SC/68C/SM/11

Sub-committees/working group name: SM

Population size diversity and distribution of Amazonian river dolphins (Inia and Sotalia fluviatilis): an extensive abundance estimation across the Amazon and Orinoco river basins

Mariana Paschoalini, Fernando Trujillo, Miriam Marmontel, Federico Mosquera-Guerra

Population size diversity and distribution of Amazonian river dolphins (*Inia* and *Sotalia fluviatilis*): an extensive abundance estimation across the Amazon and Orinoco river basins

Mariana Paschoalini*[1,2,4], Fernando Trujillo[3], Miriam Marmontel[4], Federico Mosquera-Guerra[3,5,6]

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

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

[3] Fundación Omacha, Calle 84 # No. 21-64 Bogotá, D.C, Colombia.[4] Research Group on Amazonian Aquatic Mammals, Mamirauá Institute for Sustainable Development, Estrada do Bexiga 2584 69553-225 Tefé AM Brazil

[5] Departamento de Biología, Laboratorio de Ecología Funcional (LEF), Facultad de Ciencias, Pontificia Universidad Javeriana, Cra. 7 #No. 40 – 62 Bogotá D.C., Colombia.

[6]Departamento de Biología, Grupo de Ecología del Paisaje y Modelación de Ecosistemas (ECOLMOD), Facultad de Ciencias, Universidad Nacional de Colombia, Cra. 45 # No. 26-85 Bogotá D.C., Colombia.

*pf.mariana@aqualie.org.br

Introduction

Freshwater cetaceans such as the Amazon river dolphin (*Inia geoffrensis*) and the tucuxi (*Sotalia fluviatilis*) inhabit complex ecosystems through their distribution range. These two river dolphins species occur in the major tropical river basins of the Amazon, Orinoco and Tocantins-Araguaia, in seven countries (Brazil, Bolivia, Colombia, Ecuador, Guiana, Peru, and Venezuela) (Best & da Silva 1989a, b, Pilleri & Gihr 1997, Rice 1998, Trujillo et al. 2010, Hrbek et al. 2014).

Tropical rivers have broad heterogeneity across a continuum of spatial scales that range from microhabitats to landscapes (Latrubesse et al. 2005). At the local level, small forest and savanna streams often show longitudinal successions of pool and riffle habitats with a variety of substrates, depths, and flow speeds (Godoy et al. 1999). In lowlands of Amazon and Orinoco, floodplains typically present a patchwork of densely vegetated and open-water habitats, which creates very dynamic micro and macro-habitats (Winemiller & Jepsen 1998, Goulding et al. 2003). Additionally, variation in the water level influences the availability of aquatic habitats and the levels of dissolved oxygen, resulting in important seasonal changes in productivity and biodiversity (Goulding 1989). This heterogeneity results in modified distribution patterns of the dolphins' prey and, consequently, the dolphin populations across the complex mosaic created (Martin et al. 2004; Gómez-Salazar et al. 2012b).

Rivers are known to be drivers of biodiversity and play a key role in distribution patterns of aquatic and terrestrial fauna (Ward & Tockner 2001, Naiman et al. 2002). Sampling for information on richness and abundance of species that inhabit these constantly changing and complex ecosystems require careful consideration because of the unique characteristics of these environments and the factors that affect distribution, habitat use, and population parameters (Blasius et al. 1999, Dale & Beyele 2001, Elmqvist et al. 2003).

Trends in distribution and abundance of a species are expected to occur in highly variable ecosystems, which can be better understood if sampling methods consider stratification of the study site to proper address environmental variability (Anganuzzi & Buckland 1993). In the case of river dolphins, methods for estimating density and population size have stratified the river into habitat types, where perceived gradient in dense-specific habitats exist (Martin & da Silva 2004, Gómez-Salazar et al. 2012a). Sometimes, however, variation of habitats along the river course due to natural hydro/geomorphology of the river basin (Sioli 2012, Junk et al. 2015) or by human interference (e.g. dams for irrigation or hydroelectric power production, mining processes, intense fishing exploitation, cattle raising, and climate change) can change riverine landscapes (Gregory 2006, Mosquera-Guerra et al. 2018, 2019a, b, Paschoalini et al. 2020) and cause shifts in the dolphins' distribution patterns.

Given the complex dynamics of the ecosystems inhabited by river dolphins, it is desirable to present estimates that take into account the specificities of each river, considering them as sample units. Therefore, the objective of this paper is to provide new population estimates for river dolphins Amazon river dolphin and tucuxi for different major rivers in the Amazon and Orinoco river basins, as well as discuss the population size diversity and distribution, in light of the complexity of the study areas.

Material and Methods

Study area

Sampling for abundance estimates of river dolphins were conducted over 8,667.81 km of rivers across Amazon and Orinoco river basins encompassing 15 rivers, which reflects in more than 11,000 km^2 of water surface (Fig. 1, Table 1). The Amazon is the largest river in the world in terms of discharge, and the Orinoco the third one (Godoy et al. 1999, Lewis et al. 2000, UNEP 2004). Both river systems have similar unit discharges (discharge/drainage area) and comparable sediment yields (Meade 1994). High run-off occurs from the Guyana Shield Region, which dominates the flow in the Orinoco, and from the Negro River in the Amazon basin (Junk & Furch 1993). The Amazon also receives high discharges from Andean rivers such as the Madeira, Napo, Purus, Putumayo/Iça, Caquetá/Japurá, among others. The Andean mountains contribute 85% to 90% of the sediment yield of both river systems (Martinelli et al. 1989, Meade et al. 1990; Meade

1994). Both the Orinoco and Amazon rivers have important floodplains (Hamilton & Lewis 1990, Sippel et al. 1994), but in terms of drainage areas, the Amazonian floodplains are most extensive.

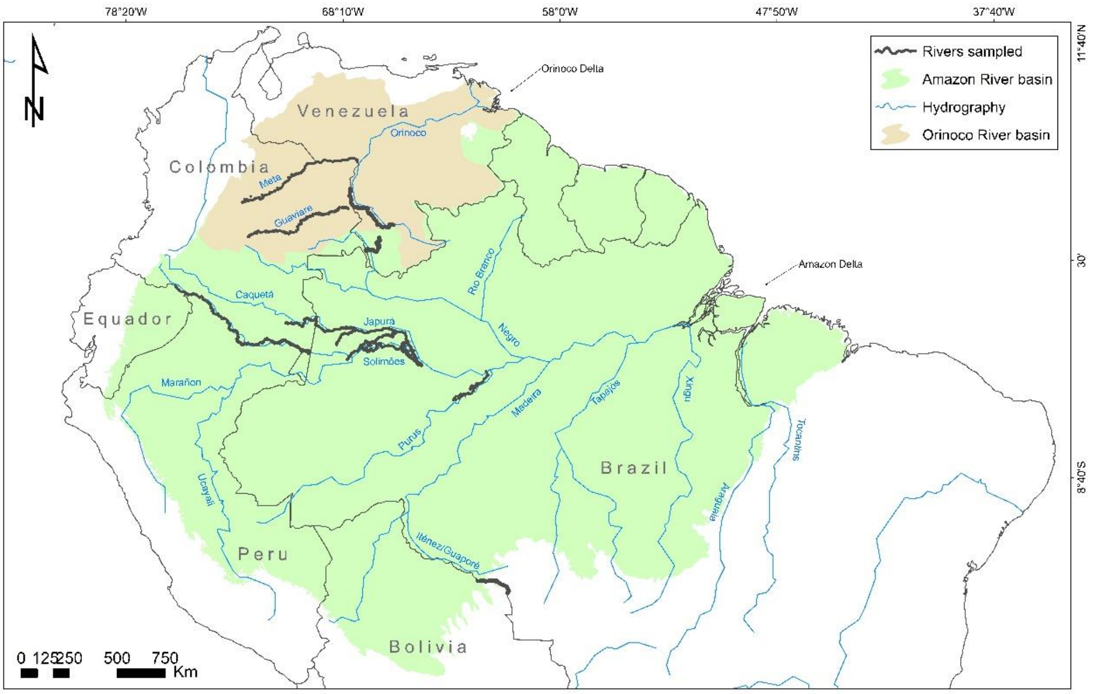

Figure 1. Rivers and stretches of rivers sampled for density and abundance estimates of South American river dolphins through Amazon and Orinoco River basins.

Table 1. Sampling effort and study areas of river dolphins abundance estimation in South America.

River	Water type	River Basin	Site	Country	Year	Effort (km)	Area (km²)
Purus	White	Amazon	Lower Purus River in the Central Amazon	Brazil	2012	512.05	538.72
Meta	White	Orinoco	Last main tributary of left side of Orinoco river	Colombia/Venezuela	2012	584.84	969
Orinoco	White	Orinoco	Cassiquiare channel	Colombia/Venezuela	2013	454	482.06
Japurá (Caquetá) + tributaries (Apaporis, Cahuinari, Miriti)	White (Japurá River), black (tributaries)	Amazon	Central Amazon and Middle Caquetá	Brazil/Colombia	2014	1027.53	1767.67
Auati-Paranã channel	Mixed	Amazon	Central Amazon - MSDR boundary	Brazil	2014	432.8	55.74
Solimões	White	Amazon	Central Amazon - MSDR boundary	Brazil	2014	581.39	1745.39
Juami and Japurá rivers	Black (Juami River), White (Japurá River)	Amazon	Central Amazon - Juami tributary river of the right side of Japurá	Brazil	2015	386.95	254
Guaviare	White	Orinoco	Ecotone river between Amazon Forest and Orinoco Savana	Colombia	2016	968	593.75
MSDR	Predominantly White with mixed water in confluences	Amazon	Central Amazon - RDSM boundary	Brazil	2016	1422.81	2652
Putumayo	White	Amazon	Upper Amazon	Cross-border: Ecuador, Colombia, Peru e Brazil	2017	1186	1108.66
Iténez (Guaporé)	Clear	Amazon	Bolivian Amazon, bordering Brazil	Bolivia	2017	481	40.18
Meta	White	Orinoco	Last main tributary of left side of Orinoco river basin	Colombia/Venezuela	2018	630.44	969

Data collection

Visual boat-based surveys took place during the rising and receding water seasons. These seasons 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), using a combination of transects running parallel (200 m strip-width transect) and cross-channel (line transect) in zigzag pattern at an average speed of 10 km/h (see Gómez-Salazar et al. 2012 for more details).

A double-decker boat (in average 7 m high and 12 m long) was used as observation platform in all surveys. A team of (at least) nine observers searched for river dolphins and rotated every hour between platforms on the bow (Platform 1) and on the stern (Platform 2). On each platform, observers alternated across three positions: port, data recorder and starboard (see Paschoalini et al. 2020 for more details). 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 and the calculation of key values for capture-recapture models to estimate detection probability (g (0)) on the trackline (Thomas et al. 2010).

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 (main river, tributary, confluence, lake, channel, and island – as described in Gómez-Salazar et al. 2012a).

Analysis

Estimates of density and abundance of river dolphins by means of habitat stratification (habitat types as proposed by Gómez-Salazar et al. 2012a) were calculated for all rivers. Data analyses were performed using the open-source statistical software R (version 3.4.3, R Core Team 2015).

Cross-channel (line transects) were analyzed using the packages *Distance* and *MRDS* following distance sampling (DS) methods (Buckland et al. 2001) to fit the detection functions, estimate detection probabilities and density and abundance for the center of the river. This analysis enabled the survey-specific detection function for those rivers (main rivers) where line transects were performed instead of using the general detection function provided in Gómez-Salazar et al. (2012a), when number of sightings were greater than 30 groups (Frias, 2019). Half-normal and hazard rate models were considered as the key functions. Model selection was conducted using Conventional Distance Sampling (CDS) (Bukland et al. 2001) and Multiple Covariate Distance Sampling (MCDS) (Marques & Buckland 2003), starting with simple models and including group size (gs), platform (pt), species (sp) and environmental condition (sightability – sg) one at a time. Model selection was performed using the Akaike's Information Criterion (AIC).

Density was estimated for each species as follows:

$$D_i = \frac{n_i \, E_i \, f(0)}{2L_i \, g(0)}$$

where, n_i is the number of groups sighted in habitat *i* (center of the river), E_i is the estimated mean group size for the population in habitat *i*, *f*(0) is the sighting probability density at zero perpendicular distance (or the inverse of the effective half strip width [ESW]), L_i is the total transect length in habitat *i*, and *g*(0) the probability of seeing a group on the transect line. Sightings collected independently by the bow and stern platforms were used to estimate the survey-specific detection probability on the trackline as: $g(0) = (1 - q^2)$ (see Gómez-Salazar et al. 2012a for more details, and improved in Frias 2019).

Parallel (strip) transects (200-m strip width) were analyzed considering the habitat stratification in field (main river, tributary, channel, confluence, lake, and island) for each river, following methods by Gómez-Salazar et al. (2012a) and improved in Frias (2019) to estimate density for each species as follows:

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

where, D_i is the estimated density in the habitat type *i*, E_i is the estimated group size for the population in habitat type *i*, L_i is the total length of the parallel transects conducted in that habitat *i*, and *W* is the strip width (200 m), g(0) estimated in line transect analysis and P_k (P_1 and P_2) the correction for undetected clusters of dolphins at each of the 50 m of the strip width regarding distance from the trackline (investigated by Gómez-Salazar and recently improved; for more details see Frias 2019).

The overall density (*D*) of both species in the whole study area of each river 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. (2012a) methods, and the overall CV was calculated as follows:

$$\text{CV} = \frac{\sqrt{\sum(SEDi^2)}}{\sum Di}$$

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

Overall abundances in each river for each species were obtained by the sum of estimated abundance ($D_i * A_i$) in each habitat type (*i*) through:

$$N_{overall} = sum(D_i\, A_i + \cdots . D_n A_n)$$

where A_i corresponds to the study area in km² in the habitat type *i*, calculated using satellite images obtained as close as possible to the time of survey execution.

Data reported in this paper focused on overall density and abundance for each river.

Results

The highest density/abundance of both species of river dolphins in South America was estimated from the survey conducted in the lower Purus River, Central Brazilian Amazon, 14.5 ind/km² (CV=0.37) and 17.14 ind/km² (CV=0.49) for Amazon river dolphin and tucuxi, respectively (Table 2). Auati-Paranã channel (Central Brazilian Amazon) and Iténez (Guaporé) River (Bolivian Amazon) were the second densest areas, with 5-6 ind/km². From Putumayo (Upper Amazon basin), Guaviare (ecotone between Amazon rain forest and Orinoco savannas), Japurá rivers, and surroundings of Mamirauá Reserve (Central Brazilian Amazon), densities varied from 2 ind/km² to 3.5 ind/km² for both species, except for Putumayo River where density of tucuxis was the lowest (0.49 ind/km², CV = 0.95). Rivers as Orinoco and its tributary Meta, presented the lowest density of Amazon River dolphins (Table 2). While optimal abundance estimate results require small CV values (< 0.4), high variation was found in some specific surveys, e.g. Cassiquiare channel (Orinoco River), Solimões River and Juami-Japurá rivers surveys (~1.35).

Table 2. Amazon river dolphins (*Inia geoffrensis*) and tucuxi (*Sotalia fluviatilis*) overall estimates of density and abundance across Amazon and Orinoco river basins.

River	River Basin	Year	Effort (Km)	Area (km²)	*Inia*			*Sotalia fluviatilis*		
					D	***N***	**Cv**	***D***	***N***	**Cv**
Purus	Amazon	2012	512.05	538.72	14.5	7672	0.37	17.14	9238	0.49
Meta	Orinoco	2012	584.84	969	1.04	972	0.56	-	-	-
Orinoco – Cassiquiare channel	Orinoco	2013	454	482.06	0.9	435	1.36	-	-	-
Japurá (Caquetá) + tributaries	Amazon	2014	1027.53	1767.67	2.19	3871	0.94	1.79	3164	0.98
Auati-Paranã channel	Amazon	2014	432.8	55.74	5.5	307	0.51	5.8	324	0.55
Solimões	Amazon	2014	581.39	1745.39	1.01	1763	1.34	1.34	2339	1.03
Juami-Japurá rivers	Amazon	2015	386.95	249.17	1.77	440	1.38	2.4	599	1.79
Guaviare	Orinoco	2016	968	593.75	3.28	1138	0.32	-	-	-
MSDR	Amazon	2016	1422.81	2652	3.17	8407	0.74	3.35	8876	0.65
Putumayo	Amazon	2017	1186	1108.66	3.49	3897	0.61	0.49	546	0.95
Iténez (Guaporé)	Amazon	2017	481	40.18	5.07	204	0.88	-	-	-
Meta	Orinoco	2018	630.44	969	1.49	1397	0.95	-	-	-

D = overall density; N = overall abundance; CV = coefficient of variation, (-) species does not occur.

Survey-specific detection probabilities at zero distance from the trackline (*g*(0)) varied from each of the rivers analyzed and by species, with tucuxi presenting higher probabilities of being detected than Amazon river dolphins for all rivers where this species occurs (Table 3). From those rivers where numbers of sightings in line transect were insufficient for proper calculation of g(0) ($n < 30$), or when only strip transects were conducted, a global g(0) was applied as suggested by Gómez-Salazar et al. (2012a) and improved in Frias (2019) for both species of river dolphins (*): Amazon river dolphin g(0) = 0.81 (CV = 0.05) and tucuxi g(0) = 0.98 (CV = 0.02). Japurá (Caquetá) plus tributaries, Auati-Paranã channel and Solimões River were sampled during the same survey, thus a single g(0) was calculated.

Table 3. Estimated survey-specific detection probability of seeing a group of dolphin at zero distance from the trackline (*g*(0)).

<table>
<tr><th rowspan="2">River</th><th rowspan="2">Water type</th><th rowspan="2">River Basin</th><th colspan="2">g(0)</th></tr>
<tr><th>Inia</th><th>Sotalia fluviatilis</th></tr>
<tr><td>Purus</td><td>White</td><td>Amazon</td><td>0.86 (0.09)</td><td>0.99 (0.008)</td></tr>
<tr><td>Meta - 2012</td><td>White</td><td>Orinoco</td><td>0.95 (0.04)</td><td>-</td></tr>
<tr><td>Orinoco - Cassiquiare channel</td><td>White</td><td>Orinoco</td><td>0.81 (0.05)*</td><td>-</td></tr>
<tr><td>Japurá (Caquetá) + tributaries (Apaporis, Cahuinari, Miriti)</td><td>White (Japurá River), black (tributaries)</td><td>Amazon</td><td rowspan="3">0.69 (0.04)</td><td rowspan="3">0.83 (0.02)</td></tr>
<tr><td>Auati-Paranã channel</td><td>Mixed</td><td>Amazon</td></tr>
<tr><td>Solimões</td><td>White</td><td>Amazon</td></tr>
<tr><td>Juami and Japurá rivers</td><td>Black (Juami River), White (Japurá River)</td><td>Amazon</td><td>0.81 (0.05)*</td><td>0.98 (0.006)*</td></tr>
<tr><td>Guaviare</td><td>White</td><td>Orinoco</td><td>0.71 (0.53)</td><td>-</td></tr>
<tr><td>MSDR</td><td>Predominantly White with mixed water in confluences</td><td>Amazon</td><td>0.79 (0.08)</td><td>0.98 (0.01)</td></tr>
<tr><td>Putumayo</td><td>White</td><td>Amazon</td><td>0.56 (0.08)</td><td>0.59 (0.13)</td></tr>
<tr><td>Iténez (Guaporé)</td><td>Clear</td><td>Amazon</td><td>0.81 (0.05)*</td><td>-</td></tr>
<tr><td>Meta - 2018</td><td>White</td><td>Orinoco</td><td>0.85 (0.11)</td><td>-</td></tr>
</table>

Discussion

Density and Population Size

In this paper, we pointed out clear differences in density and abundance across rivers sampled through the distribution range of South American river dolphins. These differences are believed to be linked to unique features of each basin and the hydro-geomorphological characteristics of each river. They are also likely related to the level of human-induced habitat modification, overfishing, pollution, direct caches and other, which might affect dolphin distribution and possibly abundance.

In the present study, there is a perceived density/population size gradient associated to (1) river basin and water type, (2) drainage position in the river basin, and (3) level and range of human activities directly affecting river dolphins' populations.

In (1) the Amazon River basin has been reported as the densest basin of South American river dolphins (Vidal et al. 1997, Trujillo et al. 2010, Aliaga-Rossel 2002, 2006, Gómez-Salazar et al. 2012a, Pavanato et al. 2016, 2019). In these previous studies, density of river dolphins was in average 2 to 6 ind./km², consistent with data presented in this paper. Most part of the rivers forming the Amazon basin are white-water type, which are rich in suspended sediments, with high primary production and consequently high biodiversity (Goulding et al. 2003, Guyot et al. 2007). The discharge of black water tributaries and marginal lakes also increase the input of organic and chemical components, creating high productive zones known as confluences. The Orinoco basin, although white water, present a lower density when compared to the Amazon. Estimates presented here (0.9 to 1.49 ind./km²) provide further strength to the hypothesis that overall density of the Amazon river dolphin in the Orinoco river basin seems to be smaller than in the Amazon River basin (Gómez-Salazar et al. 2012a). These differences are thought to be associated mainly to watershed features and productivity (Hamilton et al. 1992, Godoy et al. 1999, Trujillo et al. 2000). The Orinoco basin has low nutrient availability, rapid flow of sediments, and sandy composition (Medina & da Silva 1990, Savage & Potter, 1991, Meade 1994). During rising and high water, there is a drastic reduction of phytoplankton biomass possibly due to the high concentration of suspended solid transports during these periods (Chitty 1994). The aquatic fauna, mainly fish assemblages, are distributed from the middle towards the lower river course, where aquatic habitat is more suitable (Lasso et al. 2016). The clear water river basins such as Tocantins-Araguaia and Tapajós, characteristically deprived of nutrients, ions and sediments (Sioli 1984, Junk & Furch 1993), present the lowest densities of river dolphins (Tocantins 0.75 ind./km², Tapajós 0.40 ind./km²) (Pavanato et al. 2016, Paschoalini et al. 2020). In the Tocantins River, it is important to mention that the river is dammed and the ecosystem quite transformed, which may also have resulted in changes on dolphins' habitat use and movements (Paschoalini et al. 2020). Clear water rivers commonly flow from shield formations, and present rapids and falls in upper and middle reaches, not being highly suitable areas for river dolphins, which does not restrict their presence. The exception to that seems to be the Iténez River in the Bolivian Amazon, where Amazon river dolphin's density was 5.07 ind./km². The Itenéz River is surrounded by several oxbow black water lakes and flooded areas, which seems to create a very suitable habitat for Bolivian river dolphin.

In (2), the flow-ecology relationship implies in nonlinear biological responses (population numbers, population dynamics and structure, movements, survival, community structure) in watersheds' physical attributes along their course (temperature, dissolved oxygen, available habitat, depth, altitude, sinuosity) (Rosenfeld 2017). As one moves from the central Brazilian Amazon, the core discharge flow of this river basin (receiving hundreds of tributary rivers from Bolivia, Peru, Ecuador and Colombia) to middle-upper reaches of rivers in Eastern Amazonia, population sizes of river dolphins decrease. This is also true when looking at a single river a unit of biodiversity. Rivers are unique units that play a key role in the estimation of population size given their unique intra-specific features and the preference of dolphins for specific habitats. We can see this "tendency" when looking at data presented in Table 2 and translated in the figure below.

Figure 2. Flow-ecology relationship scheme of density and population size of river dolphins through rivers and river basins. Arrows gradient goes from green (lower density/population size) to red (higher density/population size).

Density estimates in Purus River are the greatest reported (14 Amazon river dolphin/km² and ~17 tucuxi/km²) until now for these species in the literature (Trujillo et al. 2010, Gómez-Salazar et. al 2012a). A small-scale study in Mamirauá Reserve (50 km effort), between the rivers Japurá and Solimões in the Central Amazon, have estimated 18 Amazon river dolphin/km² and a population size of Amazon river dolphin around 13.000 individuals (Martin & da Silva 2004). However, the mentioned study adopted a different methodology to calculate the area covered by the effort and did not use habitat stratification to compute abundance, making comparisons difficult.

Purus River is located in the most central part of the Amazon Basin and is characterized by a meandering aspect and muddy water (Goulding et al. 2003). Surrounded by the Amazon Rainforest, it also presents large-scale hydrologic characteristic and hydrodynamics, which stimulate the flow and renewal of nutrients, fertilizing the ecosystem with each water level variation (de Paiva et al. 2013). In Purus, the river margin as well as the confluences may present similar conditions (e.g., high productivity), which could explain the homogenous distribution of dolphins along the margins (clearly perceived in field). In addition, in this region, prey migration occurs near the river margin (Sioli 1984, Best & da Silva 1989, Trujillo et al. 2010), justifying a more frequent use of this habitat. In such environment of high fish biodiversity, Amazon river dolphin and tucuxi seem to be distributed according to this abundant source (fish).

In (3) the level and range of human activities directly affecting river dolphins' populations can represent an important factor when assessing density and population size. Despite being in the central Brazilian Amazon, the Solimões River and the stretch of Japurá River plus its tributary the Juami River presented lower densities of rivere dolphins (1-1.77 ind./km²), compared to the other Amazonian rivers sampled. These areas were historically reported as dolphins-fishermen conflicts in the "piracatinga" fishery. The piracatinga fishery has been considered one of the main current threats to Amazon river dolphin's populations (Mintzer et. al 2013, 2015, Iriarte & Marmontel 2013a, b, Salinas et al. 2014, Brum et al. 2015, Consentino & Fisher 2016, Pimenta et al. 2018). Declines in dolphins' populations were attributed to this practice, mainly in the lower Solimões and Japurá rivers (da Silva et al. 2018). In the Purus River, a survey conducted in 2017 in the same area covered in this study estimated densities of 9 Amazon river dolphins/km² and 16 tucuxi/km² (CEPAM unpublished data). The estimates presented here and those computed by CEPAM are five years apart, and the surveys reported here were conducted (in 2012) during a period of intensive fishinig for the piracatinga. Because the survey conducted by CEPAM in 2017 followed the same sampling and analytical methods, estimates produced by the two studies are comparable and suggest a decline in the density of Amazon river dolphins (from 14.5 ind/km² in 2012 to 9 ind/km² in 2017) and tucuxi (17 ind/km² in 2012 to 16 ind/km² in 2017). Despite these findings, a longer time series is needed to assess population trends reliably and new surveys in the lower Purus River are recommended to continue monitoring river dolphins' populations in this area. The Purus River should be included at a monitoring program to estimate impacts of the piracatinga moratorium during it extension so as a control area.

Other important threats warrant further research, such as bycatch levels (Campbell et al. 2020), mercury and other contaminants (Mosquera-Guerra et al. 2019a), and habitat modification (e.g. hydroelectric dams, Pavanato et al. 2016, Mosquera-Guerra et al. 2018, Paschoalini et al. 2020), and will therefore expand our knowledge on impacts of dolphins' populations.

Survey-specific g(0) and Variances

The survey-specific g(0) represents an improvement on density estimates precision, since it encompass singularities from each of the samplings, i.e. type of vessel used, observer team, environmental conditions, behavior of the local river dolphins population in the sampled area, as well as river features. Except for the Guaviare River, coefficient of variation for g(0)s were very low (<0.1), highlighting the importance to consider the specificities of each survey and area to compute reliable abundance estimates, whenever possible.

The CVs presented for density/abundance estimates are ended high, however they are pretty much associated to the high variation in encounter rates due to dolphins distribution in specific habitats and river's sub-regions. Post-stratification of survey data likely produced more robust and reliable estimates of abundance for region with high variation in density as in Tocantins River (Paschoalini et al. 2020), reducing CV as much as 70%. This approach should be further employed in some of the rivers that CV were large (e.g. Cassiquiare channel and Solimões River).

Conclusions

Estimating density and population size of river dolphins in South America is challenging. The large extent of distribution range, the lack of information on animal movement and population structure, logistical limitations and the unique and complex environmental features of the Amazon require great financial effort and long periods of data collection. Despite these difficulties, the extensive effort across many river basins in South America to estimate density and abundance of river dolphins was substantially improved.

Data presented in this paper also contributed to better understand dolphins' distribution and concentration along different river basins, sub-basin and intra-river variations. The comprehensive analysis also provides insights on the strong correlation between environmental characteristics and human impacts on dolphins' distribution. This was fundamental to see rivers as unique units and therefore, the hydro geo-morphological aspects, each river has different levels and kind of threats, which will impact differently direct or indirectly on river dolphins' populations.

Density estimates at fine scales might be good indicators of ecosystem transformation or degradation. Changes in density over time may reflect the effect of anthropogenic activities such as overfishing, deforestation, and water development projects, as well climate changes. Large-scale changes in the Amazonian ecosystem are approaching fast and shifts in population parameters (e.g., trends) may not be detected before populations are at dangerously low levels. We strongly recommend the continuity of studies at large and small scales in order to provide enough information to establish structured monitoring programs and foment management and policy actions and consideration of new methods that could improve estimates of abundance and trends of river dolphins in the Amazon.

Acknowledgements

We would like to thank the observer's team involved in field efforts in each of the expeditions (Brazil, Bolivia, Colombia, and Venezuela), local field assistants for their help during expeditions, the GIS assistance of Instituto Aqualie to assess study areas using satellite imagery, all institutions and organizations involved in data collection and financial support. This is a working paper, that will further compose an integrate article under SARDI (South American River Dolphins Initiative) strategy, reinforcing the commitment of transboundary efforts in conservation and high quality Science.

References

Aliaga-Rossel E. (2002). Distribution and abundance of the pink river dolphin, bufeo (*Inia geoffrensis*) in the Tijamuchi River, Beni-Bolivia. *Aquatic Mammals,* 28:312–323.

Anganuzzi A. A., Buckland S. T. (1993). *Post-Stratification as a Bias Reduction Technique. The Journal of Wildlife Management, 57(4), 827.*doi:10.2307/3809085

Araújo C. C., Wang J.Y. (2012). Botos (*Inia geoffrensis*) in the upper reaches of the Tocantins River (central Brazil) with observations of unusual behavior, including object carrying. *Aquatic Mammals*, 38, 435–440.

Araújo, C. C., Wang, J. Y. (2015). The dammed river dolphins of Brazil: impacts and conservation. *Oryx*, 49(1), 17-24.

Best R. C., da Silva V. M. F. (1989a). *Amazon river dolphin, boto Inia geoffrensis (de Blainville, 1817)*. Pages 1–23 in S. H. Ridgway and R. Harrison, eds. Handbook of marine mammals. Volume 4. River dolphins and the larger toothed whales. Academic Press, London, U.K.

Best R. C., da Silva V. M. F. (1989b). Biology, status and conservation of Inia geoffrensis in the Amazon and Orinoco river basins. *Occasional Papers of the IUCN Species Survival Commission SSC* 3:23-33.

Brum S. M., da Silva V. M. F., Rossoni F., Castello L. (2015). Use of dolphins and caimans as bait for Calophysus macropterus (Lichtenstein, 1819)(Siluriforme: Pimelodidae) in the Amazon. *Journal of Applied Ichthyology*, *31*(4), 675-680.

Buckland ST, Anderson DR, Burnham KP, Laake JL (1993). *Distance sampling: estimating abundance of biological populations*. Chapman & Hall, London, UK

Buckland S. T., Anderson D. R., Burnham K. P., Laake J. L., Borchers D. L., Thomas L. (2001). *Introduction to Distance Sampling*. Oxford University Press, New York, USA.

Chitty, C. H. I. (1994). Phytoplankton in the upper and middle Orinoco river. Internationale Vereinigung für theoretische und angewandte Limnologie: *Verhandlungen*, 25(3), 1856-1861.

da Silva V. M., Martin A. R. (2018). Amazon river dolphin: Inia geoffrensis. In *Encyclopedia of marine mammals* (pp. 21-24). Academic Press.

Dale V. H., Beyeler S. C. (2001). Challenges in the development and use of ecological indicators. *Ecological indicators*, *1*(1), 3-10.

de Paiva R. C. D., Buarque D. C., Collischonn W., Bonnet M. P., Frappart F., Calmant S., Bulhoes Mendes C. A. (2013). Large-scale hydrologic and hydrodynamic modeling of the Amazon River basin. *Water Resources Research*, 49(3), 1226-1243.

Elmqvist T., Folke C., Nyström M., Peterson G., Bengtsson J., Walker B., Norberg J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, *1*(9), 488-494.

Godoy J. R., Petts G., Salo J. (1999). Riparian flooded forests of the Orinoco and Amazon basins: a comparative review. *Biodiversity & Conservation*, *8*(4), 551-586.

Gómez-Salazar C., Trujillo F., Portocarrero-Aya M., Whitehead H. (2012a) Population, density estimates, and conservation of river dolphins (*Inia* and *Sotalia*) in the Amazon and Orinoco river basins. *Marine Mammal Science,* 28: 124-153.

Gómez-Salazar C., Coll M., Whitehead H. (2012b). River dolphins as indicators of ecosystem degradation in large tropical rivers. *Ecological Indicators*, 23: 19-26.

Goulding M. (1989). *Amazon: The flooded forest*. BBC Books, London, UK.

Goulding M., Barthem R. B., Ferreira E. J. G. (2003). *The Smithsonian Atlas of the Amazon. Washington,* DC: Smithsonian Institution Press.

Gregory K. J. (2006) The human role in changing river channels. *Geomorphology*, 79(3-4), 172-191.

Guyot J. L., Jouanneau J. M., Soare, L., Boaventura G. R., Maillet N., Lagane C. (2007). Clay mineral composition of river sediments in the Amazon Basin. *Catena, 71(2), 340–356.*doi:10.1016/j.catena.2007.02.002

Hamilton S., Lewis W. (1990). Physical characteristics of the fringing floodplain of the Orinoco River, Venezuela. *Interciencia,* 15: 491–500.

Hamilton S. K., Lewis W. M., Sippel S. J. (1992). Energy sources for aquatic animals in the Orinoco River floodplain: evidence from stable isotopes. *Oecologia*, 89.3: 324-330.

Hrbek T., da Silva V. M. F., Dutra N., Gravena W., Martin A. R., Farias I. P. (2014). A new species of river dolphin from Brazil or: how little do we know our biodiversity. *PloS* one 9: e83623.

Iriarte V., Marmontel M. (2013a). Insights on the use of dolphins (boto, *Inia geoffrensis* and tucuxi, *Sotalia fluviatilis*) for bait in the piracatinga (Calophysus macropterus) fishery in the western Brazilian Amazon. *Journal of Cetacean Research and Management*, *13*(2), 163-173.

Iriarte V., Marmontel M. (2013b). River dolphin (*Inia geoffrensis*, *Sotalia fluviatilis*) mortality events attributed to artisanal fisheries in the Western Brazilian Amazon. *Aquatic Mammals, 39*(2), 116.

Junk W. J., Furch K. (1993). A general review of tropical South American floodplains. *Wetlands Ecology and Management*, *2*(4), 231-238.

Junk W. J., Wittmann F., Schöngart J., Piedade M. T. (2015). A classification of the major habitats of Amazonian black-water river floodplains and a comparison with their white-water counterparts. *Wetlands Ecology and Management*, 23(4), 677-693.

Lasso C. A., Machado-Allison A., Taphorn D. C. (2016). Fishes and aquatic habitats of the Orinoco River Basin: diversity and conservation. *Journal of fish biology*, 89(1), 174-191

Latrubesse E. M., Stevaux J. C., Sinha R. (2005). Tropical Rivers. *Geomorphology*, *70*(3), 187-206.

Lewis W. M., Hamilton S. K., Lasi M. A., Rodríguez M., Saunders J. F. (2000). Ecological determinism on the Orinoco floodplain: a 15-year study of the Orinoco floodplain shows that this productive and biotically diverse ecosystem is functionally less complex than it appears. Hydrographic and geomorphic controls induce a high degree of determinism in biogeochemical and biotic processes. *AIBS Bulletin*, *50*(8), 681-692.

Marques F. F., Buckland S. T. (2003). Incorporating covariates into standard line transect analyses. *Biometrics*, 59(4), 924-935.

Martin A. R, da Silva V. M. F. (2004). River dolphins and flooded forest: Seasonal habitat use and sexual segregation of botos (Inia geoffrensis) in an extreme cetacean environment. *Journal of Zoology*, 263: 295–305.

Martin A. R., da Silva V. M. F., Salmon D. (2004). Riverine habitat preferences of botos (*Inia geoffrensis*) and tucuxis (*Sotalia fluviatilis*) in the central Amazon. *Marine Mammal Science*, 20: 189–200.

Martinelli L., Reynaldo V., Devol A., Richey J., Folsberg B. (1989). Suspended sediment load in the Amazon basin: an overview. *GeoJournal*, 19: 381–389.

Meade R, Weibezahn F, Lewis W Jr and Perez Hernandez D (1990) Suspended sediment budget for the Orinoco River. In: Weibezahn F, Alvarez H and Lewis W. Jr. (eds) The Orinoco River as an Ecosystem, pp 55–79. Universidad Simón Bolívar, Caracas, Venezuela.

Meade R. (1994). Suspended sediments of the modern Amazon and Orinoco Rivers. *Quaternary International*, 21: 29–39.

Medina E., Silva, J. F. (1990). Savannas of Northern South America: A Steady State Regulated by Water-Fire Interactions on a Background of Low Nutrient Availability. *Journal of Biogeography*, *17(4/5)*, *403.* doi:10.2307/2845370

Mintzer V. J., Martin A. R., da Silva V. M., Barbour A. B., Lorenzen K., Frazer T. K. (2013). Effect of illegal harvest on apparent survival of Amazon River dolphins (Inia geoffrensis). *Biological Conservation*, *158*, 280-286.

Mintzer V. J., Schmink M., Lorenzen K., Frazer T. K., Martin A. R., da Silva V. M. (2015). Attitudes and behaviors toward Amazon River dolphins (Inia geoffrensis) in a sustainable use protected area. *Biodiversity and conservation*, *24*(2), 247-269.

Mintzer V. J., Lorenzen K., Frazer T. K., da Silva V. M., Martin A. R. (2016). Seasonal movements of river dolphins (Inia geoffrensis) in a protected Amazonian floodplain. *Marine Mammal Science*, *32*(2), 664-681.

Mosquera-Guerra F, Trujillo F, Parks D, Oliveira-da-Costa M, Marmontel M, Armenteras-Pascual D, Usma S, Willems D, Carvajal-Castro JD, Mantilla-Meluk H, Franco N, Amorocho D, Maldonado R, Berg K, Sainz L, Van Damme PA, Campbell E (2018) Analysis of distribution of river dolphins (Inia and Sotalia) in protected and transformed areas in the Amazon and Orinoco basins. Scientific Committee / Meetings / SC67B | Slovenia 2018 / SM

Mosquera-Guerra F, Trujillo F, Parks D, Oliveira da Costa M, Van Damme PA, Echeverria A, Franco N, Carvajal-Castro JD, Mantilla-Meluk H, Marmontel M, Armenteras-Pascual D (2019a) Mercury in Populations of River Dolphins of the Amazon and Orinoco basins. EcoHealth 4(16):743-759

Mosquera-Guerra F, Trujillo F, Aya-Cuero C, Franco-León N, Valencia K, Vásquez A, Duran-Prieto C, Morales MDJ, Pachón-Bejarano GA, Mantilla-Meluk H, Armenteras- Pascual D

(2019b) Population estimate and identification of major conservation threats for the river dolphin (Inia geoffrensis humboldtiana) at the Colombian Orinoquia. Therya 11(1):9-21

Naiman R. J., Bunn S. E., Nilsson C., Petts G. E., Pinay G., Thompson L. C. (2002). Legitimizing fluvial ecosystems as users of water: an overview. *Environmental management*, *30*(4), 455-467.

Paschoalini, M., Almeida, R. M., Trujillo, F., Melo-Santos, G., Marmontel, M., Pavanato, H. J., ... & Zerbini, A. N. (2020). On the brink of isolation: Population estimates of the Araguaian river dolphin in a human-impacted region in Brazil. *Plos one*, *15*(4), e0231224.

Pavanato H. J., Melo-Santos G., Lima D. S., Portocarrero-Aya M., Paschoalini M., Mosquera F., et al. (2016). Risks of dam construction for South American river dolphins: a case study of the Tapajós River. *Endangered Species Research*, 31: 47-60

Pavanato, H. J., Salazar, C. G., Lima, D., Paschoalini, M., Ristau, N., & Marmontel, M. (2019). Density, abundance and group size of river dolphins (Inia geoffrensis and Sotalia fluviatilis) in central Amazonia, Brazil. *J. Cetacean Res. Manage.*, *20*(1), 93-100.

Pilleri G., Gihr M. (1977). Observations on the Bolivian (Inia geoffrensis boliviensis d'Orbigny, 1834) and the Amazonian bufeo (*Inia geoffrensis geoffrensis* de Blainville, 1817) with description of a new subespecies (*Inia geoffrensis humboltiana*). *Investigation on Cetacea*, 8:11-76.

Pimenta N. C., Barnett A. A., Botero-Arias R., Marmontel M. (2018). When predators become prey: Community-based monitoring of caiman and dolphin hunting for the catfish fishery and the broader implications on Amazonian human-natural systems. *Biological Conservation*, *222*, 154-163.

R Core Team (2015) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna

Rice D. W. (1998). *Marine mammals of the world: Systematics and distribution*. Special Publication Number 4, The Society for Marine Mammalogy. Available at http://www.marinemammalscience.org.

Rosenfeld, J. S. (2017). Developing flow–ecology relationships: Implications of nonlinear biological responses for water management. Freshwater Biology, 62(8), 1305-1324.

Salinas C., Cubillos J. C., Gómez R., Trujillo F., Caballero S. (2014). "Pig in a poke (gato por liebre)": The "mota" (*Calophysus macropterus*) Fishery, Molecular Evidence of Commercialization in Colombia and Toxicological Analyses. *EcoHealth*, *11*(2), 197-206.

Savage K. M., Potter, P. E. (1991). Petrology of modern sands of the rios Guaviare and Inirida, southern Colombia: tropical climate and sand composition. *The Journal of Geology*, 99.2: 289-298.

Sioli H. (1984). The Amazon and its main affluents: hydrography, morphology of the river courses, and river types. In: Sioli H (ed) *The Amazon: limnology and landscape ecology of a mighty tropical river and its basin*. Dr. W. Junk Publishers, Dordrecht, p 127-165.

Sioli H. (2012). *The Amazon: limnology and landscape ecology of a mighty tropical river and its basin* (Vol. 56). Springer Science & Business Media.

Sippel S., Hamilton S., Melack J., Choudhury B. (1994). Determination of inundation area in the Amazon River floodplain using SMMR 37GHz polarization difference. *Remote Sensing of* Environmen,t 48: 70–7

Thomas L., Buckland S. T., Rexstad E. A., Laake J. L., Strindberg S., Hedley S. L., et al. (2010) Distance software: design and analysis of distance sampling surveys for estimating population size. *Journal of Applied Ecology*, 47: 5-14

Trujillo F. (2000). *Habitat use and social behaviour of the freshwater dolphin Inia geoffrensis (de Blainville 1817) in the Amazon and Orinoco basins*. Ph.D. thesis, University of Aberdeen, Aberdeen, Scotland. 157 pp.

Trujillo F., Portocarrero M., Gómez-Salazar C., Diazgranados M. C., Castellanos-Mora L., Ruíz-García M., et al. (2010). Status and conservation of river dolphins Inia geoffrensis and Sotalia fluviatilis in the Amazon and Orinoco basins in Colombia. *The action plan for South American river dolphins 2020* p. 29-57, SOLAMAC, Bogota, DC, Colombia.

UNEP (2004). Barthem, R. B., Charvet-Almeida, P., Montag, L. F. A., Lanna, A.E. Amazon Basin, *GIWA Regional assessment 40b*. University of Kalmar, Kalmar, Sweden.

Vidal O., Barlow J., Hurtado L., Torre J., Cendon P., Ojeda Z. (1997). Distribution and abundance of the Amazon river dolphin (*Inia geoffrensis*) and the tucuxi (*Sotalia fluviatilis*) in the upper Amazon River. *Marine Mammal Scienc,e* 13:427–445.

Ward J. V., Tockner K. (2001). Biodiversity: towards a unifying theme for river ecology. *Freshwater Biology*, *46*(6), 807-820.

Williams R., Moore J. E., Gomez-Salazar C., Trujillo F., Burt L. (2016). Searching for trends in river dolphin abundance: Designing surveys for looming threats, and evidence for opposing trends of two species in the Colombian Amazon. *Biological Conservation*, *195*, 136-145.

Winemiller K. O., Jepsen, D. B. (1998). Effects of seasonality and fish movement on tropical river food webs. *Journal of fish Biology*, 53, 267-296.