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Population size estimates of pink river dolphins (*Inia geoffrensis*) using mark-recapture methods on photo-identification

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Abstract

Population size estimates of pink river dolphins (*Inia geoffrensis*) are critical to assess the conservation status of this species and the impacts of increasing human stressors in freshwater ecosystems. Photo-identifications of *Inia* dolphins were collected between February 2007 and August 2009 in two locations of the Colombian Amazon and Orinoco river basins. Population sizes of *Inia* were obtained by using the closed (Petersen) mark-recapture model on photo-identifications. The total population size estimate for right/left side individuals was 129/71 *Inia* dolphins (CV=0.36/0.35) in the Amazon location and 125/58 *Inia* dolphins (CV=0.77/0.69) in the Orinoco location. The survey and analysis protocols were designed to try to meet mark-recapture assumptions. However, photo-identification was incomplete in both study areas, and there could be recruitment and unequal probabilities of capture due to preferences of individuals for certain areas. Further effort should be focused towards expanding the photo-identification catalogues and creating long-term monitoring programs.

Resumen

Estimaciones del tamaño poblacional de los delfines rosados (*Inia geoffrensis*) son fundamentales para evaluar el estado de conservación de esta población y el impacto que pueda tener el incremento de estresores humanos en ecosistemas de agua dulce. Delfines *Inia* fueron foto-identificados durante febrero de 2007 y agosto de 2009 en dos localidades de la Amazonía y Orinoquía colombiana. El tamaño poblacional de *Inia* fue obtenido a través de la técnica de marca-recaptura, utilizando el modelo de poblaciones cerradas (Petersen). La estimación del tamaño de la población para individuos identificados por los lados derecho/izquierdo fue de 129/71 *Inia* (CV = 0.36/0.35) en la Amazonía y 125/58 *Inia* (CV = 0.77/0.69) en el Orinoco. Este estudio utilizo un protocolo estandarizado para cumplir con las suposiciones del modelo de marca-recaptura. Sin embargo, la foto-identificación fue incompleta en ambas áreas de estudio, y es posible que en la población hay reclutamiento y diferentes probabilidades de captura debido a preferencia de los delfines por ciertas áreas. Futuros esfuerzos deben enfocarse en ampliar el catálogo de foto-identificación y en la creación de programas de monitoreo.

Introduction

Pink river dolphins (*Inia geoffrensis*), listed as *Data Deficient* by the IUCN, are widely distributed in the Amazon and Orinoco river basins, except for areas of rapids or extensive ecosystem degradation (Best and da Silva, 1993; Reeves *et al.*, 2008; Gomez-Salazar *et al.*, 2012a). These river basins are facing a range of human stressors that are expected to increase, such as reduction of water quantity and quality, habitat modification and climate change (Revenga *et al.*, 2000; UNEP, 2004). In addition, there are other human stressors that directly threaten river dolphin populations such as tourism (which focuses on dolphin watching), the killing of river dolphins due to entanglements in fishing nets, and harvesting body parts as aphrodisiacs and amulets, or as bait for the mota (*Calophysus macropterus*) fishery (Loch *et al.*, 2009; Trujillo *et al.*, 2010). Standardized monitoring programs are needed to evaluate the size of dolphin populations, to monitor the consequences of increasing human stressors, and ultimately to target areas for recovery (*e.g.* Gomez-Salazar *et al.*, 2012c).

The size and trends, movement patterns, survival and recruitment rates of cetacean populations can be obtained by using data from photo-identification surveys (Hammond *et al.*, 1990; Hammond, 2009; 2010). Photo-identification, using digital cameras in areas where dolphins are conspicuous when surfacing, is a reliable and non-invasive tool to study *Inia* dolphins (Trujillo, 1994; Gomez-Salazar *et al.*, 2011). For example, photo-identification effort is recommended when surveying *Inia* dolphins in high-density areas (*e.g.* lakes) (Gomez-Salazar *et al.*, 2012a). Typically, these high-density areas are also characterized by large group sizes of dolphins (Gomez-Salazar *et al.*, 2012b), and so they attract dolphin-watching activities that so far are not regulated. Population size estimates using data on photo-identifications can be used to develop long-term monitoring programs in these critical, high-density areas that need to be carefully monitored.

Mark-recapture methods applied to photo-identification data can provide accurate estimates of population size and trends, which are both useful in assessing the impacts of human stressors on dolphin populations over time (Wilson *et al.*, 1999; Gowans *et al.*, 2000; Hammond 2009). Hence, the objective of this paper is to investigate population sizes of pink river dolphins (*Inia geoffrensis*) in two locations of the Colombian Amazon and Orinoco basins by using mark-recapture methods on photo-identifications.

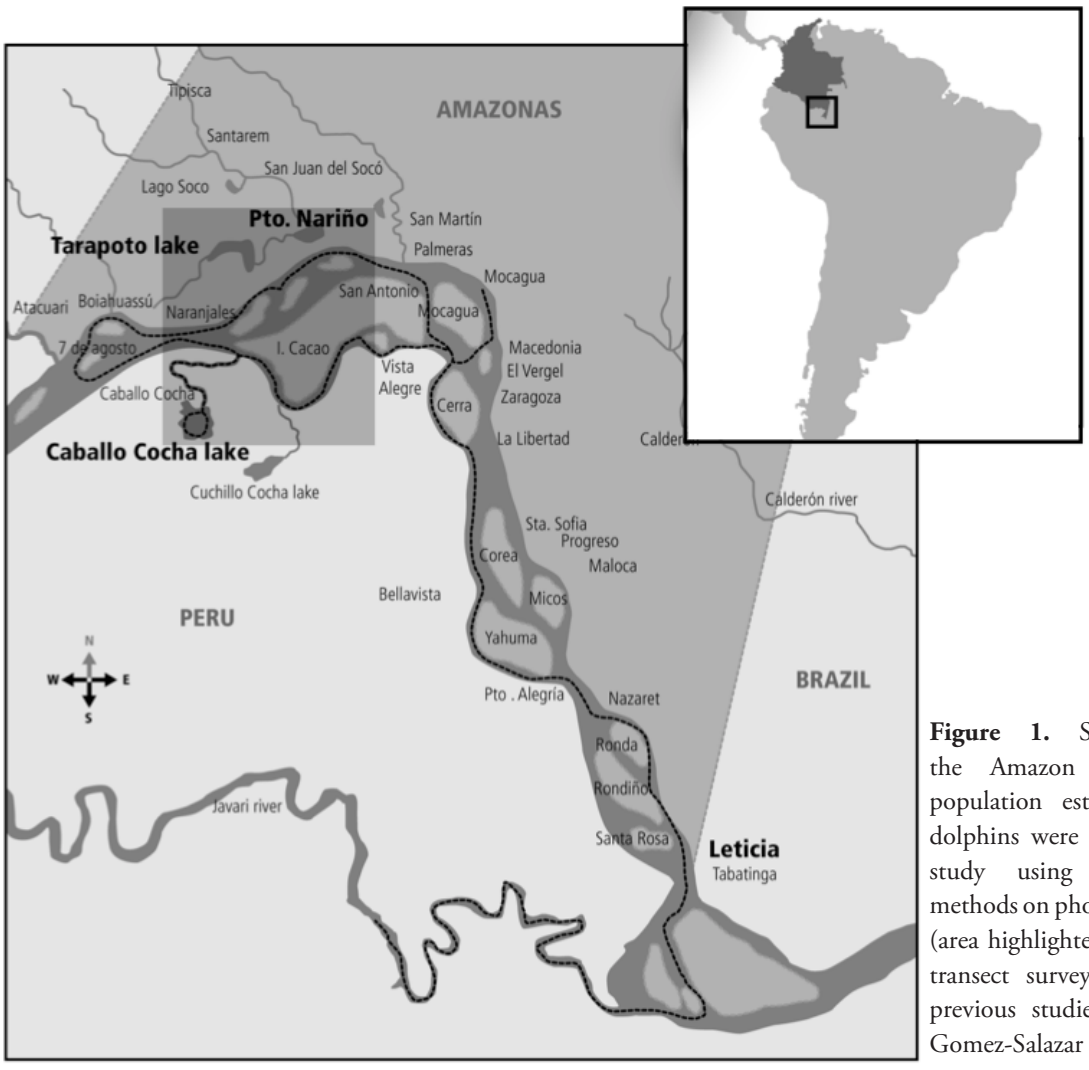

Figure 1. Study area in the Amazon location were population estimates of river dolphins were obtained in this study using mark-recapture methods on photo-identification (area highlighted) and line-strip transect surveys conducted in previous studies (dotted lines, Gomez-Salazar *et al.* 2012a).

Figure 2. Study area in the Orinoco location were population estimates of river dolphins were obtained in this study using mark-recapture methods on photo-identification (area highlighted) and line-strip transect surveys conducted in previous studies (dotted lines, Gomez-Salazar *et al.* 2012a).

Materials and Methods

Data Collection

Detailed information on survey design, data collection, and photographic analysis is given in Gomez-Salazar *et al.* (2011). Field surveys were conducted in two locations of the Amazon (Figure 1) and Orinoco river basins (Figure 2). The Amazon study area is located in the southern portion of Colombia (3º46'S, 70º22'W; 60 linear km of river surveyed) and the Orinoco basin study area is located in the northeast region of Colombia (6º11'N, 67º28'W; 120 linear km of river surveyed).

Digital photographs of *Inia* dolphins were collected during nine surveys between February 2007 and August 2009 (Table 1). Groups of *Inia* dolphins were located visually from a 6m boat, with a 25-hp outboard engine, at approximately 2m observation height. Photographic effort started only if individuals remained within 100 m of the boat (Gomez-Salazar *et al.*, 2011). Photographs collected were rated on quality of the image regardless of the markings of each individual. A quality rating (*Q*) of 1 - 5 was given to each photograph. The rating of 1 was used for very poor photographs, which were not useful and the rating of 5 was used for very good quality – ideal for photo-identification (Gomez-Salazar *et al.*, 2011). We selected those good quality photographs (rated $Q \geq 3$) that showed images of *Inia* dolphins containing at least two marks of a type considered reliable. Reliable mark types (pigmentation patterns, nicks, bends, and wounds) are those that last for a period of at least 22mo, and are located on *Inia*'s dorsal ridge and flank (Gomez-Salazar *et al.*, 2011). Based on these criteria, a photo-identification catalogue was created and used in this study.

Data Analysis

Analyses were conducted separately for left and right side photographs (Wilson *et al.*, 1999), given photographs from each side are known for only some individuals (only seven dolphins in the Amazon and two dolphins in the Orinoco were matched with corresponding left and right side photographs). Analyses were also conducted separately for each of the areas surveyed (Amazon and Orinoco), using software SOCPROG 2.4 (Whitehead 2009). A discovery curve (the cumulative number of individuals identified against the cumulative number of identifications) was plotted to investigate how complete the photo-identification coverage was.

Table 1. Number of days surveyed and photographs taken for each survey (modified from Gomez-Salazar *et al.* 2011).

Survey Dates	# days	Amazon		Orinoco	
		# of photographs			
		total	Q≥3	total	Q≥3
Feb-07	3	301	47		
Jul-07	1			142	46
Dec-Jan 08	9	865	125		
Feb-08	2	149	23		
May-08	1			281	96
Jun-08	3	154	37	145	17
Jul-08	1		4	186	36
Oct-08	1	213	69		
Nov-08	1	58	31		
Dec-Jan 09	8	1206	256		
Apr-09	1	31	8		
Aug-09	1	3			
Total	32	2980	600	754	195

Table 2. Number of dolphins identified by right/left sides (diagonal) and number of re-sighting records between years in two locations in the Colombian Amazon and Orinoco basins. Data were not collected in the Oricono study area in 2007.

Amazon location			
	2007	2008	2009
2007	7/6		
2008	3/4	30/20	
2009	0/0	1/0	4/2
Orinoco location			
	2007	2008	2009
2008	-	6/2	
2009	-	0/0	10/10

Our samples were not large enough to fit open mark-recapture models effectively (Table 2). Thus, population sizes of *Inia* dolphins and variance estimates were obtained using the closed (Petersen) mark-recapture model (Seber, 1982), with years as the sampling period, and the weighted sample variance estimate, assuming independence between years (Wilson *et al.*, 1999). The closed (Chapman) mark-recapture estimate ($\hat{N}$) is:

$$\hat{N} = \frac{(n_1 + 1)(n_2 + 1)}{m_2 + 1} - 1$$

where n_1 is the number of individuals captured and marked during sampling period 1, n_2 is the number of individuals captured and examined for marks during sampling period 2, and m_2 is the number of individuals captured during sampling period 2 that were marked during sampling period 1 (Seber, 1982).

Estimates of the population sizes of the reliably marked dolphins were divided by the proportion of reliably marked animals in the population to estimate the total population (Wilson *et al.*, 1999; Read *et al.*, 2003). Hence, following Wilson *et al.*, (1999), the total population size $\hat{N}_{total}$ was estimated as:

$$\hat{N}_{total} = \frac{\hat{N}}{\theta}$$

where $\hat{N}$ is the closed (Chapman) mark-recapture estimate using individuals identified by at least two reliable marks. θ was obtained by examining high quality photographs, ($Q \geq 4$), to estimate the proportion of well-marked (presence of at least two reliable marks), individuals in the population, (55%) (Gomez-Salazar *et al.*, 2011).

Estimated variances were obtained using the delta method (Wilson *et al.*, 1999):

$$\text{var}(\hat{N}_{total}) = \hat{N}^2_{total}\left(\frac{\text{var}(\hat{N})}{\hat{N}^2} + \frac{1-\theta}{n\theta}\right)$$

where n is the number of animals from which θ was estimated.

Results

Data consisted of individuals photo-identified by Gomez-Salazar *et al.* (2011): 41 individuals identified by the right side and 28 by the left side in the Amazon; 16 individuals identified by the right side and 12 by the left side in the Orinoco (Table 2) (see Methods in Gomez-Salazar *et al.*, 2011). The photo-identification was incomplete in both study areas as indicated by neither discovery curve leveling off (Figure 3). There were no matches between the study areas (Table 2). The total population size estimate for right side individuals was 129 *Inia* dolphins (CV=0.28) in the Amazon and 125 *Inia* dolphins (CV=0.77) in the Orinoco (Table 3). As there were no recaptures between the Orinoco samples, the upper bound of the population estimate is infinite.

Discussion

Mark-Recapture Assumptions

Violations of the assumptions of mark-recapture models can result in biased abundance estimates (Hammond, 2009; 2010). Here, we evaluate potential violations of each assumption.

1. *The closed (Petersen) model assumes no immigration, emigration, birth and/or death between each pair of consecutive sampling intervals.* We expect that these populations are nearly geographically closed given the enclosed nature of the study

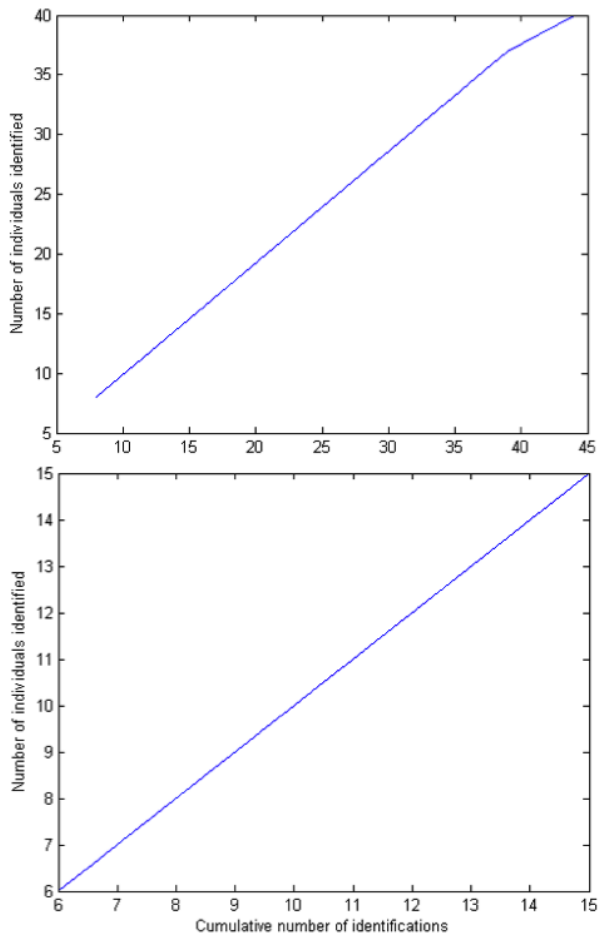

Figure 3. Discovery curves for *Inia* dolphins identified from the right side in the Amazon (above) and Orinoco (below). Similar curves were found for individuals indentified from the left sides.

areas (Figures 1 and 2) and that previous studies of this species have demonstrated only short-distance movements and high fidelity of individuals to areas where they are born (Trujillo, 1994; Martin and da Silva, 2004; Martin *et al.*, 2004; McGuire and Henningsen, 2007; Ruiz-Garcia *et al.*, 2007). Cetaceans have low reproductive and high survival rates, with a population grow rate ranging between 2-10% per year (Connor *et al.*, 2000; Barlow and Reeves, 2009). Thus, the population estimates might be biased upwards by about these amounts because of recruitment, and thus a lack of closure.

2. *Marks used to identify individual dolphins are unique, are not lost, and are correctly recorded.* A detailed protocol specifically for photo-identification of *Inia* dolphins was developed with the aim of ensuring that marks used to identify individuals are unique, are not lost, and are correctly recorded or reported (Gomez-Salazar *et al.*, 2011). For instance, to avoid mismatching individuals and assure that marking is unique, at least two reliable marks in *Inia*'s dorsal ridge and or flank are required for identification. To avoid marks being lost, only reliable marks were used, characterized by low rate of loss and lasting for the entire length of the study (22mo). To reduce the probability of errors while identifying, matching and recording individuals, we followed a standardized protocol, which includes using only good quality photographs ($Q \geq 3$), and at least two people with experience in photo-identification confirming matches (Gomez-Salazar *et al.*, 2011).

3. *Marking does not affect future survival or catchability.* Photo-identification is non-invasive, and will only cause short-term disturbance at the worst. It is thus unlikely to affect survival or the probability of recapture (Wilson *et al.*, 1999; Hammond, 2009). In addition, the study areas surveyed have a nearly constant presence of boats and thus we consider unlikely that the survey boats might have altered the dolphins' behavior between sampling occasions.

Table 3. Population size ( ), total population size ( ), standard error (SE), and coefficient of variation (CV) for all reliably-marked individuals using the closed (Petersen) mark-recapture model. Estimates were obtained for each location (Amazon and Orinoco), per each sample period and for the overall period of this study.

	Side of dolphin	2007-08		2008-09		Overall					
Amazon		$\hat{N}$	SE	$\hat{N}$	SE	$\hat{N}$	SE	CV	$\hat{N}$total	SE	CV
	Right	95	37.1	63	21.4	71	18.5	0.26	129	45.93	0.36
	Left	39	8.2	39	11.7	39	6.7	0.17	71	24.62	0.35
Orinoco											
	Right	69	43.2	-		69	43.2	0.63	125	96.90	0.77
	Left	32	17.9	-		32	17.9	0.56	58	40.16	0.69

4. *Animals have an equal probability of being captured within each sampling occasion.* Our field survey design was developed to minimize unequal probabilities of capture given potential differences in the behaviour of individuals. For example, to maximize the probability of photographing all individual dolphins within a group, photographic effort ended only when individuals could no longer be followed, or after 30 minutes of taking photographs. Moreover, to minimize the probability of identifying animals with more distinctive marks, photographs were taken of all individuals regardless of presence or conspicuousness of marks. Field surveys were also designed to give adequate coverage to all habitat types in each study area (*e.g.* main river, tributary, lake, confluence). However, there are several aspects of river dolphin ecology that might lead to more recaptures in certain areas. For instance, lakes and confluences generally contain higher densities and larger group sizes of dolphins, and in these areas dolphin are more conspicuous when surfacing and thus more efficiently photographed (Gomez-Salazar *et al*., 2011; Gomez-Salazar *et al*., 2012a,b). If particular animals use these areas preferentially, this could introduce heterogeneity. In addition, other studies have found differences in habitat preferences for male and female *Inia,* with females and calves entering the flooded areas and males remaining in the main rivers (Martin and da Silva, 2004). These kinds of heterogeneity could have produced negative biases in our estimates.

In summary, this study used a well-designed survey and standardized protocol for data analysis to try to meet mark-recapture assumptions. Recruitment and unequal probabilities of capture due to preferences of individuals for certain areas are the most likely potential violation of the assumptions. Future work should take this into consideration, increasing effort generally so that robust models can be employed, and, in particular, increasing effort in areas where *Inia* are more challenging to photograph.

Abundance estimates: Mark-recapture vs. Line-transect methods

Using mark-recapture methods, (right side individuals), this study estimated 129 *Inia* (CV=0.36) in the Amazon study area (60 linear km) and 125 *Inia* (CV=0.77) in the Orinoco study area (120 linear km). For comparison using line-strip transect methods, a study conducted 13 years ago in the Amazon study area estimated a population size of 346 *Inia* (CV = 0.12, 120 linear km, Vidal *et al.* 1997), and a more recent study in 2007 estimated a population size of 1,115 *Inia* in the Amazon study area (CV=0.78, 315.2 linear km) and 1,016 *Inia* dolphins (CV=0.85) in the Orinoco study area (461.7 linear km) (Gomez-Salazar *et al.,* 2012a).

Differences in survey methods, data analysis, study period and areas surveyed make comparisons difficult. For instance, in the Amazon study area, line-strip transects surveyed an area of 592.6km^2 during the transitional water period (Gomez-Salazar *et al.,* 2012a), and the mark-recapture study surveyed 140km^2 within that area during different months of the year (Figure 1). In the Orinoco study area, line-strip transects surveyed an area of 1,231.1km^2 during the transitional water period (Gomez-Salazar *et al.,* 2012a), and the mark-recapture study surveyed 260km^2 within that area during different months of the year (Figure 2). Hence, the areas surveyed during this mark-recapture study are obtained during multiple water periods and are within the boundaries of significantly larger areas surveyed during the line/strip transect surveys (Figure 1 and 2).

Conclusions and Recommendations for potential future work

This study presents an attempt to obtain a population size estimate of *Inia* dolphins using mark-recapture methods on photo-identifications. Given that photo-identification was incomplete in both study areas, we recommend further effort to photo-identify individuals with the goals of expanding the photo-identification catalogue and creating long-term monitoring programs. This is particularly important given that human stressors in the Amazon and Orinoco are only expected to increase. Mark-recapture methods can be a cost-effective tool providing estimates of population parameters, movement patterns, and social structure of river dolphins, which will inform conservation policy and management actions.

This and previous studies (*e.g.* Gomez-Salazar *et al.,* 2012a) have used two main methods to study river dolphins: line-strip transects and mark-recapture methods on photo-identifications. The decision regarding which method should be used will depend upon the duration and the main goals of the projects. For instance, this and previous studies (*e.g.* Gomez-Salazar *et al.,* 2012a) have suggested that mark-recapture methods on photo-identification can be an efficient tool for long-term monitoring programs of pink river dolphins. Increasing photo-identification effort and observation in the field will generally lead to an increase in the number of dolphins identified, provide information regarding the sex of individuals based on their close associations with calves (*e.g.* Read *et al.,* 2003), and allow results to be compared with other similar catalogues already existing in the Amazon and Orinoco (*e.g.* McGuire and Henningsen, 2007).

In some instances, however, long-term monitoring programs cannot be established due to constraints in logistics and funding availability. As a result, when some study areas can only be surveyed opportunistically and it is not possible to develop photo-identification, line-strip transect methods are a cost-effective tool for estimating group sizes, sighting rates, density and population sizes of river dolphins (*e.g.* Vidal *et al.,* 1997; Martin *et al.,* 2004; Gomez-Salazar *et al.,* 2012a).

While the population estimates obtained through mark-recapture methods correspond to the number of animals that use the area, the estimates obtained through distance sampling methods correspond to the animals that were present in the area during the time of the survey (Hammond 2009; 2010). Further studies could compare both methods

by conducting surveys in areas where monitoring programs are being developed (*e.g.* Colombian Amazon and Orinoco) with the goal of improving abundance and density estimates of river dolphins.

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