Universidade Federal de Juiz de Fora
Programa de Pós-Graduação em Ecologia

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ESTIMAÇÃO DOS PARÂMETROS POPULACIONAIS DE DENSIDADE E ABUNDÂNCIA PARA OS GOLFINHOS DE RIO DA AMÉRICA DO SUL BOTO (*Inia spp.*) E TUCUXI (*Sotalia fluviatilis*): APERFEIÇOAMENTO DO MÉTODO E ABORDAGENS ECOLÓGICAS

[Estimating density and population size for South American river dolphins boto and tucuxi: improving methods and ecological approaches]

Juiz de Fora, Minas Gerais - Brasil

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Mariana Paschoalini Frias

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“Você nunca sabe que resultados virão de suas ações. Mas, se você não fizer nada não existirão resultados.”

[Mahatma Gandhi]

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RESUMO

O boto (*Inia spp.*) e o tucuxi (*Sotalia fluviatilis*) são pequenos cetáceos de água doce endêmicos da América do Sul. Os golfinhos de rio estão entre as espécies de cetáceos mais ameaçadas pelas atividades antrópicas crescentes e desordenadas, tornando essencial o conhecimento de seus parâmetros populacionais. Esforços para estimar dados de abundância para as espécies de golfinhos de rio da América do Sul aumentaram nos últimos anos, fazendo-se necessário o refinamento dos métodos empregados. Um protocolo de amostragem misto utilizando transecções lineares (*Line Transect*) e de banda (*Strip Transect*), via método de amostragem de distancias *Distance Sampling* (*DS*), vem sendo aplicado nos estudos com golfinhos de rio na América do Sul. No presente estudo, foram analisados 10 anos de conjuntos de dados coletados em 31 diferentes expedições pelas bacias Amazônica, do Orinoco e do Tocantins-Araguaia para a estimação de parâmetros populacionais de boto e tucuxi. Adicionalmente, um experimento de calibração de distancias permitiu inferir sobre a acurácia dos observadores quanto à medida de distâncias aos grupos de golfinhos detectados. Por meio de um GLM – Modelo Linear Generalizado, um *slope* de 0.952 ($p<2e-16$) indicou alta acurácia na medição de distâncias, não havendo diferença estatística na estimação de abundância entre distâncias estimadas e distâncias reais. Modelos sem a utilização de variáveis (*Conventional Distance Sampling – CDS*) e com a inserção de uma ou múltiplas variáveis (*Multi Covariate Distance Sampling – MCDS*), foram testados para avaliação do modelo com a melhor curva de detecção. O método MCDS apresentou-se como o melhor modelo para a curva de detecção para ambas espécies (*Inia* $p = 0.39$ (CV = 0.12), *Sotalia* $p = 0.27$ (CV = 0.20)), utilizando em ambos as variáveis: tamanho de grupo e plataforma de observação (proa ou popa). A avaliação conjunta de dados de proa e popa via método de marcaçãorecaptura (*Mark-Recapture Distance Sampling*) permitiu estimar a probabilidade de detecção à distância horizontal zero, g(0), 0.814 (CV = 0.053) para boto e g(0) = 0.989 (CV = 0.006) para tucuxi. As estimativas de cálculo das funções de detecção f(0) e da probabilidade de detecção g(0) de forma unificada para aplicação em dados de amostragens de rios em diferentes bacias provou não ser a abordagem mais precisa. Quando possível, f(0) e g(0) devem ser calculados para amostragens específicas, pois diferentes fatores (bióticos e abióticos) e características morfo-hidro-geográficas interferem diretamente no cálculo destas variáveis. estas características parecem direcionar a distribuição e o tamanho populacional dos golfinhos de rio na América do Sul. Neste sentido, uma análise de pós-estratificação em sub-regiões de um mesmo rio (Rio Tocantins), resultou em redução de 70% no coeficiente de variação na estimação da abundância. Uma população relativamente pequena de botos foi estimada para o curso baixo-médio do Rio Tocantins (736, CV = 0.52) e, para o Rio Guaviare (1138, CV = 0.32); ao contrário do Rio Purus, onde foram estimados 7672 botos (CV = 0.37) e 9238 tucuxis (CV = 0.49). Além das características intrínsecas das bacias hidrográficas, a gama de atividades humanas em diferentes níveis de escala em cada região interfere diretamente na avaliação da estimativa de abundância para cada rio. O refinamento das análises apresentadas neste estudo aumenta a precisão dos resultados e pode contribuir para o melhoramento na estimativa do tamanho populacional para boto e tucuxi em estudos futuros.

ABSTRACT

The boto (*Inia spp.*) and tucuxi (*Sotalia fluviatilis*) are freshwater small cetaceans endemic of South America. The river dolphins are among the species of cetaceans most threatened by growing and disorderly human activities, making it essential to know the population parameters for these species. Efforts to compute estimates of abundance for South American River dolphins have increased in the last several years and refinements of the methods employed to estimate population size are required. A mixed protocol of line and strip transects via Distance Sampling (DS) methods have been applied in the studies carried out with river dolphins in South America. In this study, we analyzed a 10-year dataset collected in 31 surveys through the Amazon, Orinoco and Tocantins-Araguaia River Basins for boto and tucuxi population estimates. Additionally, a distance calibration experiment allowed to infer about observer accuracy in sampling distances to the object detected. Through a GLM – Generalized Linear Models analysis, a slope of 0.952 ($p<2e-16$) shown high accuracy of distances sampled, there was no statistical difference in abundance estimates between estimated and real distances. Models with no covariates (*Conventional Distance Sampling – CDS*) and one or multiplex variables (*Multi Covariate Distance Sampling – MCDS*) were performed to evaluate best detection curve of detection function. MCDS methods were the best model for detection function of both species (*Inia* p = 0.39 (CV = 0.12), *Sotalia* p = 0.27 (CV = 0.20)), taking into account group size and sighting platform (bow and stern) as covariates. Using data for both sighting platforms, the detection probability at zero distance (g(0)) was estimated by *Mark-Recapture Distance Sampling* for boto 0.814 (CV = 0.053) and tucuxi 0.989 (CV = 0.006). Estimates of general detection function f(0) and detection probability g(0) to apply in samplings in different rivers has proved not to be the most accurate strategy.

When possible, f(0) and g(0) should be estimated as sampling-specific since biotic and abiotic factors, and hydro-geomorphology features directly influence in the parameters estimation. Hydro-geomorphology appears to acts as unit of distribution and population size of river dolphins in South America. Therefore, post-stratification analysis in sub-regions of the same river (Tocantins River) reduced by 70% the CV's in the estimates. A relatively small population of boto was estimated to the lower-medium Tocantins River (736, CV = 0.52), and for Guaviare River (1138, CV = 0.32); otherwise, the Purus River were estimated 7672 boto (CV = 0.37) e 9238 tucuxi (CV = 0.49*).* Despite intrinsic features of river basins, several human activities at different levels, directly interferes in the interpretation of abundances estimates of each river. Refinements in analytical methods presented in this study increase the precision of results and can contribute to the improvement of population size estimates of boto and tucuxi in future studies.

CHAPTER 1: INTRODUCTION AND SYNOPSIS

1. GENERAL INTRODUCTION

The conservation of biological diversity is not reason for recent concern. Human activities, especially habitat modification and degradation, have caused global biodiversity declines for a long time (Newbold et al. 2015). Some studies suggest the loss of biodiversity as one of the most critical and current environmental problems, threatening valuable ecosystem services and human wellbeing (Ceballos et al. 2015). There is growing evidence that human demands on natural resources are accelerating and could be undermining the stability of ecosystems, suggesting that humans are now responsible for an ongoing sixth mass extinction (Pimm et al. 1995, 2014, Wake & Vredenburg 2008, Barnosky et al. 2011). In face of that, the need for development of conservation and management plans for wildlife and their habitats has never been so urgent.

Tropical ecoregions are known to be hotspots of biodiversity, and comprise territories of many emerging countries where human activities have been increasing, but wildlife management in these areas is often ineffective. The most important driver of biodiversity in these zones is the water, a natural resource that shape evolutionary and ecological processes in aquatic and terrestrial ecosystems (Naiman et al. 2002, Cowie & Holland 2006). Animal species distributed in wetlands were, and still are, the most affected by human activities (Malmqvist & Rundle 2002). Freshwater ecosystems provide resources for food (including fishery, irrigation and aquaculture), power generation, transport, and sanitation for human societies (Myers & Worm 2003, Vörösmarty et al. 2004, Dudgeon et al. 2006, Poff et al. 2007). Therefore, freshwaters are drivers of development and, consequently, are subject to multiple anthropogenic stressors (Vörösmarty et al. 2010).

Freshwater cetaceans are dolphins only found in riverine ecosystems of South America and Asia (Reeves & Martin 2009). These dolphins constitute a particularly vulnerable group of aquatic mammals. In regions where human use of natural resources overlaps with the distribution of river dolphins, disturbance and threat for these species often occur (Smith & Reeves 2012), including: habitat loss and degradation, incidental mortality (e.g., bycatch), food depletion, bioaccumulation of chemical contaminants, fragmentation and/or reduction of the distribution range, intensive boat-traffic, and acoustic pollution (Whitehead et al. 2000, Trujillo et al. 2010, da Silva et al. 2011, Smith & Reeves 2012, Gómez-Salazar et al. 2012b, Araújo & Wang 2012, Braulik et al. 2014, Gravena et al. 2014, 2015, Paudel et al. 2015, Pavanato et al. 2016).

Predicting impacts, measuring the scale and effects of threats, and proposing management and conservation actions require baseline information about the population parameters of a species. One of the most important and intriguing questions in ecology relates to the size of a certain population: "How many are there?". A crucial issue is that the answer has implications to intrinsic ecological processes of a population, and depends on the application of appropriate field-analytical techniques (Buckland et al. 2015). Knowing how many animals are in a specific place may represent a challenging task from an applied perspective. This challenge is particularly great for freshwater cetaceans due to the complexity of their habitats (Dawson et al. 2008).

The impacts of any threats to river dolphins cannot be assessed quantitatively without robust and reliable abundance and trend data. Standardized and well-designed methods that take into consideration habitat characteristics and ecological needs of each species should be employed if good quality data and robust estimates of population size are to be developed and used for management and conservation.

In light of the important ecological issues related to the pressure over the riverine ecosystems and the processes that affect animal populations of these zones, this thesis has focused on population estimates of South American river dolphins *Inia spp*. and *Sotalia fluviatilis*. In the next topics we provide a short description of the river dolphins group, cetacean population study methods, population estimates of *Inia* and *Sotalia* and major threats identified for these species.

1.1. RIVER DOLPHINS DESCRIPTION

River dolphins are small cetaceans (Odontoceti - toothed cetaceans) exclusively adapted to freshwater ecosystems (Cassens et al. 2000). This non-monophyletic group of dolphins, includes six dolphin species and one porpoise that are distributed in the watersheds of the Subcontinent of South Asia and Northern South America (Reeves et al. 2000, 2003, Reeves & Martin 2009). Almost all of them are classified as Endangered or Data Deficient regarding population conservation status by the IUCN – International Union for conservation of Nature.

River dolphins in South America are represented by species of the genera *Inia,* commonly known as boto or Amazon river dolphin, and the species *Sotalia fluviatilis*, known as tucuxi (Best & da Silva 1993, Best & da Silva 1996, Caballero et al. 2002, Cunha et al. 2005) (Fig. 1). These dolphins are distributed in three river basins (Amazon, Orinoco and Tocantins-Araguaia) across seven countries (Brazil, Bolivia, Colombia, Ecuador, Guiana, Peru and Venezuela) (Best & da Silva 1989a, b, Pilleri & Gihr 1997, Rice 1998, Trujillo et al. 2010). Within the genera *Inia*, the species *Inia geoffrensis*

geoffrensis is found in the entire Amazonian basin, *Inia boliviensis* occurs in the Bolivian Amazon basin and the upper Madeira River in Brazil (da Silva 1994, Hamilton et al. 2001, Gravena et al. 2014), and the subspecies *Inia geoffrensis humboldtiana* is restricted to the Orinoco River basin. *Sotalia fluviatilis* are sympatric species with *Inia geoffrensis* occurring in the central of Amazon River basin.

Figure 1. River dolphins species boto *Inia geoffrensis* (a) and tucuxi *Sotalia fluviatilis* (b). Author: Fernando Trujillo

A third species of *Inia* has been proposed for the Tocantins-Araguaia River basin in Brazil: *Inia araguaiasensis* (Hrbek et al. 2014) (Fig. 2). *Inia* dolphins found in the Tocantins-Araguaia (hereafter, Araguaian botos) are spatially isolated from those inhabiting the Amazon River basin, restricted to some tributaries of the Tocantins and inhabiting a complex transition between two major Brazilian biomes, the Cerrado savanna and the Amazon rainforest (Hrbek et al. 2014). Some morphological aspects are still required by the The Committee on Taxonomy of the Society for Marine Mammalogy regarding to recognize the Araguaian boto as a new species because of the small sample size of morphometric data used in the species description (Committee of Taxonomy, 2019). Thus, we refer to Araguaian boto in this thesis as a population of *Inia* distinct from that found in the Amazon River basin.

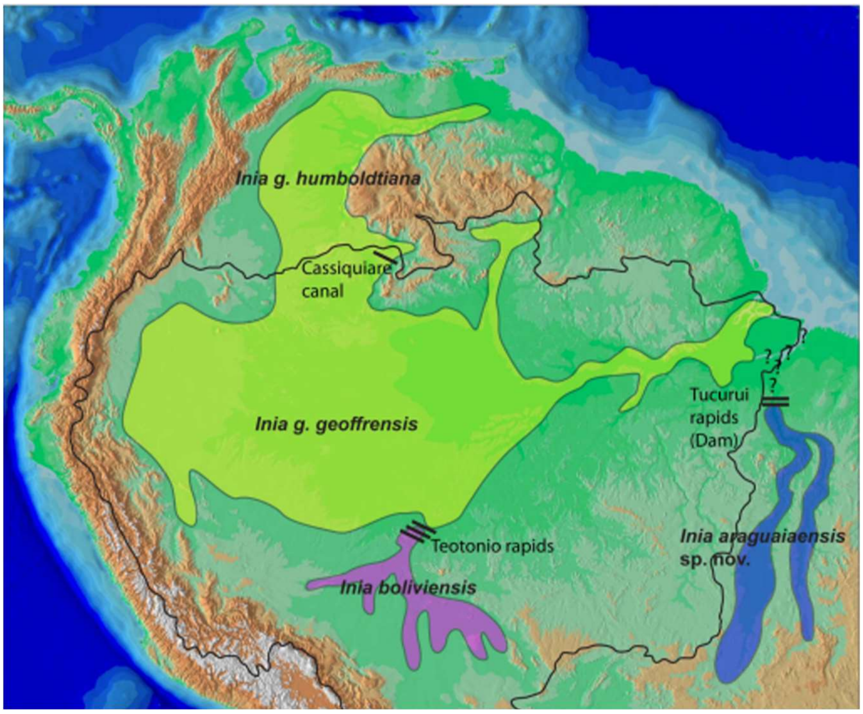

Figure 2. Distribution of species and subspecies of *Inia*. Black outline denotes the limit of the Amazon basin. Question marks denote uncertainty as to which species occurs in the Tocantins River downstream of the Tucuruí dam which potentially delimits the distributions of *I. geoffrensis* and *I. araguaiaensis*. Bars on the Madeira River represent a series of rapids that delimit the distribution of *I. geoffrensis* and *I. boliviensis*. The single bar on the northern limit of the Amazon basin represents the Casiquiare canal which connects the Amazon and Orinoco basins, and is thought to delimit the *I. g. humboldtiana* subspecies from *I. g. geoffrensis.* Adapted from Hrbeck et al. (2014).

1.2. CETACEAN POPULATION ABUNDANCE METHODS

Animal populations can be determined in two manners: a census or sampling. Census occurs when all individuals of a given population are enumerated and sampling occurs when the population size is computed based on counting a fraction (sample) of the population (Buckland et al. 2000). Sampling is the most used method because census is rarely feasible or, if feasible, it is typically prohibitively expensive (Borchers et al. 2002).

Several sampling methods have been developed to estimate population size of cetaceans, including visual surveys (the animals or part of their body are sighted), cue counting (splash, mainly), mark-recapture via tagging or photo-id, passive acoustic detections (Seber 1982, Buckland et al. 2000, Borchers et al. 2002, Evans & Hammond 2004, Zerbini et al. 2006, Mellinger et al. 2007). Among the sampling methods by visual counting, one of the most common is known as Distance Sampling (DS) (Buckland et al. 2001, 2015), which can be divided in two categories: line and point transect. The most widely used form of distance sampling is line transect sampling (Thomas et al. 2010).

Through line transect sampling, a survey region is sampled by placing a number of lines at random in the region or, more commonly, a series of systematically spaced with a random start point (Buckland et al., 1993). Perpendicular distances are collected from the detected “object” (dolphin or a group of dolphin) to the transect line and used to estimate the proportion of animals missed within the sampled area (Buckland et al. 2001, 2004). Density within this area is computed by dividing the number of groups seen by the probability of detecting them and multiplied by the study area size to compute population size/abundance (Thomas et al. 2010, Buckland et al. 2015). Line transect is a well-established method to estimate density and abundance and is applicable to a broad range of cetacean species. It has been recently used to estimate the population size of *Inia* and *Sotalia*.

1.3. STUDYING ABUNDANCE OF SOUTH AMERICAN RIVER DOLPHINS – PAST, PRESENT AND DEVELOPMENTS

The first attempts to estimate the number of dolphins belonging to the genera *Inia* and *Sotalia* occurred in the 1950s, reporting only the encounter rates instead of density or population size (Layne 1958, Kasuya & Kajihara 1974, Pilleri & Gihr 1977, Meade & Koehnken 1991, da Silva 1994, Herman et al. 1996).

In the mid-1990s, a mixed sampling protocol using strip and line transect methods was implemented by Vidal et al. (1997) to achieve the best sampling coverage considering the complexity of the Amazon region and the ecology of the river dolphins. This study was carried out in 120 linear km in the Amazon River, at the border between Colombia, Peru and Brazil. Vidal's study set the stage for subsequent work, which followed a similar protocol (McGuire 2002, Aliaga-Rossel 2002, Martin & da Silva 2004, Martin et al. 2004). These studies demonstrated that river dolphins aggregate in productive environment such as river confluences and lakes, where the diversity and abundance of prey is high and the water flow is relatively low. Aggregation in these areas are believed to benefit dolphins because they can optimize energy expenditure during foraging (Martin & da Silva 2004, Martin et al. 2004).

In the 2000s, the sampling protocol developed by Vidal et al. (1997) was improved by Gómez-Salazar et al. (2012a). Gómez-Salazar et al. (2012a) study showed that detection of river dolphins is not perfect in strip transects (as assumed before) and provided estimates of detections probabilities at different distance bins from the survey line, taking into account both the uneven distribution of the animals across the strip as well as the imperfect detection by observers. In addition, their study was developed based on a larger dataset (seven rivers) and encompassed a substantially broader area (5,708 km^2) compared to Vidal et al (1997) (250 km^2).

Before Gómez-Salazar et al. (2012a), population estimates of river dolphins in South America were obtained sporadically and surveys were conducted in a relatively small scale, contributing limited information about the density and population size of both genera. In addition, the low spatial resolution of the early studies (Layne 1958, Kasuya & Kajihara 1974, Pilleri & Gihr 1977, Meade & Koehnken 1991, da Silva 1994, Herman et al. 1996) added to differences in sampling and analytical methods made density comparisons across studies difficult.

Since Gomez-Salazar et al. (2012a), a more standard sampling protocol has been used. A large dataset has been built by the efforts of researches from seven countries within the distribution range of river dolphins in South America (Bolivia, Brazil, Colombia, Ecuador, Guiana, Peru, and Venezuela). Besides the important improvements developed by Gomez-Salazar et al. (2012a), many factors suggest the current methods require improvements by taking into account the complexity of the sampling regions, logistical and operational limitations, the need for consistent and well-trained observers team, potential violations of distance sampling assumptions and lack of information on population structure and animal movements. Therefore, a review of sampling and analytical methods is required to improve robustness of river dolphins population estimates.

2. RESEARCH PRESENTED AND ORGANIZATION OF THESIS DISSERTATION

This thesis is organized in 5 Chapters. The first chapter presented a brief introduction of the topics covered in the thesis. Chapter 2 describes the general analytical framework used to compute density and abundance estimates of river dolphins, including a discussion of possible logistical and analytical limitations. Chapter 3 provides results of an investigation of the effect of measurements errors in sampling distances using data from a field calibration experiment. Chapter 4 presents improvements in the analytical methods for estimation of river dolphin abundance using distance sampling methods, and provides population size estimation of three major rivers in Amazon, Orinoco and

Tocantins-Araguaia basin within an ecological and conservation perspective. Chapter 5 present broad final conclusions of the study.

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CHAPTER 2. LOGISTICAL AND ANALYTICAL LIMITATIONS IN ASSESSING DENSITY AND ABUNDANCE OF AMAZONIAN RIVER DOLPHINS

Abstract: The impacts of threats to any species cannot be assessed qualitatively without robust and reliable population abundance data. Standardized and well-designed methods according to the habitat and the biological characteristics of the species should always be employed. Then, the information generated will be capable of determining the size of a population in values, as soon as management and conservation strategies. Even when robust analytical methods are used, environmental complexity of the sampled area can show restrictions difficult to predict. Survey the abundance of riverine dolphins are especially difficult due to the challenges imposed by the habitats. In the Amazon and Orinoco, dolphins are seasonally affected by the hydrological pulses driven by the Andes. The transformation of habitats in some areas is huge. Variations in the scale of 11-15 meters can occur at the vertical dimension (river level) and hundreds of kilometers in the horizontal dimension (flooding area) of a river, causing quick changes in land-scape, and demanding adaptive and malleable methodologies. This chapter brings a review of estimating Amazonian River Dolphins (*Inia sp.* And *Sotalia fluviatilis*) abundance pointing out the field methods and analytical limitations, arguing about the study area complexity (access limitation), design survey, logistical operations and adaptation, team training, cross country efforts, statistical approaches, and applicable solutions when possible.

1. INTRODUCTION

The conservation status of small freshwater cetaceans, particularly the Amazonian river dolphins of the genera *Inia* and the species *Sotalia fluviatilis*, has been under concern for many years (Reeves & Leatherwood 1994, Trujillo et al. 2010, Barreto et al. 2011). This concern has stemmed from substantial incidental catches in artisanal fishing activities (Vidal 1993, da Silva & Best 1996, Loch et al. 2009, Iriarte & Marmontel 2013), intentional killing for use as bait in *Piracatinga* fishery (da Silva et al. 2011, Mintzer et al. 2013, Brum et al. 2015); from declines in the population numbers (Mintzer et al. 2013, Williams et al. 2016, da Silva et al. 2018), possible risks for contaminants including heavy metals from gold mining (Best & da Silva 1989a, Monteiro-Neto et al. 2003, Lailson-Brito et al. 2008, Gómez-Salazar et al. 2012a), habitat fragmentation and population isolation by the construction of hydroelectric dams (Portocarrero-Aya et al. 2010, Araújo & Wang 2015, Gravena et al 2014, 2015, Pavanato et al. 2016, Latrubesse 2017).

Because of the exposure to so many threats, there is an urgent need for baseline information on the abundance and trends of river dolphins to formulate proper management and conservation actions. Quantitative data have been used to estimate relative or absolute abundance of boto and tucuxi (da Silva 1994, Pilleri & Gihr 1977, Layne 1958, Kasuya & Kajihara 1974, Meade & Koehnken 1991, Herman et al. 1996, Vidal et al. 1997, Trujillo 2000, McGuire 2002, Aliaga-Rossel 2002, Martin & da Silva 2004, Martin et al. 2004, Gómez-Salazar et al. 2012, Aliaga-Rossel et al. 2012, Pavanato et al. 2016, Coimbra et al. 2016, Williams et al. 2016, Campbell et al. 2017, Oliveira et al. 2017, da Silva et al. 2018). However, except perhaps for Gómez-Salazar et al. (2012a), these studies have focused on relatively small geographic areas (less than 100 linear kilometers of river) and have applied different methodologies (e.g., photo-identitication/capture-recapture, passive acoustics, direct counting, and distance sampling) often in manners that are not comparable. The most used method to estimate density and abundance in recent years has been those based on distance sampling theory from visual surveys, and for this reason this work will focus on this specific approach.

Spatial, temporal, and environmental differences in surveys limit comparability of the estimates across the areas sampled. Furthermore, there are important logistical issues related to data collection that must be considered and explored, as the restrains imposed by environmental features that may quickly change the land-scape (habitat types), the vessels type used to conduct the surveys, and the formation of a well-trained field team.

The need for accurate and precise estimates of abundance of *Inia sp.* and *Sotalia fluviatilis* throughout the Amazon-Orinoco river basin has been recognized by the International Union for Conservation of Nature (IUCN), the Action Plan for South American River Dolphins (Trujillo et al. 2010), national actions planes included in distribution range of these species, and by the International Whaling Commission (IWC 2018). They specifically recommend that South American river dolphins abundance must be estimated using dedicated sightings surveys, in long-term time series, using standardized methods, and the improvement and/or development of alternative methods to achieve a robust field methodology applicable to shuch a complex ecosystem.

Since 2006, the SARDPAN project (South American River Dolphin Protected Area Network) has been conducting extensive vessel surveys in six of the seven countries (Brazil, Colombia, Bolivia, Venezuela, Peru, Ecuador) within the distribution range of *Inia sp.* and *Sotalia fluviatilis.* These surveys have been using a combination of line transect and strip transects sampling methods as described in Gómez-Salazar et al. (2012a). These species are difficult to survey because of their small size and because their cryptic behavior (*Inia sp.* in particular) at the water surface make them difficult to be detected. Also, the characteristics of their habitats are such that traditional line/strip transect methods can be difficult to apply both from a logistical as well as methodological standpoint. For these reasons, the potential bias in abundance estimation must be addressed in the surveys design.

This chapter provides a detailed review of the current sampling protocol used to estimate population size of Amazonian River Dolphins by the SARDPAN project, with the goal of identifying methodological and analytical limitations, possible source of bias in the estimates and potential actions that could help improving methods and, consequently, estimates. The discussion is presented based on the study area complexity (limited access and environmentally dynamics), survey design, logistical, team training, cross country efforts, statistical approaches, and applicable solutions when possible.

2. CURRENT SAMPLING PROTOCOL USED TO ESTIMATE DENSITY AND ABUNDANCE OF AMAZONIAN RIVER DOLPHINS

During the past few decades population size estimates for South American river dolphins (boto and tucuxi) based on visual surveys, have been computed using a mixed protocol of strip and line transect sampling (Gómez-Salazar et al. 2012a, Aliaga-Rossel 2012, Pavanato et al. 2016, Pavanato et al. 2018 (in press)). According to Martin and da Silva (2004) and Gómez-Salazar et al. (2012a, 2012b), groups of boto and tucuxi are distributed along the river following a concentration gradient from the margins to the main river channel (Fig. 3). The combination of line and strip transects was designed to cover the widest sampling area possible, taking into account the distribution gradient of dolphins and the different habitats in the river system (main river, tributary rivers, lakes, channels, islands, and confluences) (Fig. 4). The protocol proposed by Gómez-Salazar et al. (2012a) consists in a series of four strip transects 2.5 km length placed 100 m parallel to the river margin (200 m strip width) followed by one line transects or cross-channel transects, crossing from one margin to another following a zigzag pattern (~45º)(Fig. 5).

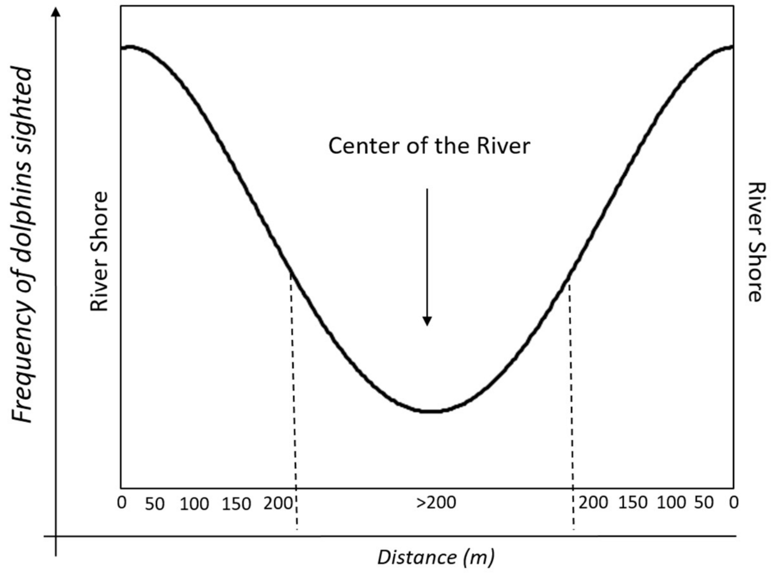

Figure 3. Theoretical distribution gradient of dolphins between river margins in Amazon river system.

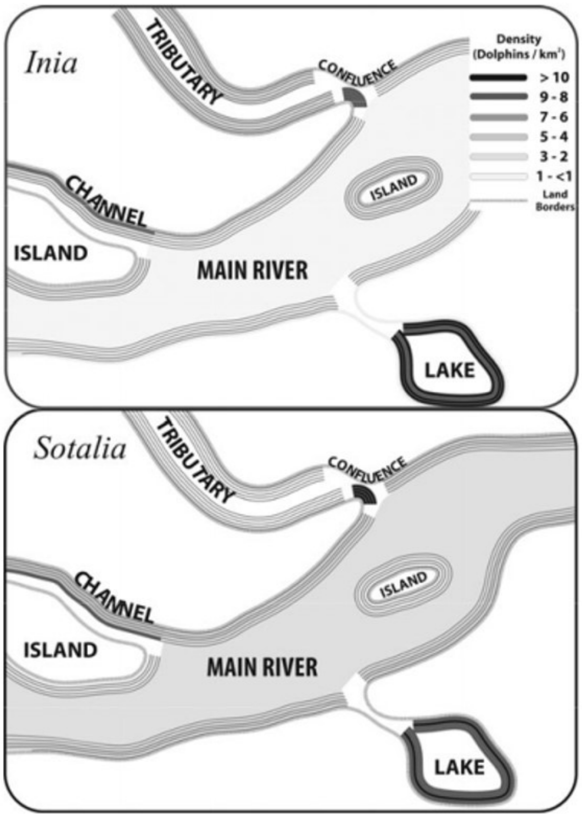

Figure 4. Scheme of a hypothetical section of a river basin, showing densities for each habitat type. Adapted from Gómez-Salazar et al. (2012a).

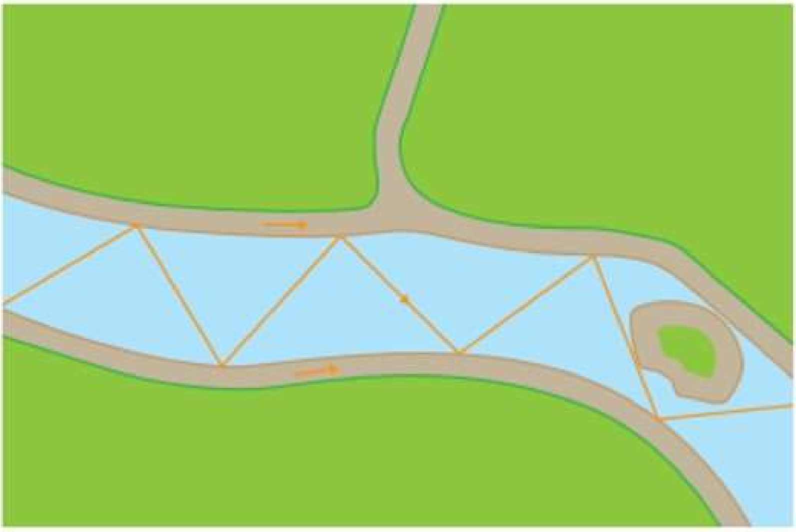

Figure 5. Sampling design with detail of line transects (cross-channel between margins – orange lines) and strip transects 200m width (parallel to the river margin – brown strip) in river system. Adapted from Trujillo et al. (2010).

The searching for dolphins is usually conducted by a team of nine observers during typically 10-hour sampling per day. Observers rotate every hour through two platforms (bow and stern). At each platform, two observers (port and starboard) actively search for dolphins from 10° on the opposite side to 90° on their own side, and a third position is responsible for data recording. Observer rotate through the following positions: port observer, data recorder, and starboard observer. After completing the rotation cycle within a platform, each researcher rests for a minimum of two hours. The overlap in the observers' searching fields was established to minimize the probability of missing animals in the vicinity of the trackline (Gómez-Salazar et al. 2012a). The observations is supposed to be independent between platforms to enable the estimation of the detection probability in the trackline, or g(0) (Lake & Borchers 2004, Gómez-Salazar et al. 2012, Pavanato et al. 2016); only sightings made for the second platform (stern) is report to the first platform (bow) via radio to correct for the missed animals.

Observers search for dolphins with naked eyes and used angle boards to measure the angle between the sighting and the trackline. Thereat, the majority of the observers had previous training and experience in estimating distances. For all sightings, the observers reported the species, group size, and presence of calves, radial distance from the observer, angle from the trackline, and distances from the dolphin group to the margin (in ranges of 50 meter intervals up to 200m, that is 0-50, 50-100, 100-150, 150-200m). Other information regarding to habitat type is also recorded (e.g., water coloration, margin composition – sand, rocky, beach, vegetation type and forest associated), as well as environmental conditions such as glare intensity, sightability, river state (Beaufour scale 0-3), rain. Off-effort observers are not involved in searching and should not report new detections.

Although important improvements in sampling of river dolphins were implemented in the sampling protocol of Gómez-Salazar et al. (2012a), some aspects of the protocol regarding the complexity of the area sampled and its implications in the logistics and analytical limitations require further evaluation. These potential limitations and how they can affect sampling, analysis, the resulting estimates and their reliability are further discussed.

3. LOGISTICAL AND ANALYTICAL LIMITATIONS OF THE AMAZONIAN RIVER DOLPHINS SAMPLING PROTOCOL

3.1. LOGISTICAL LIMITATIONS

3.1.1. Environment complexity

In the Amazon and Orinoco river basins, dolphins are seasonally affected by the hydrological pulses mainly driven by the precipitation and thaw in the Andes (Junk et al. 1988, Junk et al. 1997). The uplift of the Andean region has a direct effect on regional climate and fundamentally changed the Amazonian landscape by reconfiguring drainage patterns and creating a vast influx of sediments into this basin (Hoorn et al. 2010). The transformation of habitats in some areas were significant, variations of up to of 11-15 meters at the vertical water level and up to hundreds of kilometers in the horizontal plane of a river occur in a seasonal basis (Goulding et al. 1996, Junk et al. 1997). During the low water period (dry season), the availability of aquatic habitats is considerably reduced and the levels of dissolved oxygen and primary productivity change, which result in modified distribution patterns of the dolphin's preys and, consequently, the dolphin populations (Goulding 1989, Neiff 1996, Martin et al. 2004, Gómez-Salazar et al. 2012b).

The rivers of Amazonian-Orinoco basin present similar geographic conformations with deep and narrow, or extensive channels and of low depth, they can display sets of islands, interlinked systems of lakes, and a wide variation in the margin composition from rocky to sediment mud (Sioli 1984, Junk & Furch1993). Sinuosity is also an importante feature of the Amazon hydro-geomorphology. These rivers have convex margins of great morphodynamic importance, granting to these environments a landscape of meanders and directly influencing the construction-deconstruction of shores through erosion processes (Sioli 2012, Wittimann & Junk 2016).

The hydromorphology of the river in the survey time is another issue that limits the optimal application of the methods designed. Amazon river dolphin' surveys are optimally conducted during the transitional water period (raising or falling waters) because most habitats are available to dolphins and to vessels during this period, maximizing chances of detection (Gómez-Salazar et al. 2012a). However, these periods have suffering great changes and to predict the exact moment to conduct the survey have been a challenge (Marengo & Espinoza 2016, Terborgh et al. 2018). Most of the times, the researchers rely on the information available in the literature regarding to limnology and hydrography to define the best time of sampling. Nevertheless, the conditions found in field can vary significantly.

Uncharted shallow channels, the emergence of beaches, the presence of rapids, and rocky margins are often found during the course of research, which forces changing in vessel's course and consequently changes in transects allocation, even when the survey design is based on the most recent available information (e.g., use of satellite imaging from periods as close as possible to the timing of sampling). In Tapajós (Pavanato et al., 2016) and Tocantins rivers (present study), for example, due to the configuration of rocky margins the mean distance of shore in strip transects were greater than 100 m (128 m and 120 m respectively), and maximum distances of shore 626 m and 549 m, respectively. The conduction of strip transects far than 200m of the margin can compromise the methodology assumption regarding to dolphins distribution gradient (Williams et al. 2016). In Guaviare and Putumayo rivers, an expedition conducted in 2016 and 2017 respectively, due to the presence of rapids and emergence beaches and shallow channels the sampling was stopped until the restoration of favorable and safe navigation conditions. In these two cases, stretches of approximately 100 km of river were navigated off-effort, compromising the collection of important information in the presence of dolphins. Thus, these conditions make it clear that traditional systematic survey designs will rarely be completed as planned and highlight the need for surveys that are adaptive and analysis methods that accommodate changes made during the survey.

3.1.2. Logistics

The most suitable method to sample rivers in Amazon is to conduct vessel surveys, using vessels that features comprises the needs of each area in terms of accessibility, regarding the ecosystem dynamics. Visual boat-based surveys are widely used for estimation of density and population size of cetaceans worldwide (Borchers et al. 1998, Buckland et al. 2001) and are particularly indicated for sampling complex regions such as rivers, estuaries and bays (Thomas et al. 2007).

To accomplish surveys in remote zones and with different levels of accessibility, keep constancy in the use of vessels with similar characteristics in terms of platform height and length is a major logistical challenge. In the Amazon, vessel fleets are concentrated in urban centers, often far from the survey areas. Travelling from these centers to some of these survey areas requires boats of sufficient sizes to accommodate a large crew and, for this reason, often represent relatively large expenses. In addition, larger vessels may not have access to certain habitats, especially shallow or narrow areas or those where rapids are present. Then, adaptability of the sampling vessels is crucial in regions where access to the dolphin's habitats is difficult. A panoramic view of the study area is presented in the Figure 6.

Different vessels were used to comply 28 different survey regions and logistical facilities, resulting in variation in platforms heights in the dataset. Survey platforms with different platforms height can result in different fields of view and will affect detection probability and potentially sighting rates (Evans & Hammond 2004). Therefore, the correction factor value (P1 and P2) proposed by Gómez-Salazar et al. (2012a), as well as the application of a unique estimated g(0) may not be realistic, since in their study all line transect of different surveys were analyzed as a single sample. A feasible solution for considering this source of variance is to explore the platform height as covariate in the analysis and models performed, as well as the *Beaufort* scale is used in the marine environment (Forney 2000, Hammond et al. 2002, Buckland et al. 2015). Therefore, new correction factor values and detection probabilities g(0) might be computed embodying this variance source.

Figure 6. Map of main rivers in Amazon, Orinoco and Tocantins river basins, highlighting areas of constrains and difficult access recognized as limits of dolphin's distributions (waterfalls), and areas of gaps.

Other survey methods and platforms such as towed-arrays (acoustics detectors) and aerial surveys (planes, drones, blimp) commonly applied in marine environment studies of cetacean abundance (Oliveira et al. 2017, Oliveira da-Costa et al. 2019) have been considered to be applied for population estimates of river dolphins in South America. Nevertheless, the adverse logistics and operational conditions limit the optimal use of these methodologies (e.g. aerial surveys – landing in remote areas, cloud cover and rain; towed-arrays – river' sinuosity and submerged objects that may broke or rolled up cables). Other studies have been exploring alternative aerial survey methodologies such as blimps (Oliveira et al. 2017) and drones (Oliveira et al. 2019). However, until now, visual boat surveys remain as the most feasible strategy to cover largest and complex areas, though necessary improvements.

3.1.3. Limitations in data collection protocols

Training of a competent observer team is paramount, but it is time consuming and demands a significant financial investment because the sampling region is a large area to cover across many countries. Further, keeping the same researchers is not always possible. As well as, the capacitation of the crew to conduct the vessel according to the survey needs is crucial since most of the time they are local people and are not habituated to scientific expeditions. Before starting any survey, the crew need to be instructed about the navigation methodology and the purpose of the research, in order to enable them to perform transects designing and to assist with navigation limitations .

The observer team also undergoes training for sighting (visual search for group of dolphins) and data record (field forms) using data of dolphins sighting, habitat type and environmental information. Additionally, the data record position has to manage the GPS used to control transects length and the point in which the dolphin (or group of dolphins) was seen. The data recording person, located in bow platform, is also responsible for communication (via radio) between platforms (correction of missed groups) and with the crew (navigation).

As information are recorded manually in field forms and the data-recording person accumulate multiple functions, problems of missing data occur frequently. The non-consistency in data record is a great issue mainly when the missing data is related to crucial information to compute density and abundance. These include group size, radial distance, transect length, and angle between a sighting and the trackline. For example, in 28 surveys missing data represented 12.8% of the all observations (n = 6177). Important variables that may interfere on detection as platform height, environmental conditions (glare strength, visibility, river state, water color), speed, and depth, must be consistently collected for use, for example, as covariates in detection probability models.

To help with data management during sighting effort, automated digital platforms coupled to GPS could provide a good tool to the data recorder. There are a few research software developed to collect data from marine mammals, the most used in Distance Sampling methods for abundance survey is *Wincruz* (Windows Real Time Sighting-Effort Event Logger, written by R. Holland, SWFSC, NOAA, USA). The *Wincruz* is an event-driven program to record sighting and effort data on ship line transect surveys and to graphically display sighting locations. The application of this kind of software may increase the efficiency of data collection during river dolphins surveys and minimize missing data events.

An important issue related to searching effort is that off-effort observers and data recorders are not expected to be involved in searching and, therefore, should not call new detections (Hammond et al. 2002). As the data recording position is close to observers position in sighting platforms they might see dolphins, but they cannot report these detections to avoid influences in the methodology (two active observers by platform). This, is substantially important since the detection probability g(0) is calculated based on the correction for missing detections made between bow and platforms, external sights could bias the g(0) estimate.

3.2. ANALYTICAL LIMITATION

3.2.1. Distance Sampling Assumptions Violation

There are several fundamental assumptions for proper application of distance sampling methods (Buckland et al. 2001). Which are (1) transect lines are randomly placed independently of the animal distribution; (2) all animals at distance zero from the transect line are detected; (3) distances are measured with exactitude; (4) animals are detected by the observers in theirs initial location, which means that they did not respond to the vessel presence.

3.2.1.1. First Assumption: Random Distribution

The survey design using line transects consists in an algorithm that places random transects across the study area. The standard methods (Buckland et al. 2001) assume that the density of animals in the area surveyed is on average equal to the density in the entire study area. Thus, if each part of the study area has the same probability of being surveyed (uniform coverage probability) this statement is true. This kind of method is called Design-Based. An adequate survey design is necessary to achieve uniform or near-uniform coverage probability and ensure that estimates of density and abundance are unbiased from a design standpoint.

Design-based methods assume sampling lines are randomly allocated with respect to the distribution of animals through transects, and consequently in the study area. Because of the hydro-morphological characteristics of the Amazon and limitations to navigation of the survey vessels, it is nearly impossible survey randomly allocated transects in the rivers. For this reason, the stratified survey design approach proposed by Gómez-Salazar et al. (2012a) where the river channels are surveyed using traditional line transect methods and the margins of the rivers are surveyed using a strip transect approach, was an important consideration to minimize bias in the estimation of river dolphin abundance in the Amazon.

In spite of the techniques employed to solve the violation of the first assumption, the environment complexity still represents challenges due to the high dynamicity of water levels, climate changes, and animal's concentration in some areas. Quick changes in water level alter the landscape and force changing in the navigation course, and consequently affect the sampling. According to Williams et al. (2016), the selection of few transects may violate the assumption of a systematic or randomized survey design, but it will be not be a big issue as long as the average portion of the population being sampled proportionally into habitat strata along large surveys.

Post-stratification analysis might be a good option for dealing with areas when constrains impose sudden changings in transect design, and also for high variance between strata. In the presence of large-scale gradients in animal density, divide the study area into small regions so as to maximize the between-stratum variation in density and minimize the within-stratum variation may lead to greatly increase precision of estimates (Thomas et al. 2007). Environmental, ecologic, and anthropogenic factor (e.g. great distances from shore, presence of rocky margins, shallow channels, beaches, boat traffic, artisanal fisheries nets, number of confluences - productive areas, changes in sediment flow) may affect dolphin's gradient of distribution and habitat use, and can be used to classify specific sub-units into the study area. Thereby, post-stratification is convenient to obtain estimates considering specific variates, allowing understanding the singularity of each sampling area (Thomas et al. 2010, Buckland et al. 2015), and producing more reliable estimates for complex areas such as for river.

3.2.1.2. Second assumption: Animals in the line are detected with certainty

Ensure 100% detection for cetacean species at zero distance from the trackline is in general an issue. Cetaceans, spend most of the time submerged, being available to be detected in the water surface for short periods (especially small odontocete), usually when breathing. In cases when is important to consider the detection probability different from one, double-platform survey are recommended (Laake & Borchers 2004). Each platform records data of animals sighting assuming de configuration of 'on-way' independency, i.e., with one platform being unaware of detection made by the other, but not vice versa. This provides a capture-recapture model, which allows estimation assuming detection probability (g (0)) is less that unity (Otis et al. 1978, Huggins 1991, Buckland et al. 1993, Laake & Borchers 2004, Fletcher & Hutto 2006, Thomas et al. 2010).

For river dolphins in Amazon, an unique g(0) was estimated by Gómez-Salazar et al. (2012a) using double platform and combining data of all line transects conducted in different rivers sampled (*Inia* g(0) = 0.947, cv 0.025*, Sotalia fluviatilis* g(0) = 0.997 cv 0.003). As mentioned in previous topics, many factors may cause bias in detection probability as high variability in observer's team, number of active observers, different platform height, vessel's type, environmental conditions. For this reason, for future surveys it would be appropriate to consider survey-specific estimates of g(0) for that rivers where it is feasible to perform line transect. For tributary rivers (width less than 400 meters) where only strip transect is conducted, however, the application of a general g(0) is useful when using density estimator proposed by Gómez-Salazar et al. (2012a). As mentioned for the issue with platform height regarding detection probability and sighting rate, to improve the calculation of the unique g(0), a model considering the incorporation of all these variables could provide good adjustment in g(0) estimate minimizing sources of bias and providing more reliable population estimates.

3.2.1.3. Third assumption: Exactitude in distances measurements

In practice, this assumption is often violated since it depends of high equipment calibration and its proper use, or the perfection and well-trained human eye. Errors in sampling distances in surveys for abundance estimates of marine mammals are a general issue, and may have a substantial impact on the bias and accuracy of distance sampling estimators (Barlow et al 2001, Borchers et al. 2010). Sampling distances accurately in freshwater environments is a special challenge due to difficulties in navigation and sinuosity (Smith et al. 2006, Williams et al. 2016). In marine environments equipment such as reticulated binoculars are used to estimate distances from the observer to the detected object, however the use of this tool in a closed environment (without continuous horizon) such as the Amazon is impractical.

In environments such as rivers and estuaries the sampling of distances are usually performed by naked eye, which devote substantial time in training and calibration of the observer's team (Schweder 1997, Hammond et al. 2002, Williams et al. 2007). Because of that, inaccuracies are expected to occur. Training of an experienced and well-calibrated observer team is limited by the availability of time for research, and financial expense in travel costs. To access the level of calibration of observer's team is necessary to create realistic experiments that simulate real conditions, which means a field distance estimation experiment under variable sun glare, wind, rain, water transparency, Beaufort scale, and glare. Considering the significance of the accuracy in distances collection, which may indicate the precision of population's estimation, we will present in the chapter 3 of this thesis results of one field experiment devoted to identify and quantify the errors associated with the distance estimation and its potential effect on final estimates.

The precision in distances may also represent a source of bias regarding to the width in the strip transect in the current method. As mentioned previously, due to the presence of beaches, sand bars and rocky margins, distances from the vessel to the river shore may not be kept at 100 m, compromising the strip width of 200 m. This can substantially affect the estimates in strip transects causing underestimation, since this method considers the gradient of dolphin's distribution as shown in the figure 1. In order to accommodate this variance, we advise the use of the mean width using distances measured with laser range finder when this variance is not too large (between 20 and 50 meters). The mean width was already used by Gómez-Salazar et al. (2012a) to calculate the strip width for tributary rivers and narrow channels. For those areas when distances become greater, it is more appropriate however to perform a line transect crossing the river to the other margin, if this margin presents better conditions for the vessel to be kept at the distance established in the protocol. If neither of these options are feasible, would be advisable close the effort until the restoration of favorable conditions.

3.2.1.4. Fourth Assumption: Animals do not respond to the survey platform before being detected.

Distance Sampling is a snapshot method in which animals are "frozen" in the initial position that they were detected (Buckland et al. 2001). Actually, animals are dynamic entities and are constantly interacting with their habitats, including migration movements. In practice, nonresponsive movement is not significant problematic provided it is slow relatively to the observation platform (Thomas et al. 2014, Glennie et al. 2015). Otherwise, responsive movements before detection are indeed problematic, and it is often difficult to determine whether it has occurred (Buckland et al. 2005). Responsive movement commonly occurs in a direction away from the observer on the line, although sometimes animals are attracted to the observer and move towards the line, and could lead to over or underestimation bias (Fewster et al. 2008).

Amazonian river dolphin of *Inia* species are curious and charismatic animals, that usually approach boats (Best & da Silva 1989a, b, Paschoalini 2014). During surveys, we have seen positive responsive movements of botos toward the vessels. In contrast, tucuxi dolphins tend to avoid vessels and present a negative responsive movement, moving away from the track line. However, we cannot ensure that these movements occurred before dolphins have being detected by the observers.

According to Dawson et al. (2008) boat surveys often result in responsive movement of animals, which is a very important issue to consider. Double-observer method (capture-recapture) can be used to account responsive movements, in which the trajectory and group composition can be compared to the first sight (Palka & Hammond 2001). To minimize the effects of responsive movements in boat surveys the use of high sighting platforms is also indicated, so that observers will be able to detect animals further away, possibly before the react to the observation platform (Dawson et al. 2008, Buckland et al. 2015). Models assuming movement's pattern using tagging technologies and training of the observer's time to report and confirm sighting data are also recommended as alternative approach (Thomas et al. 2014).

It is important that field experiments be developed to investigate river dolphins' movements in response to vessels during surveys. In addition to double-observer methods, drones can also be used flying concomitantly with visual boat-survey, allowing the visualization of dolphin movement regarding to the vessel and at what distance they start to react.

3.2.2. STUDY AREA: COMPLEXITY FOR DELIMITATION

The definition of the study area is one of the first steps when designing a survey. Distance Sampling works on spatial scales dependent on precise metrics, and has interface with Geographic Information Systems (GIS) by using ArcView (or ArcGis) (ESRI 2000, 2004, Strindberg et al. 2004, Buckland et al. 2015). The correct calculation of the size of the sampled area and the whole study area will directly affect the estimates and may cause overestimation or underestimation of population size (Strindberg et al. 2004). In the case of river dolphins this is a significant factor because they are seasonally affected according to water period being more aggregated in dry season, disperse in flooded season and more random distributed in transitional periods (raising and falling waters) (Trujillo 2000, Martin & da Silva 2004, Gómez-Salazar et al. 2012b). So, to proper calculate sampled and study area for this species the period must be taken into account, which is also highlighted by Williams et al. (2016).

The surveyed areas of the river dolphins sampling are currently computed *a posteriori* of the study conduction, using remote sensing. Satellite images obtained for free open-access software as Google Earth, are used to draw polygons of water surface in the stretch of the river sampled. The polygons are drawing by habitat types (main river, channels, confluences, lake, and tributary) respecting the features of each habitat type described by Gómez-Salazar et al. (2012a). To compute just the area occupied by water surface, the polygons of islands is discounted to exclude land mass.

Satellite images and remote sensing techniques have been widely used in ecological studies to characterize landscape dynamics, zoning and ecological-economic mapping, delimitation and characterization of river basins, climate changes, deforestation, animal movement patters, habitat use, among others (Asner et al. 1998, Gould 2000, Achard et al. 2002; Parmesan & Yohe 2003, Sawunyamaet al. 2006, Handcock et al. 2009, Palmer et al. 2015). This variety of ecological applications require data from broad spatial extents that cannot typically be collected using field-based methods. For tropical areas, notwithstanding, the satellite coverage does not provide systematic and long-term time series of images, there is no continuity of scenes within the same year for many places (Hansenn et al. 2008). In some cases, the coverage is so inefficient that temporal difference between images can reach up to 10 years, as in remote area of Amazon.

The high water levels dynamics through Amazon ecosystems is a fundamental factor that rapidly change the shape of habitats, oftentimes remodeling the river course by construction-deconstruction of shores through erosion processes (Sioli 2012, Wittimann & Junk 2016). The use of images far from the period of the survey conduction may be subjected to substantial errors that can be difficult to overcome. For this reason, discontinuity of information intra-year time series is determinant for representing source of error in accuracy assessment of measurements delimitation (Mertes et al. 1995, Smith 1997, Frappart et al. 2005, Pettoreli et al. 2014). Data continuity needs to preserve and to improve existing long-term archives of satellite remote sensing products (Kerr et al. 2003), as well making them available easily to be able to contribute and to develop a robust method to understand trends and future impacts on biological diversity (Millennium Ecosystem Assessment 2005, Turner et al. 2015).

Another important issue addressed to remote sensing in tropical forests is persistent cloud cover. The cloud cover precludes the correct visualization of the image, that confounds efforts to operationalize land cover (Asner 2001, Powell et al. 2004, Helmer & Ruefenacht 2005), and change characterizations in the case of river dolphins, for habitat types and limits of the margins. Thus, to generate reliable data on the surface of the study area it is necessary to access high-resolution satellite images from the exact time when the survey was carried out and to ensure that the habitats where the sampling occur are visible in the images.

Despite the recent advances in remote sensing techniques as higher resolution sensors, operability, high-tech softwares (Kennedy et al. 2014, Asner 2015, Tang & Shao 2015), multi-decadal continuous Earth observation information is only available from a very few satellite systems and the images are high costly to obtain. Then, difficulties to precisely calculate the extent of the study area remain. Especially for river dolphins, that are significant affected by water level in terms of distribution pattern, and the lack of continuity information in intra-year time series.

A feasible solution that could provide accurate assessment of study area and habitat types can be explored using field data (GPS), remote sensing imagery of Landsat and Copernicus sensor free open-access in Google Earth software (Kennedy et al 2014), and georeferenced analysis tools within Arview or Arcmap software. In addition, data can be interpreted using inundation models and precipitation data available, which may be able to give a scale and magnitude of water level variation on area measurements.

For future studies, another way to obtain high-resolution images very accurately is the use of drones to get these imagens at the time of survey is conducted. Drones have built-in georeferenced systems and produce images that can be imported into visualization, management, processing and analysis of geographic data. A labor-intensive work will be need to create a mosaic of the drone images and consequently calculate the study area; nevertheless, the results would be more realistic and precise. Methods to make the area calculation process faster and more efficient are encouraged to improvement the reliability of the estimates, and to speed up the publication of data for managements decisions and conservation actions.

4. FINAL STATEMENTS AND RECOMMENDATIONS

This work highlights dificulties inherent in designing an effective monitoring program to obtain river dolphins population estimates and trends using visual boats surveys. The water level dynamics and the largest area to sample, presents several logistical and environmental constrains, that address different source of variances. Additionally, with the lack of available information on population structure and dynamic, and animals movements patterns, it is unlikely estimate the absolute abundance of river dolphins in the Amazon.

The great effort employed to get abundance estimates for river dolphins across different rivers in the Amazon, provided support information that enabled to identify the replicability of the method for different settings, and the needs of alternate or complimentary methods in some cases. Given the limited resources for long-term monitoring surveys in remote and largest areas, we should maximize inferences in strategic areas feasible to implement a consistent monitoring program.

Instead of obtain absolute abundance estimates in all surveys, index for relative abundance might be obtain in areas of relative easy access where surveys can be implemented periodically, in an intensive and low-cost way. Thus, robust and cost-effective methods should provide more reliable estimates of abundance trends, allowing elucidating river dolphins *Inia* a *Sotalia* conservation status. Passive acoustics monitoring (PAM) is one of the more recent and promising tool that have shown reasonably estimates of trends in abundance of cetaceans in important cases such as the decline of Vaquita (*Phocoena sinus*) population (Jaramillo-Legorreta et al. 2017) and in effective conservation actions for Harbor porpoise (*Phocoena phocoena)* in the Baltic Sea (Calén et al. 2018).

Another tool that have been used and explored as alternative methodology is the use of drone as a survey platform. The World Wide Fund for Nature (WWF) in association with the Mamiraua Institute for Sustainable Development (IDSM) have used drones in small areas to improve detections and to future development of an algorithm able to identify dolphin's clues and conduct estimate surveys. Reliable information of dolphins geographic distribution and movement patterns are also important for decision-makers and abundance estimates. The South American River Dolphin Initiative led by Omacha Foundation, WWF, IDSM, and institutions of Bolivia, Peru and Ecuador, are working toward filling this gap using satellites transmitters in Amazonia river dolphin *Inia*, which will allowing incorporating spatial data in both analysis and conservation planning.

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CHAPTER 3. INVESTIGATING THE POTENTIAL BIAS IN DISTANCES MEASUREMENTS ERRORS IN IN SOUTH AMERICAN RIVER DOLPHINS ABUNDANCE SURVEYS

Abstract: Distance Sampling methods requires distances between the survey platform and the objects of interest to be measured accurately in order to compute unbiased estimates of density and abundance. However, in practice, this assumption is often violated due to measurement error. This occurs, for example, because of poor equipment calibration, lack of observer training, variable environmental conditions, and habitat complexity. Because estimates of population size are important to assess the conservation status of endangered species, it is important to ensure proper and accurate data collection. Because distance is estimated by eye in river dolphin surveys, it is important to investigate whether measurement error is an issue and whether they could lead to bias in estimates of abundance. In this study, distance estimation experiments were conducted to explore relationships between estimated and measured distance and assess potential for bias. Results shown that while heteroscedasticity was observed in the data, estimated and true distances showed a linear correlation. The most supported model estimated a slope of 0.952 ($p < 0.001$), suggesting that distance is slightly overestimated, but still relatively accurate. Estimates of detection probability (P) using observed perpendicular distances obtained during an actual survey and distances corrected by the most supported model in this study were, as expected, not statistically different. Values of P for corrected (p=0.52, CV=0.068) and estimated (p=0.49, CV=0.073) were nearly identical. We conclude that river dolphins estimation in South America are reliable with respect to potential biases in estimation of distance. Nevertheless, the continued training of observers is always recommended to refine and consolidate sampling methods and, consequently, to continue computing unbiased estimates of density.

1. INTRODUCTION

A basic assumption of Distance Sampling (DS) methods is that the perpendicular distances of objects of interest and the transect line are estimated without error (Chen 1998, Buckland et al. 2001, Palka & Hammond 2001). However, this assumption is often violated and distance estimation is subject to measurement errors when obtained by an observer without the aid of instruments (e.g., reticled binoculars) (Thompson & Hiby 1985, Alldredge et al. 2007) especially if no training or calibration occurs. In line transect sampling, detection probability is estimated by fitting models to the perpendicular distance obtained by observers (see Buckland et al. 2001, for basic theory).

Under or overestimation of distance leads to, respectively, negative or positive bias in estimates of density. Four types of error have been identified in distances measurements within a DS context: (1) recording/data handling errors, (2) rounding errors, (3) biased random errors, (4) unbiased random errors. The two first kinds are expected to be solved by working with an experienced and well-trained team of observers and can be minimized before using data in analysis. The two other types, random errors, are most concerning. These type of errors have been explored in earlier studies and some additive and multiplicative models were developed to incorporate measurement errors in population size estimates (Hiby et al. 1989, Alpizar-Jara 1997, Chen 1998, Chen & Cowlling 2001, Schweder 1996, 1997). Advanced models more applied to distance sampling methods and analysis were published by Barlow et al. (2001), Marques (2004), and Borchers et al (2010), describing the ways in which errors are generated and different factors that influences perpendicular measurements.

No measurement is exact and random errors arise from the inability to record precise distances. According to Marques (2004) there is always some kind of additive error in any distance measurement, but given proper field methods and a well-trained observer team it is plausible to assume that this additive error is negligible if bias is random when compared with other potential sources of bias (e.g. availability).

Distance measurements in river dolphins surveys are performed by naked eye, without the aid of instruments. Since the rivers do not present a horizon, has many curves, and differences in margin height, it is difficult to use binoculars to help measuring distances. Additionally, constant changings in the environment (habitats) provides some level of difficult in training and calibration, so measurement errors in estimating distances are expected to occur.

Given the importance of the effect of distance measurements in reliability of density estimation in line transect surveys, we investigate the proportion of errors in sampling distances in Amazonian river dolphins surveys, and its potential effect in fit detection function for density estimates.

2. MATERIAL AND METHODS

2.1. FIELD METHODS

A distance estimation experiment was conducted to assess potential errors in determining perpendicular distances in river dolphin abundance surveys. The study was conducted with the team of observer responsible for the surveys in two rivers, Guaviare (Colombia) and Juruá (Brazil), in 2016. The experiment was conducted using regional Amazonian double-deck boats used in the survey of the two rivers. Both boats were similar in size and height of the observer platform (20 m length, 7 m eye height).

The experiment consisted in estimating radial distances to a fixed and continuously visible target, which the true position was determined with the aid of a GPS (Garmin 73S) and known only by the person leading the experiment and recording the data. Care was taken so that no observer was colorblind. In the first survey (Guavirare River), the experiment was conducted with six observers and a set of 25 random distances (n = 150 samples). In the second survey (Juruá River), the experiment was conducted with 12 observers and a set of 10 random distances (n = 120 samples). Overall sample size were 270 estimated distances and its respective true distances. Distances for both experiments was generated using the minimum and maximum distances recorded in real surveys of Amazon river dolphins between 2006 to 2015 (from the dataset of abundance surveys of the South America River Dolphin Protected Area Network – SARDPAN).

A "passing mode" design was adopted to reproduce real survey conditions . The boat was positioned at each one of the know distances from the fixed object, and each of the observers was asked to estimate distance in just a few seconds. Angle position was measured by the leader of the experiment in each station of distance, in order to minimize any effect of this variable in distances estimation and to standardized perpendicular distances calculation. Thus, angles were assumed to be collected with exactitude. The observers were advised not to communicate with each other and no feedback regarding their performance was provided by the data recorder during the trails to avoid a distance-training exercise. In both rivers, the experiment was conducted during good sighting conditions (*i.e.* no rain, no sun glare, river state in Beaufort scale 0) in order to maintain the target visible.

2.2. ANALYTICAL METHODS

a. Estimation of Error Model

Data collected were compiled from paired observations of radial distances, i.e. those measured by observers (estimated) versus those calculated (true) using the GPS for each one of the distance stations in a single dataset. Data analysis was performed in program R (R Core Team, 2015) using packages car, MASS, rcompanion, nlme, AICcmodavg. As first step, an exploratory analysis was conducted to investigate different distributions in the data: Gaussian, Gamma, Poisson, Gaussian Inverse. The Gamma distribution was the one that best fitted model for data distribution chosen by the smaller Akaike's information criterion (AICs) (Akaike 1973).

Generalized linear models (GLM) were used to assess potential biases in radial distance estimation. Residuals were modelled with a Gamma distribution family with an identity link function. Estimated distances were used as the response variable (y) and measured values as the explanatory variable (x). The error structure was investigated based in the additive model for random errors (Chen, 1998; Marques, 2004), by modeling the equation:

$$E(Y|x) = \beta_0 + \beta_1 * x_i + \varepsilon_i$$

Where E () is the model prediction of the distance Y on the basis of x, (β_0) is the intercept, (β_1) is the angular coefficient or slope, (x_i) is the each distances measured/observed in meters, and ε_i represents all residual factors plus possible measurement errors for each distance measured/observed in a Gamma error distribution. Following the Gamma distribution, the model parameters vector were **Ø** = (shape, scale, α) and variance calculated as CV = $1/\sqrt{\alpha}$.

b. Estimation of Detection Probability

Overall radial distances measured by observers (estimated) and by GPS (true) were transformed in perpendicular distances to fit models of detection functions. Hazard-rate (HR) and half-normal (HN) models with no adjustments were considered as key function forms to fit the estimated and the true distances using Conventional Distance Sampling (CDS) methods (Buckland et al. 2001). For model selection, the AICs was applied to choose the best-fitted model. Using the Multi Covariate Distance Sampling

(MCDS) methods (Marques et al. 2004), observers were added to the best-fitted model to investigate the random effect of them in the detection function, and AICs was used to compare models performed. These analysis were conducted in R program (R Core Team 2015), using the packages mrds and Distance.

3. RESULTS

The exploratory analysis to investigate the distribution family in the data showed that Gamma family is the best model of distribution comparing the AICs (Table 1). Results of GLM suggested that while heteroscedasticity was observed in the data, the relation-ship between estimated and measured distances showed a linear correlation (confirming gamma-distributed errors appropriated - dispersion parameter for the Gamma family was 0.174). The model fit to the data is shown in Figure 7(a) and residual diagnostics can be seen in Figure 7(b). Model parameters are provided in table 2. The fit indicates that observers tend to overestimate distances to animals starting approximately from 200 m. From the whole sample (n = 270), 48% (n = 131) of distances were overestimated ranging from 0.5% to 166.66%, with a standard deviation of $\sigma = 24.55$ and CV = 87%.

Table 1. Results of models distribution family investigated compared by AICs.

Models	**AIC**	**ΔAIC**	**ACIcwt**	**Cum.wt**	**LL**
Gamma	2885.9	0	1	1	-1439.9
Poisson	2962.91	77.0199	0	1	-1478.4
Guassian	2986.94	101.05	0	1	-1490.4
Gaussian Inverse	3079.83	193.94	0	1	-1536.9

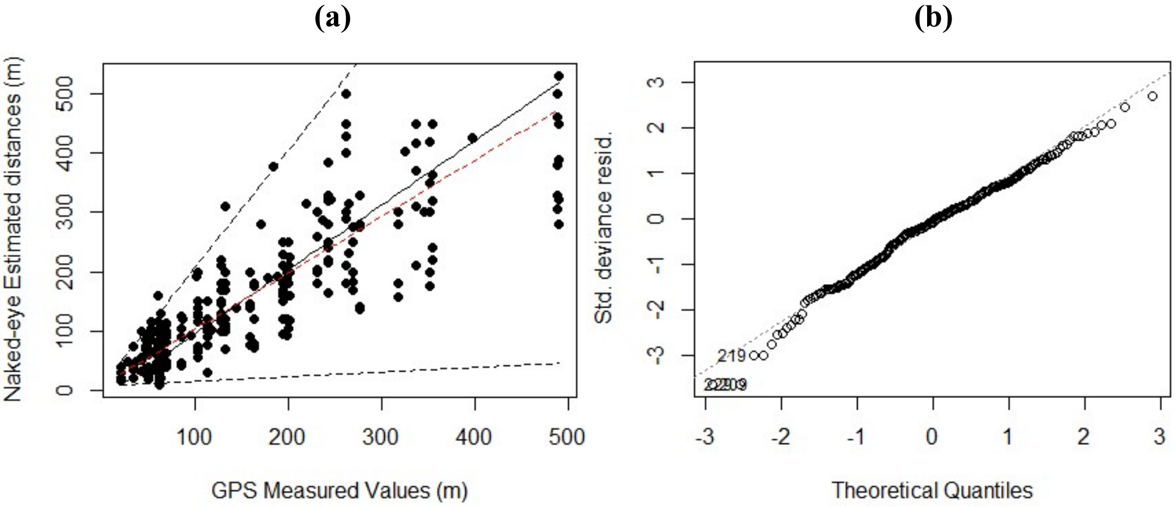


Figure 7. (a) Estimated and measured distances. A solid heavy line represents the linear correlation, the fitted model is shown as a dashed red line, and the confidence interval of 95% of the data as dashed black lines. **(b)** QQ-Plot for the fitted model.

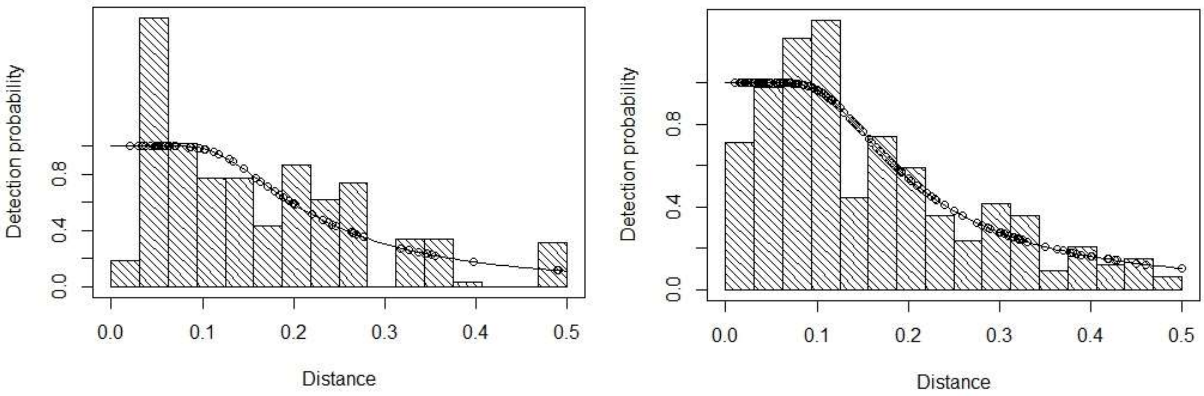
Table 2. Generalized Linear Model (GLM) parameters for the model selected, Gamma distribution with link "identity".

	Estimate	**SE**	***z***	***p***
Intercept	7.22	3.39	2.13	0.03
True distance	0.95	0.04	22.62	<2e-16

The best-fitted model of detection function was that using Hazard-rate as key function for both true and estimated distances (Table 3), and are shown in the detection probability curve in Figure 8. At Hazard-rate model, we added the 19 observers as covariate to the detection function of estimated distances, which gave an AIC = -463.64 and a ΔAIC = 32.28, showing no significant effect observer in model performance. Estimates of detection probability (p) using estimated perpendicular distances and distances measured by the most supported model in this study were not statistically different, values of p were identical ($X^2 = 0.0008$, $p = 0.976$).

Table 3. Results of the models performed for selection of the best-fit detection function for measured and estimated distances.

Model	AIC	ΔAIC	*p*	CV
True Distances				
Hazard-rate	-490.03	0	0.52	0.07
Half-normal	-489.84	0.19	0.51	0.04
Estimated Distances				
Hazard-rate	-495.93	0	0.49	0.07
Half-normal	-493.77	2.15	0.50	0.04



(a) **(b)**

Figure 8. Hazard-rate detection function for **(a)** true distances and **(b)** estimated distances. Distances are presented in kilometers.

4. DISCUSSION

Differences in distances estimated by naked eye and GPS were expected to occur; nevertheless our results have shown that the most supported model estimated a slope quite close to one, suggesting estimated distances by observers were relatively accurate. Despite the heteroscedasticity, the model provided a fit whose residuals were spread in a linear relation-ship along the x and y-axes, suggesting that this model could provide corrected radial distances estimate that were unbiased on average.

Measurement errors found in range of distances starting in 200 m does not seem to be substantial to induce large differences in abundance estimation but were significant to contribute to the heteroscedastic error structure observed. The reason for this might be the effect of the analysis compiling pairs observations of 'true' (measured) and estimated distances, that smoothed overall bias. Observers vary the way that they estimate distances, due to individual perceptions. The perception bias attributed to individual observers is subjective and associated to the manner that each one generally perceives distances (depth sense, reference points). Individual differences in sighting distance is so difficult to interpret as individual differences in sighting rates (Buckland et al. 2001, Thomas et al. 2014), making difficult to modelling observer specific error.

Given the high number of observers and the inconsistent manner in which they participate in the abundance surveys for Amazonian river dolphins it would be difficult to produce a model that considered individual differences among observers. An analysis combining all observers is indicated in this case, rather than pairs of independent estimated distances (Butterworth 1982, Chen 1998, Barlow et al. 2001, Fuller 2006, Williams et al. 2007, Borchers et al. 2009, Leaper et al. (unpublished)). Would be appropriate and indicated to conduct the distance-training experiment for each survey, in order to compute observer-individual error.

An important point of this study was that the distance-estimation experiment was conducted in passing mode differently of the common practice of using fixed platforms. Williams et al. (2007) in a similar study conducted during a river dolphin survey in 2002 in Amazon river found substantial variation in the way six observers estimated distance to 22 fixed objects from a static platform, high heteroscedasticity and a non-linear function. There is evidence that in experiments were fixed targets and static platforms are used, observers have a longer period of time to judge ranges converting the estimation process into a distance training exercise (Hammond et al. 2002), increasing variances. When trying to calibrate and adjust the estimation of distances, observers increases the time processing this information, and often increase the chances of errors. Distance experiments using a passing platform may be more realistic because they reproduce near-real conditions for time reporting of the "sighting", minimizing chances of using the experiment as a calibration exercise and providing more realistic information regarding errors associated with visual distance estimation in the field. Additionally, we carefully call attention to the fact that our dataset is wide and the range of 'true' distances was not arbitrary/opportunistic spanning the range of true distances in real surveys, which can potentially increase the robustness of our results.

Although no differences were found in the results of the estimation of detection probabilities measured and estimated distances, some models of detection function, especially Hazard-rate even when fitting well, may be influenced by observations very near the trackline and/or in the tail of the distribution of perpendicular distances (Buckland 1985, Burnham & Anderson 1998, Buckland et al. 2001). As the probability of detection decrease at greater distances, observations made far from the trackline would contribute with few detections that could be excluded from the analysis to increase robustness in fitting the detection (Buckland et al. 2001), and minimize the source of bias caused by different measurement errors at different range of distances. Truncation of distant sightings is recommended in conventional distance sampling methods around 5% of distances for line transect sampling or when detection probability drops quickly (Thomas et al. 2009). Distances for detection of river dolphins in Amazon are not too large since the environment impose visual restrains as narrow and sinuous margins. Gómez-Salazar et al. (2012a) have seen that detection probability of river dolphins, mainly for botos, is significantly reduced from 200 m. Truncate data at 200 m might help to exclude larger distances for which estimates may present greater error. This would be appropriated to increase accuracy in the estimation of detection probability and minimize the measurement error effect.

It is important to remember that errors in perpendicular distances (*x*) also depends of errors in record radial angles. It is possible that in boat based surveys, observers round radial angles close to convenient values (e.g., 0, 10, 50, 100º) (Barlow et al. 2001, Marques 2004). This rounding is particularly important for detections at relatively large distances and narrow angles, especially at zero, potentially causing positive bias in estimation of abundance. The extent to which bias in radial distance will affect bias on perpendicular distance so will be influenced by the distribution and accuracy of sighting angles measurements. However not addressed in the present study, that was focused in distance estimation, we highlight the importance to explore this potential source of bias in future works.

The experiment conducted in this study is relatively simple and easy to replicate. While it demonstrated that observers in the Guaviare and Jurua rivers were relatively accurate in their estimates of distance, this may not be the case for other studies. Therefore, replicating the experiment in the future may be appropriate as a calibration exercise or to potentially correct distance estimates for observers for which bias may be detected. The experiment design is relatively simple and the time spent conducting it is relatively short in terms of the overall survey period, especially considering the potential benefits to improve data reliability. We recommend the continuity of distance training exercises for observers who are involved with Amazonian river dolphin abundance estimates, and particularly increase effort and sample size for range of distances greater than 200 m, in order to continue improving the precision of distances measurement.

5. CONCLUSION

River dolphins' estimation in South America using data from SARDPAN surveys are reliable with respect to potential biases in visual estimation of radial distance. There are remaining distances in which measurement errors were detected to be great, and for that continue training of observers are recommended to improve the quality of sampling distances. Obtaining accurate distance measurements will improve data reliability and, consequently, the quality of the estimates of density and abundance computed with those distances.

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CHAPTER 4. DENSITY AND ABUNDANCE ESTIMATES OF SOUTH AMERICAN RIVER DOLPHINS: HYDRO-GEOMORPHOLOGY AND HABITAT INTEGRITY DRIVES OF DISTRIBUTION AND POPULATION SIZE.

Abstract: Estimating density and abundance of river dolphins in South America is challenging because the riverine ecosystem is complex and is subject to constant changes. Understanding rivers as units and drivers of biodiversity is the first step to plan and to conduct well designed surveys to better assess distribution and to estimate population size of river dolphins. In addition, the use of appropriate methods is needed to accommodate challenges associated with the heterogeneity of riverine habitats, which may influence distribution and density. In this study, density and population size is estimated for river dolphins *Inia spp.* (Araguaian boto), *Inia geoffrensis humboldtiana* and *Sotalia fluviatilis* in three major different rivers: Purus, Tocantins and Guaviare (Amazon, Tocantins-Araguaia and Orinoco basins, respectively). The highest density of Amazonian river dolphins was estimated for the Purus River: 7,672 *Inia geoffrensis* (CV = 0.37) and 9,238 *Sotalia fluviatilis* (CV = 0.49). In Tocantins and Guaviare rivers, the population of boto and tucuxi were smaller (736 (CV = 0.52) and 1,000 (CV = 0.32) individuals, respectively) and density was associated to a latitudinal and longitudinal gradients in the characteristics of the rivers. Smaller density and population size in Tocantins River was attributed to possible effects of the Tucuruí Dam, and in Guaviare River to the watershed features. The use of post-stratification techniques minimized the influence of spatial heterogeneity across the study areas, and resulted in a substantial reduction in the CV (as much as 70%) of the estimates. This study provides improvements in analytical methods and contributed with new estimates of abundance in new regions for both species of river dolphins in South America.

1. INTRODUCTION

Freshwater cetaceans such as the boto (*Inia spp*) and the tucuxi (*Sotalia fluviatilis*) inhabit complex ecosystems through their distribution range. These two river dolphins occur in the major tropical river basins: 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 result in modified distribution patterns of the dolphin's preys 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 key role in distribution patterns of aquatic and terrestrial fauna (Naiman et al., 2002, Ward & Tockner 2001). 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 process, intense fishing exploitation, cattle raising) can change riverine landscapes (Gregory 2006) and cause shifts in the dolphin's distribution patterns. Thus, geographic stratification of the study area, in the case of river dolphins, can improve precision of the estimates and be beneficial for management (Thomas et al. 2010).

Considering the complex dynamics of the ecosystems inhabited by river dolphins, it is desirable to implement analytical and sampling methods that take into account the specificities of each river, taking them as sample units. Therefore, the objective of this chapter is providing new population estimates for river dolphins boto and tucuxi for three different major rivers in the Amazon, Orinoco and Tocantins-Araguaia basins, as well as propose improvements in analytical methods used, seeing the complexity of the study areas.

2. MATERIAL AND METHODS

2.1 STUDY AREA

Between May 2006 and June 2018, 31 surveys were conducted in large rivers of six countries in South America (Fig. 9; Table 4), covering more than 30.000 km in the three major river basin of the tropical rainforest: Amazon, Orinoco and Tocantins-Araguaia. 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 Guayana 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. 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 relation to their drainage areas, the Amazonian floodplains are most extensive. Details on each survey are presented in the Table 4.

The Tocantins-Araguaia river basin is the largest hydrographic basin entirely in Brazilian territory, flowing from the Brazilian Shields into the Atlantic Ocean alongside the Amazon River (Goulding et al. 2003). The two basins have become disconnected during the transition of the Pliocene to the Pleistocene period, remaining linked by a narrow channel in the Amazon delta where the Tocantins River drains (Rossetti & Valeriano 2007). This basin is formed by the Araguaia and Tocantins Rivers, being Tocantins the largest clear-water river in Brazil (length ~ 2600 km) characteristically deprived of nutrients, ions, and sediments (Sioli 1984, Junk & Furch 1993).

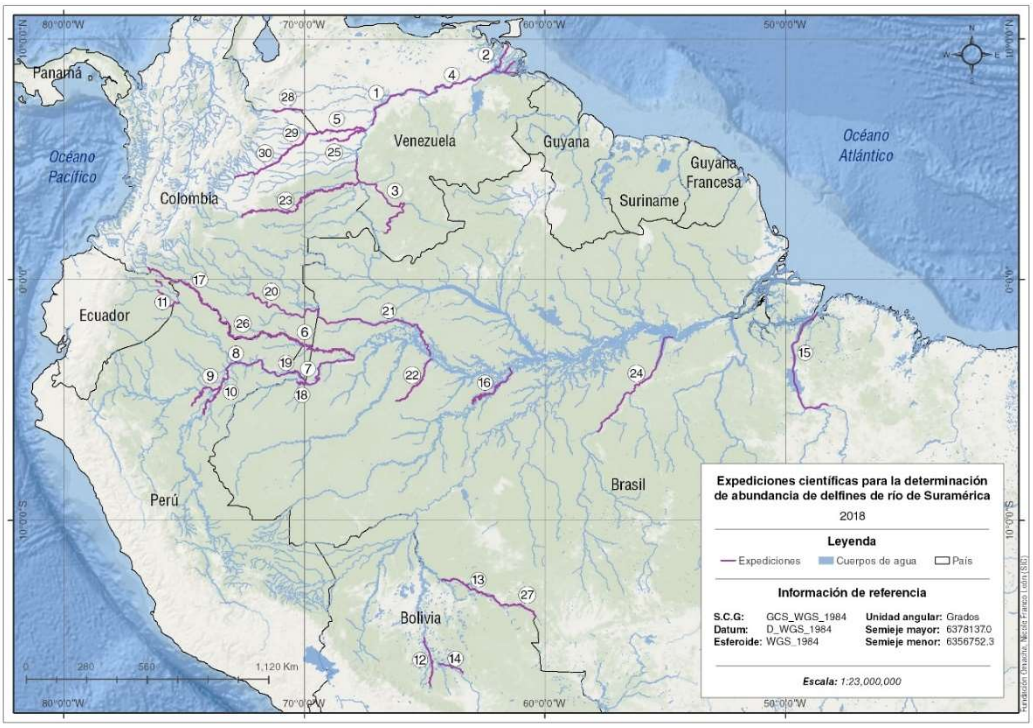

Figure 9. Map of surveys conducted in rivers of the Amazon, Orinoco, and Tocantins-Araguaia basins. **Source:** Fundación Omacha (2018).

Table 4. Surveys conducted detailed by region and time of study conduction.

River	*Basin*	*Country*	*Date*
Orinoco Middle	Orinoco	Venezuela	2006
Samiria and Marañon	Amazon	Peru	2006
Ucayali	Amazon	Peru	2006
Napu, Yasuni, Guayabero	Amazon	Ecuador	2006
Amazonas	Amazon	Colombia - Peru - Brazil	2007
Mamore	Amazon	Bolivia	2007
Itenez	Amazon	Bolivia	2007
Grande	Amazon	Bolivia	2007
Javaria	Amazon	Brazil	2007
Loretayacu	Amazon	Colombbia	2007
Meta	Orinoco	Colombia	2008
Orinoco Delta	Orinoco	Venezuela	2009
Putumayo	Amazon	Colombia	2009
Putumayo Middle	Amazon	Colombia	2010
Purus	Amazon	Brazil	2012
Orinoco South	Orinoco	Venezuela	2013
Tefé	Amazon	Brazil	2013
Orinoco Middle	Orinoco	Venezuela	2014

Tocantins	Tocantins-Araguaia	Brazil	2014
Japura and Caquea	Amazon	Colombia - Brazil	2014
Tapajós	Amazon	Brazil	2014
amazonas - Iquitos	Amazon	Peru	2015
Caqueta	Amazon	Colombia	2015
Guaviare	Orinoco	Colombia	2016
Bita	Amazon	Colombia	2016
Putumayo, Amazonas	Amazon	Colombia, Peru, Brazil, Ecuador	2017
Itenez	Amazon	Bolivia	2017
Arauca	Orinoco	Colombia - Venezuela	2017
Arauca	Orinoco	Colombia	2018
Meta	Orinoco	Colombia	2018-I
Meta	Orinoco	Colombia	2018-II

2.2 SURVEY DESIGN

Visual boat-based surveys were carried out to compute abundance estimates for river dolphins boto and tucuxi. Using standardized methods, sampling was performed using a combination of transects running parallel (200 m strip-width transect) and cross-channel (line) to the shore as proposed by Gómez-Salazar et al. (2012a) and detailed in the chapter two. A field stratification of the study area into seven habitat types (main river margin, main river channel, tributary river, channel, island, lake, and confluence) was delineated in order to incorporate variation of distribution and trends in density of animals in the complex riverine ecosystem (Vidal et al. 1997, Martin & da Silva 2004, Gómez-Salazar et al. 2012a, Pavanato et al. 2016).

2.3 DATA ANALYSIS

Data analyses were performed using the packages Distance and MRDS in R Program version 3.4.3 (R Core Team 2015). The analyses were conducted in four steps as follows:

(i) Estimation of detection probability in line transect for:

  a. Global detection function: develop of new general detection function curve and models, for each species, testing covariates not tested in the traditional method proposed;

  b. River-specific detection function;

(ii) Estimation of detection probability in strip transects;

(iii) Estimation of global and river-specific g(0);

(iv) Density and abundance estimates for Purus, Tocantins and Guaviare (Fig. 10) evaluating post-stratification for including variance and trends in density as function of hydro-geomorphology, when needed.

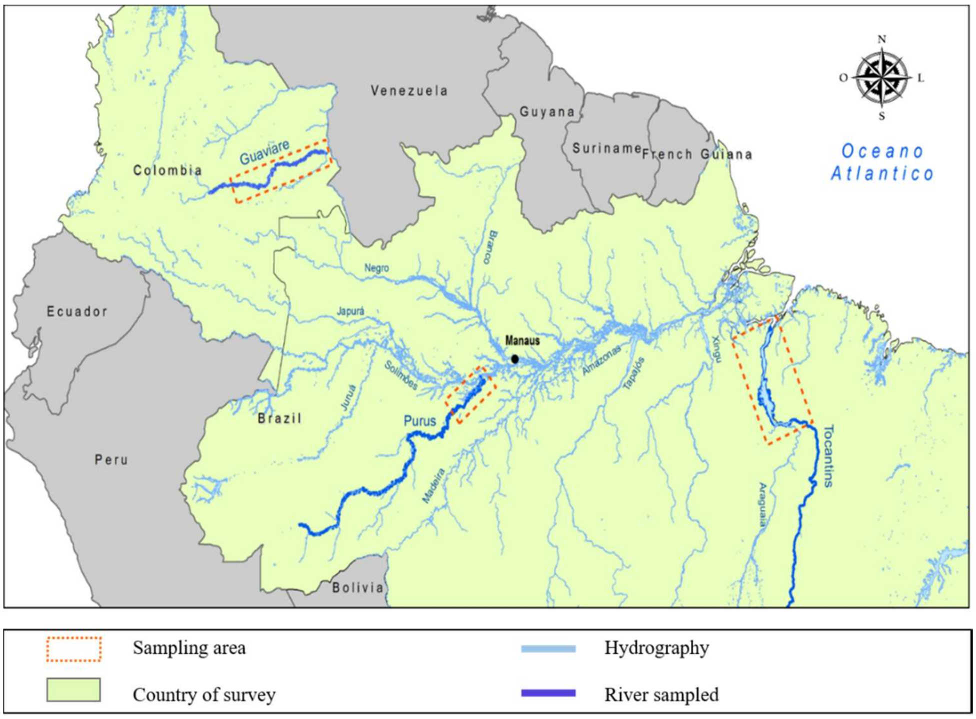

Figure 10. Study areas used to estimate density and abundance of the boto and the tucuxi.

General cross-channel dataset of all rivers surveys were used in the items (i)a, (ii) and (iii). Apart from the estimation of a common detection function, density and abundance of Purus, Tocantins and Guaviare rivers were computed separately. Cross-channel transects were used to estimate density for the habitat type “river channel” (center of the river), and parallel transects (strip transects) were used to estimate density in the other habitat types (river margin, channel, island, confluence, lake, tributary).

(i) Estimation of detection probability in line transect (cross-channel)

Data from all rivers for which cross-channel line transects were conducted were pooled for analysis. The effort for this analyses comprises 1085 linear transects in 1727.5 km, 340 and 251 sighting of botos, and tucuxis (Table 5). The overall number of sightings was reduced to 544 observations (325 boto and 219 tucuxi records) after checking for inconsistences (missing data of sight and inconsistence in covariates collection).

Table 5. Summary of line transect data conducted across 22 surveys from 2006 to 2017, where (k) is number of transects, (L) realized effort, (n boto) and (n tucuxi) number of sighted groups of each species, (n) the overall number of sights – join species.

River	*Basin*	*Country*	*Date*	*River Seasoning*	*Water Type*	*k*	*L*	*n Inia*	*n Sotalia*	*n*
Maranon	Amazon	Peru	2006	Dry	Branca	46	45.9	18	12	30
Orinoco	Orinoco	Colombia/Venezuela	2006	Dry	Branca	89	103.4	7	9	16
Napo	Amazon	Ecuador	2006	Flooded	Branca	10	13.13	0	0	0
Javari	Amazon	Colombia	2007	Rasing	Branca	18	18.5	2	1	3
Amazonas	Amazon	Colombia	2007	Rasing	Branca	19	29.3	2	2	4
Orinoco	Orinoco	Venezuela	2009	Dry	Branca	44	68.3	5	8	13
Meta	Orinoco	Colombia	2012	Falling	Branca	91	196.1	14	0	14
Purus*	Amazon	Brazil	2012	Flooded	Branca	27	69.6	60	93	153
Cassiquiare	Orinoco	Venezuela	2013	Dry	Mista	43	37.4	5	0	5
Orinoco	Orinoco	Venezuela	2013	Dry	Branca	148	125.5	8	0	8
Tefé	Amazon	Brazil	2013	Rasing	Mista	20	29.25	10	5	15
Apaporis	Amazon	Colombia	2014	Rasing	Preta	5	4.5	1	1	2
Aranapu	Amazon	Brazil	2014	Rasing	Mista	1	1.14	2	0	2
Caqueta	Amazon	Colombia	2014	Rasing	Branca	10	10	1	1	2
Orinoco	Orinoco	Venezuela	2014	Rasing	Branca	60	110.5	9	29	38

Tapajós	Amazon	Brazil	2014	Falling	Clara	37	89	7	19	26
Tocantins*	Tocantins-Araguaia	Brazil	2014	Rasing	Clara	133	276	96	3	99
Solimões	Amazon	Brazil	2014	Rasing	Branca	38	102.1	13	15	28
Japurá	Amazon	Brazil	2014	Rasing	Branca	69	101.3	20	46	66
Guaviare*	Orinoco	Colombia	2016	Falling	Branca	89	133.7	32	0	32
Napo	Amazon	Ecuador	2017	Rasing	Branca	19	47.27	1	0	1
Putumayo	Amazon	Colombia	2017	Falling	Branca	69	115.6	27	7	34

*(*) Data of cross-channel line transect used for fitting detection function for Purus, Tocantins and Guaviare Rivers in the item (*iv*) of the analysis.*

a. Global detection function

Cross channel transects were analyzed following distance sampling (DS) methods (Buckland et al. 2001, Marques & Buckland 2003). Exploratory analyses were performed in the dataset to assess appropriate truncation distances and to evaluate whether binning the data into pre-specified distance intervals would improve the fit of detection probability models. Truncation distance was defined as 200 m by visually inspection of the perpendicular distances histogram and by the results presented in chapter three. Detection probability was estimated by fitting half-normal and hazard-rate models to perpendicular distance with no adjustments using Conventional Distance Sampling analysis (CDS) or Multiple Covariate Distance Sampling (MCDS). In the latter, covariates were included in candidate detection probability model individually or in combination. Covariates considered in these models are listed in Table 6. Model selection was performed using the Akaike's Information Criteria (AIC).

Table 6. Candidate covariates teste in the detection function models

Candidate covariates tested in the detection function models		
Covariate	**Factor/Numeric**	**Description (range of values)**
Group size (*gs*)	Numeric	*Inia geoffrensis* (1-15), *Sotalia fluviatilis* (1-22)
Sighting Platform (*pt*)	Factor	Bow (1) and Stern (2)
River Season (*rs*)	Factor	Razing waters, Flooded, Falling waters, Low waters
River State (*r*)	Factor	Mirror (Beaufort scale 0), calm (Beaufort scale 1), moderated (Beaufort scale 2), ripple (Beaufort scale 3)
Water Type (*w*)	Factor	White (W), Black (B), Clear (C), Mixed (M)
Glare Strength (*gl*)	Factor	No glare (0), low (1), moderated (2), intense (3)
Sightability (*sight*)	Factor	low (1), moderated (2), good (3), optimal (4)

b. River-specific detection function

River-specific detection function were performed for the rivers Tocantins, Purus and Guaviare. For these river, line transect were optimal conducted with more than 60 sightings in Tocantins and Purus Rivers and at least 30 sighting in Guaviare River, allowing fitting a detection probability curve. Evaluation whether binning the data into pre-specified distance intervals resulted in distances grouped in bins of 30 m to improve the fit of detection probability models. Detection probabilities models were performed for each river following the same steps described in item a.

(ii) Estimation of detection probability in strip transects

The estimated parameters of the best-fitted model of detection function were used to update the estimated mean proportion of animals detected in different sections of the strip (P_k) as in Gómez-Salazar et al. (2012a). P_k (P_1 and P_2) corresponds to the detection probability (P) within each 50 m of the strip, where k = 1 for the perpendicular distances within 0-50 m (eq. 1) and k = 2 for distances within 50-100 m (eq. 2). These values are computed based on the detection functions to correct for undetected animals within each section of the strip.

$$P_{0-50} = \frac{\int_0^{50} g\,(x)}{50} \quad (eq. 1)$$

$$P_{50-100} = \frac{\int_{50}^{100} g\ (x)}{50} \quad (eq.2)$$

Where, *g(x)* is a detection probability function of estimated parameters (shape and scale) of the best fitted model of general cross-channel line transect analyses.

A third *P* value ($k = 3$) was calculated for those rivers where the mean width is ~ 300 m. In these rivers, dolphins are distributed similarly to the gradient observed in the strip width of 200 m, and the navigation in these regions are best conducted in the center of the river. P_3 was calculated as the probability of estimating dolphin groups between 100 and 150m from the trackline $\mathrm{P}_{100-150}$.

(iii) Estimation of g(0)

A previous 'global' g(0) was estimated by Gómez-Salazar et al. (2012a) as 0.947 (CV = 0.025) for Inia species and 0.994 (CV = 0.003) for *Sotalia fluviatilis*. These estimates were updated here with the addition of 24 new surveys conducted since the work of Gomez-Salazar et al. (2012a) was completed.

The new 'global' g(0) was estimated for the boto and the tucuxi using double-platform detections in a capture-recapture framework (Laake & Borchers 2004) using general cross-channel line transects as proposed by Gómez-Salazar et al. (2012a):

$$\mathrm{g}(0) = \left(1 - \left({}^{\mathrm{n01}}/_{\mathrm{n1}}\right)^2\right) \quad (\mathrm{eq.}\,3)$$

where n_1 is the number of groups sighted from the second platform within 50 m of the transect line, and n_{01} the number of these that were missed by the first platform. An estimate of the coefficient of variation of this estimation also follow Gómez-Salazar et al. (2012a) methods. River-specific g(0) were computed for Tocantins, Purus and Guaviare Rivers.

(iv) Density and Abundance Estimates

Density and abundance estimates were computed for the lower Purus River, the Tocantins River, and the Guaviare River. The comprised effort and area covered by each survey is shown in the table 7.

Table 7. Summary of effort (km) and area (km²) covered in the surveys conducted in Purus, Tocantins and Guaviare rivers.

River	Date	River Basin	Effort	Area
Purus	2012	Amazonas	512.05	355.95
Tocantins	2014	Tocantins-Araguaia	585.81	2657.4
Guaviare	2016	Orinoco	986	593.75

a. Post-Stratification

When field stratification in habitat types was not enough to explain high variance in density, post-stratification was used to minimize the effect of the significant heterogeneity of densities across the study area. This was the case of Guaviare and Tocantins rivers. For these rivers, sets of transects were grouped in sub-regions (strata) as recommended by Thomas et al. (2007, 2010) and Fewster et al. (2009). In the Guaviare River, three sub-regions were proposed as lower, middle and upper river, considering the river length; and in the Tocantins River three sub-regions were establishedas downstream, reservoir (artificial lake) and upstream of the Tucuruí dam, that changed the natural river course

Density and abundance for river channel (center of the rivers) where line transect were performed, were calculated as follow:

$$D_{ij} = \frac{n_{ij}\, E_{ij}\, f(0)}{2L_{ij}\, g(0)} \quad (eq. 4)$$

where *n* is the number of groups sighted in habitat type *i* and strata *j*, *E* is the estimated mean group size in habitat type *i* and strata *j*, *f(0)* is the sighting probability density at zero perpendicular distance (or the inverse of the effective half strip width [*ESW*] → $f(0) = 1/\mathrm{ESW}$), *L* is the total transect length in habitat type *i* and strata *j*. and *g(0)* the probability of seen a group of distance zero on the transect line. Empirical variances, standard errors and CV's were estimated in DS methods (Buckland et al. 2001, Thomas et al. 2010, Fewster et al. 2009).

The method proposed by Gomez-Salazar et al. (2012a) was used to estimate density in strip transects by habitat types and strata as follows:

$$D_{ij} = \frac{E_{ij}\left[\frac{n_{0-50}}{P_2} + \frac{n_{50-100}}{P_1} + \frac{n_{100-150}}{P_1} + \frac{n_{150-2}}{P_2}\right]}{WL_{ij}g(0)} \quad (eq. 5)$$

where *D* is the estimated density in habitat type *i* and strata *j*, E is the estimated group size for the population in habitat type *i* and strata *j*, *L* is the total length of the parallel transects conducted in habitat type *i* and strata *j*, and *W* is the strip width (200 m). P_1 and P_2 (P_k) were estimated in the general cross-channel line transect analyses in the equations 2. The overall density is the mean of stratum-specific density estimates, weighted by the effort carried out in each strata.

b. Population Size and variances

Finally, we obtained abundance by habitat type and strata through:

$$\mathrm{N_{ij} = D_{ij}\,A_{ij}} \quad \text{(eq. 6)}$$

where A_{ij} corresponds to the area (in km^2) of each habitat type and in each stratum (when applicable).

Areas were calculated using satellite images in a period of the year as close as possible to the season the survey was conducted. The satellite images of each area (from Purus, Tocantins and Guaviare) were imported to ArcView software version 10.3 (ESRI 2000). Polygons for each of the habitat type in each the river system were then created to calculate the region-specific area.

Standard errors (SE) and coefficient of variations (CV) were obtained for each habitat type following Gómez-Salazar et al. (2012a) for each region. The overall population size (N_t) was calculated as the sum of abundance in each habitat type or strata (depending of the river), and the coefficient variation (CV) of the total estimate was calculated as:

$$\mathrm{CV(N_t)} = \frac{\sqrt{\sum \mathrm{SE(N_i)}}}{\sum \mathrm{N_i}} \quad \text{(eq. 7)}$$

where: N_i is the abundance in each region/stratum and SE(N_i) is the standard error associated with N_i.

3. RESULTS

3.1 GENERAL CROSS-CHANNEL LINE TRANSECTS: UPDATING GLOBAL DETECTION PROBABILITIES AND GLOBAL G(0).

A total of 283 unique groups (n = 184 for bow platform detections and n = 99 of new stern detections) were used to fit the detection function for the boto after accounting for groups detected by both platforms (total of 325 sightings). The number of detections made from the two platforms (confirmations/duplicates) for boto was 42 groups. A total of 189 unique groups (*n* = 163 for bow platform detections and *n* = 26 of new stern detections) were used to fit the detection function for the tucuxi after accounting for duplicate sightings (219 groups in total).

Detection probability models proposed by the boto and the tucuxi are given in Tables 8 and 9. The hazard-rate was the most supported model according to the AIC for both the boto and the tucuxi. The most supported model for the boto was the hazard-rate with platform (*pt*) and group size (*gs*) as covariates. But a model that incorporated sightability (sight) was also well supported (within 2 AIC units, Table 8). Irrespective of the model used, however, the detection probability estimated for all models within two AIC units was similar (*P* ranged from 0.37 and 0.39)

For tucuxi the most supported model was that one combining the covariates river season (*rs*), platform (*pt*) and group size (*gs*) (Table 9). However, sightability was also included in combination with some of these covariates in models with AIC within 2 units of the most-supported model. As observed for the boto, detection probability estimated for all models with delta AIC >= 2 were similar (*P* ranged from 0.26 to 0.27) (Table 7).

Models are listed in tables 8 and 9 are in ascending order of ΔAIC values. Plots of the detection function for the best model and Q-Q goodness of fit plots are shown in figure 11.

Table 8. Conventional Distance Sampling (CDS) and Multi Covariate Distance Sampling (MCDS) models for boto (*Inia*) with Hazard-rate (hr) distributions and covariates. Corresponding AIC's criterion, ΔAIC, detection function probability (Average (*P*)) and coefficient of variation (CV (*P*)) are shown. The best fitted model is shown in **bold** and supported models within 2 AIC units delimited with dashed lines.

Modelo	*AIC*	Δ*AIC*	*P*	*CV*
hr + *pt* + *gs*	**-907.26**	**0.00**	**0.39**	**0.12**
hr + *pt* + *sight*	-906.58	0.68	0.38	0.12
hr + *pt* + *r*	-906.22	1.04	0.37	0.12
hr + *pt* + *gs* + *sight*	-905.98	1.28	0.39	0.12
hr *null*	-905.23	2.03	0.39	0.12
hr + *gs*	-904.62	2.64	0.40	0.11
hr + *pt* + *gs* + *rs*	-904.42	2.84	0.39	0.11
hr + *pt* + *rs*	-904.21	3.05	0.39	0.11
hr + *pt* + *gl*	-903.72	3.54	0.36	0.13
hr + *r*	-903.48	3.78	0.39	0.12
hr + *pt* + *w*	-902.49	4.77	0.35	0.14
hr + *rs*	-902.43	4.83	0.40	0.11
hr + *pt* + *gs* + *w*	-902.41	4.85	0.35	0.14
hr + *w*	-901.56	5.70	0.35	0.14
hr + *gl*	-901.33	5.93	0.36	0.13
hr + *sight*	-882.96	24.30	0.54	0.04

Table 9. Conventional Distance Sampling (CDS) and Multi Covariate Distance Sampling (MCDS) models for tucuxi (*Sotalia fluviatilis*) with Hazard-rate (hr) distributions and covariates. Corresponding AIC's criterion, ΔAIC, detection function probability (Average (*P*)) and coefficient of variation (CV (*P*)) are shown. The most supported model is shown in **bold** and supported models within 2 AIC units delimited with dashed lines.

Modelo	*AIC*	Δ*AIC*	*P*	*CV*
hr + *rs* + *gs* + *pt*	**-733.21**	**0.00**	**0.27**	**0.20**
hr + *rs* + *gs*	-732.01	1.20	0.27	0.18
hr + *rs* + *gs* + *sight*	-732.00	1.21	0.26	0.18
hr + *rs* + *gs* + *pt* + *sight*	-731.37	1.83	0.27	0.19
hr + *rs* + *gs* + *pt* + *w*	-730.66	2.55	0.26	1.31
hr + *rs* + *gs* + *r*	-730.50	2.70	0.27	0.18
hr + *rs* + *gs* + *pt* + *sight* + *w*	-730.46	2.74	0.26	0.37
hr + *rs* + *gs* + *w*	-729.47	3.74	0.26	0.81
hr + *rs* + *gs* + *pt* + *gl*	-729.30	3.90	0.26	0.19
hr + *rs* + *sight*	-729.20	4.00	0.27	0.18
hr + *rs* + *pt*	-728.87	4.34	0.27	0.20
hr + *rs* + *gs* + *pt* + *r*	-728.71	4.49	0.27	5.28
hr + *rs*	-728.01	5.20	0.27	0.19
hr + *rs* + *gs* + *gl*	-727.92	5.28	0.26	0.18

hr + *rs* + *r*	-727.30	5.91	0.27	0.19
hr + *rs* + *gl*	-725.62	7.59	0.26	0.19
hr + *rs* + *w*	-724.56	8.64	0.27	0.19
hr + *w*	-721.83	11.37	0.27	0.18
hr *null*	-685.53	47.67	0.28	0.16
hr + *gs*	-684.15	49.06	0.30	0.15
hr + *pt*	-684.05	49.16	0.27	0.16
hr + *r*	-683.92	49.29	0.28	21.79
hr + *gl*	-680.40	52.81	0.26	0.17
hr + *sight*	-674.53	58.68	0.46	0.05

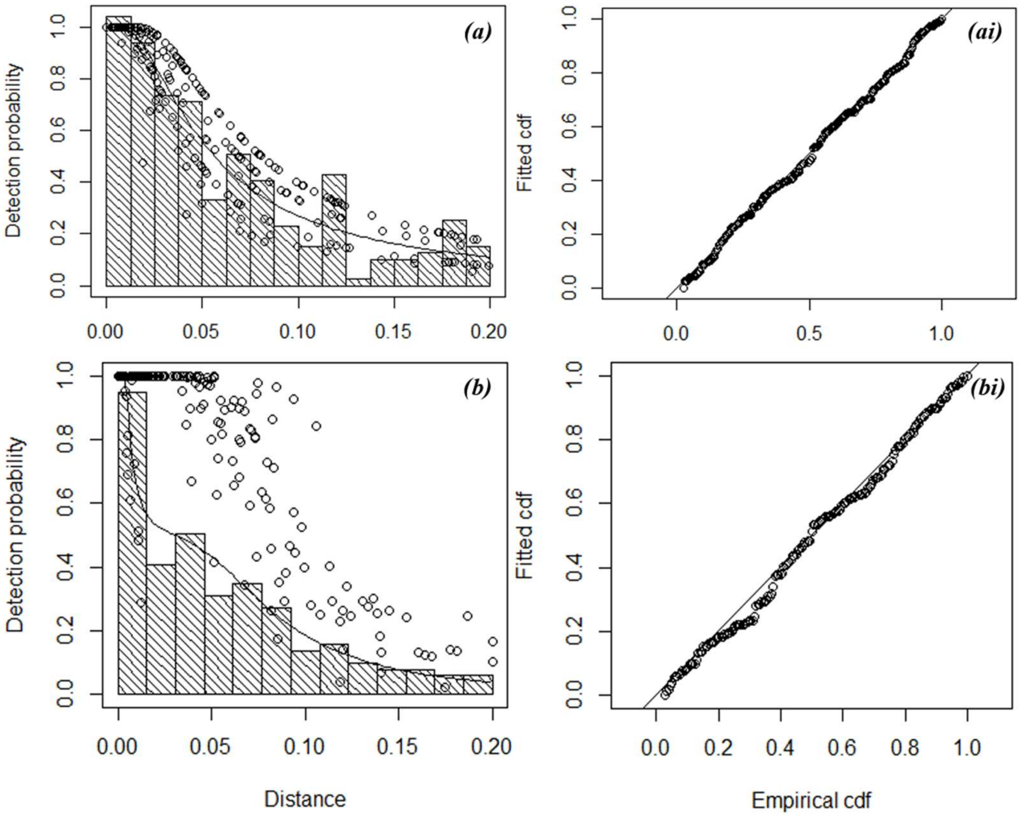

Figure 11. Detection function for the most supported model for ***(a)*** boto and ***(b)*** tucuxi. The line corresponds to the average detection probability (Hazard-rate model), ***(ai)*** Q-Q plot of cumulative distribution function (CDF) of the fitted detection function to the distribution of the data (empirical distribution function or EDF) for boto and ***(bi)*** Q-Q plot for tucuxi.

The probability of missing dolphins on the trackline estimated from the equation 3 was g(0) = 0.814 (CV = 0.053) for boto and g(0) = 0.989 (CV = 0.006) for tucuxi. New P_k parameters were estimated for boto as P_1 = 0.960 and P_2 = 0.630 (*shape* = 0.37 (SE =

0.12), *scale* = -2.61 (SE = 0.42)) and scale parameters , and for tucuxi as P_1 = 0.998 and P_2 = 0.893 (*shape* = 0.99 (SE = 0.15), *scale* = -2.24 (SE = 0.41)). Detection probability estimated for groups between 100 and 150m from the trackline *(*P_3*)* as 0.375 for boto and 0.485 for tucuxi.

3.2 DENSITY AND ABUNDANCE ESTIMATES

Purus River

The total effort covered in Purus River was 512.05 km, from which 75.44 km was in line transects and 436.61 km in strip transects. Overall number of sightings in the river channel (line transect effort) was 153, from which 60 (*n* = 125 individuals) were observation of boto species and 93 (*n* = 307 individuals) tucuxi. The majority of boto and tucuxi sightings were obtained while conducting strip transects, ~ 85% (330 observations, *n* = 644) and 76% (438 observations, *n* = 1597) for each species respectively.

From the 153 groups sighted in line transect, 127 were bow platform detections and 26 new stern platform detections. The number of detections made from the two platforms (confirmations/duplicates) was high n = 101 groups, and new detection from the stern platform contributed with an increment of 17% in detections. The g(0) was estimated for boto as 0.862 (CV = 0.09) a probability of missing dolphins in the trackline of 18%, and for tucuxi as 0.991 (CV = 0.008) or less than 1% of probability of missing this species in the trackline.

The hazard-rate model of detection function considering group size as covariate was the best fitted model according to the AIC (Table 10, Fig. 12). This model was then used to estimate density in the river channel for both taxa. Models with platform and river state covariates were also supported models within the 2 units of AIC, evidencing the contribution of the second platform and the good condition of the river in the detection efficiency. The higher ranking model with species as covariate had a delta AIC value of 3.54, suggesting that species had a small effect in the detection probability of river dolphins in the Purus River.

The population sizes estimated in Purus River for the boto and the tucuxi, were, respectively, 7672 individuals (CV = 0.37) and 9238 individuals (CV = 0.49) (Table 11). The estimated abundance for both of these river dolphins species in this river is high as a result of the greatest densities reported for these species. Highest density for boto was found for the habitat type river margin, while for tucuxi was de river channel (Table 11). In addition, density of botos in the tributary and islands was substantially higher than the density of tucuxi in these same habitats (Table 11), demonstrating a clear partitioning of the habitat by these species. The small area sampled in the habitat type tributary resulted in high stratum-specific CV, as it did for confluences (Table 11).

Table 10. Conventional Distance Sampling (CDS) and Multi Covariate Distance Sampling (MCDS) models for joint detections of river dolphins in Purus River with Hazard-rate (hr) distributions and covariates. Corresponding AIC's criterion, ΔAIC, detection function probability (Average (*P*)) and coefficient of variation (CV (*P*)) are shown. The best fitted model is shown in **bold** and supported models within 2 AIC units delimited with dashed lines.

Model	*AIC*	Δ*AIC*	*P*	*CV*
hr + *gs*	**-528.75**	**0**	**0.28**	**0.13**
hr + *gs* + *p*	-530.66	1.91	0.28	0.13
hr + *gs* + *r*	-530.74	2.00	0.28	0.13
hr *null*	-531.78	3.03	0.27	0.12
hr + gs + r	-532.18	3.43	0.28	0.13
hr + *sp*	-532.28	3.54	0.28	0.11
hr + *r*	-533.69	4.95	0.27	0.12
hr + *p*	-533.77	5.02	0.27	0.12
hr + *gs* + *p* + *r*	-534.06	5.31	0.28	0.13
hr + *sp* + *p*	-534.27	5.53	0.28	0.11
hr + *r* + *p*	-535.68	6.93	0.27	0.12

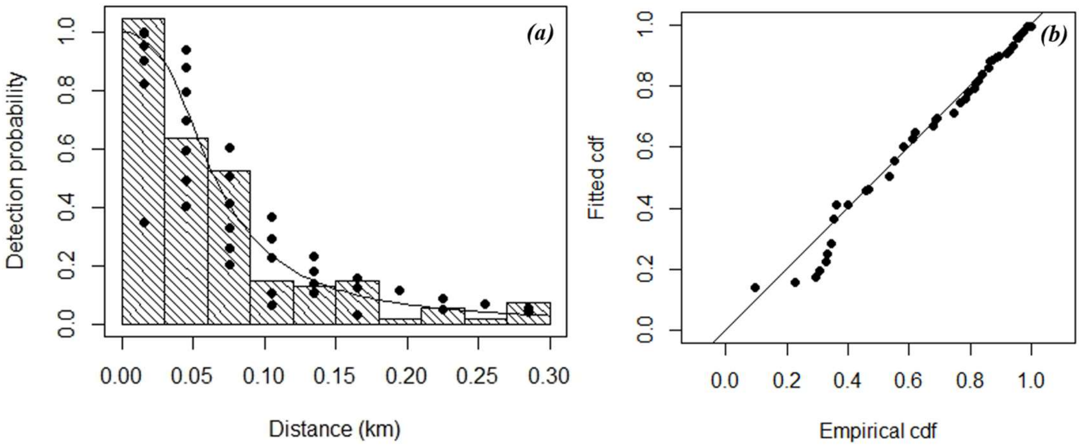

Figure 12. **(a)** Detection function for the most supported model. The line corresponds to the average detection probability (Hazard-rate model), ***(b)*** Q-Q plot of cumulative distribution function (CDF) of the fitted detection function to the distribution of the data (empirical distribution function or EDF).

Table 11. Estimates (overall and by habitat/stratum) of groups size (E[s]), encounter rate (Er), density (D), abundance (N), coefficient of variation (CV) and area of inference (km²) for boto and tucuxi in the Purus River.

Habitat	***E(s)***	***Er***	***D***	***N***	***CV***	***Area***
Boto						
River Margin	1.89	1.99	33.88	5959.15	0.3	175.89
River Channel	1.88	0.75	7.95	1165	0.21	146.64
Channel	1.86	1.49	12.79	30.31	0.77	2.37
Island	1.75	1.49	8.63	61.27	0	7.1
Confluence	2.22	2.22	23.91	66.46	0.7	2.78
Tributary	2.5	0.56	13.72	203.33	1.16	14.82
Lake	2.31	0.61	0.95	186.409	0.88	196.22
Total	2.05	1.30	14.54	7672	0.37	538.72
Tucuxi						
River margim	3.31	4.3	21.69	3815.05	0.52	175.89
River Channel	3.3	1.23	34.96	5126	0.4	146.64
Channel	2.93	0.82	17.95	42.54	0.68	2.37
Island	2	0.37	0.69	4.89	0	7.1
Confluence	3.55	2.23	12.1	33.63	1.27	2.78
Tributary	4	0.19	0.69	10.2258	1.89	14.82
Lake	3.91	0.94	1.05	206.03	0.91	196.22
Total	3.29	1.44	12.73	9238	0.49	538.72

Tocantins River

Total trackline effort in the Tocantins River was 585.81 km, with 275.58 km surveyed in line transects and 309.24 km in strip transects. The study area was post-stratified into three sub-regions (strata) due to the presence of a hydroelectric dam (the Tucuruí Dam). Search effort carried out in each strata is shown in Table 12.

A total of 138 groups of botos (*n* = 198 individuals) and nine groups of tucuxi dolphins (*n* = 17 individuals) was observed. The population size of tucuxi dolphins could not be calculated with any accuracy due to the low number of sightings, and because they were concentrated in a small region of the river. The results presented here, therefore, focus on Araguaian boto.

From the 138 groups, 92 (n = 131 individuals) were sighted in river channel (line) transects and 46 (*n* = 67) in strip transects (Table 12). More groups were sighted in line transects because this methods were performed in the entire habitat type reservoir – the artificial lake created by Tucuruí dam where navigation in complicated close to the shores and to conduct strip transects, besides the largest area covered by this lake (Table 12).

Table 12. Search effort conducted across the Tocantins River by strata, where (*k*) is number of transects, (*L*) realized effort and (*n*) number of sightings. Area is expressed in km² and (-) indicates no effort.

Strata	**Area**	**Line**			**Strip**		
		L	***k***	***n***	***L***	***k***	***n***
Downstream	1169.4	67.8	34	4	184.8	81	21
Reservoir 1	404	42.1	17	4	-	-	-
Reservoir 2	699	94	43	52	-	-	-
Upstream	385	72.7	39	32	124.5	58	25

From the 92 groups sighted in line transects, 81 (*n* = 29 for bow platform detections and *n* = 52 of new stern detections) were used to fit the detection function after accounting for groups detected by both platforms. The number of detections made from the two platforms (confirmations/duplicates) was low (*n* = 11 groups, 13%), thus new detections from the stern platform contributed with more than 60% of detections of all groups detected.

The estimated g(0) was 0.659 (CV = 0.262), suggesting a probability of missing dolphins on the trackline higher than 30%. Data were truncated to 300 m in this analyses because in this survey dolphins were sighted in greater numbers within a wider strip than usual. The hazard-rate model with platform as covariate was most supported detection probability model according to the AIC (Table 13, Fig. 13). This model was then used to estimate density in the habitats river channel and dam reservoir (artificial lake), where line transects were surveyed.

The overall abundance of Araguaian botos was estimated at 736 individuals (CV = 0.52). The initial habitat stratification made prior to the survey, with sampling divided in six habitat types resulted in high stratum-specific and overall CVs (Table 14). Geographic post-stratifying the data to incorporate the high latitudinal and longitudinal trends in density in distinct areas of the study regions, including those under Tucuruí dam influence zone, reduced the CV by 70% (Table 14). However, the overall CV of the estimates was still high.

Densities decreased from the margin to the center of the river in all sections (downstream and upstream), but dolphins were concentrated at the center in the reservoir. High densities were observed in channels and near islands both downstream and upstream (Table 14). In general, lower densities were found downstream of the Tucuruí dam for all habitat types except for channels (Fig. 14). Density in the river margin was more than 60% higher upstream than downstream of the dam, and the resulting abundance estimation upstream of the dam was nearly twice the one downstream. Density within the reservoir habitat was highly variable, with point estimates decreasing gradually towards the dam (Fig. 15).

Table 13. Distance Sampling (DS) models for Araguaian boto with Hazard-rate (hr) distributions and covariates. Corresponding AIC's criterion, ΔAIC, detection function probability (Average (*P*)) and coefficient of variation (CV (*p*)) are shown. The best fitted model is shown in **bold** and supported models within 2 AIC units delimited with dashed lines.

Modelo	***AIC***	**Δ*AIC***	***P***	***CV***
hr + *pt*	**-343.01**	**0**	**0.20**	**0.22**
hr + *gs* + *pt*	-344.11	1.09	0.20	0.23
hr *null*	-345.01	2.00	0.19	0.22
hr + *gs*	-345.69	2.68	0.19	0.24

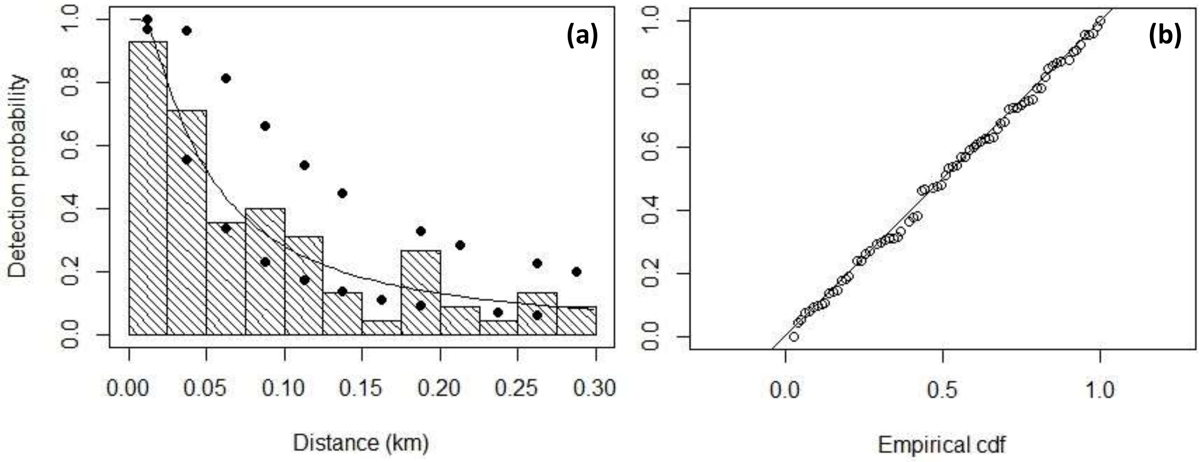

Figure 13. (a) Detection function for the most supported detection probability model for the boto in the Tocantins river. The line corresponds to the average detection probability (hazard-rate model) and dots the covariate levels for platform (*pt*). **(b)** Q-Q plot of cumulative distribution function (CDF) of the fitted detection function to the distribution of the data (empirical distribution function or EDF).

Table 14. Estimates (overall and by habitat/stratum) of groups size ($E[s]$), encounter rate (Er), density (D), abundance (N), uncertainty (CV) and area of inference (km²) for Araguaian boto in the Tocantins River.

Habitat	*E(s)*	*Er*	*D*	*N*	*CV*	*Area*
No post-stratification						
River margin	1.5	0.1	0.21	195	2.7	927.76
River channel	1.56	0.64	0.94	300	0.4	318.9
Reservoir	1.3	0.67	1.35	1489	0.3	1103
Channel	2	0.29	1.86	139	2.12	74.97
Island	1.1	0.74	0.7	163	0.36	232.76
TOTAL	1.41	0.40	1.16	2286.0	1.78	2657.40
With post-stratification						
River margim downstream	1.86	0.07	0.23	30	0.92	133.5
River channel downstream	1.44	0.05	0.02	16	0.67	794.2
Channel downstream	2	0.25	1.68	96	1.27	57.3
Island downstream	1.08	0.18	1.24	228	0.50	184.4
Reservoir part 1	1.25	0.09	0.02	8	0.56	404.0
Reservoir part 2	1.96	0.44	0.19	133	0.39	699.0
River margim upstream	1.40	0.10	0.72	63	0.53	87.4
River channel upstream	1.45	0.38	0.13	30	0.40	231.6
Channel upstream	1	0.10	1.06	19	1.81	17.7
Island upstream	1	0.21	2.32	112	0.27	48.4
TOTAL	1.44	0.19	0.76	736	0.52	2657.4

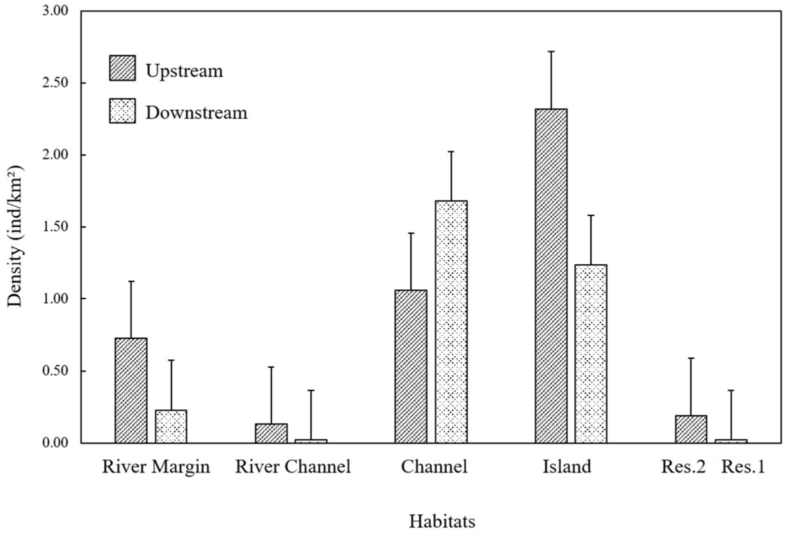

Figure 14. Decreasing of density by habitat type surveyed regarding the post-stratification towards the Tucuruí dam in Tocantins River. Bars represent the standard error (*SE*).

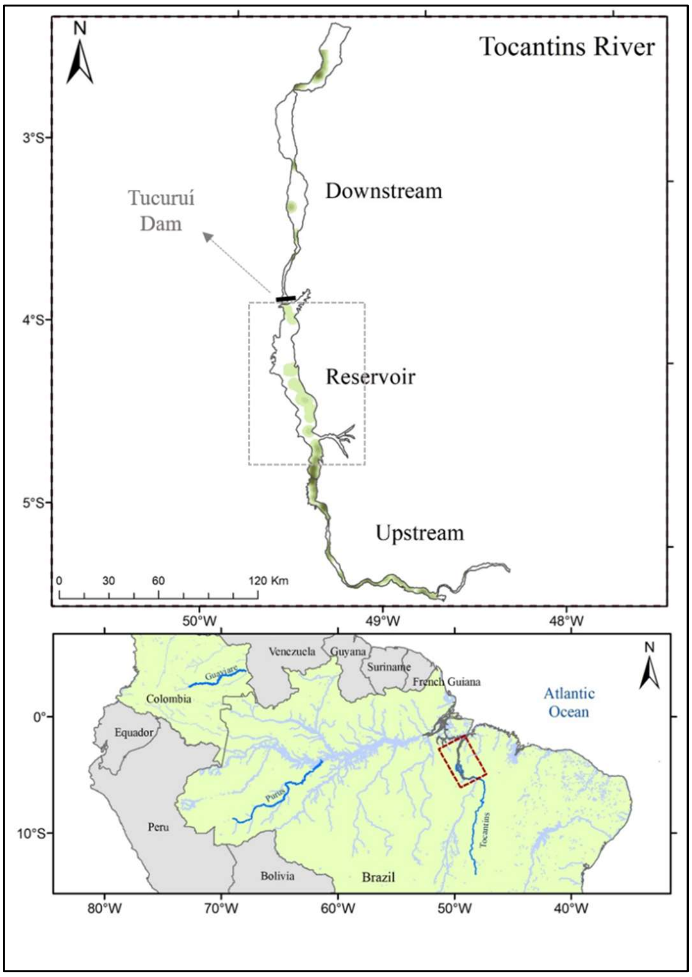

Figure 15. Map highlighting the gradually decreasing of density towards the Tucuruí dam in Tocantins River. Color gradient represents the plotted density across the study region.

Guaviare River

The total effort covered in Guaviare River was 986 km, from which 135 km was in river channel (line) transects and 851 km in strip transects. Overall number of sightings of boto was 261 groups ($n = 422$ individuals), with 32 groups ($n = 50$ individuals) detected in the river channel and 229 ($n = 372$ individuals) in strip transects. Searching effort carried out in each strata is shown in table 15. Tucuxi does not occur in this river.

Table 15. Searching effort conducted across the study area by strata, where (k) is number of transects, (L) is the realized effort and (n) the number of groups seen. Area is expressed in km^2.

Strata	**Area**	**Line**			**Strip**		
		L	***k***	***n***	***L***	***k***	***n***
Lower	187	36.7	28	9	319.7	127	96
Middle	304	64.2	41	23	489	194	126
Upper	100	33.9	21	0	139.9	60	7

From the 32 groups sighted in line transects, 24 (*n* = 14 and 10 for the front and stern platforms, respectively) were used to fit the detection function after accounting for groups detected by both platforms.

The hazard-rate model of detection function considering group size as covariate was the best fitted model according to the AIC (Table 16, Fig. 16). However, the simplest model with no adjustments (hr *null*) was considered for supported models (2 AIC units), and presented a smallest CV. Thus, the hr *null* was used to estimate density in the habitat river channel.

The number of detections made from the two platforms (confirmations/duplicates) was low *n* = 8 groups, new detection from the stern platform contributed with 33% of all groups detected. G(0) was estimated 0.71 (CV = 0.53) a probability of missing dolphins in the trackline of ~30%. The data were not truncated in this analyses so that no observations were missed, given the low number of sightings, one of the main reasons of the relatively high CV of g(0) estimates.

Table 16. Distance Sampling (DS) models for boto with and Hazard-rate (hr) distributions and covariates. Corresponding AIC's criterion, ΔAIC, detection function probability (Average (*p*)) and coefficient of variation (CV (*p*)) are shown. The best fitted model is shown in **bold** and supported models within 2 AIC units delimited with dashed lines.

Modelo	*AIC*	*ΔAIC*	*P*	*CV*
hr + *gs*	**-69.89**	**0**	**0.33**	**0.36**
hr + *gs* + *pt*	-68.90	0.99	0.31	0.43
hr *null*	-68.98	0.91	0.37	0.24
hr + *pt*	-67.38	2.51	0.36	0.35

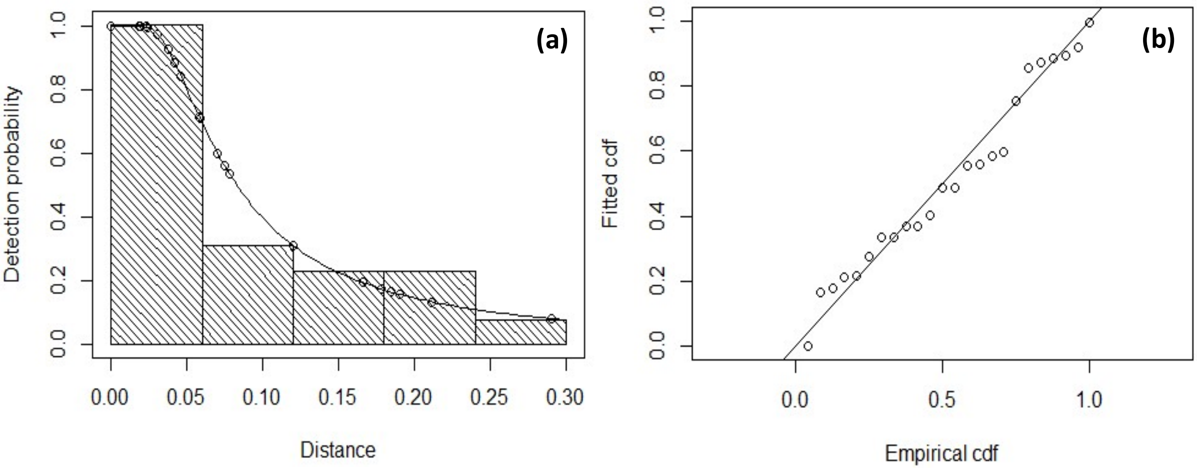

Figure 16. (a) Detection function for the most supported model. The line corresponds to the average detection probability (Hazard-rate model). **(b)** Q-Q plot of cumulative distribution function (CDF) of the fitted detection function to the distribution of the data (empirical distribution function or EDF).

The estimated abundance of botos was 1138 individuals (CV = 0.32), with N = 35 in the upper, N = 874 in the middle and N = 229 animals in the lower Guaviare River. The highest density was observed for the habitat type confluence for both middle and lower course of the Guaviare River. Confluences were absent in the upper region of the river. The only transect performed in the tributary river resulted in no sightings.

The initial habitat stratification made prior to the survey, with sampling divided in six habitat types considering the river as a whole unit resulted in relatively high stratum-specific and overall CV's (Table 17). Geographic post-stratification accounted for the relatively high variance in density in distinct areas of the river, and the estimate of abundance presented a CV nearly 30% lower (Table 17). No dolphin groups were sighted during 46 consecutive transects conducted in the upper Guaviare river and that was reflected in the CV of the no post-stratified analyses, mainly in the habitat type river margin (Table 17). Improvements in precision were also observed for the habitat channel from the middle to the lower course of the river, evidencing the heterogeneity of the ecosystem. High CVs have also resulted from the relatively small sample size in this habitat, but after post-stratification CVs of the estimated density and abundance were improved by more than 70% (Table 17).

Table 17. Estimates (overall and by habitat/stratum) of groups size (E[s]), encounter rate (Er), density (D), abundance (N), uncertainty (CV) and area of inference (km²) for boto Guaviare River.

Habitat	***E(s)***	***Er***	***D***	***N***	***CV***	***Area***
No post-stratification						
River margin	1.56	0.17	4.30	1885	1.76	438.43
River Channel	1.38	0.13	0.96	94	0.35	97.68
Channel	2.14	0.09	2.66	56	2.68	21.04
Island	0	0	0	0	0	33.58
Confluence	1.90	3.59	8.93	8	0.38	0.90
Tributary	0	0	0	0	0	2.12
TOTAL	1.16	0.66	2.81	2043	0.75	593.75
With post-stratification						
Upper						
River margin	1.2	0.05	0.46	34	0.46	75.03
River channel	0	0	0	0	0	9.74
Channel	0	0	0	0	0	3.07
Island	0	0	0	0	0	12.17
Confluence	0	0	0	0	0	75.03
Middle						
River margim	1.5	0.56	3.51	777	1.06	221.38
River channel	1.62	0.31	1.39	71	0.23	51.19
Channel	1.66	0.28	1.76	22	0.62	12.36
Island	0	0	0	0	0	19.06
Confluence	1.85	9.91	26.62	5	0.59	0.17

Lower						
River margin	1.56	0.38	1.43	203	0.35	142.04
River channel	1.5	0.27	0.11	4	0.19	36.75
Channel	2.5	0.54	1.91	11	0.64	5.61
Island	0	0	0	0	0	2.35
Confluence	1.89	4.33	15.37	11	0.61	0.73
Tributary	0	0	0	0	0	2.12
TOTAL	0.95	1.04	3.28	1138	0.32	593.75

4. DISCUSSION

This study provided new insights into sampling and analytical methods to estimate abundance of river dolphins in the South America. Buckland et al. (2001) recommended a minimum of 60-80 sightings for accurate estimation of detection functions. We used a larger dataset of 283 sightings for the boto and 219 for the tucuxi, which provided an opportunity to improve estimation of detection functions, detection probability on the trackline and overall abundance of river dolphins in many locations along their distributional range.

4.1 ESTIMATION OF DETECTION FUNCTIONS

An implicit assumption of standard line transect methodology is that detection probabilities depend solely on the perpendicular distance of detected objects to the transect line (Buckland et al. 2001). The use of MCDS (Marques & Buckland 2003) has shown that covariates can improve models of detection probability and estimates of density. The use of MCDS methods allow for an assessment of factors that influence the detection of river dolphins, something that had not been broadly considered in other river dolphins studies (Marques et al. 2004). Models considering platform, group size and sightability were generally more supported for both boto and the tucuxi. Good environmental conditions, sightability (e.g. glare strength, river state) in particular, as expected, contributed to detectability in the supported models with two units of AIC.

Season appears to strongly influence detection of tucuxi, but not boto. Water level at the time of the survey was an important covariate in the detection of the former. River dolphins are seasonally influenced by water levels, being more gregarious in the dry season, more dispersed in flooded season, and more randomly distributed in transitional periods (raising and falling waters) (Trujillo 2000, Martin & da Silva 2004, Gómez-Salazar et al. 2012b, Williams et al. 2016). Tucuxi responds to water level variation first than boto due to morphological aspects, since its body assume a fusiform shape they perform displacement movements through the main channel of the river avoiding obstacles (Martin et al. 2004, Mintzer et al. 2016). During the water-razing season, tucuxi prey displaces towards lakes and future flooded forest (várzea - igapó), being this period the best time to survey conduction to achieve good detection of this species. During this seasoning tucuxis are commonly seen displaying a variety of aerial behaviors (Best & da Silva 1993, da Silva & Best 1996, Flores & da Silva 2009) allowing detection from the bow and stern sighting platforms at larger distances. Otherwise, water level did not showed to be an important factor in the detection of botos. This might be due to the fact that botos movements are not so affected by obstacles as tucuxis, and that during breathing only a small proportion of its body emerges (Best & da Silva 1989, 1993, Gómez-Salazar et al. 2012b).

4.2 CORRECTION FACTORS FOR DETECTION IN STRIP TRANSECTS

Most river dolphin abundance surveys have been conducted using strip transects (Gómez-Salazar et al. 2012a). However, detection is not 100% in the strips because some animals are known to be missed away from the transect line, a violation of the assumption of perfect detection in strip transects. For this reason, Gomez-Salazar et al. (2012a) computed correction factors referred to as "P_k*'s*". In this study, new detection functions were calculated with a greater sample and a more diverse dataset (*n* = 283 groups of boto and *n* = 198 tucuxi using 22 river surveys in the present study compared to *n* = 38 groups of boto and 27 tucuxi using 7 river surveys in Gómez-Salazar et al. (2012a)). The updated "P_k*'s*" can be useful to improve abundance estimates of river dolphins in future surveys when sample sizes are insufficient to compute survey-specific detection functions.

In this study, in addition to updating P_1 and P_2, a third P_k (P_3) was calculated. This value, can be applied in tributary river and narrow channel that did not exceed 300 m, and in which dolphins distribution are similar to the strip-width of 200 m.

4.3 DETECTION PROBABILITY ON THE TRACKLINE

The use of double platform was essential to correct for the number of missed groups on the trackline by the first platform and it also influences the detection function. The second platform (stern) has more time to detect these missed groups, since the search area remains in the field vision longer due to the curves of the river. These results suggest that adding stern platform significantly improve the detection efficiency. It also highlights the need for estimating g(0) for river dolphins, using, for example capture-recapture models (Buckland et al 2001, 2015; Marsh & Sinclair 1989, Laake & Borchers 2004, Gómez-Salazar et al. 2012a, Pavanato et al. 2016).

The stern platform was more important for boto than for tucuxi because a greater proportion of the former (~35%) was detected by the observers located in the rear of the boat. This difference might be associated to the cryptic behavior of this species, which shows small fractions of their body when surfacing and also short breathing intervals and is typically found in small groups (Best & da Silva 1989a). Such features make these animals more difficult to detect. The stern platform also contributes with new detections (~14%) for tucuxi, but a greater proportion of groups of this species were seen by the front observers when compared to the boto. Tucuxi are gregarious and are commonly seen in larger groups *(*Best & da Silva 1993, da Silva & Best 1996, Flores & da Silva 2009*)*. Also, this species displays aerial behavior more frequently and are easier to be detected (Best and da Silva 1989a, b, Martin et al. 2004, Gómez-Salazar et al. 2012b).

This study used a higher and more diverse sample to estimate the 'global' trackline detection probability that the values previously computed by Gomez-Salazar et al. (2012a). The numbers presented in this study ($g(0)$ = 0.814, CV = 0.05 for boto and $g(0)$ = 0.989, CV = 0.006 for tucuxi) were slightly lower for both species than those calculated by Gómez-Salazar et al. (2012a) ($g(0)$ = 0.947, CV = 0.025 for boto and $g(0)$ = 0.997, CV = 0.003 for tucuxi). Assuming a normal distribution, the confidence intervals do not overlay resulting in a significative difference between the values calculated by Gómez-Salazar et al. (2012a) (95% CI = 0.901-1) and those presented in this study (95% CI = 0.743-0.897). The difference can be addressed to the great variability in aggregated surveys, variating the detection probability as function of many observers, distinct platforms, sightability, river shape, animal's behavior. However, it suggests that the probability of missing dolphins in the trackline can be higher than previously thought for river dolphins, as expected for other small cetaceans (Otis et al. 1978, Huggins 1991, Buckland et al. 1993, Laake & Borchers 2004, Fletcher & Hutto 2006, Thomas et al. 2010).

In Amazonian biome, rivers systems are composed by either larger rivers (reaching more than 5 km between shores) and tributary rivers (mean 300-400 m width) (Sioli 1984). Line transect sampling methods were designed to be applied in rivers where mean width is at least 1 km, so that lines are equal distant and provide a homogeneous coverage area. As *g(0)* is computed using detection from line transects, and in tributary rivers does not allow optimal conduction of line transect to compute survey-specific *g(0)*, global *g(0)* previous estimated from Gómez-Salazar et al. (2012a) were suggested to be used to compute density in those rivers. We recommend to replace the previous *g(0)* to updated value provided in this study, which can improve the reliability of future estimates in tributary rivers.

4.4 POST STRATIFICATION

High latitudinal and longitudinal trends in density were identified in Tocantins and Guaviare rivers, with apparent different reasons: the possible effect of the Tucuruí Dam in the former and watershed features in the latter. The initial stratification across habitats, as proposed by Gomez-Salazare et al. (2012) resulted in estimates of density and abundance with high CV's. Geographic post-stratification of the sighting data reduced CVs by as much as ~70%, increasing the reliability of our results. However, in most instances CVs (e.g. Tocantins-Araguaia) are still relatively high (>0.30) and new approaches to sample and analyze abundance of river dolphins will need to be developed. Despite that, this study shows that geographical stratification, in addition to habitat stratification, is a valuable approach to improve estimates of abundance of river dolphins and should be incorporated in planning future surveys (see discussion of Chapter 2).

4.5 INDIVIDUAL RIVER DENSITY AND ABUNDANCE ESTIMATES

This study has estimated group size, encounter rates, density and abundance for river dolphin populations in new areas of the Amazon, Orinoco and Tocantins-Araguaia River basins. Confluences and channels have been confirmed as high-density (Gómez-Salazar et al. 2012a, Pavanato et al. 2016, Pavanato et al. in press). The highest density and abundance for both the boto and the tucuxi ever throughout these species range was documented in the Purus River, located in the central of Amazonian river basin. A relatively small population of Araguaian botos was estimated in Tocantins River, one of the riverine ecosystems most affected by anthropogenic activities inside in the Amazon and the one where a large dam has caused many changes in river flow, biochemistry and landscape. The Guaviare River was sampled for the first time, an important river in an ecotone area between the Orinoco and the Amazon River basins, and a site with limited access for many years due to regional conflicts in Colombia.

The results provided in this study pointed out to clear differences in density and abundance across the three rivers. These differences are believed to be related 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, which has affected dolphin distribution and possibly abundance.

Purus River

Density estimates in Purus River are the greatest reported (14 boto/km² and ~13 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 boto/km² and a population size of boto 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 used habitat stratification for compute abundance, making comparisons difficult.

In general, estimates of density for boto and tucuxi in the Amazon River basin are of about 2 to 5 botos/km² and 4 to 6 tucuxis/km² in tributary rivers (Vidal et al. 1997, Trujillo et al. 2010, Aliaga-Rossel 2002, 2006, Gómez-Salazar et al. 2012a, Pavanato et al. (in press)). In this study, habitat-specific and overall density were substantially higher, but relative density across species were consistent with those from previous studies. These findings support habitat partitioning theory between boto and tucuxi, since the higher densities for botos were related to the river margin while for tucuxi was the center of the river (river channel) *(*Martin et al. 2004, Pavanato et al. 2016).

Besides habitat partitioning, botos and tucuxis were more associated with the river margin in the Purus River, differing from other studies (Gómez-Salazar et. al 2012a, Pavanato et al. 2016). Purus River is located in the most central part of the Amazon Basin and is characterized by the meandering aspect and muddy water, rich in Andean sediments, conferring to this river great richness of nutrients and biodiversity (Goulding et al. 2003, Guyot et al. 2007). Surrounded by the Amazon Rain Forest, it also present large-scale hydrologic and hydrodynamic, 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 a more homogenous distribution along the margins. 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, boto and tucuxi seems to be distributed influenced by this abundant source.

Purus River is the last great tributary of the right bank of the river Solimões (name given to the Amazon river before the encounter with the Negro river). Because of its high species richness and high productivity, this river is an important fishing ground (Batista & Júnior 2003). Approximately 40% of the landings in Manaus come from the River's lowland lakes (Batista & Júnior 2003, Santos et al. 2006). One of the fish species responsible for a considerable amount of fishery production in lower Purus, in the last five years, is the piracatinga (Brum et al. 2015). The piracatinga (*Calophysus macropterus*) is a scavenging catfish, which have been historic catch in Brazil since the later 90's to replace an overfished species in the Colombian market (Trujillo et al. 2010, Mintzer et al. 2013).

The piracatinga fishery in the Central Amazon has been of great concern in recent years. Botos have been illegally killed in the Central Amazon and their blubber and meat have been used as bait in this fishery (Loch et al. 2009, da Silva et al. 2011, Alves et al. 2012, Mintzer et al. 2013). For this reason, the piracatinga fishery has been considered one of the main current threats to boto'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, Martin & da Silva 2018, da Silva et al. 2018). Based on the scientific information, the Brazilian government established a moratorium prohibiting the commercialization of piracatinga for five years, starting in 1st January 2015 (MMA 2016) as an attempt to reduce the illegal hunting and develop strategies, monitor river dolphins populations – botos mainly, and to obtain information about population structure (Franco et al. 2016).

After publication of the normative instruction, monitoring programs were created by the Centro Nacional de Pesquisa e Conservação da Biodiversidade Amazônica do Instituto Chico Mendes de Conservação da Biodiversidade (CEPAM/ICMBio) (MMA 2016). Surveys in areas where piracatinga fishery occurs have been implemented in order to assess potential declining trends in the abundance of boto population as a consequence of the illegal hunting. In the Purus River, a survey conducted in 2017 in the same area covered in this study estimated densities of 9 boto/km² and 16 tucuxi/km² (CEPAM unpublished data). The estimates presented here and those computed from CEPAM are five years apart and the surveys reported here were conducted (in 2012) during a period of intensive fisheries 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 botos (from 14 ind/km2 in 2012 to 9 ind/km2 in 2017) and a relatively stable population of tucuxi (13 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 population in this area.

Recent studies in the Central and Upper Amazonian river basin, have identified decline in population numbers of river dolphins (Mintzer et al. 2013, Williams et al. 2016, da Silva et al. 2018). Besides piracatinga fishery, river dolphins in South America are threatened by a range of human activities that put them in danger (water population – heavy metals, habitat loos and degradation, food resource exploitation, dams construction, population fragmentation), making difficult to assign one main cause of the perceived reduction in numbers. Compare data from one area to another, should also be done with caution regarding to methods, regional scale and as highlighted in our study the river basin.

Tocantins River

This is the first effort to estimate population size of the Araguaian boto. Our study demonstrates that Araguaian boto have a relatively small population for the sampled area covered in the present study in the Tocantins River. For an equivalent effort employed in the Tapajós River (Pavanato et al. 2016) comprising a smaller area, boto population was 50% larger. Density in all habitats sampled was substantially smaller for Tocantins River than Tapajós River, with river margin and island habitat types presenting the highest differences (more than 70% smaller downstream the Tucuruí dam). Comparing boto density in river margin habitat between upstream Tucuruí dam in Tocantins River and the Tapajós River, this difference is only 15% (0.72 and 0.87 respectively). In Tapajós River botos were recorded in higher densities in islands (5.7 ind/km^2) in the lower course of this river basin, where there is much availability of this habitat type, similarly of Tocantins river's shape. However, we found the highest density for islands in upstream the Tucuruí dam (2.32 ind/km^2) in the middle Tocantins River, where this habitat is less available compared to the lower course.

The Tocantins and Tapajós rivers are similar in terms of shape and features (clear waters, low concentrations of nutrients, ions, and sediments), margin composition (rocky), and presence of rapids (Sioli 1984, Junk & Furch 1993). These rivers also have their headwaters in the Central Brazilian Shield and are important waterways for agricultural exports (Fearnside 2015). Due to similar conditions between the two river basin features, one might expect the density and population size of boto to be similar as well. However, the Tapajós River basin is relatively more pristine and we did expect a small population in Tocantins River since it is intensively altered by several long-term human activities (large cities, farms, boat traffic, fishing and agricultural exploitation, hydroelectric dams, and mining).

The estimated survey-specific detection probability on the trackline for Tocantins River (g(0) = 0.659, CV = 0.26) is quite similar to that found in the Tapajós River (g(0) = 0.648, CV = 0.27, Pavanato et al. 2016). This means that more than 30% of groups were not detected on the survey trackline. This estimate is considerably greater from the 'global' g(0) estimated by Gómez-Salazar et al. (2012a) (g(0) = 0.947, CV =0.02), where only nearly 5% of dolphins are missed on the survey line, much less than the presented in this study (19%). Water transparency was raised as the most probable hypothesis to explain the reduced detection probability in the Tapajós River (Pavanato et al. 2016), since solar reflection is greater in transparency water than white and black, and dolphins shown a small proportion of its body (dorsum) when breathing. However, we did not found this relation in the detection function adding water type covariate. In addition, the survey was conducted during the rainy season, when transparency decreases in Tocantins River, making it difficult to assert the real effect of this variable.

The Tucuruí dam, placed in the lower course of the Tocantins River, is likely a key factor causing contrasting boto density and abundance when comparing Tapajós and Tocantins rivers. The Tucuruí dam substantially altered hydrological cycles up and downstream on the Tocantins River. The amount of water is directly influenced by the frequency of dam floodgates opening. Notably, the dam has dramatically altered the frequency and duration of downstream high and low pulses, as well as the rate and frequency of water condition changes (Timpe and Kaplan 2017). These changes in hydrology, in addition to changes in water quality, are typically detrimental to downstream biota and biodiversity (Lytle and Poff 2004, Richter et al. 1998, Nilson & Berggren 2000, Pringle et al. 2000).

Although there are no previous studies in the area, preventing to affirm assertively the effect of the Tucuruí dam on Araguaian botos, we believe based in the Chinese and Asian river dolphins recent historic, that Tucuruí dam is what that caused differences found in density and distribution of boto in that area. Our survey suggests that the Araguaian boto population was affected by the Tucuruí dam. As demonstrated in previous studies (Vidal et al. 1997, Martin & da Silva 2004, Martin et al 2004, Trujillo et al. 2010, Gómez-Salazar et al. 2012a, b, Pavanato et al 2016, Pavanato et al. (in press)) across other river basins, boto densities are higher in habitat types such as channels and islands, and this occurred both downstream and upstream the dam (Table 14). However, our post-stratification of the data indicates that densities are lower downstream (Fig. 14) which is corroborated by the visualization of density gradients along the study area (Fig. 15).

Araguaian boto densities were 68% smaller in river margins downstream, another possible impact of the dam construction. The Tocantins River is wider and presents more islands and smaller channels along its lower reaches, so differences in density might also occur due to the relationship between area and probability of detection. Nevertheless, the increased availability of these downstream habitats does not modify the pattern demonstrated by downstream trend data. The river margin is an important habitat for botos. Dolphin preys typically migrate along the margins, where there is also a major concentration of nutrients, providing habitats with higher productivity (Sioli 1984, Dudgeon 1992, FAO 2001, Luz-Agostinho et al. 2018). The Tucuruí dam may have affected distribution of dolphin preys as result of margins flow changes and decreased sediment load (Barrow 1987, Ribeiro et al. 1995).

We observed spatial heterogeneity within the Tucuruí reservoir. Our results indicate that densities decrease as one moves from upstream areas (Fig. 15). A previous study in the same region of our sampling investigated limnological aspects of the lower and middle Tocantins River (Espíndola et al. 2000). In this study, the authors identified the existence of three compartments with different limnological characteristics determined as a function of the system's hydro-geo-morphometry with upstream-downstream spatial distribution and density of zooplankton. The gradient described for the zooplanktonic community overlaps the gradient found for botos inside the reservoir, the first part of this section is considered an “aquatic desert” deeply changed in ecological structure. This spatial trend has been attributed to physical and chemical differences caused by horizontal circulation of the reservoir's water, which caused thermal and oxygen stratification, with larger anoxic bottom layers as one moves towards the dam

(Espíndola et al. 2000). The impacts of the Tucuruí hydroelectric dam are considerable in terms of habitat transformation, biodiversity loss, productivity, and ecosystem service provisioning (Fearnside 1990, Fearnside 2001, Mérona et al. 2001).

In addition to habitat transformation that affected the distribution and habitat use for dolphins along the Tocantins River, the Tucuruí dam was responsible for the first major break in connectivity in the basin, which fragmented the boto population and disrupted fish migrations. By disrupting the river flow, the Tucuruí dam isolated groups of dolphins in two stretches of the river, possibly interrupting gene flow and generating subpopulations (da Silva & Martin, 2010, Araújo & Wang, 2012).

Hrbek et al. (2014) proposed that Araguaian boto (*Inia araguaiaensis*) only occurs upstream of the Tucuruí dam, however recent findings demonstrate the presence of the Araguaian boto downstream of the dam extending the known distribution range to the border of Marajó's Island (Siciliano et al. 2016). Notwithstanding, analysis of both nuclear and mitochondrial DNA revealed that animals inhabiting waters below the Tucurui Dam are hybrids between Inia araguaiaensis and Inia geoffrensis, therefore limits of distribution of the two species remain unknown (Hrbek personal comm). This finding supports the boto's population fragmentation of the sampled region covered in our study. According to the IUCN, the distribution of botos include the whole extension of the Tocantins River (IUCN 2013). In the upper reaches of the Tocantins River, six other small dams also overlap with boto distributions. Araujo & Wang (2015) suggested that the Araguaian boto population is currently fragmented into eight groups in the Tocantins River. It is known that fragmentation decreases genetic diversity and increases inbreeding (Turvey 2007, Gravena et al. 2015), which can significantly reduce populations and ultimately lead to extinctions (Turvey et al. 2012).

Hundreds of hydroelectric dams have been proposed throughout the Amazon, including the Tocantins-Araguaia Basin (Kahn et al. 2014, International Rivers 2015, Lees et al. 2016, Winemiller et al. 2016). Considering those that are either under construction, planned or inventoried, a total of 24 dams overlap with the distribution of both river dolphins (*Inia* spp. and *Sotalia fluviatilis*) (International Rivers 2015, Araújo & Wang 2014, Pavanato et al. 2016). Of those, 11 are located in the Tocantins-Araguaia, making dolphins in this basin potentially the most impacted by dam construction. Building all dams would further fragment the boto populations into as many as 12 groups in the Tocantins River. In addition, it would permanently break the connectivity between dolphins in the Tocantins and Araguaia river, further contributing to isolation of smaller sub-groups of river dolphins in this major Brazilian river basin.

The panorama set for Araguaian botos is quite similar to that faced by Indus river dolphins (*Platanista gangetica minor*), whose population was fragmented into eight groups in a river blocked by 17 dams (Kreb et al. 2010, Braulik et al. 2012a, 2012b, 2014). Habitat transformation, food depletion, and genetic isolation have caused sharp declines in the populations of Indus river dolphins (Huang et al. 2012, Braulik et al. 2014). Hydropower development in the Tocantins-Araguaia basin must be planned strategically. If more dams are to be constructed at all, future projects should be placed in upstream reaches where botos are absent to avoid large-scale reductions in Araguaian boto populations.

River dolphins are top predators and are considered indicators of freshwater ecosystem degradation (Gómez-Salazar et al. 2012b, Turvey et al. 2012). Evaluation of their distribution and density can be informative to understand patterns or trends in changes of regional biodiversity and habitat transformation. Hydroelectric dams reduce the environmental structure and complexity, and the fragmentation of river corridors by multiple dams could lead to the decline of the Araguaian boto population in the near future.

This study highlights the urgent need to re-evaluate the model that South American governments are adopting to obtain energy in the Amazon. This is particularly important for the Tocantins-Araguaia river basin, where many dams have been proposed. Further research is imperative to better assess distribution, density, habitat use and trends in abundance of the Araguaian boto in the Tocantins and Araguaia rivers. This endemic species is under major threats and conservation actions are required to prevent it from having the same fate as that of the Asian river dolphins.

Guaviare River

Efforts to investigate the abundance of river dolphins on the Guaviare River were substantially delayed due to armed conflicts in Colombia, which prevented access to this region for environmental research for many years (Vargas 1998, Álvarez 2003). This is one of the first scientific studies conducted in a large extension of the Guaviare River following the cessation of armed conflict, and the first to estimate boto density and population size (tucuxi dolphin's does not occur in this river). Estimates presented here provide further strength to the hypothesis that overall density of the boto in the Orinoco river basin seems to be smaller than in 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 headwater of the Guaviare River is in the Colombian Andes and is formed by the rivers Ariari and Guayabero in the upper lift Andean region (Junk 1993, Godoy et al. 1999). It flows through the Colombian Llanos, the savannas of Northern South America to the Amazon Rainforest into the Lower Orinoco; in the upper reaches, it has low nutrient availability, rapid flow of sediments, and sandy composition (Medina & da Silva 1990, Savage & Potter, 1991, Meade 1994). During raising and high water, there is a drastic reduction of phytoplankton biomass possible due to the high concentration of suspended solids that Guaviare River transports during these periods (Chitty 1994). The aquatic fauna, mainly fish assemblage, are distributed from the middle towards the lower river course, where aquatic habitat is more susceptible (Lasso et al. 2016). River dolphins seems to follow this gradient from the middle towards the lower Guaviare River.

The transitional biome in which Guaviare River flows through works as ecotone driving process of biodiversity speciation (Hoorn et al. 2010). Dolphins found in this region are possible evolutionary units *Inia geoffrensis humboldtiana*, the only subspecies currently recognized for *Inia geoffrensis*, and restricted to Orinoco basin (Banguera-Hinestroza et al. 2002, Martínez-Agüero et al. 2010). Gómez-Salazar et al. (2012a) in large effort conducted in Meta River (1,321.1 km^2) and in Orinoco river (1,684 km^2) estimated the population size of *I. g. humboldtiana* at 1016 (CV = 0.85) and 1779 (CV = 0.87) individuals respectively. The present estimate for the Guaviare river has adds another 1138 individuals (CV = 0.32) to this population, thought the numbers may not be all added together because of the time difference in which the estimates were computed (2006 and 2016). The present survey comprised the entire navigable area of Guaviare River using the same methodology applied in the Meta and the Orinoco rivers. Because the remaining area to be covered it is not extensive (small and narrow tributary rivers), the population of botos in the Orinoco river basin is thought to be small (~5,000 individuals).

No population trends is available for dolphins in Orinoco river basin, repeated surveys have been conducted in Meta River for the years 2006, 2012 and recently in 2018. The threats for this species are the same faced by the river dolphins in Amazon and

Tocantins-Araguaia river basins. Estimates of trends in abundance is one of the next steps of the SARDIPAN initiative.

5. CONCLUSIONS AND FINAL CONSIDERATIONS

This chapter provided improvements in estimates of various abundance parameters for river dolphins in the Amazon and additional analytical approaches that could help increasing accuracy of estimates computed with abundance surveys in complex and difficult to survey areas. As mentioned before, the population estimates presented here can be used as baseline for monitoring programs directed to assess trends in river dolphin's population in the rivers Purus, Tocantins and Araguaia, and therefore can contribute to management decisions.

The extensive effort applied across river basins in South America to estimate density and abundance of river dolphins by SARDIPAN initiative was substantially important to develop a holistic ecologic view of the factors that influence distribution, density and abundance of *Inia spp.* and *Sotalia fluviatils*. This large dataset was essential to evaluate the methods employed and to propose improvements in estimates using existing data or improvements in future surveys. A comparison of the results provided here with those from other areas allowed the identification of key areas to be resampled in the future, especially in regions where growing threats may impose risk to dolphins (e.g., Tocantins River).

Sampling the entire range of distribution of river dolphins in South America is a difficult task and it is quite improbable that the range of all populations will be surveyed simultaneously in order to obtain population-wide estimates of abundance. However, efforts such as those described above can be directed to specific areas and a consistent monitoring along with information on potential seasonal movements of dolphins should prove essential to enhance the conservation and management of river dolphins in South America.

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CHAPTER 5. GENERAL 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 by SARDIPAN initiative was substantially important.

Reviewing fieldwork and analytical methods, allowed us to identify changes needed and to propose alternate or supplementary methods to estimate population size of *Inia spp.* and *Sotalia fluviatilis*. Ongoing projects are developing and exploring new tools to maximize efforts and reduce cost-time in assessing population parameters of river dolphins in South America, such those mentioned in the final statements in chapter 2. Results of the new technologies of drones and satellite transmitters should help planning surveys regarding dolphin's movements during water seasonality periods, dimensioning the study scale, and the proper calculation of the study area. Development of these projects was only possible because of the previous experience gathered from conducting visual boat surveys during the last 10 years.

The research presented in this thesis suggests that distances measurement error is not likely resulting in bias in the estimation of abundance of river dolphins. Analytical improvements in the estimation of detection probability (*e. g*, through the use of multiple covariate distance sampling methods), survey-specific estimates of g(0) and post-stratification of survey data likely produced more robust and reliable estimates of abundance for the regions considered in this study. This research also contributed to better understand dolphin's distribution and concentration along different sub-regions of the rivers. This was fundamental to see rivers as unique units that playing a key role in the estimation of population size given their unique features and the preference of dolphins for specific habitats. 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. These activities represent threats to the ecosystem, causing profound changes in the environment. In terms of biodiversity and ecological processes, the construction of dams in particular, can fragment populations, reduce river flow, affect river pulses, change the water quality, and ultimately contribute to the reduction or extinction of many species, including river dolphins. In the chapter 4, discussion presented about Tocantins River strongly suggests that the Tucuruí hydroelectric dam shifted river shape and the aquatic ecological structure. Although there are no previous studies in the area, preventing to affirm assertively the effect of the dam on Araguaian botos, we believe based in the Chinese and Asian river dolphins recent historic, that Tucuruí dam is what that caused differences found in density and distribution of boto in that area. The implementation of other 11 hydroelectric projects in this basin will likely cause population fragmentation of the Araguaian boto habitat and may have devastating impacts to a population that is relatively small population.

Given the potential for population fragmentation and changes in abundance, further studies should survey areas not previously sampled in the upper reaches of Tocantins-Araguaia River basin before any other hydroelectric dam construction is developed. As well as, other areas where dams overlap river dolphins distribution in South America should be monitored to investigate the effect of dam constructions of boto and tucuxi dolphis. Such studies will allow for an assessment of the effects of the dams in the population of dolphins and their habitats.

Data from an additional 12 surveys are under analysis and results should be published in due course for a better description of the density and abundance patterns across the range of river dolphins in the Amazon. Estimates of population size and, most importantly, trends in abundance should be given priority given the need to assess impacts of ongoing threats to *Inia spp*. and *Sotalia fluviatilis*. 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.