Latin American Journal of Aquatic Research
E-ISSN: 0718-560X
lajar@pucv.cl
Pontificia Universidad Católica de Valparaíso
Chile

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Latin American Journal of Aquatic Research, vol. 45, núm. 5, noviembre, 2017, pp. 957-969
Pontificia Universidad Católica de Valparaíso
Valparaíso, Chile

Available in: http://www.redalyc.org/articulo.oa?id=175053482011

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Research Article

Abundance estimate of the Amazon River dolphin (*Inia geoffrensis*) and the tucuxi (*Sotalia fluviatilis*) in southern Ucayali, Peru

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ABSTRACT. We present results of the first simultaneous visual and acoustic surveys for Amazon River dolphins (*Inia geoffrensis*) and tucuxi (*Sotalia fluviatilis*), undertaken in Yarinacocha Lagoon, Ucayali, Peru (length = 20 km, area = 12 km^2). A total of 324 *Sotalia* observations and 44 *Inia* observations were recorded in boat-based transects. Based upon total survey effort, we estimated *Sotalia* had a mean density ± SE of 1.98 ± 4.6 ind km^{-2} and an overall abundance of 34 individuals (95% CI: 28 to 40). Average density for *Inia* was 0.2 ± 1.2 ind km^{-2} with overall abundance estimated at 3 individuals (95% CI: 0 to 8). Visual surveys observed more *Sotalia* individuals during earlier hours of the day and during the dry season. Preliminary acoustic surveys undertaken with C-POD passive acoustic monitoring devices indicated a diel pattern in detections of cetacean vocalization with more diurnal activity and with detections ceasing from midnight until 10:00 AM. This work highlights the possibility of monitoring both river dolphin species through visual and acoustic surveys to generate baseline information on abundance trends and distribution patterns in the Ucayali region, an area with high levels of human disturbance.

Keywords: *Inia geoffrensis*, *Sotalia fluviatilis*, boto, tucuxi, population, abundance, seasonality, echolocation loggers, C-POD.

INTRODUCTION

To facilitate effective species conservation, a good understanding of population density is crucial (Nichols & Williams, 2006; Gómez-Salazar *et al.*, 2012). Population assessments play a critical role in informing conservationists about the effects of anthropogenic impacts (Legg & Nagy, 2006). Although abundance data are key, there are difficulties considering when monitoring aquatic mammals should be carried out (Taylor *et al.*, 2007). First, these species spend most of their time underwater and many are widely distributed and highly mobile. Second, effort hours are restricted by optimal weather conditions and daylight. Third, funding can be a limitation, as surveys require vessels or aircrafts and are relatively expensive (Aragonés *et al.*, 1997; Dawson *et al.*, 2004; Taylor *et al.*, 2007). Nevertheless, visual surveys utilizing distance sampling and mark-recapture methodologies are still used regularly (Würsig & Jefferson, 1990; Barlow *et al.*, 2005; Prado *et al.*, 2013).

More recently, passive acoustic monitoring (PAM) has been developed as an alternative or complement to visual surveys. PAM can detect cetaceans solely by recording the distinctive acoustic signals they make (Mellinger *et al.*, 2007; Miller *et al.*, 2015). PAM has advantages over visual surveys as they record cetaceans when submerged, reach areas where visual surveys are difficult (*e.g.*, Arctic ecosystems) and are independent of weather conditions and daylight (Marques *et al.*, 2009). PAM is also capable of generating information on habitat use and seasonal variation (Legorreta & Hinojosa, 2013). This technology also has certain disadvantages. Animals can only be detected when making noise, analysis of acoustic data files can be difficult and time consuming, and positive species identification is often challenging (Rayment *et al.*, 2009; Sousa-Lima *et al.*, 2013). A variety of equipment, such as hydrophones, Acoustic tags (A-Tags), and Cetacean Porpoise Click Detectors (C-PODs) have been developed to meet different study needs (Nielsen & Møhl, 2006; Marques *et al.*, 2009; Gallus *et al.*, 2012).

Corresponding editor: Enrique Crespo

The freshwater tucuxi (*Sotalia flluviatilis)* (hereafter referred to as *Sotalia)* and the Amazon River dolphin, also known as boto (*Inia geoffrensis)* (hereafter referred to as *Inia)* are both endemic to the Amazon-Orinoco river basin (Jefferson *et al.*, 2008). The total population sizes of both species are currently unknown. As riverine species, these dolphins are greatly affected by human disturbance (Turvey *et al.*, 2007; Kelkar *et al.*, 2010), yet both are still listed as Data Deficient by the IUCN (Reeves *et al.*, 2011; Secchi, 2012) highlighting one of the key research gaps in cetacean conservation (Parsons *et al.*, 2015). Line and strip transects and mark-recapture techniques have been used to estimate population sizes for both species in certain areas of their distribution but methods have not been standardized, making total estimates unreliable and hard to compare (Gonzalez, 1994; Martin & da Silva, 2004b; Aliaga-Rossel *et al.*, 2006; Gómez-Salazar *et al.*, 2012a, 2012b, 2014; Mcguire & Aliaga-Rossel, 2014) The most extensive survey to date covered the main rivers in six countries resulting in significant insights into habitat preferences for both species (Gómez-Salazar *et al.*, 2012). Bolivia and Peru had the highest overall densities, and confluences and lakes were highlighted in terms of habitat preferences (Gómez-Salazar *et al.*, 2012). These areas have previously been reported as abundance hot spots for both species (Martin *et al.*, 2004; Martin & da Silva, 2004b; Mcguire & Aliaga-Rossel, 2014).

In this study we set out to describe the spatial distribution and relative abundance of *Inia* and *Sotalia* in Yarinacocha lagoon, on the Ucayali River in Peru using both visual observations and passive acoustic monitoring. This research could help better understand *Inia* and *Sotalia* population trends and to develop effective management strategies in this under-studied, human impacted environment near the southern limit of these species' distributions.

MATERIALS AND METHODS

Study site

Yarinacocha Lagoon (Landing port: 8.35°S, 74.58°W; West border: 8.25°S, 74.63°W; East border: 8.32°S, 74.56°W) is situated 6 km from Pucallpa, the second largest city in the Peruvian Amazon, in the Ucayali Region (Suppl. 1). The oxbow lagoon width varies, but is as much 1000 m wide depending upon section and season. The lagoon is about 17 to 20 km long, with an area of approximately 12 km^2, and a maximum depth of 25 m, depending upon rainfall. For our dolphin abundance estimations, we used the mean width of 800 m and the area of 12 km^2. There are two seasons, wet: high-water (January-March), falling-water (April-June), and dry: low-water (July-September), and rising-water (October-December). During wet season the lagoon is connected at both ends to the Ucayali River and acts as a riparian highway for commerce and transportation. The lagoon has 35 registered artisanal fishers and 50 tour boat operators.

Field methods

Ten boat-based surveys were conducted in Yarinacocha lagoon between June 2013 and January 2015 (Fig. 1). Surveys were conducted at least once per season. The study site was divided into 17 sections, each 1 km long (Fig. 1). The boat drove along the middle of the lagoon with variable strip width, up to 500 m on each side. As the lagoon is also used as a riverine highway, other sampling methods (*e.g.*, zig-zag transects) were also considered, but these were not possible because they would have been dangerous and disruptive to boat traffic.

Transects were undertaken between 08:00 and 18:00 h. The 12-m research boat travelled at an average speed of 11 km h^{-1}. There were two observers for each transects a data collector and the boat driver; all observations were carried out by naked-eye. A Global

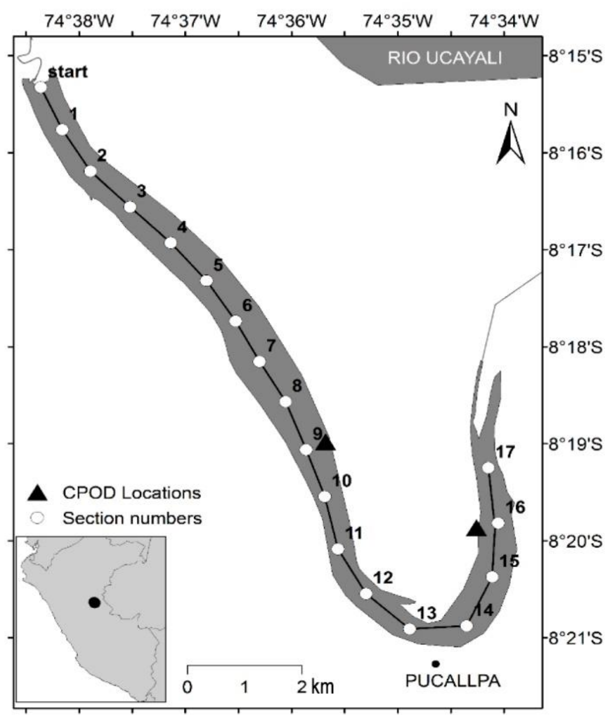

Figure 1. Study site showing the distribution of all 17 surveys transects in Yarinacocha Lagoon, Ucayali, Peru. Numbers represent the end of all transects, each *ca.* 1 km long. Black triangles display the locations of C-POD deployments (Near 8.31°S, 74.59°W; Near 8.33°S, 74.57°W).

Positioning System (GPS) recorder provided information on location, date and time, vessel location, and vessel speed. When dolphins were sighted, species, position, group size, and perpendicular distance from the boat were estimated and recorded. Estimation of perpendicular distance was practiced and improved prior to surveys with laser range finders.

In tandem with visual surveys, two C-PODs were deployed in two locations: Jungla Ecolodge (8.32°S, 74.59°W) and Pandisho Shelter (8.33°S, 74.57°W). AC-POD is an autonomous data logger that detects echolocation trains produced by odontocetes (Roberts & Read, 2015; Chelonia Ltd., 2011). Each C-POD contains an omnidirectional hydrophone, ten D-cell alkaline batteries and a 4 GB memory card specifically formatted for each pod. C-PODs were deployed at a minimum depth of 5 m for a total of eight successful recording bouts.

Data analysis

Density estimates were made by counting only observations that were within 100 m perpendicular distance from the boat on either side where detection probability for both species was considered to be highest (See Fig. 2c-2d; Suppl. 2). Effective transects' width was 0.2 km and transect area for each 1 km transect was 0.2 km^2. Density for each 1 km transect was estimated, then extrapolated to cover the average width (800 m) of the lagoon.

A General Linear Model (GLM) with a Quasipoisson error structure was used for both species to assess the relationship between the number of individuals observed, hour of day, month and transect location (R Core Team 2013). Statistical results were considered significant for $P \leq 0.05$. A Spearman's rank-order correlation between *Inia* and *Sotalia* observations was also performed to determine if there is a correlation between observations of both species. Descriptive statistics are presented as mean ± SE unless specified otherwise.

Logged C-POD data were analyzed using C-POD.exe software (Chelonia Ltd., 2011). The C-POD has different classification schemes for click trains, used by odontocetes for echolocation. 'Click train' is operationally defined as "a long series of similar echolocation signals made with similar time gaps between successive clicks" (Chelonia Ltd., 2011). Each click train is classified by the C-POD to one of the following: high, moderate, low, and doubtful. This study only exported detections classified as high and moderate quality.

C-PODS can also classify click trains by group of species that emit "Narrow Band High Frequency (NBHF)", "other cetaceans", "and sonars (ship sonars, biosonars) and unclassified". We considered clicks that were classified as "NBHF" and "other cetacean". Click trains were analyzed with the KERNO classifier. These filters reduce the amount of sonic interference and false positives originating from boat and human activities. Detection Positive Minutes (DPMs), a standard measure of how much time animals are present, were calculated to indicate the presence of dolphins (Chelonia Ltd., 2011). Potential diel patterns were explored by calculating the mean number of clicks recorded for eight 3 hour windows over the course of a 24 h day. Currently, C-PODs are not able to discriminate between *Inia* and *Sotalia* click trains. Therefore, acoustic data relate to detections of both species.

All spatial analyses and maps were prepared using ESRI ArcMap 9.2, and Hawth's Tools (Beyer, 2004) Quartic Kernel and 50%, 75% and 90% probability contour analyses were performed using 500 m grid spacing and a smoothing factor of 100 m for dolphin observation points.

RESULTS

Detection probability and group size

A total of 324 observations of 686 individuals of *Sotalia* were recorded, of which 226 observations were at a distance of 100 m or less (67%; Fig. 2). For *Inia*, we had a total of 44 observations of 69 individuals, 29 (68%) of these detections had distance estimations of 100 m or less (Fig. 2). *Sotalia* had significantly larger groups (KW test, $P < 0.05$, df = 1), with a mean group size of 2.1 ± 1.5 (range = 1 to 9) compared with *Inia*, with a mean group size of 1.4 ± 0.8 (range = 1 to 4). Both species had higher frequency of observations where individuals were alone or partnered, 73% for *Sotalia* and 94% for *Inia* (Fig. 2).

Hotspots and density

The average density for *Inia* was 0.2 ± 1.1 ind km^{-2} and its overall abundance was calculated at 3 individuals (95% CI: 0-8). *Sotalia* had an average density of 1.98 ± 4.60 ind km^{-2} with an overall calculated abundance of 34 ind (95% CI: 24-40). Although the densities of both species were higher in transect numbers 4, 7, 14, 17 (Fig. 3; Suppl. 3), there was no overall inter-specific correlation in abundance (Spearman's correlation $R_{1,17} = 0.02$, $P = 0.37$). No autocorrelation was found in *Sotalia* counts (D-W value = 1.95) nor *Inia* (D-W value = 1.45), therefore samples are independent. Average density of *Sotalia* during the dry season was 2.4 ± 5.3 ind km^{-2} (n = 374, during the wet season (January-June) it was 1.7 ± 3.9 (n = 312), a significant difference (KW

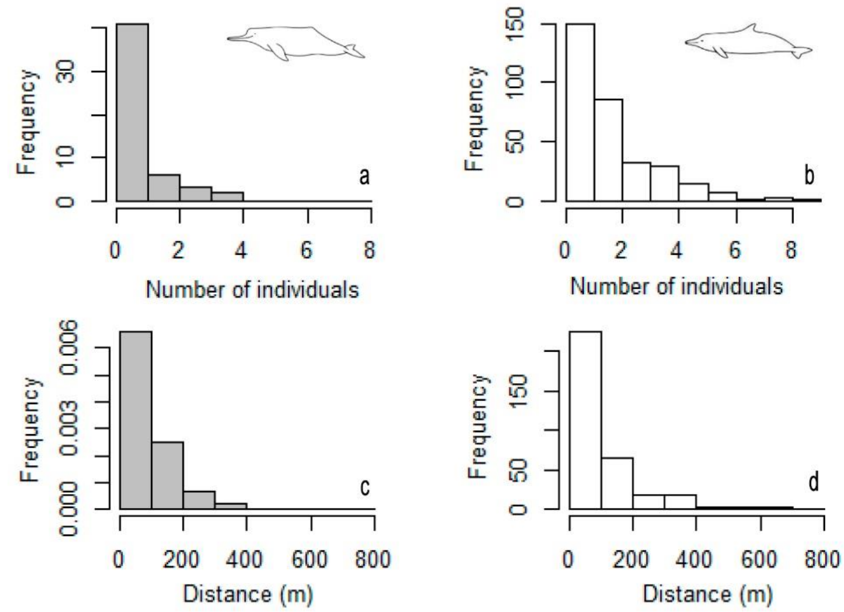

Figure 2. Group size and observation distance. Group size for a) *Inia* was generally significantly smaller than for b) *Sotalia*. Most observations of both species were within 100 m perpendicular distance of the boat. c) *Inia* and d) *Sotalia.*

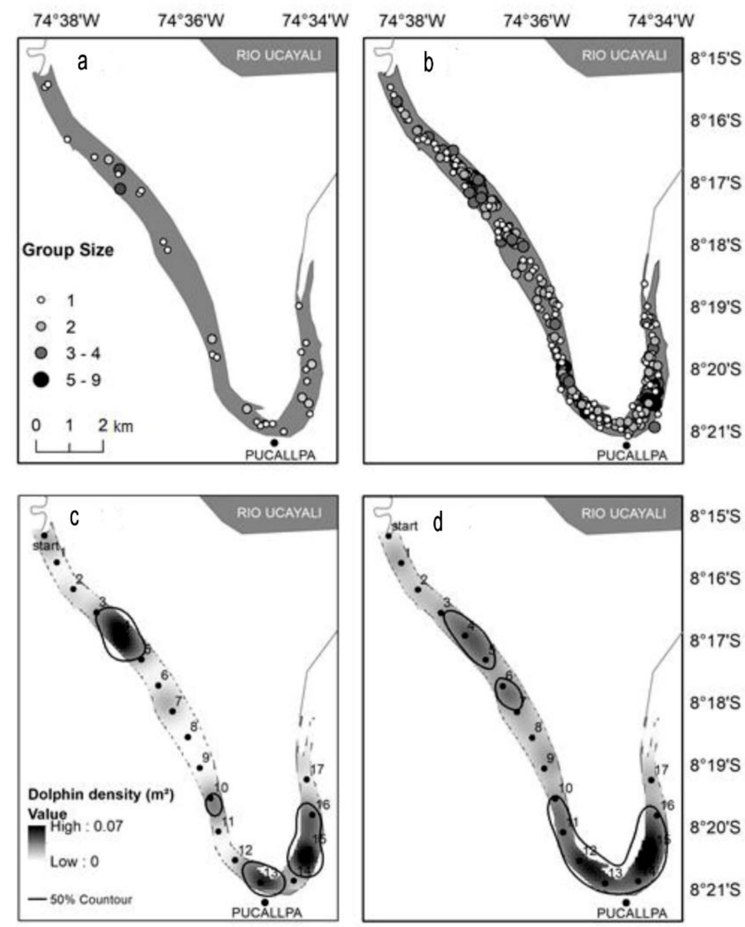

Figure 3. Maps representing all *Inia* a) and *Sotalia* b) observations, by group size. Kernel density estimations for *Inia* c) and *Sotalia* d) show areas with higher densities of individuals m^{-2}. High values are presented in dark grey, with ≥0.07 individuals m^{-2}.

test, $P = 0.03$, df = 1). *Inia* had an estimated density of 0.3 ± 1.5 (n = 43) during the dry season and 0.14 ± 0.9 (n = 26) during the wet season although this difference was not statistically significant (KW test, $P > 0.05$, df = 1).

Seasonality and diurnal activity

We ran three Generalized Linear models with a Quassipoisson error distribution to determine if environmental variables affected species detections (Table 1). The first model analyzed what affected the detectability of river dolphin species in the lagoon. We found that the number of river dolphins observed varied by time of day (*GLM*, $F_{1,12} = 5.87$, $P = 0.01$) and month (*GLM*, $F_{1,7} = 6.95$, $P = 0.05$). The second model found that the number of *Sotalia* individuals observed in Yarinacocha lagoon varied by month ($F_{1,7} = 6.49$, $P = 0.01$), time of day ($F_{1,12} = 9.77$, $P = 0.001$) and transect number ($F_{1,16} = 4.35$, $P = 0.03$). This means that *Sotalia* individuals were seen in early hours of the day, and later months of the year (coinciding with dry season) (Suppl. 4). The third model was with data on *Inia* observations. *Inia* on did not present a significant interaction or relationship with any variable ($F_1 = 2.44$, $P = 0.11$).

C-POD recordings were made on four occasions at La Jungla, and four occasions at El Pandisho (Fig. 5; Suppl. 5). Logged click trains indicated more acoustic activity during the day and until midnight at both locations (between 10:00 and 23:59). Detection positive minutes (DPM) were also calculated for each monitoring point. La Jungla had more detections (2.8 DPM ± 2.6, n = 4) than El Pandisho (0.8 DPM ± 0.8, n = 4). Two deployments recorded at both sample sites showed higher DPMs at La Jungla, although this difference was not statistically significant (Mann-Whitney, U = 2238.5, Z = 0.07, $P > 0.05$). This coincides with our observed density estimations, as transects closer to La Jungla had higher densities than transects closer to El Pandisho.

DISCUSSION

This study was successful at conducting abundance and density estimations for *Inia* and *Sotalia* species in Yarinacocha lagoon, Peru. We surveyed both dolphin populations across all four seasons and identified factors affecting their distribution, including location in lagoon, time of day and month. This area is important for *Inia* and *Sotalia* as it is the southernmost point of their distribution in Peru (Vidal *et al.*, 1997; Aliaga-Rossel, 2002; Gómez-Salazar *et al.*, 2010) and for the subspecies *I. g. geoffrensis* (Banguera-Hinestroza *et al.*, 2002). Our successful deployment of C-PODS also provided valuable information on river dolphins in the lagoon. The use of acoustic devices such as the C-POD proved to be a useful methodology for rapidly determining presence/absence of river dolphins and also provide insights into the habitat use of both species.

Results from this study coincide with previous studies in other areas of the Amazon-Orinoco basin. Our model results indicated that more *Sotalia* were seen at earlier hours of the day and during the dry season. Our results are similar to those obtained from previous work in areas where dolphin densities were observed as highest when river levels were at their lowest (Aliaga-Rossel, 2002; Martin & Da Silva, 2004b; Kelkar *et al.*, 2010). This variation in dolphin density can be attributed to seasonal movements, as individuals that prefer to inhabit tributaries have to move to areas that do not dry out (Martin & Da Silva, 2004b). Differences in species detectability could, however, skew results, as *Sotalia* often forms larger groups and have more conspicuous behavior than *Inia*. As the lagoon shrinks, detections are easier and densities could possibly be higher. Group sizes for *Inia* have previously been reported to be relatively small (Gómez-Salazar *et al.*, 2012, 2014), with reported means of approximately 2 individuals although *Sotalia* groups have been reported to be as big as 37 individuals (Gómez-Salazar *et al.*, 2012). In Yarinacocha lagoon, most observations were of 1 to 2 individuals for both species. The largest group of *Sotalia*, in Yarinacocha was of 8 individuals. Small group sizes could be explained by the lack of natural predators for both species (Gómez-Salazar *et al.*, 2014)). Food availability may also be more important (Smith & Reeves, 2000; Gómez-Salazar *et al.*, 2012). As the lagoon is only 12 km^2, food availability may be limited and dolphins also have to compete with extensive fisheries that operate year-round.

There was no statistical correlation in the abundance of the two dolphin species along the water course, suggesting that they may be well adapted to co-existence, perhaps by exploiting slightly different foraging niches or strategies. Both species have been shown to have a generalist diet, consisting of as many as 43 species for *Inia* and 28 species for *Sotalia* (Da Silva, 1983).

Our observed *Sotalia* density of 1.98 ± 4.6 individuals km^{-2} was high compared to other studies (Aliaga-Rossel, 2002; Vidal *et al.*, 1997) where densities were close to 1 or fewer ind km^{-2}. Other parts of the Amazon River basin with higher *Sotalia* densities (up to 10 ind km^{-2}) have been reported to support this level of abundance due to a combination of hydro-logical and physical features (possibly temperature, nutrients, currents) that result in high species richness

Table 1. Results of the Quasipoisson GLM testing. Output of the minimal adequate Generalized Linear Model (GLM) with quasipoisson distribution using 3 covariates (Transect number, Time of day and Month) for *Sotalia* observations, *Inia* observations, and both species observation.

	Estimate	SE	*t*-value	*P*-value
Sotalia				
Transect number	0.03	0.01	2.07	0.04
Time	-0.07	0.02	-3.10	0.00
Month	-0.03	0.01	-2.47	0.01
Inia				
Intercept	-2.98	0.16	-19.03	<2e-16
Both				
Time	-0.06	0.23	-2.50	0.01
Month	-0.03	0.01	-2.81	0.01
Degrees of freedom for best fit model				
	Null Deviance	**df**	**Residual Deviance**	**df**
Sotalia	2276.4	1383	2215.8	1380
Inia	476.38	1383	476.38	1383
Both	2405.9	1383	2365	1381

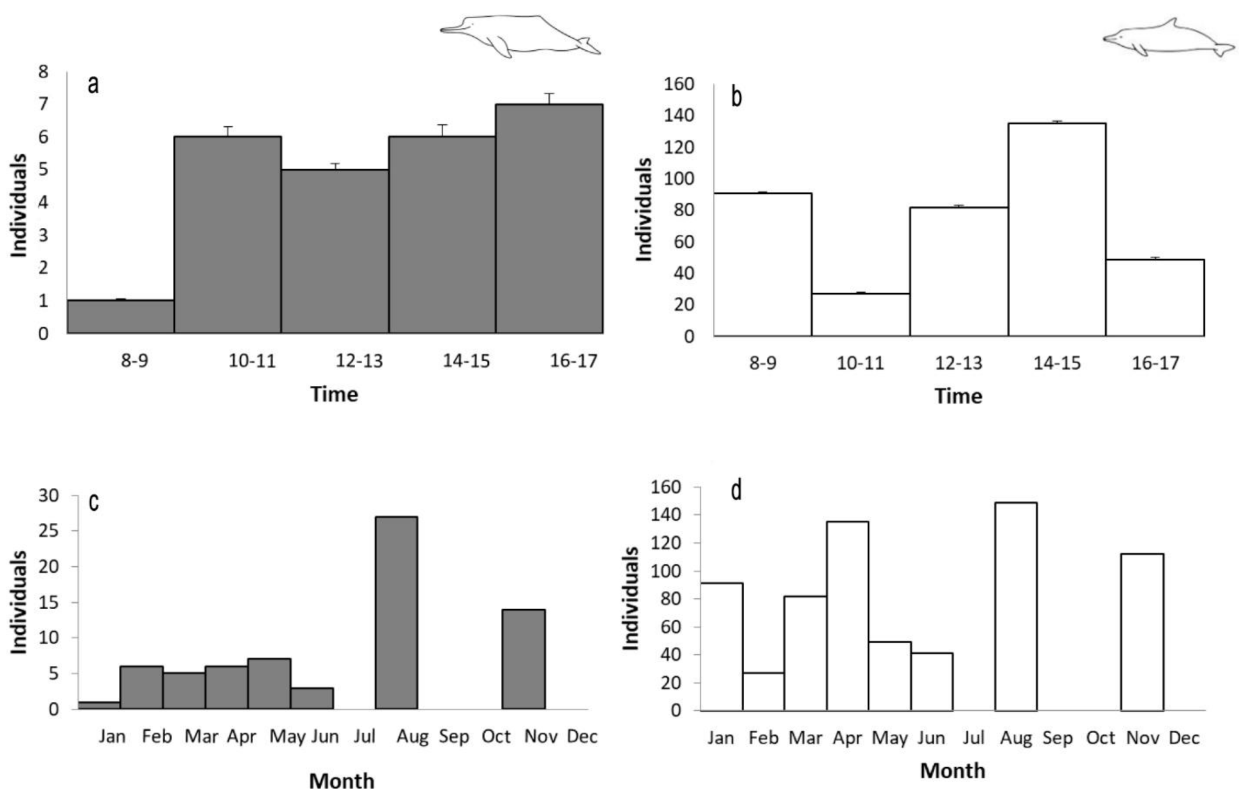

Figure 4. Mean number of individuals observed by time: in two-hour blocks for a) *Inia* and b) *Sotalia* and by month for c) *Inia* and d) *Sotalia.* Wet seasons are from January to June and dry seasons are from July to December. All graphs are presented with Standard Error. No data are available for July, September, October and December.

and abundance as well as habitat qualities that make the area more suitable for reproduction and resting (Vidal *et al.*, 1997). *Sotalia* has shown an apparent preference to lakes instead of tributaries and confluences (Vidal *et al.*, 1997) in other parts of the Amazon-Orinoco basin which could also help to explain the relatively high estimated density in Yarinacocha lagoon. In contrast, we observed *Inia* at lower densities than has been reported in other studies in the Marañon River, Samiria River and close to the Peruvian-Colombian border (Vidal *et al.*, 1997; Gómez-Salazar *et al.*, 2012). The highest densities recorded for *Inia* have been in protected

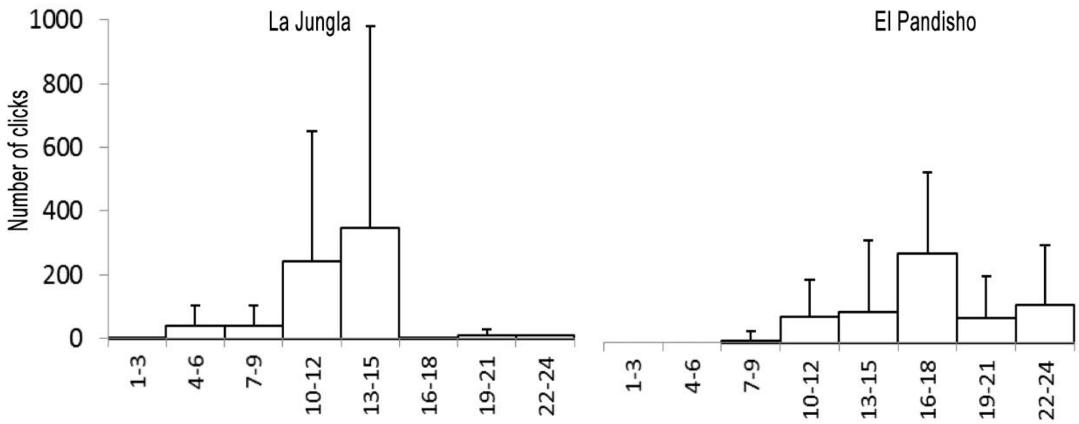

Figure 5. Mean number of clicks by three-hour bins recorded at each C-POD deployment location.

areas in Mamiraua Sustainable Development Reserve, Brazil (up to 18 dolphins km^{-2} in floodplain channels) (Martin & da Silva, 2004b) and in Pacaya-Samiria Natural Reserve Iquitos, Peru (5.94 *Inia* km^{-2}) (Gómez-Salazar *et al.*, 2012).

As there are no total counts or estimates of river dolphin populations in Peru, we cannot assess the importance of the Yarinacocha Lagoon populations for these species as a whole. This study is, however, the first for river dolphins in the lagoon, and represents the southernmost study about river dolphins in published records on the Ucayali River. Additionally, insights from the study could be used to help inform and design river dolphin conservation and management measures that also promote the lagoon's burgeoning tourism sector which includes dolphin watching tour operators.

Even with relatively few deployments, C-PODs were successful at detecting the presence of dolphins at both deployment locations (Fig. 4). Results suggest a diurnal/crepuscular pattern where dolphins in the Yarinacocha lagoon are more active acoustically during the day and possibly into the early evening. This concurs with visual surveys, where most of the observation was in earlier hours of the day. Diurnal acoustic patterns have been found in marine cetaceans (Todd *et al.*, 2009) and other river dolphin species such as the Ganges river dolphin *Platanista gangetica* (Sasaki-Yamamoto *et al.*, 2012), but few acoustic studies have been conducted on *Inia* and *Sotalia* (Ding *et al.*, 2001; May-Collado & Wartzok, 2010). Differences in the type, incidence, frequency range, and duration of acoustic cues have been reported between these species (Ding *et al.*, 2001; Podos *et al.*, 2002; May-Collado & Wartzok, 2007, 2010). Data from Brazil for *Inia* and *Sotalia* showed a significant increase in echolocation activity around dusk that continued throughout the night (Tregenza *et al.*, 2007). Our results may arise from a variety of factors including a change in predator behavior as a response to prey activity patterns (Todd *et al.*, 2009; Leeney, 2011) or movement to other areas at dawn when there is less anthropogenic activity (boat traffic, fishing), leading to fewer acoustic detections (Sasaki-Yamamoto *et al.*, 2012). In tandem, given how acoustically dependent these species are, consideration should be given to understanding the impacts of anthropogenic sound (McKenna *et al.*, 2016).

Our project encountered several limiting factors. Observers travelled down the middle of the lagoon. This could mean that we potentially missed seeing dolphins close to riverbanks and shallow waters, and therefore may be underestimating densities. Furthermore, detectability may vary depending upon season. When the lagoon is at a lower level, the lagoon shrinks, resources are concentrated and individuals are easier to detect. Comparatively, when the lagoon is at a higher level *Inia* and *Sotalia* have shown preference for flooded forests, and tributaries that reduces their detectability (Martin & da Silva 2004b). In terms of habitat preference there is evidence that shows both species present higher densities within 100 m to the shore (Martin & Da Silva, 2004a; Gómez-Salazar *et al.*, 2012). However, these surveys have been done in main rivers that are much wider and deeper than the Yarinacocha lagoon (Vidal *et al.*, 1997; McGuire, 2010). Additionally, we are presenting abundance estimations that were generated as means from detections at different lagoon widths. When the lagoon was at a low water level, the boat was closer to the margins then when it was high water level. Also, at this stage of the project we were not able to develop species discrimination for the acoustics data collected using C-PODs. This may be possible with the concurrent deployment of the C-POD and observers, in the same location and at the same time allowing for species discrimination. This would greatly empower future work.

Considerations for future work

Information on the distribution and habitat use by *Inia* and *Sotalia* is limited, particularly in some regions of the central-west Amazon basin. Most studies have focused on protected areas closer to the Amazon River in northern Peru (Gómez-Salazar *et al.*, 2010, 2012; Mcguire & Aliaga-Rossel, 2014). For the Yarinacocha Lagoon we discerned areas and hours of peak activity that could be avoided by boat operators (cargo, human transport) and fishing activities. We also recommend additional efforts to monitor individuals acoustically and to expand surveys to other areas of the Ucayali River. To aid in the conservation of both species it is important to continue with population studies that generate baseline information for this and other areas of the Ucayali. A study of the relationship between dolphin distribution and water depth could shed light on habitat preferences of each species in the lagoon, as well as working in other parts of the Ucayali River with a mixed-transect method. Additional research and monitoring is particularly important given that human stressors on the Amazon basin are only expected to increase. Results from this study could be used in region-specific management plans and can provide baseline information for river dolphin species that can inform future environmental impact assessments in the face of rapidly developing threats (Paudel *et al.*, 2016; Pavanato *et al.*, 2016).

ACKNOWLEDGEMENTS

This work was supported by the Rufford Small Grants Foundation, Conservation Leadership Programme, World Wildlife Fund, ProDelphinus and University of Exeter. Many thanks to Nick Tregenza, Robert Palacios, Andrea Pasara, Luis Miguel Barron and all researchers that participated in the collection of data used in this study. We would like to thank the communities of Pucallpa, and Yarinacocha lagoon for their generous hospitality to the researchers that were involved in the study. We are also very appreciative of the three reviewers of this manuscript; we thank them for their detailed comments and suggestions.

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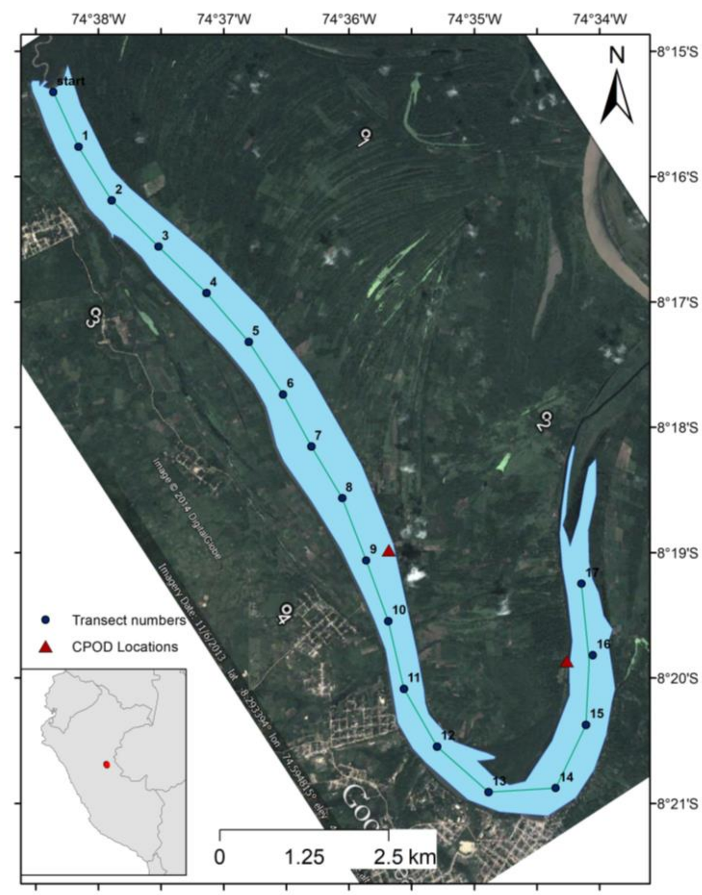
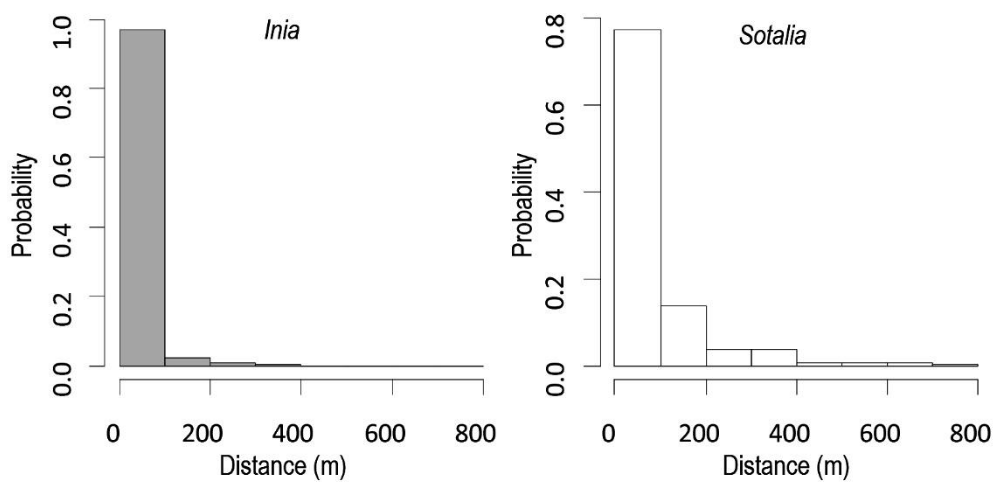
Received: 26 May 2016; Accepted: 3 June 2017

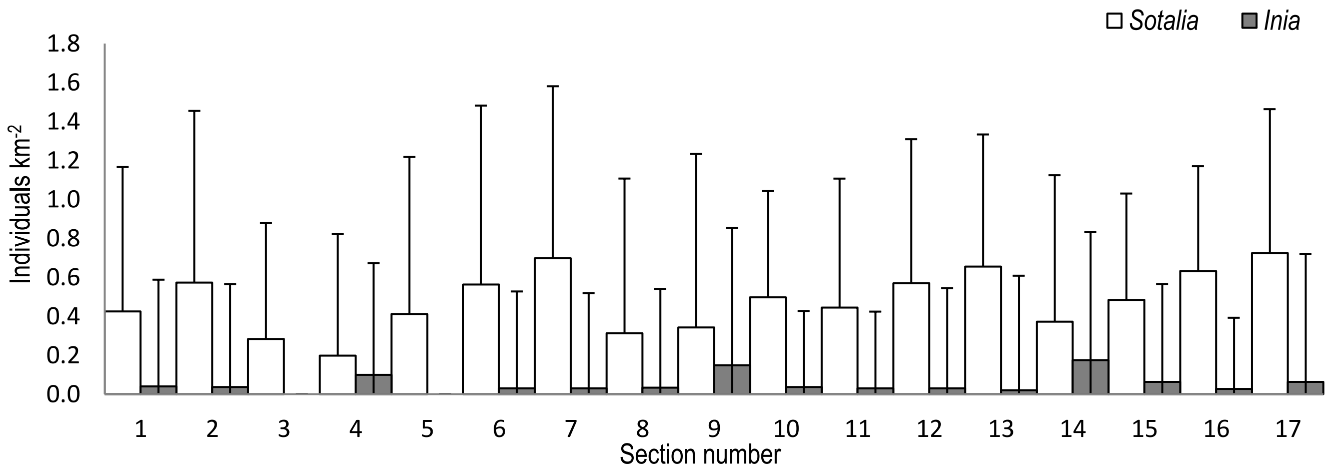
Supplemental figures



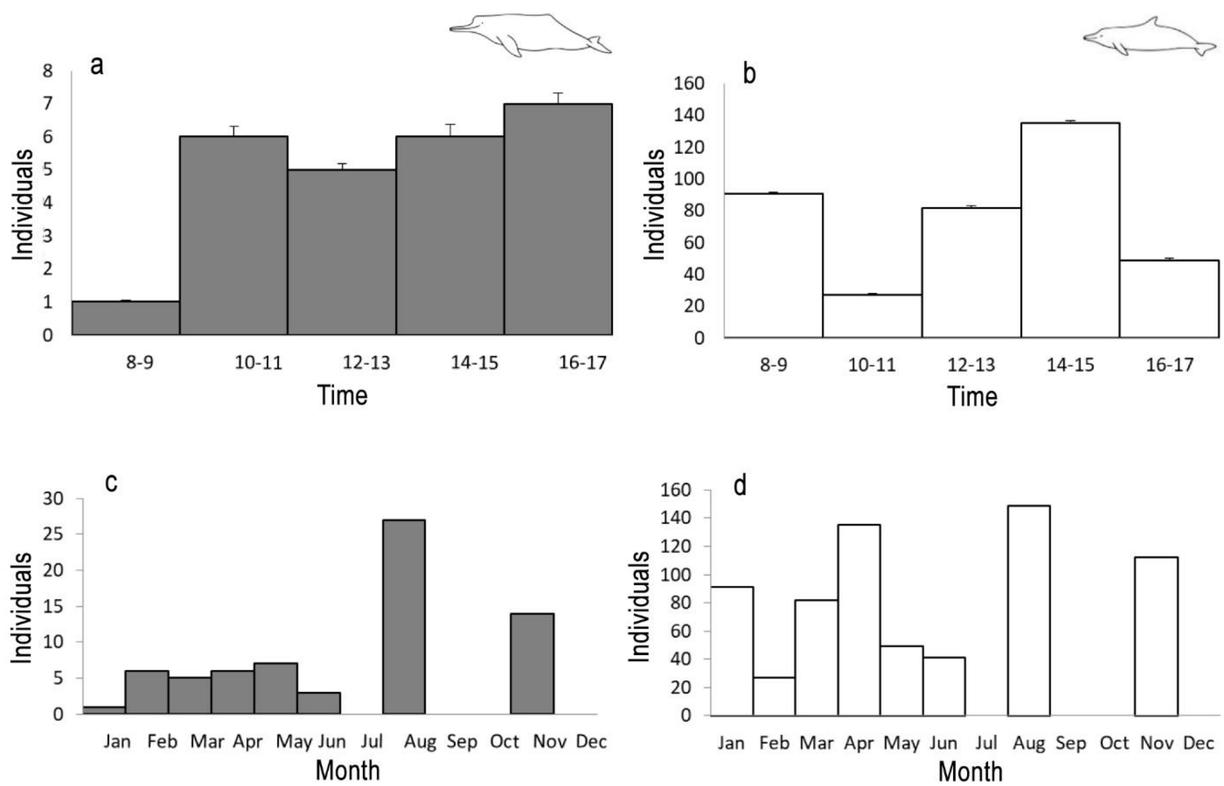
Supplemental 1. Overview map including location and distribution of Pucallpa City, Ucayali.



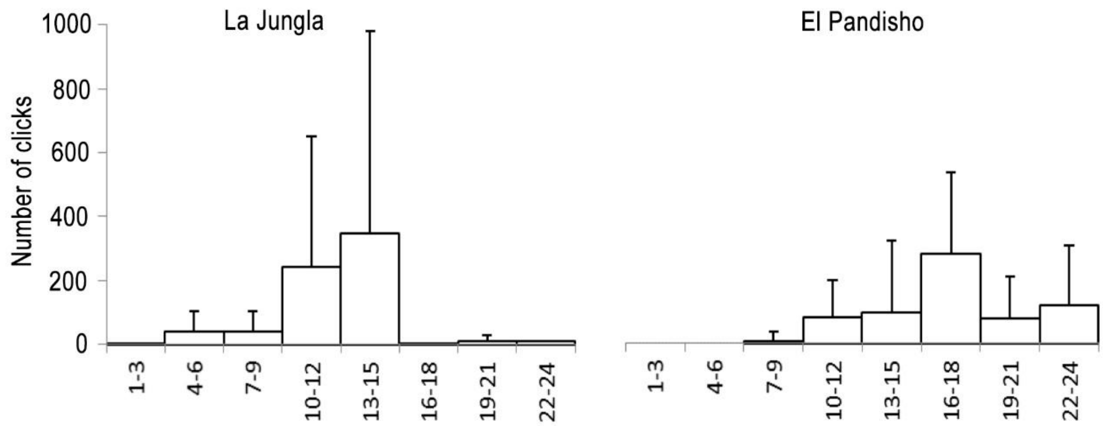
Supplemental 2. Detection probability estimates for *Inia* and *Sotalia* detections.



Supplemental 3. Mean density estimation by transect route for both *Inia* and *Sotalia*. Results presented with standard error bars estimated for each transects number.



Supplemental 4. Mean number of individuals observed by time: in two-hour blocks for a) *Inia* and b) *Sotalia* and by month for c) *Inia* and d) *Sotalia.* Wet seasons are from January to June and dry seasons are from July to December. All graphs are presented with Standard Error. No data are available for July, September, October and December.



Supplemental 5. Mean number of clicks by three-hour bins recorded at each C-POD deployment location.