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Passive acoustic monitoring of river dolphin (*Inia geoffrensis* and *Sotalia fluviatilis*) presence: A comparison between waters near the city of Iquitos and within the Pacaya-Samiria National Reserve

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Abstract

All river dolphin species are in decline as a direct result of intensified anthropogenic activity along river systems. In South America, the size and geographical complexity of their range pose a challenge to status assessment. Passive acoustic monitoring offers a cost-effective, scalable, and readily standardized method for determining species distribution and can augment the spatiotemporal coverage of visual survey efforts currently underway. A passive acoustic survey of dolphin presence was conducted in two areas of the Amazon River subject to different degrees of human use; the inland port city Iquitos and the Pacaya-Samiria National Reserve, in Peru. Surveys were based on acoustic detection of biosonar activity. Recorders were distributed at 17 sites along 61 linear km of river habitat for durations of 46 to 148 h. Dolphin presence was 45% lower near the city than in the reserve. This study demonstrates the efficacy of acoustic monitoring as a method for testing dolphin redistribution and/or decline hypotheses in the context of anthropogenic development. The methods are applicable to continuous future monitoring and status assessment of river dolphins in South America as well as in Asia.

Keywords:
boto, tucuxi, survey, distribution, Amazon

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Introduction

Water development projects, land use change, contamination, and intensified fishing practices are known factors contributing to the probable extinction of the Yangtze river dolphin (*Lipotes vexillifer*) and declining populations of the South Asian river dolphin (*Platanista* spp.), Irrawady dolphin (*Orcaella brevirostris*), and finless porpoise (*Neophocaena a. asiaeorientalis*). Although not yet as extensive, river system development in South America is following a similar path as that of Asia, with analogous impacts on dolphin species likely to follow. Recently, both the Amazon river dolphin or boto (*Inia geoffrensis*) and the the tucuxi dolphin (*Sotalia fluviatilis*) were categorized as endangered (da Silva *et al*., 2018*b*; 2020). There is limited information regarding the population sizes of these species, their distributions, and potential changes to habitat suitability. Recent studies, however, indicate that declines and redistributions of populations related to anthropogenic activity are occurring in specific locations of the Amazon and Orinoco river systems (Gómez-Salazar *et al*., 2012*a*; Pavanato *et al*. 2012*a*; Araújo and Wang, 2015; da Silva *et al*., 2018*a*). Although these studies cover only a small fraction of the species' ranges, it is likely that they are representative of population trends occurring throughout the Amazon and Orinoco river systems (da Silva *et al*., 2018*a*, *b*).

Broad-scale population monitoring is needed in order to prioritize, direct, and evaluate conservation efforts. To be effective, monitoring methods should be relatively easy to implement, standardized, reliable, cost-effective, sustainable over large spatial and temporal scales, and provide timely turnaround of data results. This study describes and demonstrates one such method, namely passive acoustics, for monitoring shifts in river dolphin distribution relative to anthropogenic development. Shifts in distribution offer an early indication of degraded habitat suitability, which is a precursor to population decline. The use of passive acoustic monitoring described here also provides a potential means to extrapolate the findings of visual abundance surveys to larger spatiotemporal scales in a standardized manner. This capability is of greatest necessity given the paucity of resources available for conducting visual surveys throughout the species' expansive ranges.

Methods

Study Areas

A passive acoustic survey of dolphin presence was conducted in two areas of the Amazon watershed of northern Peru: Iquitos and the Pacaya-Samiria National Reserve (PSNR) (Fig. 1). Iquitos and the PSNR are representative of urban and rural river habitats of the Amazon Basin. Iquitos is characterized by a high degree of anthropogenic presence (*e.g*. vessel traffic, standing gillnets, and urban waste) while the PSNR is a pristine area where a baseline reference of dolphin populations in their natural state can be obtained. These areas share similar physical geography and are located in the same meso-level ecosystem. Moreover, there are no physical obstructions to dolphin movement between Iquitos and the PSNR. With human population being the primary difference between study areas, Iquitos and the PSNR provide an ideal case study for acoustically monitoring river dolphin presence in the context of anthropogenic development of the Amazon River.

The City of Iquitos and its contiguous metropolitan districts Belén, Punchana, and San Juan Bautista, referred to hereafter simply as Iquitos, comprise an urbanized area of approximately 360 km^2 with a human population[1] of more than 471,000. The city is located on the main channel of the Amazon River and is bordered by the Nanay River to the northwest, and Itaya River to the southeast (Fig. 2). It is an important port for shipping and transportation between South America's interior and the Atlantic Ocean, and a major center of commerce for the petroleum, natural gas, timber, fishing, and tourism industries.

Conversely, the PSNR is a protected area of 20,800 km^2 with a human population of 24,000 dispersed among 92 separate villages. It is bordered by the Marañón River to the north and the Ucayali River - Puinahua Canal to the south, which join to form the Amazon River (Fig. 1). The economy is comprised of limited floodplain agriculture, subsistence hunting, fishing, and forest product gathering (Barham *et al*., 1999; Coomes *et al*., 2004), with ecotourism as a growing contributor to local livelihoods (Monteferri and Carpio, 2007).

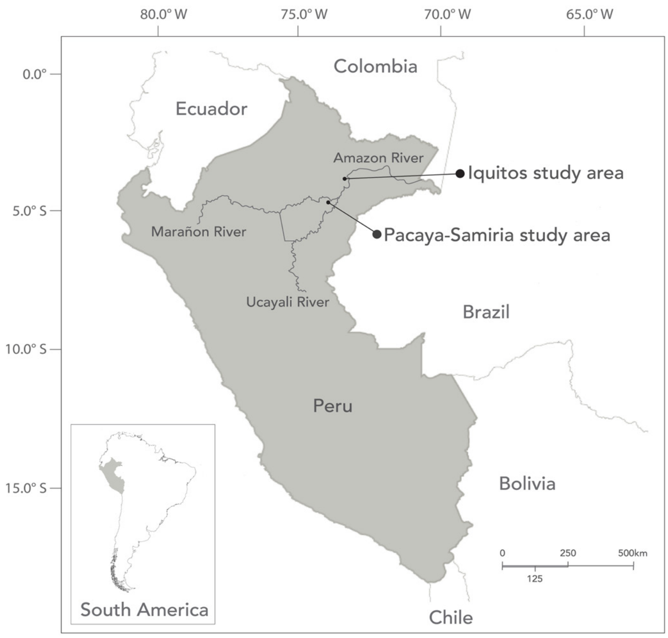

Figure 1: Iquitos and Pacaya-Samiria study areas in northern Peru. Iquitos is located on the Amazon River 170 km downstream from the Pacaya-Samiria study area.

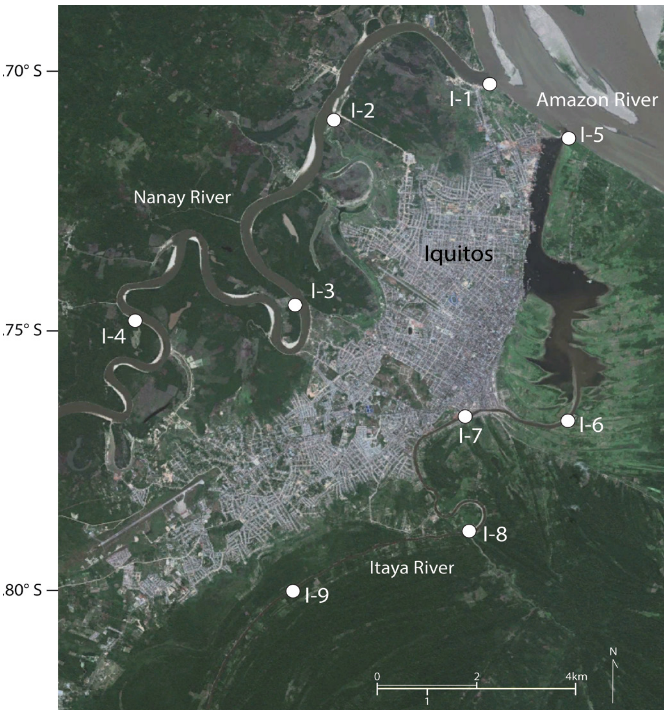

Figure 2: Recording sites situated around the City of Iquitos. Sites Iquitos-1 (I-1) through I-4 were located in the Nanay River to the northwest of the city. Sites I-5 through I-9 were located in the Itaya River to the southeast of the city. The large river to the northeast is the Amazon main channel.

Both Iquitos and the PSNR lie within the ranges of boto and tucuxi (McGuire and Aliaga-Rossel, 2010; Trujillo *et al*., 2010; Gómez-Salazar *et al*., 2012*b*). During the wet season, boto may disperse among flooded forests, small tributaries, and otherwise isolated lakes depending on calving status and/or prey availability (Martin and da Silva, 2004). They return to main channels in the dry season as receding water levels necessitate. Tucuxi are found primarily in mainstream channels and lakes of sufficient year-round volume. While boto are known to travel farther from mainstream channels than tucuxi (Martin *et al*., 2004), the two species are sympatric in the waters surveyed in this study (Gómez-Salazar *et al*., 2012*b*). Moreover, this acoustic survey was conducted during the dry, low-water season when boto retreat from peripheral areas to reside in main channels alongside tucuxi, where prey density is high.

Data Collection

The Iquitos study area consisted of nine survey sites stationed within the main waterways surrounding the city (Fig. 2). Site Iquitos-1 (I-1) was located at the confluence of the Nanay and Amazon rivers, while sites I-2 through I-4 were located upstream in the Nanay River at the confluences of smaller tributaries. Site I-5 was located at the confluence of the Itaya and Amazon rivers. During the survey, the recorder at this site was accidentally retrieved by a local gillnet fisherman. This recorder was subsequently relocated to Site I-6, where the Itaya River mainstream broadens to a lake before passing the Iquitos Port

1. INEI (Instituto Nacional de Estadística e Informática) (2009) *Perú, Estimaciones y Proyecciones de población por sexo según departamento, provincia y distrito 2012-2015*. Lima, Peru.

2. SERNANP (Servicio Nacional de Áreas Naturales Protegidas) (2009) *Plan maestro Reserva Nacional Pacaya Samiria para la conservación de la diversidad biológica y el desarrollo sostenible de la Reserva Nacional Pacaya Samiria y su zona de amortiguamiento 2009-2013*. Iquitos, Peru.

Terminal and entering the Amazon. Sites I-7 through I-9 were located upstream in the Itaya River at the confluences of smaller tributaries. Stream depth at the recording sites ranged from 3.5 m to 7.0 m and varied by < 0.5 m during the survey. Stream width ranged from 60 m to 339 m. Spacing between adjacent recording sites ranged from 1.9 km to 7.7 km. Recorders were deployed on 20 August 2014 and retrieved on 25 August 2014, yielding recordings that ranged in length from 46 hr 12 min to 119 hr 20 min. The recorder at Site I-5 was relocated to Site I-6 on 23 August (Table 1).

The PSNR study area consisted of eight survey sites in waterways comparable to the Nanay and Itaya rivers in width and depth. These sites were clustered within the northeast region of the reserve, 170 km upstream from Iquitos (Fig. 3). Site Pacaya-Samiria-1 (PS-1) was located at the confluence of the Yanayacu-Pucate River and Marañón River. Site PS-2 was located at the confluence of the Yanayacu and Pucate rivers. Sites PS-3 through PS-5 were located upstream in the Pucate River at the confluences of smaller tributaries. Sites PS-6 and PS-8 were located upstream in the Yanayacu River at the confluences of smaller tributaries, while site PS-7 was located between sites PS-6 and PS-8 but not near a confluence. Stream depth at the recording sites ranged from 3.3 m to 8.0 m and varied by < 0.5 m during the survey. Stream width ranged from 61 m to 240 m. Spacing between adjacent recording sites ranged from 3.4 km to 6.0 km. Recorders were deployed on 27, 28, 29, and 31 August 2014 and retrieved on 2 and 3 September 2014, yielding recordings that ranged in length from 72 h 37 min to 148 h 02 min (Table 1).

Recorders were placed at river confluences in order to increase the probability of dolphin detection. The affinity of river dolphins to confluences is well documented by a number of studies showing higher density and abundance in such areas (Vidal *et al*., 1997; Martin *et al*., 2004; Kreb and Budiono, 2005; Smith *et al*., 2009; Braulik *et al*., 2012; Gómez-Salazar *et al*., 2012*b*; Araújo and da Silva, 2014). Spacing between recorders in the Iquitos study area was largely dependent upon confluence location, but was also intended to provide evenly spaced coverage surrounding the city with a limited (n=8)

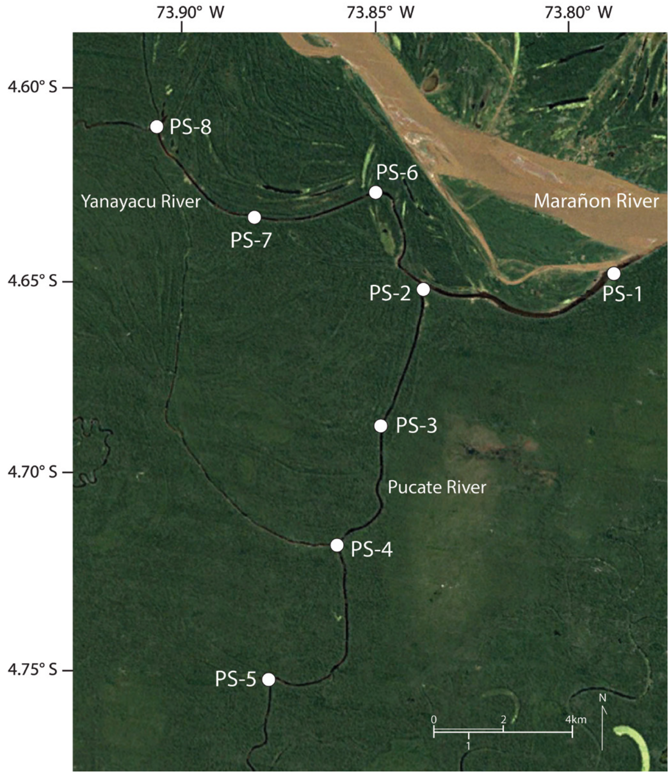

Figure 3: Recording sites situated in the Pacaya-Samiria National Reserve (PSNR), 170 km upstream from Iquitos. Sites Pacaya-Samiria 1 (PS-1) and PS-2 were located in the Yanayacu-Pucate River. Sites PS-3 through PS-5 were located in the Pucate River. Sites PS-6 through PS-8 were located in the Yanayacu River. All recording sites except PS-7 were located at the confluences of smaller tributaries. The large river to the northeast is the Marañón main channel.

number of recorders. Recording sites in the PSNR study area were intended to match those near Iquitos in spacing, proximity to tributaries, and in river depth and width.

Acoustic surveys were conducted using shore-based Song Meter SM2 digital recording units (Wildlife Acoustics, Inc.) attached to HTI-96-min hydrophones (High Tech, Inc.) via 20 m cables. The HTI-96-min hydrophones had a sensitivity of -164.3 dB (re: 1V/μPa) and flat frequency response (±2.2 dB)

Table 1: Recording site locations, hydrology, period, and percent time during which biosonar was present. 'Stream' refers to the Yanayacu, Pucate, Itaya, and Nanay rivers in which recorders were placed. Tributaries were unnamed. 'Main Channel' refers to the Amazon and Marañon rivers. 'Yn-Pc' refers to the Yanayacu-Pucate River.

		Site	Latitude	Longitude	Stream Depth (m)	Stream Width (m)	Tributary Depth (m)	Tributary Width (m)	Distance from Main Channel (m)	Survey Start Date | Time	Survey End Date | Time	Duration	Biosonar Presence
Iquitos	Nanay	I-1	-3.702	-73.246	7.0	325	---	---	0	20 Aug 2014 12:21:50	25 Aug 2014 10:55:50	118 h 34 min	57%
		I-2	-3.709	-73.275	3.9	339	2.8	36.8	4,700	20 Aug 2014 11:23:53	24 Aug 2014 23:49:53	108 h 26 min	5%
		I-3	-3.745	-73.282	5.8	230	3.2	31.3	9,700	20 Aug 2014 10:46:37	25 Aug 2014 10:06:37	119 h 20 min	2%
		I-4	-3.748	-73.312	5.3	212	2.9	24.0	17,300	20 Aug 2014 09:20:02	24 Aug 2014 23:45:02	110 h 25 min	2%
	Itaya	I-5	-3.713	-73.232	5.7	134	---	---	0	20 Aug 2014 12:59:52	23 Aug 2014 12:08:52	71 h 9 min	71%
		I-6	-3.767	-73.232	4.5	107	---	---	6,200	23 Aug 2014 15:37:24	25 Aug 2014 13:49:24	46 h 12 min	0%
		I-7	-3.766	-73.251	5.2	87	0.6	18.0	8,400	20 Aug 2014 16:35:38	25 Aug 2014 13:24:38	116 h 49 min	0%
		I-8	-3.788	-73.250	3.5	90	3.4	21.1	13,000	20 Aug 2014 14:50:32	25 Aug 2014 12:59:32	118 h 9 min	0%
		I-9	-3.800	-73.283	5.7	60	1.0	11.5	16,900	20 Aug 2014 15:44:54	25 Aug 2014 12:32:54	116 h 48 min	0%
Pacaya-Samiria	Yn-Pc	PS-1	-4.648	-73.788	8.0	240	---	---	700	28 Aug 2014 14:19:10	03 Sep 2014 02:55:10	132 h 36 min	99%
		PS-2	-4.652	-73.837	12.2	108	3.6	83.0	6,700	28 Aug 2014 09:34:51	03 Sep 2014 02:53:51	137 h 19 min	93%
	Pucate	PS-3	-4.687	-73.848	11.0	71	1.0	7.2	11,000	27 Aug 2014 16:14:36	01 Sep 2014 16:24:36	120 h 10 min	57%
		PS-4	-4.718	-73.860	5.5	79	2.3	31.0	15,000	27 Aug 2014 16:51:04	01 Sep 2014 17:00:04	120 h 9 min	56%
		PS-5	-4.753	-73.877	8.8	62	2.0	22.9	20,800	27 Aug 2014 17:30:45	02 Sep 2014 21:32:45	148 h 2 min	29%
	Yanayacu	PS-6	-4.627	-73.849	3.5	77	0.7	4.5	10,100	29 Aug 2014 09:25:33	03 Sep 2014 09:56:33	120 h 31 min	80%
		PS-7	-4.633	-73.881	3.3	69	---	---	13,800	31 Aug 2014 14:51:04	03 Sep 2014 15:28:04	72 h 37 min	44%
		PS-8	-4.610	-73.906	5.5	61	2.8	20.0	17,900	28 Aug 2014 10:57:49	03 Sep 2014 06:00:49	139 h 3 min	84%

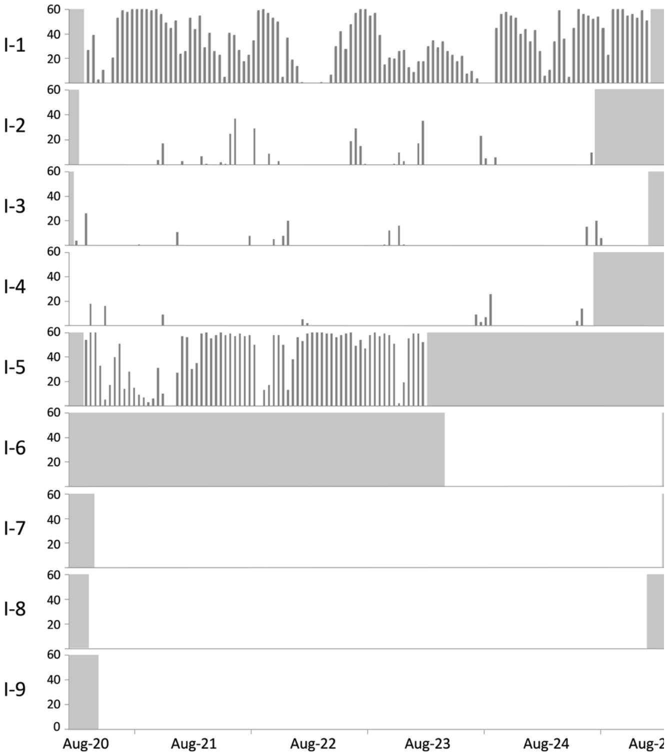

Figure 4: Number of 1-min samples containing echolocation clicks during each hour of recording at each site in the Iquitos study area. The vertical axis represents 60 minutes. The horizontal axis represents 123 hours. Dark grey blocks represent periods with no recorded audio data. The change of recording location from site I-5 to I-6 occurred midway through the survey.

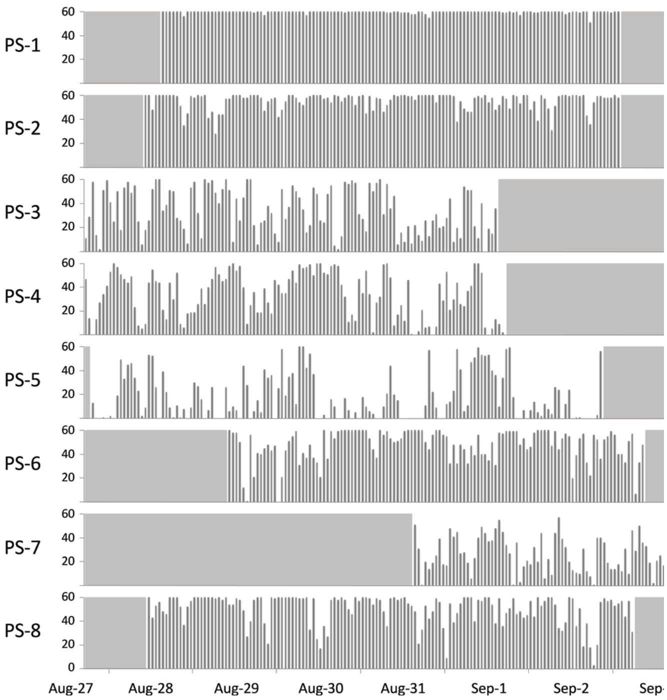

Figure 5: Number of 1-min samples containing echolocation clicks during each hour of recording at each site in the PSNR study area. The vertical axis represents 60 minutes. The horizontal axis represents 158 hours. Dark grey blocks represent periods with no recorded audio data.

from 2 Hz to 30 kHz. Sensitivity decreased to -184 dB and varied by ±3.7 dB between 30 kHz and 48 kHz. All units were set to record at a sample rate of 96 kHz, gain of 24 dB, and bit depth of 16. At these settings, the complete system had a sensitivity of -140.3 dB (±2.2 dB from 2 Hz-30 kHz) and -160.0 dB (±3.7 dB from 30-48 kHz), dynamic range of 96 dB, and Nyquist frequency of 48 kHz. Although the HTI-96-min hydrophone frequency response is not flat above 30 kHz, all units shared the same frequency response curve. Therefore, no sensitivity bias was introduced between recording sites.

Data Analysis

The recordings were analyzed visually and aurally using Raven Pro[3] v. 1.5. Spectrograms were set to span 16-48 kHz with a 1024-point FFT, Hann window, and 50% overlap (frequency resolution of 93.8 Hz, time resolution of 5.3 ms). The full duration of each recording was divided into 1-min time slices. Each time slice was then assessed for the presence of echolocation signals (hereafter referred to as biosonar) characteristic of boto and tucuxi (Kamminga *et al*., 1993; Yamamoto *et al*., 2015). A binary value of 1 (if present) or 0 (if absent) was assigned without differentiating between the two species. Results were then plotted using time series graphs displaying the number of 1-min samples during each hour of recording that contained biosonar at each survey site (Figs. 4 and 5). The percent of 1-min samples that contained biosonar during the full recording period at each survey site was also calculated (Table 1, column 12).

A nonparametric Wilcoxon rank-sum test using MATLAB[4] statistical toolbox was conducted to compare presence between the Iquitos and PSNR study areas. The Wilcoxon rank-sum test is an appropriate alternative to the Independent Samples *t*-Test when analyzing data sets that are small in sample size and lack normality in their distribution (Larsen and Marx, 2006). The comparison was based on the percent of 1-min samples that contained biosonar during the full recording period at each of the 17 survey sites (Table 1, column 12). Using these same data points, the relationship between presence and river depth, river width, and upstream distance from the Marañon and Amazon main river channels was investigated. This was done through simple linear regression analyses. River measurement values are displayed in Table 1.

Because ambient noise from rain, boat motors, and non-target species varies across time and space, potential detection bias due to acoustic masking (Clark *et al*., 2009) of biosonar may have been introduced between survey areas. To address this, the equivalent continuous sound level - Leq (dB re 1μPa) within the 16-48 kHz frequency band for each 1-min sample was determined using Raven Pro v. 1.5 signal calibration and waveform measurement tools[5]. Then, all 1-min samples were categorized by Leq in 1 dB increments and the percent of 1-min samples that contained biosonar at each Leq value was compared between Iquitos and the PSNR. Results for both survey areas were plotted in a graph displaying the percent of 1-min samples that contained biosonar at each Leq value (Fig. 6).

3. Center for Conservation Bioacoustics (2014) *Raven Pro: Interactive Sound Analysis Software Version 1.5* [Computer software]. The Cornell Lab of Ornithology, Ithaca, NY, USA.

4. MathWorks Inc. (2016) *MATLAB* and *Statistics Toolbox Release 2016a*. [Computer software]. Natick, Massachusetts, USA.

5. Charif, R.A., Strickman, L.M. and Waack, A.M. (2010) *Raven Pro 1.4 User's Manual*. The Cornell Lab of Ornithology, Ithaca, NY, USA.

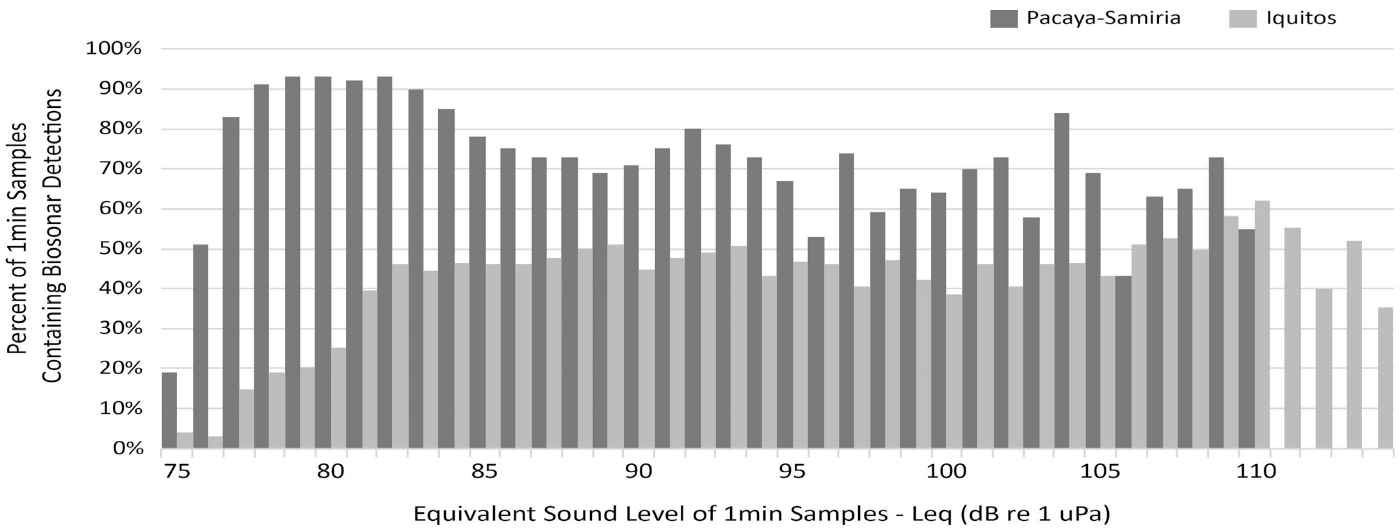

Figure 6: Percent of 1-min samples containing biosonar for each noise level measured in the PSNR and Iquitos study areas cumulatively. Presence was greater in the PSNR at all but two noise levels (106 dB and 110 dB).

Results

There was great heterogeneity in dolphin presence across recording sites. Presence ranged from 0% (upstream in the Itaya River proximal to Iquitos) to 99% (at the confluence of the Marañon River in the PSNR). In both the Iquitos and PSNR study areas the confluences with main channels (Amazon and Marañon, respectively) were most heavily occupied by dolphins, while in all four rivers studied (Nanay, Itaya, Yanayacu, and Pucate), presence was lower at upstream recording sites than at their downstream outlets.

Biosonar detections in the Iquitos survey area occurred only at sites located in the Amazon and Nanay Rivers (Figs. 2 and 4). Detection rate decreased significantly (from 57% to 2%) in the Nanay River upstream from its confluence with the Amazon River. No biosonar was detected in the Itaya River upstream from its confluence with the Amazon River.

Biosonar was detected at all recording sites along the Yanayacu, Pucate, and Marañón rivers of the PSNR survey area (Figs. 3 and 5). Highest detection rates occurred at the Marañón River confluence (Site PS-1, 99%) and where the Yanayacu and Pucate rivers merge to create the Yanayacu-Pucate River (Site PS-2, 93%). Detection rate decreased upstream from these sites (to as low as 29%), but the decrease was not as extensive as that observed in the Nanay River of Iquitos.

Overall, dolphin presence was greater in the PSNR than near Iquitos. Recorders detected biosonar in 40,952 (69%) of the cumulative 59,387 1-min samples recorded in the PSNR survey area and 7,693 (17%) of the cumulative 45,687 1-min samples recorded in the Iquitos survey area. When excluding recording sites upstream in the Itaya River where no detections occurred, cumulative presence in the Iquitos survey area was 7,693 (24%) of 31,670 samples.

The difference in presence between the Iquitos and PSNR study areas was statistically significant (Wilcoxon rank-sum test; p = 0.0073, α = 0.05). The negative correlation between presence and upstream distance from river main channels was linear and statistically significant in the PSNR study area (β_1 = -0.03 Presence km^{-1}, 95% CI = [-0.06 -0.00], p = 0.036) (Fig. 7a). In the Iquitos study area, the correlation was nonlinear with presence dropping off precipitously as distance from the

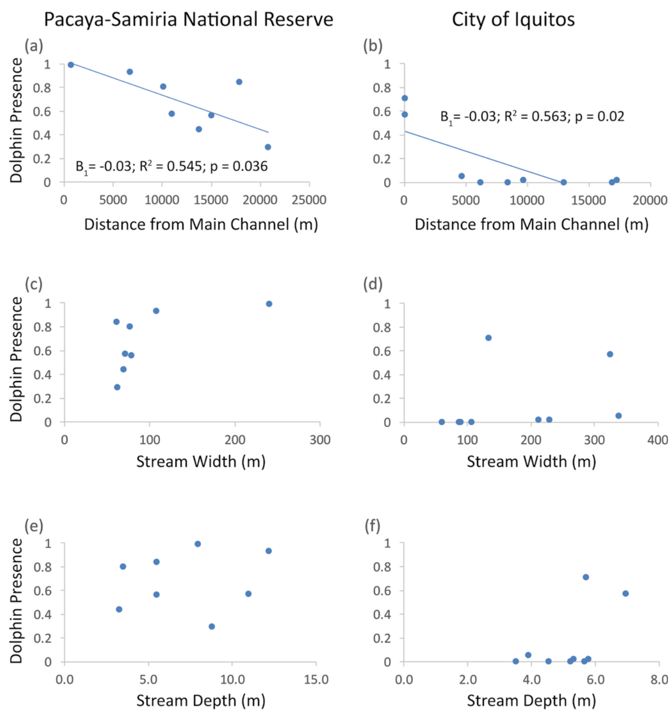

Figure 7: Linear regression plots of dolphin presence at each survey site vs hydrological measures in the PSNR study area (left) and Iquitos study area (right). Y-axes represent the portion of 1-min samples that contained biosonar during the survey. X-axes represent distance from main river channels, stream width, and stream depth in meters. Best-fit lines, and values for ß1, R^2, and p are given for plots in which ß1 differed from 0 with statistical significance (α = 0.05).

Amazon main channel increased (Fig. 7b). No correlation was found between presence and river depth or river width in either study area (Fig. 7c, d, e, f).

Leq of 1-min samples ranged from 75 to 110 dB at the PSNR recording sites and from 75 to 114 dB at the Iquitos recording sites. This resulted in 36 Leq levels at which biosonar presence between the two survey areas was compared. The percent of 1-min samples containing biosonar detections was higher in the PSNR at all of the Leq levels measured except for 110 dB and 106 dB (Fig. 6).

Discussion

Findings

This study demonstrates the efficacy of acoustics for monitoring dolphin distribution in relation to human presence. Overall, dolphin presence was lower in the Iquitos study area than in the PSNR study area. Although these results were statistically significant, longer recordings are needed to examine seasonality of presence and to compare change over time in each study area. One of the many advantages of acoustic monitoring is that studies can easily be scaled up with limited additional effort. In this case, leaving recorders deployed for a longer duration would effectively increase the temporal scale of the project (with little or no additional field work) allowing for an examination of relative change over time and increasing the statistical strength of the results.

The negative relationship between anthropogenic activity and dolphin presence has been observed through visual surveys of river dolphins in other areas of the Amazon and Orinoco river basins (Gómez-Salazar *et al*., 2012*a*). And though this acoustic monitoring study was conducted on a limited scale which greatly reduced its statistical power, the results nonetheless agree with previous studies and should be considered in the context of known effects of anthropogenic presence on dolphin populations.

The complete absence of biosonar upstream in the Itaya River coincided with the densest human presence among study sites. The majority (6.5 of 9.5 km) of the west bank of the Itaya River, from its mouth at site I-5 to 1.9 km upstream of site I-7, is comprised of urban infrastructure including the Iquitos Port Terminal, lumber mills, and the densely populated district of Belén. This section of river also experiences the most boat traffic of all recording sites in the study. High ambient noise levels in this area may have prevented the detection of infrequent biosonar signals if they occurred. Yet the comparison of this section of river to others at equal noise levels reveals a relative minimum in dolphin presence along the Itaya River. Moreover, there was an absence of biosonar at sites farther upstream in the Itaya River (*i.e*. I-8 and I-9) where noise levels were reduced and probability of detection was not diminished.

It is unclear however, if the physical geography of the lake situated between sites I-5 and I-6 affected the presence of dolphins upstream of site I-5. The use of lakes by boto and tucuxi has been documented extensively. McGuire and Aliaga-Rossel (2010) documented both species in lakes as shallow as 1.5 m in the PSNR. During the study period, the center-of-channel depths of the lake inlet (site I-6) and lake outlet (site I-5) were 4.5 m and 5.7 m, respectively. Stream depth and width upstream of the lake at sites I-7, I-8, and I-9 were similar to those in the PSNR at sites PS-6, PS-7, and PS-8, where dolphins were present.

Dolphins were in fact present upstream in the Nanay River on the north side of Iquitos, although their occurrence was infrequent and dispersed. The detection of dolphins occupying this river in particular demonstrates the value of continuous acoustic monitoring in areas with transient and/or low-density populations. During daylight hours between sunrise (06:10:00 h PET) and sunset (18:10:00 h PET), dolphin presence at sites I-2, I-3, and I-4 amounted to 4%, 1%, and 1% respectively. It would not be unlikely for a daytime visual survey (conducted at boat speeds set to outpace dolphins in order to prevent double counts) to entirely miss the presence of dolphins in this area.

Dolphin presence decreased with upstream distance from main river channels in both study areas. This decrease however, was more pronounced in the Iquitos study area (Fig. 7a, b) indicating a diminished preference for habitat in tributaries bordering the city. Dolphins appear to have approached Iquitos via the Amazon main channel but rarely ventured nearer than the Nanay and Itaya confluences (Figs. 2 and 4). Stream width and depth at the survey sites were not significantly correlated with dolphin presence. A broader range of width and depth values is needed to fully examine the relationship between dolphin presence and these parameters.

While noise levels ranged from 75 to 110 dB in both study areas, levels exceeding 110 dB occurred only in the Iquitos study area (Fig. 6). Overall dolphin presence may have been lower near Iquitos due to higher vessel traffic and noise levels. Boat avoidance behavior has been documented in the Yangtze finless porpoise (Li *et al*., 2008; Wang *et al*., 2015) and Irrawaddy dolphin (Kreb and Rahadi, 2004). Dey *et al*. (2019) modeled increased metabolic stress in Ganges river dolphins as they changed behavior in response to vessel noise.

The influence of noise in complex river habitats warrants further investigation. However, it is likely that multiple, scale-dependent environmental factors influenced the distribution of dolphins. For example, overall dolphin presence was lower near Iquitos, which had higher noise levels, compared to the PSNR. But when comparing sites along the Nanay River inside the Iquitos study area, the opposite trend can be seen. There was less dolphin presence at quieter sites upstream (Sites I-2, I-3, and I-4) than near its confluence with the Amazon main channel (Site I-1) where more vessel traffic occurred. Here, factors affecting dolphin preference for downstream sites may have overridden the local effect of noise. The cause of downstream preference is not clear. The trend was apparent in both the PSNR and Iquitos study areas (Fig. 7a, b). Distribution along tributaries may have been driven by prey density (Martin *et al*., 2004).

The comparison of biosonar detection between study areas at equal ambient noise levels was included in the methods to eliminate potential bias stemming from uneven variations of noise throughout the surveys. When comparing study areas at equal ambient noise levels, presence was greater in the PSNR than in Iquitos (Fig. 6). This demonstrates that the reduced presence observed in Iquitos was not due simply to masking of biosonar by anthropogenic noise.

The negative relationship between biosonar detection and noise that would be expected due to masking was obscured in Fig. 6 because data for multiple sites was plotted cumulatively. In general, sites farthest from the main river channels had less boat traffic and less dolphin presence than sites near main channels where both dolphins and boat traffic occurred most. In effect, upstream sites were quieter and had fewer echolocation clicks compared to downstream sites. Combining data across upstream and downstream sites within each study area led to a leveling out of the negative relationship that would be apparent at each site individually. These differences between upstream and downstream sites were more prominent in the Iquitos study area. Note in Fig. 6 that a downward trend can be seen in the PSNR data but not in the Iquitos data.

Although the number of individual dolphins present was not determined in this study, these methods nonetheless provide a cost-effective means to determine relative abundance and distribution over time and space. Moreover, when paired with visual abundance surveys, passive acoustic monitoring can be used to interpolate temporally continuous results between visual efforts or to extrapolate spatial results beyond the geographic areas covered by visual efforts. These capabilities are important to population assessments as boto and tucuxi distribution changes dynamically by season and covers vast geographic ranges.

While it is possible to differentiate between biosonar from boto and tucuxi through visual inspection of waveforms (Kamminga *et al*., 1993), it would be cost-prohibitive to do so for large datasets covering ecologically meaningful spatiotemporal scales. Future research should focus on automated detection and classification algorithms to streamline analysis efforts as has been done for other odontocete species (Gillespie and Caillat, 2008; Klinck and Mellinger, 2011; Roch *et al*., 2011).

Optimizing Recording Parameters

In this study, recorders were set at a sample rate of 96 kHz for an effective recording frequency of 48 kHz. This was the highest sample rate capability of the SM2 recorders and was sufficient to capture the lower frequency component of boto and tucuxi biosonar. However, the peak frequencies (*i.e*. the frequencies of maximum power) of biosonar from boto and tucuxi fall within the 47 to 125 kHz and 47 to 137 kHz bandwidths, respectively (Yamamoto *et al*., 2015). Ideally, recorders in this study would have sample rates high enough to capture the frequencies at which biosonar has the greatest power. This would increase the probability of detection when ambient noise levels are high and increase the distance over which dolphins could be detected.

However, recording at higher sample rates draws more power and requires more storage space. This necessarily reduces the time that the recorders can operate autonomously in the field (as they require more frequent battery and data-drive changes). The tradeoff between recorder deployment time and sample rate should be considered carefully when designing an acoustic monitoring study. If the recorders are readily accessible and attended regularly enough to keep up with data storage and power demands, then taking advantage of a high sample rate will provide biosonar recordings with the highest signal to noise ratio. If, however, the advantage of acoustic monitoring to cover large (sometimes remote) areas for long periods of time is to be fully exploited, then recorders may need to be left in the field unattended for as long as possible. In such a case, a sample rate of 96 kHz (effective recording frequency of 48 kHz) is sufficient to capture boto and tucuxi biosonar, as demonstrated here.

Due to constraints on funding and travel time, in-situ propagation tests to determine the detection ranges at each recording site were not conducted. Previous studies have detected Ganges river dolphins and Yangtze finless porpoises in river environments at distances of 80 m (Sasaki-Yamamoto *et al*., 2013), 275 m (Akamatsu *et al*., 2001), 300 m (Akamatsu *et al*., 2008), and 478 m (Li *et al*., 2009). Detection distance is affected by biosonar source level, background noise level, recording frequency, and hydrophone sensitivity. River depth, substrate, and meanders also influence sound propagation and can therefore have an impact on detection distance. Although the exact detection ranges in this study are unknown, factors affecting detection range were controlled at all recording sites to prevent bias. All recorders had the same gain setting, hydrophone sensitivity, and recording frequency, and were deployed in locations of similar river depth and substrate. All hydrophones were positioned 0.5 m above the river bottom. All recording sites (except site I-8) were distanced from meanders by a mid-channel sightline minimum of 375 m in the upstream and downstream directions. Site I-8 was obstructed by a meander in the downstream direction at a distance of 275 m. Background noise was accounted for as described above. Future acoustic monitoring studies should include propagation measurements to quantify site-specific detection distances. This is especially important when comparing presence across different habitat types where depth, width, bottom type, and proximity to meanders differ between recording sites.

The location and spacing between recording sites in the Iquitos and PSNR study areas were chosen to maximize the probability of detecting dolphins. River dolphins prefer stream confluences, where prey density is high and where they can conserve energy by avoiding swift downstream currents in the confluences' eddies. Confluences were relatively evenly spaced around the City of Iquitos, and given a limited number of recorders to work with, Sites I-1 through I-9 were the best suited to encircle Iquitos. In the PSNR study area, confluence sites of similar spacing, depth, and width were chosen for comparative purposes.

With limited project resources, a visual survey during the recording period was not organized. Previous studies that implemented simultaneous visual and acoustic surveys revealed positive linear relationships between the number of echolocation clicks detected and number of finless porpoises observed in the Yangtze River (Wang *et al*., 2005; Kimura *et al*., 2010). Correlating visual and acoustic detections of river dolphins may offer a baseline from which increasing, decreasing, modulating, or steady-state trends can be acoustically determined during periods when visual surveys are not conducted. This type of analysis would be particularly valuable near *varzea* habitats where dolphin distribution changes dynamically with seasonal rise and fall of water levels.

This study demonstrates the feasibility of passive acoustic monitoring in river environments to investigate the distribution of river dolphins relative to anthropogenic presence. While the location and the duration of surveys in future studies will vary according to research objectives, the methods presented here illustrate in general terms the fundamentals of passive acoustic monitoring and key considerations for increasing the probability of dolphin detection. These methods are readily scalable and are applicable to continuous future monitoring and status assessment of river dolphins in South America as well as in Asia.

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