Juiz de Fora, Rua
José Lorengo Kelmer s/n, 36036-900 Juiz de Fora MG Brazil

⁴ Department of Mathematic and Statistics, University of Otago, Dunedin, New Zeland

Introduction

Abundance is one of the paramount parameters to assess, measure and predict impacts on animal population dynamics. National and regional action plans (ICMBio 2019, Trujillo *et al.* 2010, 2014; Utreras *et al.* 2013), the International Union for Conservation of Nature's Red Lists, and the International Whaling Commission (IWC 2018) stimulate the improvement of density, abundance and trend estimates for Amazon endemic *Inia* and *Sotalia*, to subsidize conservation or recovery planning processes. However, population size of either species for the whole area is presently unknown.

Efforts to estimate numbers of South American river dolphins based on visual surveys date back to the middle 1950s, based on encounter rates, and remained so for almost 40 years (Layne 1958, Pilleri and Gihl 1977, Magnusson *et al.* 1980, da Silva *et al.* 1984, Best and da Silva 1989, Herman *et al.* 1996). Following Vidal *et al.*'s (1997) use of a protocol combining line and strip transects to account for river particularities in the region, other studies followed suit, expanding our knowledge on the matter (Aliaga-Rossel 2002, McGuire 2002, Martin and da Silva 2004, Martin *et al.* 2004). The protocol was further developed by Gómez-Salazar *et al.* (2012a) and in the last few years efforts have been further increased and generated a new suite of analyses (Pavanato *et al.* 2016, Williams *et al.* 2016, Mosquera-Guerra *et al.* 2020, Paschoalini 2019, Pavanato *et al.* 2019, Paschoalini *et al.* 2020).

Amazonian river dolphins distribution overlaps with that of humans in the most productive areas, and a number of threats have been listed as a consequence of such proximity (*i.e.*, incidental and directed mortality, increased boat traffic, noise, plastic, oil, heavy metal and chemical pollution, habitat degradation, dams, food resources reduction, and climate change (Estupiñan *et al.* 2003, Loch *et al.* 2009, Trujillo *et al.* 2010, Gómez-Salazar *et al.* 2012, Smith and Reeves 2012). This is only partially the case with dolphins at Mamirauá Sustainable Development Reserve (MSDR). Being distributed in one of the most protected floodplain areas, which has been recognized as providing protection and shelter to river dolphin populations (Mintzer *et al.* 2013, 2015), current threats are likely more restricted to human-related mortality than any of the other factors.

Until 2011 *Inia geoffrensis* was considered data deficient (DD) due to lack of enough information to enable risk assessment. Da Silva *et al.* (2011) suggested a 10% annual population reduction in a portion of the Mamirauá Reserve and by 2018 *I. geoffrensis* was upgraded to IUCN's Endangered category (da Silva *et al.* 2018), with a suspected reduction of at least 50% in its total population within 75 years (as of 2000). The decline was mostly attributed to the use of dolphins as bait in the piracatinga fishery, identified in the area in the early 2000s (Estupiñan *et al.* 2003, Silveira and Viana 2003). Starting in January 2015, the Brazilian government established a 5-year ban on

fishing and commercialization of piracatinga in order to halt the illegal hunt while generating information about the dolphin population (Franco *et al.* 2016).

Since 1993 the Mamirauá Institute has been collecting data on dolphin mortality (including associated with piracatinga), and more recently conducting boat, canoe and drone surveys, both in and around MS DR and other river systems as well, under a regional effort named SARDI (South American River Dolphin Initiative). This study was originally designed to monitor areas of graded effect of piracatinga fishery in order to evaluate its possible effects on dolphin populations along the Mamirauá and Amanã reserve borders. Here we present preliminary results for three years of monitoring.

Material and methods

Study area

The study was conducted in the 120,000 ha southeastern most portion of the MS DR. The MS DR has a total area of 1,124,000 ha and is located in the Central Amazon surrounded by Solimões and Japurá rivers (south and east limit: 03°09'35" S 64°47'37" W; north limit: 01°50'05" S 65°42'19" W; west limit: 02°32'50" S 67°22'08" W). It is inserted in an alluvial dense rain forest ecosystem (floodplain) with humid equatorial climate with monthly thermal amplitude of 8 - 10° C. Seasons are defined according to the water cycle, based on dry, flood, rising and receding regimes. Water level variation can reach vertically 10.6 m and hundreds of meters in the horizontal plan (Plano de Gestão MS DI, 2010). The flood and dry peaks occur in June and between October and November, respectively, and transitional periods of rising from December to May and falling waters from July to September (Ramalho *et al.* 2009).

The monitoring of river dolphins occurred in three key sub-regions of the Mamirauá Reserve: (1) Horizonte, (2) Ponto X, (3) Jarauá (Figure 1). These areas were selected because they concentrated a high effort of piracatinga fishery along the Solimões and Japurá rivers. Two of these sub-regions were known as high and moderate incidence of piracatinga activity (areas 2 and 3) and area 1 represented a 'control area' with lower piracatinga fishing.

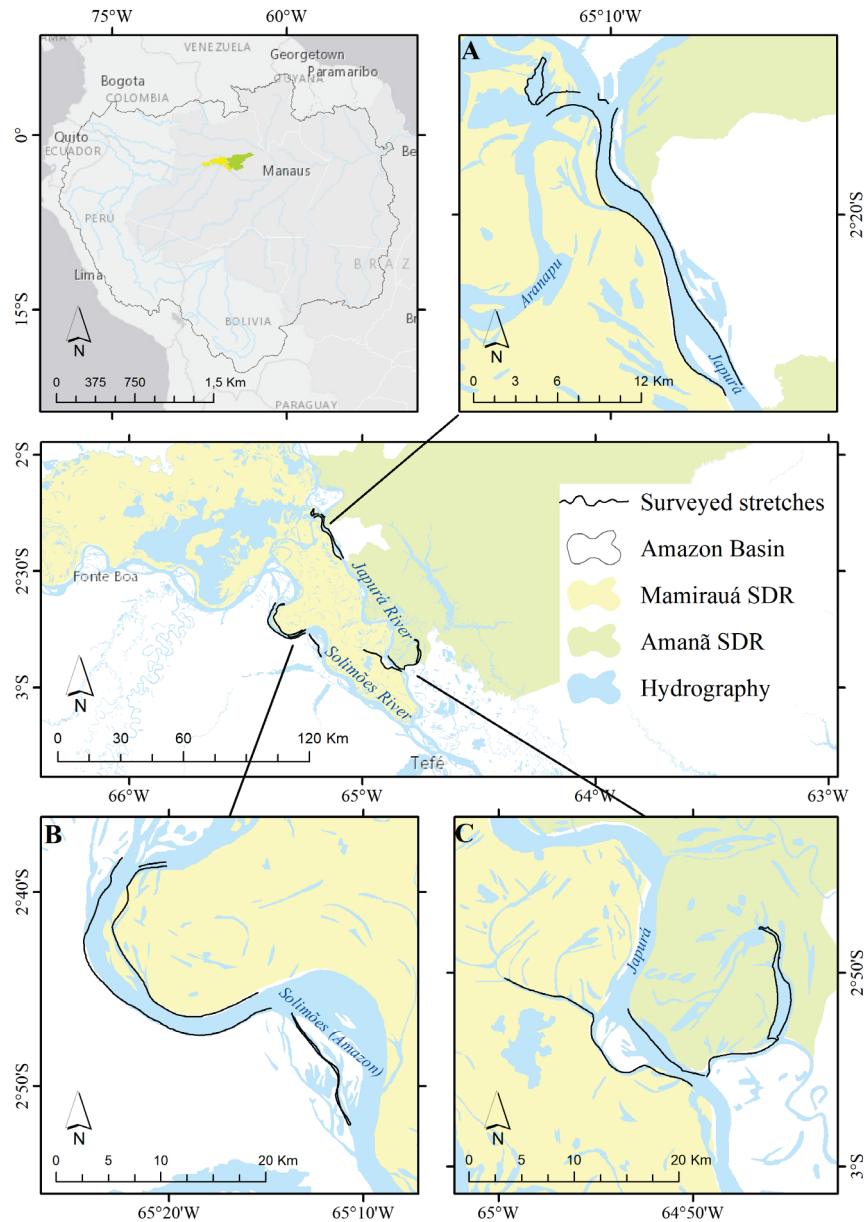


Figure 1. Mamirauá Sustainable Development Reserve and the sub-regions sampled: Ponto X (A), Horizonte (B), and Jarauá (C).

Ponto X is located on the Japurá River ($02^{\circ}26'37''$ S $65^{\circ}04'54''$ W) at the entrance of the Aranapu Channel, and is bounded on the right margin by the MSDR and on the left side by the Amanã Sustainable Development Reserve. This area is composed by a mixture of white and black waters, the latter coming from the marginal lakes and inland creeks. Ponto X habitats comprise the main Japurá River, Jutaí Lake, confluence, channel, and island channel. The Horizonte sub-region is located on the Solimões River ($02^{\circ}45'11''$ S $65^{\circ}15'24''$ W) and its water bodies are typically white water. The sampled habitats in Horizonte comprised the main river, small channels and islands. The third sub-region sampled is Jarauá ($02^{\circ}50'19''$ S $64^{\circ}59'37''$ W), which is predominantly composed by flooded forests throughout the year. In this area, the main Japurá River, channel, island channel, and Pantaleão Lake habitats were sampled.

Data collection

Surveys took place during the rising (January and February) and receding (July and August) water seasons from 2017 to 2019, totalizing six sampling efforts. These dates were chosen to standardize the water transition periods, in which most part of the habitat types are available and dolphins are theoretically randomly distributed (Gómez-Salazar *et al.* 2012).

The sampling protocol followed methodology proposed by Gómez-Salazar *et al.* (2012), surveying strip transects parallel to the river margin at a pre-established distance of 100 m (*i.e.*, a 200 m strip width) using a small boat 6 to 8 m length traveling at an average speed of 10 km/h. The total effort comprised 233 km along the three areas surveyed (Horizonte: 97.8 km; Ponto X: 67 km; Jarauá: 68.2 km). Data collection was conducted using double platform configuration with three observers (two at the bow and one at the stern). The observations of both platforms were assumed to be independent, *i.e.* the observers of the stern platform were unaware of detections made by those on the bow ('one-way' independence), to enable the correction of missed sightings.

Sighting effort was conducted under good environmental conditions, and at each sighting the observers reported species, group size, presence of calves, radial distance between the sighting and the vessel, the radial angle, distances from the dolphin groups to the margin, habitat type (river, confluence, lake, channel or island channel), position of the group through GPS, and presence and type of fishing gear.

Data analysis

Our goal was twofold: i) estimate density by habitat type, season and year averaged across the three sites; ii) estimate an overall density by season and year. Data analyses were performed using the statistical software R (version 3.4.3, R Core Team 2015).

As dolphins are not 100% detected during visual boat surveys, a general detection function was used to estimate the proportion of individuals that were not detected, and, from there, an estimate of the population density was obtained (Buckland *et al.* 2001; Thomas *et al.* 2002). The detection function for Amazonian river dolphins was investigated by Gómez-Salazar *et al.* (2012) through line-transect sampling efforts, and recently improved by Paschoalini (2019). Considering a strip width of 200 m from the shore, the probability detection for groups sighted between 0 and 50 m from the trackline (P_1) at perpendicular distances is the same of 50–100 m and 100–150 m from the shore; and the probability detection for groups sighted between 50 and 100 m from the trackline (P_2) is the same of 0–50 m and 150–200 m from the shore. We used the estimated P_k parameters for *I. geoffrensis* as $P_1 = 0.960$ and $P_2 = 0.630$ (*shape* = 0.37 (SE = 0.12), *scale* = -2.61 (SE = 0.42)) and for *S. fluviatilis* as $P_1 = 0.998$ and $P_2 = 0.893$ (*shape* = 0.99 (SE = 0.15), *scale* = -2.24 (SE = 0.41)) according to Paschoalini (2019).

We used a general probability of detection on the trackline ($g(0)$) for each species as 0.81 (CV = 0.05) for *I. geoffrensis* and 0.99 (CV = 0.006) for *S. fluviatilis*.(updated in Paschoalini (2019) following methods proposed by Gómez-Salazar *et al* (2012)).

Density was estimated by means of stratification for each habitat type (river margin, island channel, channel, confluence, lake) as follows:

$$D_i = \frac{E_i \left[\frac{n_{i\ 0-50}}{P_2} + \frac{n_{i\ 50-100}}{P_1} + \frac{n_{i\ 100-150}}{P_1} + \frac{n_{i\ 150-200}}{P_2} \right]}{W L_i g(0)}$$

where E_i is the estimated group size at habitat type i , L_i is the total transect length at habitat i , and W is the strip width (200 m).

The overall density (D) of both species in the whole study area was calculated as the weighted average obtained by dividing the estimated abundance (sum of the abundance for each habitat type) by the area in squared km. Variances were obtained following Gómez-Salazar *et al.* (2012) methods, and the overall CVs was calculated as follows:

$$CV = \frac{\sqrt{\sum (SE_i^2)}}{\sum D_i}$$

where SE_i is the standard error of the density at habitat i .

The overall density along the years and across seasons was plotted using the *stat_smooth* function of *ggplot2* package (Wickham 2016), assuming the "lm".

Results

We surveyed 1309.37 km of strip transects in the study area of MSDR, 681.04 km of which during the rising water, and 628.33 during the receding water season. The mean number of sightings per expedition during rising waters was 196 ± 76.08 (min = 118, max = 270) and during receding waters was 229.67 ± 10.97 (min = 221, max = 242). The mean of individuals sighted during the rising water was 145.75 (min = 55, max = 96) *I. geoffrensis* and 253.75 (min = 150, max = 334) *S. fluviatilis*. In the receding water period we saw a mean of 194.67 (172 – 209) individuals of *I. geoffrensis* and 288.33 (min = 233, max = 325) *S. fluviatilis* (Table 1).

Table 1. Number of sightings (n) and number of individuals sighted of *Inia geoffrensis* and *Sotalia fluviatilis* by season in the Mamirauá Reserve. Means \pm standard deviation are also represented. Total effort for each expedition: 233 km.

Year	Season	n	<i>Inia geoffrensis</i>	<i>Sotalia fluviatilis</i>
2017	Rising	118	55	150
	Receding	226	203	233
2018	Rising	270	196	334
	Receding	242	209	325
2019	Rising	200	173	264
	Receding	221	172	307
Mean	Rising	196 \pm 76.08	145.75 \pm 76.65	253.75 \pm 92.87
	Receding	229.67 \pm 10.97	194.67 \pm 19.86	288.33 \pm 48.76

According to Tables 2 and 3, confluences and island channels are the preferred habitat types for both species during the rising water. In the receding water, *I. geoffrensis* was recorded more frequently using channels instead of island channels, and *S. fluviatilis* shifted from island channels to main rivers. However, confluences remained as the habitat type where dolphins were more densely found.

The greatest density of *I. geoffrensis* occurred in the receding water season of 2018 (7.95 inds/km², CV = 0.77), while the rising water of 2017 had the lowest density (2.01 inds/km², CV = 0.44, Table 4). For *S. fluviatilis*, the highest density was in rising season of 2018 (14.37 inds/km², CV = 0.78) and the lowest in the receding water period of 2017 (4.62 inds/km², CV = 0.91, Table 4). The mean group size of *I. geoffrensis* varied from 1.06 (CV = 0.95) in the rising period from 2017 to 1.74 (CV = 0.33) in the receding period from 2019. For *S. fluviatilis*, it varied from 1.89 (CV = 0.2) in the rising period from 2017 to 3.12 (CV = 0.23) in the receding period from 2018 (Table 4).

We can notice differences between the rising and receding water seasons, however, there is no evidence of a clear population trend (Figure 2).

Table 2. Effort and density estimates by habitat for *Inia geoffrensis* and *Sotalia fluviatilis* during the rising water from 2017 to 2019 in three key areas of the Mamirauá Reserve.

Year	Habitat	Area (km ²)	L (km)	k	<i>Inia geoffrensis</i>					<i>Sotalia fluviatilis</i>				
					E(r)	E(s)	CVgs	Di	SE	E(r)	E(s)	CVgs	Di	SE
2017	River	23.46	113.9	47	0.38	1.31	0.48	2.09	3.23	0.62	2.32	0.65	3.27	5.6
	Channel	8.16	47.2	20	0.20	1	0	1.86	3.31	0.28	1.5	0.47	1.4	2.7
	Confluence	0.77	3.05	5	1.14	1	NA	6.18	NA	3.50	1.8	0.46	18.31	22.7
	Island C.	4	24.49	11	0.22	1	0	1.28	2.46	0.29	2.25	0.84	4.28	3.45
	Lake	6.59	33.2	14	0.24	1	0	1.89	2.47	0.41	1.6	0.56	3.52	5.57
2018	River	23.46	116.4	47	1.03	1.2	0.34	4.91	4.38	1.03	1.2	0.34	3.97	3.51
	Channel	8.16	69.7	29	0.17	1	NA	1.44	NA	0.24	1.4	0.5	1.82	4.37
	Confluence	0.77	3.05	5	2.67	1	0	18.53	28.49	2.00	5	0.78	41.61	48.67
	Island C.	4	18.5	8	1.42	1.43	0.45	14.26	18.02	1.74	3.86	0.79	17.56	25.03
	Lake	6.59	33.2	14	1.34	1.36	0.41	10.97	11.94	0.67	2.85	0.84	6.88	9.42
2019	River	23.46	116.4	47	0.89	1.44	0.46	4.80	5.44	1.05	3.23	0.64	7.85	7.83
	Channel	8.16	47.2	20	0.35	1.1	0.29	3.06	3.91	0.23	3.22	1.08	3.01	5.51
	Confluence	0.77	3.05	5	0.39	1.5	0.47	10.30	15.18	0.23	4	0.71	14.98	24.49
	Island C.	4	18.5	8	0.72	2.5	0.93	13.58	19.86	0.30	2.6	0.52	2.47	3.3
	Lake	6.59	33.2	14	0.85	1.44	0.72	8.51	11.29	0.25	2.25	0.39	1.99	2.71

L = realized effort; k = number of transects; E(r) = encounter rate (number of groups sighted per km); E(s) = group size; CVgs = coefficient of variation of group size; Di = density in habitat i (number of individuals/km²); SE = standard error

Table 3. Effort and density estimates by habitat for *Inia geoffrensis* and *Sotalia fluviatilis* during the receding water from 2017 to 2019 in three key areas of the Mamirauá Reserve.

Year	Habitat	Area (km ²)	L (km)	k	<i>Inia geoffrensis</i>					<i>Sotalia fluviatilis</i>				
					E(r)	E(s)	CVgs	Di	SE	E(r)	E(s)	CVgs	Di	SE
2017	River	23.46	116.4	47	0.86	1.46	0.43	4.05	4.98	1.01	2.49	0.82	5.71	7.73
	Channel	8.16	42.9	18	1.21	1.39	0.46	13.03	19.51	0.33	3.5	0.65	4.97	10.8
	Confluence	0.77	3.05	5	0.83	1.33	0.43	6.18	11.04	0.77	2	0	11.65	16.21
	Island C.	4	18.5	8	0.29	1	0	1.36	1.30	0	NA	NA	0.00	NA
	Lake	6.59	33.2	14	0.63	1.38	0.63	6.05	6.85	0.17	1.33	0.39	0.76	1.2
2018	River	23.46	116.4	47	1.06	1.54	0.68	6.58	8.75	1.13	3.28	0.82	8.11	9.68
	Channel	8.16	42.9	18	1.02	1.44	0.52	11.13	15.02	0.53	3.93	0.87	6.98	11.77
	Confluence	0.77	2.64	4	1.31	1	0	23.79	38.27	1.17	3.4	0.33	26.92	32.52
	Island C.	4	19.49	9	0.64	1.28	0.59	5.80	4.89	0.17	2	0.5	1.3	2.21
	Lake	6.59	25.6	11	0.96	1.08	0.27	8.34	8.24	0.1	3	0.47	0.00	0.00
2019	River	23.46	107.8	44	0.72	1.38	0.54	4.02	4.47	0.95	2.75	0.74	5.94	7
	Channel	8.16	44.7	19	0.95	1.81	0.39	12.08	17.63	0.8	2.72	0.37	9.42	10.99
	Confluence	0.77	3.05	5	0.71	2.5	0.28	16.47	25.63	1.35	4	0.87	23.07	34.08
	Island C.	4	18.5	8	0.8	2	0.6	10.52	14.74	0.52	3	0.38	3.02	6.28
	Lake	6.59	33.2	14	0.25	1	NA	0.95	NA	0.56	1.5	0.47	5.42	3.22

L = realized effort; k = number of transects; E(r) = encounter rate (number of groups sighted per km); E(s) = group size; CVgs = coefficient of variation of group size; Di = density in habitat i (number of individuals/km²); SE = standard error

Table 4. Group size and total density of *Inia geoffrensis* and *Sotalia fluviatilis* for the rising and receding water seasons from 2017 to 2019 in the Mamirauá Reserve.

Year	Season	<i>Inia geoffrensis</i>				<i>Sotalia fluviatilis</i>			
		E(s)	CVgs	D	CV	E(s)	CVgs	D	CV
2017	Rising	1.06	0.13	2.01	0.44	1.89	0.20	6.16	0.79
	Receding	1.31	0.14	5.85	0.78	2.33	0.39	4.62	0.91
2018	Rising	1.20	0.17	6.29	0.72	2.86	0.57	14.37	0.78
	Receding	1.27	0.18	7.95	0.77	3.12	0.23	8.66	0.83
2019	Rising	1.60	0.33	5.95	0.70	3.06	0.22	6.06	0.88
	Receding	1.74	0.33	5.91	0.79	2.79	0.32	9.37	0.79

E(s) = group size; CVgs = coefficient of variation of group size; D = overall density (number of individuals/km²); CV = coefficient of variation of density

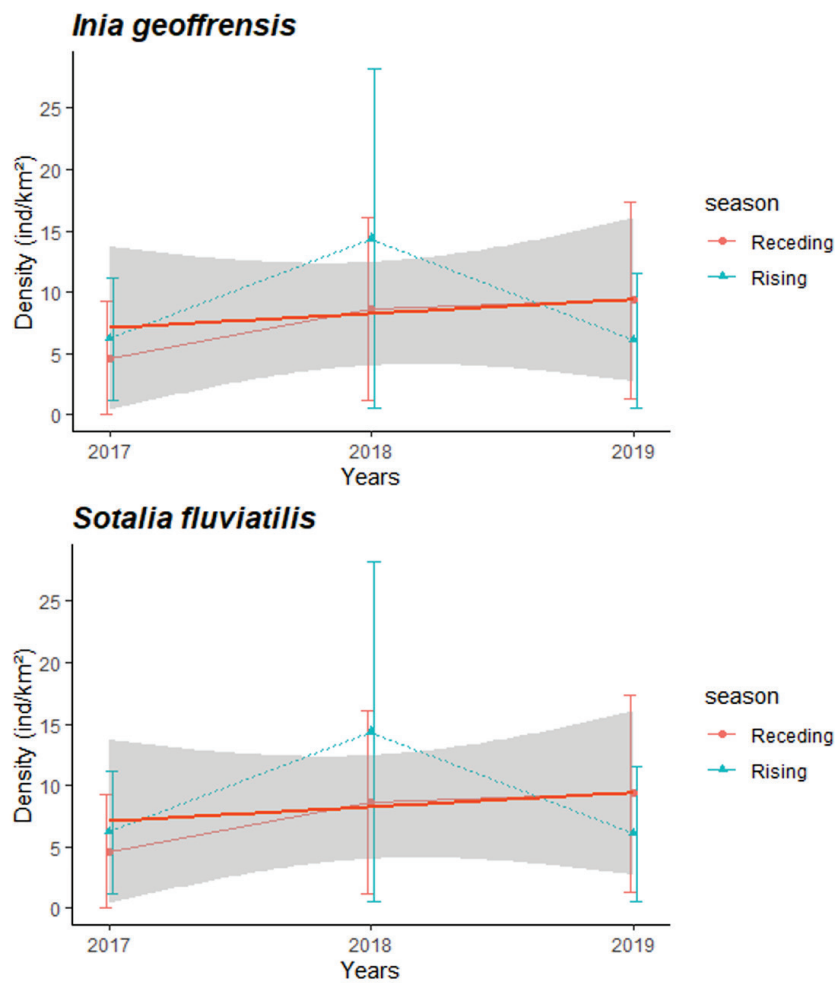


Figure 2. Time series density of *Inia geoffrensis* and *Sotalia fluviatilis* populations in the Mamirauá Reserve from 2017 to 2019 during the receding and rising water seasons.

To get a sense of the fishing pressure in the sampled areas, we recorded all fishing gear during the surveys. According to Table 5, the monofilament nets were the most used gear, followed by other catfish corrals (*i.e.*, a siege or corral), longlines and piracatinga fishing boxes.

Table 5. Fishing gears recorded during all sampling in the study area of Mamiraua Reserve.

	Monofilament nets	Corral	Piracatinga box	Longline
Rising 2017	14	5	-	1
Receding 2017	49	4	1	2
Rising 2018	23	22	1	4
Receding 2018	22	20	-	8
Rising 2019	6	12	3	13
Receding 2019	10	4	-	7
TOTAL	124	67	5	35

Discussion

This is the first effort to assess the density of *I. geoffrensis* and *S. fluviatilis* around the MSDR considering different areas within the reserve. The methodology chosen here is the most used throughout the Amazon to obtain density of river dolphins. The preliminary density results presented here in a three-year-survey suggest that there is no clear population trend for either species.

I. geoffrensis density reflected an increase from 2017 to 2018, and then remained apparently constant, as shown in Figure 2. The 2017 rising water season sample was a pilot expedition to validate the logistical conditions and recognition of the study area, which may have had an effect on the result of lower density of botos. Preference for confluences had already been detected in previous works (McGuire 2002, Martin and da Silva 2004, Martin *et al.* 2004).

The study sites (Horizonte, Ponto X and Jarauá) differ from each other. Horizonte is located on the Solimões River, with great water speed flow and suspended materials. The main river at this region is wide (>1.5 km) and we can note that there is a smaller number of dolphin in this area. Ponto X is located at the meeting of the Solimões and Japurá rivers, with greater fishing pressure, and notably a high incidence of piracatinga fishery prior to the ban; the numbers of dolphins seem to be higher than in Horizonte. Finally, Jarauá is composed mainly of channels and narrower rivers, where we can record an even greater number of dolphins. These specificities among the areas can explain the high CV. Nevertheless, this is a preliminary analysis and a stratified analysis in small areas shall be carried out in the near future to assess the population size of each sampled area, which could improve the CVs and consequently the estimates. In addition, a future analysis aims to compare the three sites in terms of density and fishery pressure. In fact, this highlights the heterogeneity of the

Mamirauá reserve and that assessments in these areas should be carried out taking into account the high variability in this area.

During all surveys, we recorded the fishing gears present in the study areas. Monofilament net was the artifact most used (Table 5), and the most dangerous to the bycatch of dolphins. Corrals for piracatinga catching were recorded in 2017, 2018 and in January 2019, in Horizonte and Ponto X sites. Although small quantities have been recorded, these records occurred only in the areas where transects were performed, so there is a great probability that this practice is still occurring in other areas. This fact is an indication that piracatinga fishing has persisted throughout the period, despite the ban 2015-2019. Other types of fishing traps, such as corrals, were registered in high numbers, especially in Ponto X sub-region. These records are important to identify which areas contain the greatest threats to the dolphins, such as the risks of bycatch and the possible return of the use of boto as bait in piracatinga fishing with the end of the moratorium in January 2020.

A larger-scale monitoring of the dolphins' population using large boats has been conducted since 2014 comprising all hydrological seasons (dry, flood, rising and receding waters). Therefore, our objective is to incorporate previous data in the analyses. Da Silva *et al.* (2018) proposed a population decrease tendency of *I. geoffrensis* and *S. fluviatilis* in the Mamirauá Reserve from 1994 to 2017. Our data do not visually show either a decreasing or increasing trend for *I. geoffrensis*, and suggest a density increment in *S. fluviatilis* (pending further investigation). Our results reflect a period after the peak of piracatinga catches and under the effect of the moratorium, after the study period of da Silva *et al.* (2018). Possibly, the populations are maintaining themselves over time since 2017, and perhaps, the piracatinga moratorium has had a positive impact by preventing more animals from being removed from the population. Notwithstanding, we highlight that results from both studies must be compared with caution since the spatial and temporal scales differ. Additionally, we call attention to the possibility of increasing bycatch in regions with great records of monofilament nets and potential conflicts with fishermen.

Conclusions

Due to the short sampling period, no trend in density estimates are possible at this time. However, the data suggest that neither species is decreasing when looking at the three sub-regions jointly.

It is quite complicated to try to extrapolate these numbers from a small area to a much larger area such as the whole reserve. The large monitoring program of abundance estimates from SARDI have shown that rivers and their complex systems act as drivers of density and abundance depending on their integrity (conservation and threats levels) and productivity, in addition to the hydro-geomorphological aspects (Pavanato *et al.* 2016, Mosquera-Guerra *et al.* 2019, 2020, Paschoalini 2019). In the same river or river system, population parameters may vary largely and the exactified analysis (considering sub-regions) seems to be the best way to understand these numbers (density and

abundance) at local, regional and large-scale (Paschoalini *et al.* 2020). Documenting trends in density or abundance of river dolphins in Amazon, Tocantins-Araguaia and Orinoco river basins is a big challenge, and even though evidences might point to a decline in some specific areas, it merits further effort of investigation along with new approaches other than visual boat surveys (as drones and acoustic monitoring, as being undertaken by SARDI).

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