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Geographic Variation in Low Frequency Narrow-Band Sounds Produced by Amazon River Dolphins (Inia geoffrensis) in Brazil and Peru

Grace Olson
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To the Dean of the Graduate School:

We are submitting a thesis written by Grace Olson entitled “Geographic Variation in Low Frequency Narrow-Band Sounds Produced by Amazon River Dolphins (*Inia geoffrensis*) in Brazil and Peru.” We recommend acceptance in partial fulfillment of the requirements for the degree of Master of Science in Biology.

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GEOGRAPHIC VARIATION IN LOW FREQUENCY NARROW-BAND SOUNDS PRODUCED BY AMAZON RIVER DOLPHINS (INIA GEOFFRENSIS) IN BRAZIL AND PERU

A Thesis
Presented to the Faculty
Of the
College of Arts and Sciences
In Partial Fulfillment
Of the
Requirement for the Degree
Of
Masters of Science
In the Department of Biology
Winthrop University

August 2017

By
Grace Olson
Abstract

Relatively few studies have investigated the sound production of Amazon River dolphins (*Inia geoffrensis*), and many questions remain regarding their social sounds. In this study, we document the presence of “low frequency narrow-band” (LFN) sounds in Amazon River dolphins. Amazon River dolphin LFN sounds were recorded in the Mamirauá Sustainable Development Reserve (Brazil), and 1,512 km away in the Área de Conservación Regional Comunal Tamshiyacu Tahuayo reserve (Peru). A quantitative comparison of LFN sounds produced by these two populations demonstrates evidence of geographic variation. The LFN sounds produced by Amazon River dolphins in Peru have a higher peak frequency (median = 3380.3 Hz) than the sounds produced by the dolphins in Brazil (median = 2805.2 Hz) (Mann Whitney U = 51,170, p = 0.00). The duration of LFN sounds produced was found to be longer by Amazon River dolphins in Peru (median = 0.103 sec) than the sounds produced in Brazil (median = 0.095 sec) (Mann Whitney U = 37,539.5, p = 0.001). In addition, the number of sounds per train and the inter-sound intervals (sec) were significantly greater in the Peruvian population of Amazon River dolphins (median number of sounds per train = 2.00, median inter-sound interval = 0.06 sec) than the sounds produced by the dolphins in Brazil (median number of sounds per train =1.00, median inter-sound interval =0.031 sec) (Mann-Whitney U =16,883.5, p =0.003, Mann-Whitney U = 15,723.5, p = 0.00). The variation found in the social sounds produced by geographically separated Amazon River dolphin populations provides valuable information about this species and the plasticity of their communication signals. The differences in the analyzed LFN parameters may reflect environments, differences in group size and composition, background noise and/or evolutionary responses to geographic isolation and may impact dispersal capabilities of these animals as the first step in evolutionary divergence and speciation.
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Introduction

Amazon River Dolphins

Amazon River dolphins (Inia spp.) are obligate freshwater species from the order Cetacea and the suborder Odontoceti. Amazon River dolphins are only found in the Amazon and Orinoco River basins of South America (Best and da Silva 1993). Amazon River dolphins are also known as botos, or pink river dolphins, due to the pink coloration that is displayed by larger adults, especially males (Best and da Silva 1993, Martin and da Silva 2006). They are the largest of freshwater dolphin species in the world and have no natural predators (Best and da Silva 1993). Male Amazon River dolphins have an average body length of 2.55 meters and weight of 160 kilograms. Amazon River dolphins are sexually dimorphic and males are larger than females (female average body length of 2.01 meters and weight of 98.5 kilograms) (Best and da Silva 1993). Males also are significantly pinker in coloration than females and show higher levels of scarring, primarily from male-male aggression (Martin and da Silva 2006).

Recent analyses have determined that Amazon River dolphins have been isolated from oceanic dolphin lineages for 12-15 million years (Hamilton et al. 2001). They are significantly different morphologically and behaviorally from oceanic dolphins and belong to their own family, Iniidae (Best and da Silva 1993, Hamilton et al. 2001). Physical characteristics such as: a prominent rostrum, large and broad pectoral fins, a keel shaped dorsal fin, and increased flexibility in the neck and pectoral fins of Amazon River dolphins make them well adapted to their riverine environment (Best and da Silva 1993).
Currently, *Inia* is divided into three species (Figure 1). “Amazon River dolphin” is the common name used for all members of the *Inia* genus. *Inia geoffrensis*, the first species in the genus to be described and the one with the largest distribution, is split into two subspecies, *I. g. humboldtiana* and *I. g. geoffrensis*. The other two species are *I. boliviensis* and a recently proposed new species, *Inia araguaiaensis* (Ruiz-Garcia et al. 2008, Hrbek et al. 2013). The Amazon River dolphin species are limited in their distributions and movements by large rapids or waterfalls (Best and da Silva 1993). A fourth dolphin species, the tucuxi (*Sotalia fluviatilis*), is a delphinid that lives sympatrically in the freshwater habitats of the Amazon basin (Vidal et al. 1997, da Silva et al. 2010).

Figure 1. Map showing the range of *Inia* species and subspecies. *Inia geoffrensis* (lime green), *Inia boliviensis* (purple), *Inia araguaianensis* (blue). The question marks indicate an area of unknown *Inia* presence. Image taken from Hrbek et al. 2013.
The various species of *Inia* are spread over six South American countries (Bolivia, Peru, Brazil, Colombia, Ecuador, and Venezuela), but the overall population size of any species is unknown. Small scale studies in some portions of their ranges show that the dolphins appear to be numerous, but data are lacking in many areas of known Amazon River dolphin occurrence. A study by Vidal *et al.* (1997) along 120 kilometers of river on the Colombia and Peruvian borders estimated a population of 348 Amazon River dolphins (*I. g. geoffrensis*) using a strip transect model. The long-term study in Mamirauá Sustainable Development Reserve estimated the population in the 11,240 km² reserve to be around 13,000 Amazon River dolphins (*I. g. geoffrensis*) (Martin and da Silva 2004b).

The flooded forest habitats in the Amazon River basin where these animals are found can be described as either várzea or igapó, based on the type of water found in the rivers. Várzea are white-water habitats that are rich in sediments and nutrients. These nutrients and sediments are carried down the rivers from the Andes, where they originate, and are often deposited along riverbanks and in lakes (Prance 1979, Kvist and Nebel 2001, Rios-Villamizar *et al.* 2014). While várzea habitats are called white-water, they are actually a muddy brownish-red in color. White-water rivers are characterized as having high turbidity, low water transparency, and a pH that is close to neutral (Rios-Villamizar *et al.* 2014). Igapó habitats are black-water and characterized as being nutrient poor. Black-water rivers originate in sandy areas and appear black (Kvist and Nebel 2001). These rivers have a higher water transparency, a more acidic pH of 4-5, and have a high humic content (Prance 1979, Rios-Villamizar *et al.* 2014).
Studies of Amazon River dolphins and the tucuxi show that both are found in a variety of habitats, but they have clear preferences for confluences of river channels and highly productive areas where white and black water mix (Vidal et al. 1997, Martin et al. 2004). Amazon River dolphins are known to range between main rivers, smaller channels, and lake systems within the várzea and igapó riverine habitats (Best and da Silva 1993). There is a bias for female Amazon River dolphins to occupy lake systems and channels, and for males to spend more time in the main rivers (Martin and da Silva 2004a). However, unlike Amazon River dolphins, tucuxi are not as flexible and do not have the ability to enter the seasonally flooded forests during high water levels. They prefer open channels and areas with deeper water (da Silva et al. 2010).

A long-term research project using freeze-branded individuals and radio tags in the Mamirauá Sustainable Development Reserve in Brazil has been influential in understanding the movement of local Amazon River dolphins. In that region, the influence of the seasonal fluctuation of flooding typical of the Amazon River basin on Amazon River dolphin movement has been studied (Martin and da Silva 2004b). The resident Amazon River dolphins spend most of the year occupying lake systems and channels off the main river, but they must leave during the low water season. Some of the residents show site fidelity and return to the same lake systems year after year, but other individuals sighted in the area appear to be more transient and are not seen for years at a time (Martin and da Silva 2004b). This shows evidence of structured populations based on floodplain lake systems. Martin and da Silva (2004b) also found that the dolphins can move tens to hundreds of kilometers.
The same seasonal influence in movement has also been observed in studies of Amazon River dolphins in Bolivia and along the Colombia-Peru border (Vidal et al. 1997, Aliaga-Rossel 2002). Research in Peru using photographic identification analyzed movement patterns of Amazon River dolphins and reported that they can travel an average of 60.8 kilometers (McGuire and Henningsen 2007). Transient and resident dolphins were observed by McGuire and Henningsen (2007) during the nine year study, similar to the work done by Martin and da Silva (2004b). The study in Peru also found evidence of site fidelity of resident Amazon River dolphins in the three river systems surveyed, with no dolphins observed moving between systems (McGuire and Henningsen 2007).

Amazon River dolphins are typically more solitary than oceanic dolphins, but they can be found at times in loosely affiliated aggregates of individuals (Best and da Silva 1993, Aliaga-Rossel 2002). Often large groups of animals (up to twenty individuals) occupy a similar part of a habitat where there may be a high density of resources, such as at the edge of a lake system off a main river channel during low water seasons (Martin and da Silva 2004b, Gomez-Salazar et al. 2011). However, it appears that long term social groups are not common (Martin and da Silva 2004b, Gomez-Salazar et al. 2011).

Mother-calf relations seem to be the most typical long-term association found in Amazon River dolphins. Martin and da Silva (2004a) suggest that lake systems are often used by mother-calf pairs as protected or nursery areas. These locations may provide
higher abundance of food sources, physical protection from aggressive males, and safety from strong currents (Martin and da Silva 2004a).

The overlap of both Amazon River dolphins and the tucuxi with human activity is of great concern for their conservation. The International Union for the Conservation of Nature (IUCN) classifies the Amazon River dolphins as “data deficient” (Reeves et al. 2013). Scientists lack information to determine overall population numbers for any of these dolphin species, and are unclear about the extent to which current threats are affecting them (Reeves et al. 2013). Common threats to these animals include habitat destruction from deforestation for agriculture, and population fragmentation caused by dam construction (Gomez-Salazar et al. 2012). Araújo and Wang (2015) conducted one of the first studies to investigate the overlap of Amazon River dolphins’ distribution and hydropower dam construction. Effects like fragmentation, isolation, and the impacts on prey species warrant concern with the increasing number of dams planned (Araújo and Wang 2015). By-catch conflict with local fisheries has had a significant impact on populations in some regions, where human hostility and accidental entanglement in fishing nets are frequent causes of dolphin mortality (Alves et al. 2012). There is also a more recent threat from intentional killing of Amazon River dolphins by local fishermen to be used as bait for catching a catfish species (*Calophysus macropterus*) (Iriarte and Marmontel 2013, Mintzer et al. 2013).

Conservation efforts for Amazon River dolphins are strongly linked to our scientific understanding of the species and their biology. Increased knowledge regarding ecological and behavioral characteristics of Amazon River dolphins is imperative to
alleviate the threats to the dolphins, and to provide effective strategies to reduce specific pressures on different populations. Acoustic research is particularly important in our understanding of this genus. As with all cetaceans, these dolphins have a tremendous dependence on acoustic signals. Murky water and unpredictable surfacing patterns make visual collection of field data difficult with Amazon River dolphins.

**Odontocete Acoustics**

Odontocete cetaceans use sound for navigation, foraging, and for communication. Their sounds are commonly classified as either echolocation, whistles, or burst pulses. Echolocation is characterized by pulsed, broadband acoustic signals produced with high intensity and high peak frequencies (Au and Hastings 2010). These pulses are emitted in trains of repeated clicks and have been shown to be used for navigation and foraging in both free-ranging and captive dolphins (Au et al. 1974). Whistles are defined as tonal signals, which are narrow-band, frequency modulated sounds. They have fundamental frequencies commonly ranging between 5-15 kHz and are primarily used as social signals (May-Collado and Wartzok, 2008). Burst pulses are similar to echolocation signals but are produced at higher pulse rates (greater than 300 pulses per second) and have been found to be used as social signals. They are strings of clicks occurring at high pulse rates and are produced over a broad frequency range (Lammers et al. 2003, Simard et al. 2008).

Other cetacean social sounds that do not fit into these categories have been documented. Several examples of low frequency sounds have been categorized under different names such as “bray calls” (Herzing 1996, Janik 2000). For this study, I will be
adapting the terminology from Schultz et al. 1995 and Simard et al. 2011, which described these other social sounds as low frequency narrow-band (LFN) sounds. Low frequency narrow-band sounds are characterized as being highly harmonic (with a series of frequencies that include fundamental frequency and integral multiples of the fundamental frequency), tonal sounds with low peak frequencies (<10 kHz), short durations (<1 sec), and are typically made in trains of multiple LFN sounds.

**Amazon River Dolphin Acoustics**

Relatively few studies have investigated the sound production of Amazon River dolphins. Early research on captive Amazon River dolphins (*I. geoffrensis*) described and categorized their sounds into 12 distinct vocalizations but found no whistles (Caldwell et al. 1966, Caldwell and Caldwell 1970). However, a wider variety of sounds have been documented in wild populations for these species, including echolocation clicks, burst-pulses, and whistles (Norris et al. 1972, Nakasai and Takemura 1975, Ding et al. 2001, Podos et al. 2002, May-Callado and Wartzok 2007, Trone et al. 2015, Yamamoto et al. 2015, Amorim et al. 2016). Several studies describing the production of whistles by Amazon River dolphins have been discredited due to questions concerning the validity of their experimental designs (Nakasai and Takemura 1975, Ding et al. 2001). This is primarily due to the presence of the delphinid tucuxi during recordings, as these dolphins are known to produce whistles, and in mixed species groups the researchers were not able to localize which species were producing the whistles (Nakasai and Takemura 1975, Ding et al. 2001). Although low frequency sounds appear to be common in Amazon River dolphins (Caldwell et al. 1966, Podos et al. 2002, Amorim et
al. 2016), as with most odontocetes, these and other social sounds have received little attention.

**Sound Variation**

As social signals are known to be structurally and functionally flexible, they are likely to show inter-population variation (Griebel and Oller 2008). Differences in social sounds can provide information on a species’ populations and social organization (Bazúa-Durán and Au 2004). Inter-population variation in communication signals has been studied most extensively in birds. Early work by Nottebohm (1969) on variation in avian social sounds described differences in communication signals as being either geographic variation or dialects. Geographic variation is defined as “the differences in the songs of birds that do not normally mix and are separated by long distances” (Nottebohm 1969, 299). Nottebohm’s study defined dialects as “variation in songs of birds found in neighboring populations that have potential for interbreeding” and found that dialects in birds act as a means of reducing gene flow (Nottebohm 1969, 299). This allows for more efficient adaptation to local environmental differences.

Another study used the terminology ‘microgeographic variation’ and ‘macrogeographic variation’ to distinguish between vocal variability in populations that are either continuous groups or separated by long distances and do not mix (Krebs and Kroodsma 1980). Conner (1982) stressed the importance of differentiating between these types of vocal variation, and the degree of isolation between populations in order to distinguish possible mechanisms behind the differences found in vocalizations.
As with other species, odontocete social sounds can serve several functions, such as providing information about the sender, location of the sender, and specific contextual information about behavior and movement within groups (Nakhara 2002). Comparative studies among five species of odontocetes (false killer whales *Pseudorca crassidens*, short-finned pilot whales *Globicephala macrorhynchus*, long-finned pilot whales *Globicephala melas*, white-beaked dolphins *Lagenorhynchus albirostris* and Risso’s dolphins *Grampus griseus*) showed significant quantitative differences in frequencies, call duration, and number of inflections in social tonal sounds (Rendell *et al.* 1999). That study found duration of social sounds to be an important acoustic call parameter in discrimination between inter-species sounds, and between population calls (Rendell *et al.* 1999).

Research has identified intra-specific variation in social sounds produced between neighboring populations in two species of odontocetes. Long-term research on resident killer whales (*Orcinus orca*) in coastal waters off British Columbia and Washington showed structural variance of calls between pods (Deecke *et al.* 2000). These pod-specific repertoires and discrete calls are considered dialects as the pods are neighboring and have potential for interbreeding. They are thought to function as signals for maintaining group contact and possibly conveying group identity. Research on sperm whales (*Physeter microcephalus*) has documented variation between the matrilineal family clans in vocal signals called ‘codas’ (Rendell and Whitehead 2003). These calls are social signals that do not propagate over large distances. Therefore, it is hypothesized
that these calls are shared only within clans and are possibly used to maintain group cohesion (Rendell and Whitehead 2003).

Variation in social sounds between groups of cetaceans from different geographic areas has also been documented. Spinner dolphins (*Stenella longirostris*) in the Hawaiian Islands do not appear to follow the same patterns of distinct call variation as sperm or killer whales, as they belong to fission-fusion societies (groups size and composition changes over time) instead of stable groups (Bazúa-Durán and Au 2004). Spinner dolphins can travel between the islands, but whistle-specific subgroups have been identified among individuals that usually spend time together (Bazúa-Durán and Au 2004).

Spatial variation has also been documented in blue whales (*Balaenoptera musculus*) in their low frequency social songs. These songs have been proposed to be useful in the identification of populations, as there are distinct differences in different global regions (McDonald *et al.* 2006). These variations represent current movement and association patterns in a species with a wide geographic distribution, as blue whales have (McDonald *et al.* 2006).

**Research Objective**

Since most research on aquatic mammal acoustics and social sound variation has been collected in the marine environment, this study aimed to investigate the presence of geographic variation in a freshwater species of dolphin (Deecke *et al.* 2000, Rendell and Whitehead 2003, Bazúa-Durán and Au 2004, McDonald *et al.* 2006). The project
examined the sound production of Amazon River dolphins (specifically *I. geoffrensis*) in two separate populations, one in the central Brazilian Amazon, and the other in the Peruvian Amazon. Low frequency narrow-band (LFN) sounds were analyzed and quantitatively compared between the two locations. Based on previous research on variation in cetacean social sounds and the long distances between the populations, I expected to find differences between Brazilian and Peruvian populations of Amazon River dolphins with respect to acoustic parameters (peak frequency, duration, and patterns of sound production) of LFN sounds (Deecke *et al.* 2000, Rendell and Whitehead 2003, Bazúa-Durán and Au 2004, McDonald *et al.* 2006).

This research is important for several reasons. First, this project increases our knowledge of the acoustic communication by Amazon River dolphins, which has received comparatively little attention from the scientific community. This study also yields important information about the influence of geographic isolation on the evolution of dolphin communication, especially among non-delphinids. In addition, this research highlights the importance of using acoustic information as a tool to better understand populations and for conservation and management.
Materials and Methods

Field Sites

Brazil: Mamirauá Sustainable Development Reserve (RDSM)

The Mamirauá Sustainable Development Reserve is located in floodplain region between the Japurá and Solimões Rivers (Amazon River). The seasonally flooded forest at this site is along white-water rivers and is described as a várzea (Prance 1979). The reserve, with an area of 1,124,000 hectares, was established in the early 1990’s and is the largest Brazilian conservation area devoted to flooded forest biodiversity (Queiroz 2011). The reserve experiences seasonal flooding that causes water levels to fluctuate between eleven and fifteen meters a year (Martin and da Silva 2004a). Peak flooding is in the months of May and June, and water levels are lowest in October and November (Martin and da Silva 2004b). The acoustic study reported here was conducted in the Mamirauá Lake system of the reserve which begins at the confluence of the Amazon (Solimões) River and its tributary the Japura River (-3.136844, -64.785968) (GPS coordinates reported in decimal degrees). This lake system is composed of a main channel with various sized lakes surrounded by dense forest that are inundated with water during the annual flood cycle. The flooded forest area is 225 square km, with a main channel approximately thirty km in length to the main Mamirauá Lake (-2.99101, -64.939425) (Figure 1). This system provided a unique opportunity for recording sounds of Amazon River dolphins in an area where long-term studies with freeze-branded individuals have provided information on seasonal movements and habitat distribution of a resident population (Martin and da Silva 2004a).
Figure 2: Map of field site in Brazil. Acoustic data were collected in the Mamirauá lake system within the Mamirauá Sustainable Development Reserve. The extent of the lake system channel surveyed is highlighted with a blue line. The image used for this map was captured during the dry season.
Peru: Área de Conservación Regional Comunal Tamshiyacu Tahuayo (ACRCTT)

The Peruvian reserve Área de Conservación Regional Comunal Tamshiyacu Tahuayo (ACRCTT) is located in the northeastern region of the country. The establishment of the reserve in 1991 was a response by local inhabitants and international researchers to prevent exploitation of natural forest resources from outside loggers, hunters, and others (Newing and Bodmer 2003). The 325,000 hectares reserve has a western border along the Tahauyo River, which is a tributary to the Amazon River. The acoustic data collection for this study took place along part of the black-water Tahauyo River, which is in an igapó flooded forest habitat (Figure 2). The peak flooding in this region occurs between March and May and the lowest water levels are found from August to October (Kvist and Nebel 2001). There is a difference of eight to ten meters in the water levels during high water and low water (Kvist and Nebel 2001). Research on Amazon River dolphins in the reserve began in 2001 (Rogers, pers. com.). The previous research in this area concentrated on mother-calf interactions, specifically focusing on surface behaviors and acoustic data collection for analysis (Fedoruk 2006, Burch 2014).
Figure 3: Map of Peru field site. Data collection was focused on the Tahuayo River near the Área de Conservación Regional Comunal Tamshiyacu Tahuayo. The extent of the river surveyed is highlighted with a blue line.
Brazil Acoustic Data Collection

In Brazil, the data were collected during a ten-month period from August 2013 to May 2014 during boat-based surveys for Amazon River dolphins. Recordings for this acoustic analysis were limited to the Mamirauá Lake System entrance, the main channel, and lakes of the Mamirauá Lake System (Figure 1). Observations were confined to this area to limit recording tucuxi, as the delphinid species are less likely to enter lake systems, especially during lower water levels. Throughout the study, daily river depth recordings were taken with a Digital Sonar handheld depth sensor at the research base to assess the water levels in the channel (-3.048614, -64.851024).

Field recordings were collected opportunistically from a five-meter aluminum boat with an outboard engine, using a calibrated Sony digital audio recorder (PCM-M10, 96 kHz-24-bit resolution anti-aliasing filter) and a single hydrophone (HTI 96 MIN, -170 dB/V). During boat-based follows of single species Amazon River dolphin groups (*I. geoffrensis*), the hydrophone was deployed at approximately 3-meters depth off the boat while the motor was off. This was done to reduce the chance of entanglement of the hydrophone in the many submerged branches in the study area and to allow for unmasked recordings of low frequency sounds. In addition, boat noise from other vessels in the area could mask the low frequency sounds. Recordings were stopped when visual contact was lost for more than two minutes, or if interfering boat noise persisted for more than one minute (in order to continue other data collection).

During each acoustic recording session the location of the group was collected using a Garmin Legend H Handheld GPS Navigator for GPS coordinates, and water
depth at the site of the animals’ original surfacing were noted. Group structure was documented for each recording, including information about the identity of individual freeze-branded animals, unmarked animals (no freeze-brand), number of animals, and age classes. Age classes for unmarked animals were determined by color and size. Male Amazon River dolphins are typically pink in coloration and larger than females in body size by up to 55% (Martin & da Silva 2006). Besides being smaller, females are characteristically greyer in color. Calves are up to one-fourth the size of the females, darker grey in color, and are seen surfacing next to a female. Juveniles are grey and are greater than one-quarter and up to one-half the size of females. In addition, photographic data were collected using a Canon EOS 200x zoom DSLR Camera to identify individuals.

During these group follows data were also being collected for the long-term research effort, Projeto Boto that is run by Dr. Vera da Silva and Dr. Tony Martin. This project has been studying Amazon River dolphins in the Mamirauá reserve since 1994 using individual identification, in order to better understand the species’ biology, ecology, and work towards conservation efforts (Projeto Boto, 2006). Behavioral data were difficult to collect during the acoustic study due to limited visibility, the unpredictability of the animals surfacing, and limited numbers of field researchers in the boat. Therefore, behaviors were not recorded consistently and certain behaviors visible at the surface were described. These behaviors included object carrying (e.g. carrying non-food objects such as sticks, grass, or rocks in their mouths that are sometimes thrashed against the water, as described by Martin and da Silva 2008), calf-mother interactions
(e.g. whether calves surfaced and swam next to the mother or separate from the mother), and foraging (if fish were seen in the mouth).

**Peru Acoustic Data Collection**

Data were collected in the along the Tahuayo River near the ACRCTT, Loreto Department, Peru from June 17, 2016, until July 31, 2016. In order to collect data that were directly comparable to the Mamirauá lake system data during the study in Peru the same acoustic recording methods were applied, and to the extent possible, identical data collection methods were used for dolphin group sighting information. Recordings were collected during the falling water season in the Tahuayo River from the village of Huasi (-4.16402, -73.18255) up the river approximately forty-seven kilometers to its confluences with the Tangarana River (-4.407177, -73.285814) (Figure 2). River depth measurements were collected daily with a handheld depth sounder (Depthmate Model SM-5), at the eco-tourist Tahuayo Lodge to track the water levels during the study (-4.31133, -73.23189).

The same recording equipment was used for data collection, including a calibrated Sony digital audio recorder (PCM-M10, 96 kHz-24-bit resolution anti-aliasing filter) and a single hydrophone (HTI 96 MIN, -170 dB/V). Data collection took place from a five-meter aluminum boat with an outboard engine. When an Amazon River dolphin (*I. geoffrensis*) was sighted, the engine was turned off and the hydrophone was deployed off the boat at approximately three meters depth. During each recording, headphones were plugged into the recorder in order to listen to what was being recorded. Any sounds produced by the dolphins, plus unknown sounds were noted, along with the time of
sounds. Acoustic recordings were stopped when visual or auditory contact was lost for more than five minutes, or if nearby boat noise interfered for longer than one minute.

The location of each group was collected using a GPS (Garmin Legend H Handheld GPS Navigator), the water depth at the approximate location of the original surfacing was collected, and both were noted in the same fashion as in Brazil. Group structure was noted during every recording, using the age and sex classes described above. Amazon River dolphins in this area are not individually freeze-branded, but photographs were taken during sightings (using a Canon EOS Rebel t5i) to be used for photographic identification on individuals based on natural marks.

The behaviors of the Amazon River dolphins along the Tahuayo River were observed and noted during acoustic recordings, including the same behaviors described for Brazil. Additionally, surface behaviors, orientation to the river’s flow, and time in between surfacings were recorded for focal animals for each group by a trained intern. Surface behaviors were categorized as either high rolls (hr), head up (hu), or skim (sk) (Table 1). The animals’ orientation in the river was categorized as parallel, or perpendicular to the flow of the river. It was further noted if an audible or visible blow (breathe) was associated with an animals’ surfacing.

Table 1. Definitions of Amazon River dolphin surfacing behaviors

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Roll (hr)</td>
<td>Melon and head breaking the surface followed by dorsal fin. Occasionally rising completely out of the water with an arched back. Associated with diving behavior.</td>
</tr>
<tr>
<td>Head Up (hu)</td>
<td>Beak and occasionally melon protruding from water, but no high roll following</td>
</tr>
<tr>
<td>Skim (sk)</td>
<td>Head and sometimes dorsal fin breaking the surface parallel to the water</td>
</tr>
</tbody>
</table>
ArcGIS Analysis of Field Sites

Geographical information systems and satellite imagery (ESRI ArcGIS 10.3) were used for determining more precise distances of the length of river surveyed at the study sites in Brazil and Peru (Landsat5). The distance between the two sites along the Amazon River was also measured. The distances were determined by digitizing the centerline of each river channel, and computing the geometry to measure the length (kilometers).

Acoustic Analysis

Definition of Low Frequency Narrow-band Sounds

In this study, the low frequency narrow-band (LFN) sounds produced by Amazon River dolphins in Brazil and Peru were analyzed. Adobe Audition (2.0, Adobe Systems Inc., San Jose, CA) was used to visually identify the low frequency sounds for analysis in each recording. In order to identify the LFN sounds as being tonal, the waveform file was utilized to determine the nature of the sound as being sinusoidal or pulsed (Figure 4). To be included in analysis these sounds had to have a fundamental peak frequency (frequency that the acoustic energy is most focused) under 10kHz. Sounds were categorized as either ‘long’ or ‘short’ LFN sounds based on their duration (length from beginning to end of the sound in seconds). Low frequency narrow-band sounds greater than 0.150 second in duration were categorized as ‘long’ sounds, and sounds less than 0.150 second were considered ‘short’ sounds (Figure 5). This break in duration was found at the 75th percentile of the duration measured for all sounds recorded, and coincided with an intuitive categorization break that could be determined visually.
Figure 4. Top panel: spectrogram of LFN sounds produced by Amazon River dolphins (recorded in Peru). Bottom panel: waveform of the first LFN in the spectrogram in the top panel (indicated by the arrow) demonstrating the sinusoidal structure of the sound.

Figure 5. Spectrogram showing three LFN sounds produced by Amazon River dolphins. ‘Long’ LFN sounds are greater than 0.150 seconds in duration (Sound A). ‘Short’ LFN sounds are less than 0.150 seconds in duration (Sound B). This file was recorded on the Tahuayo River, Peru in June 2016.
Peak Frequency and Duration of Sounds

The parameters of peak frequency and duration of low frequency narrow-band sounds produced by Amazon River dolphin were measured. The sounds need to be clearly defined (no overlap with other sounds that could confuse the analysis), and have a high signal-to-noise ratio (SNR) to be included in peak frequency and duration analysis. Once identified, sounds were isolated from the file using the cursor tool on Adobe Audition. A sound was isolated by using the zoom tool to expand the window and to visually identify the beginning and the end of each sound. Then the sounds were analyzed using a custom Matlab (2007b, Mathworks Inc., Natick, MA) routine that is able to accurately and precisely quantify the physical structures of sounds (as used by Simard et al. 2008). The sound analysis of the peak frequency (Hz) and duration (seconds) of all low frequency narrow-band sounds was conducted using this routine (Figures 6 and 7).

Figure 6. Spectrogram showing three LFN sounds produced by Amazon River dolphins. Peak frequency (frequency at which acoustic energy is most focused) of the first LFN sound is indicated by the arrow. Recording collected on the Tahauyo River, Peru in July 2016.
Figure 7. Spectrogram of two LFN sounds produced by Amazon River dolphins. The duration (measured from the beginning of a sound to the end in seconds) is indicated on the first LFN with the arrow. Recording collected on the Tahauyo River, Peru in July 2016.

Sound Pattern Analysis

Sound pattern analysis was also conducted in Adobe Audition for the low frequency narrow-band sounds. The typical pattern of low frequency narrow-band sounds is defined by trains, or a series of closely spaced LFN sounds. To be included in sound pattern analysis, sounds needed to be clearly defined (with a clear beginning and end) and have a high SNR (signal-to-noise ratio). The difference in intensity at the beginning of each sound was used to distinguish the clear beginning of sounds. For each train, the number of sounds was determined (Figure 8) and the inter-sound intervals (milliseconds) were measured (Figure 9). The inter-sound interval was measured using the screen cursor tool in Audition to measure the time from end of one sound to the beginning of the subsequent sound. Inter-sound intervals were used to define trains: if the inter-sound interval between two sounds was less than twice the average interval in milliseconds for the train so far, the sound was considered part of a new train.
Figure 8. Spectrogram of eleven LFN sounds in a train (indicated by the bracket) produced by Amazon River dolphins. Recording collected on the Tahuayo River, Peru in July 2016.

Figure 9. Spectrogram of two LFN sounds produced by Amazon River dolphins. The inter-sound interval (measured from the end of one sound to the beginning of the subsequent sound) is indicated on with the arrow. Inter-sound interval was measure in milliseconds. Recording collected on the Tahuayo River, Peru in July 2016.
Figure 10. Spectrogram showing a train of LFN sounds produced by Amazon River dolphins with inter-sound interval of zero milliseconds. Individual sounds are distinguished by difference in intensity at the beginning of each sound.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 23, copyright 2015). Since the data were not normally distributed, a non-parametric statistical test was used, and when reporting descriptive statistics for data collected at both sites, both the median and mean values are reported. In order to determine whether there are differences in the physical parameters of low frequency narrow-band sounds produced by Amazon River dolphins at the sites in Brazil and Peru, the duration and peak frequency of sounds from each location were compared. An Independent Samples Mann-Whitney U was performed to determine if there were differences between median peak frequencies of the sounds produced at each site and to test whether the median duration (seconds) of ‘long’ and ‘short’ sounds differed between sites. In addition, a Moses Test for Extreme Reaction was used to determine if there were differences in the range of peak
frequency and duration (seconds) of the LFN sounds produced by Amazon River
dolphins at the two sites.

To compare sound pattern production of low frequency narrow-band dolphin
sounds in Brazil and Peru the number of sounds produced in a train and the inter-sound
interval (milliseconds) were used. An Independent Samples Mann-Whitney U test was
used to compare the number of sounds produced per train from each location. To test for
differences in the inter-sound interval (milliseconds) of LFN trains two separate analysis
were completed. An Independent Samples Mann-Whitney U test was used to compare
the inter-sound interval of trains from Peru and Brazil. A separate Mann-Whitney U test
was used to compare the interval data not including effective inter-sound intervals of zero
(milliseconds) (Figure 10). To determine if extreme values influenced the range of
number of LFN sounds produced in a train by Amazon River dolphins in Brazil and Peru
a Moses Test for Extreme Reaction was used. I tested the inter-sound interval
(milliseconds) with and without zero second inter-sound interval data separately using the
Moses Test for Extreme Reaction.
Results

ArcGIS Analysis of Field Sites

The Mamirauá Lake system area in Brazil where I collected acoustic recordings was 30.582 km long based on ArcGIS analysis (refer to Figure 1). The program also determined the length of the surveyed portion of the Tahauyo River in Peru, from the Tangarana confluence to Huaisi was 50.708 km (refer to Figure 2). The distance between the study site in Brazil (mouth of the Mamirauá Lake system) to the study site in Peru (Huaisi channel) was 1,512.816 kilometers along the Amazon (or Solimões River) (Figure 11). This distance measured indicates that the two populations studied are separated by a long distance and probably do not normally mix, and any differences found in the sound production would be evidence of geographic variation.

Figure 11. Map showing the distance along the Amazon (Solimões) River between the field site in Brazil and the field site in Peru.
Acoustic Analysis

Recordings in Brazil

In the Brazil study area, 791 minutes of recordings were collected on 30 groups of free ranging Amazon River dolphins. These groups had a mean group size of 12.85 animals (group size range of 2-28 animals). These groups included all male groups, mother-calf groups, as well as mixed groups (with adult females and males, juveniles, and calves present). Twenty-two groups (73%) produced LFNs, and all of those groups comprised freeze-branded and unmarked animals. Sixty-eight percent of the groups with LFN sounds in recordings, had at least one mother-calf present.

A total of 589 LFN sounds were identified in the recordings of Amazon River dolphins in Brazil. These LFN sounds had harmonic structure. The recording equipment abilities cut off at 50,000 kHz, so the extent of the harmonics could not be determined. The sample sizes for each parameter measurement may vary from the total number of LFN sounds identified, as some sounds did not meet the criterion required for analysis.

Recordings in Peru

In Peru, a total of 1,700 minutes of acoustic recordings of Amazon River dolphins were collected from 61 groups of animals, which ranged from one to six animals. All groups included a mix of females, calves and juveniles. Analysis of the recorded files determined that 36% of the Amazon River dolphin groups (22 of 61 groups) produced LFNs. LFN sounds were only found in recordings when there was more than one animal present. Fifty-five percent of the recordings of groups with LFN calls present had
mother-calf pairs. A total of 420 LFN sounds were identified in the recordings collected at this location.

Peak Frequency Analysis

A total of 392 LFN sounds were found to be suitable for analysis and were analyzed for peak frequency (67%). The mean peak frequency (Hz) of sounds produced in Brazil was 3,030.01 Hz (SD = 1951.27), and the median peak frequency was 2,805.15 Hz. The peak frequencies of the LFN sounds ranged from 207.05 Hz to 9,507.31 Hz (Table 2).

Of the 420 LFNs recorded in Peru, 325 (77%) met the criteria for peak frequency analysis of the peak frequency. The mean peak frequency of the sound recorded was 3,712.10 Hz (SD = 2,208.58), and the median peak frequency was 3,380.28 Hz. The LFN sounds peak frequencies ranged from 327.42 Hz to 9,428.92 Hz (Table 2).

Table 2. Descriptive statistics for the peak frequency in hertz for LFN sounds produced by Amazon River dolphins in Brazil and Peru.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Low Frequency Narrow-band sounds</th>
<th>Peak Frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Brazil</td>
<td>392</td>
<td>3030.01</td>
</tr>
<tr>
<td>Peru</td>
<td>325</td>
<td>3712.10</td>
</tr>
</tbody>
</table>
Sound Duration Analysis

For the analysis of the duration of ‘short’ LFN sounds, 320 sounds were deemed suitable (54% of total sounds). The mean duration of short LFN sounds in Brazil was 0.096 second (SD = 0.027), and the median duration was 0.095 second, with a range from 0.035 second to 0.149 second (Table 3). A total of 84 ‘long’ LFN sounds were used in duration analysis (14% of total sounds). The mean duration was 0.217 seconds (SD = 0.026), and the median duration was 0.184 second. The range of duration of long LFN sounds was 0.150 to 0.769 second (Table 4).

The duration analysis of short LFN sounds produced in Peru used 280 sounds (67% of identified sounds). The mean duration of short sounds was 0.103 second (SD = 0.027) and the median was 0.103 second (Table 3). The range of duration of short sounds was 0.011-0.149 second. I analyzed 106 long LFN sounds (25%) for duration analysis. The mean duration of long sounds was 0.197 second (SD = 0.569), and the median was 0.175 second. The range of duration of long sounds were 0.150 to 0.418 second (Table 4).

Table 3. Descriptive statistics for the duration in seconds of ‘short’ LFN sounds produced by Amazon River dolphins in Brazil and Peru.
Table 4. Descriptive statistics for the duration in seconds of ‘long’ LFN sounds produced by Amazon River dolphins in Brazil and Peru.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Low Frequency Narrow-band Sounds</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>84</td>
<td>0.217</td>
<td>0.095</td>
<td>0.184</td>
<td>0.150-0.769</td>
</tr>
<tr>
<td>Peru</td>
<td>106</td>
<td>0.197</td>
<td>0.569</td>
<td>0.175</td>
<td>0.150-0.418</td>
</tr>
</tbody>
</table>

*Sound Pattern Analysis (Number of Sounds per Train)*

Regarding the sound pattern analysis of Amazon River dolphin LFN sounds in Brazil, 264 trains were appropriate for analysis. Single LFN sounds were observed in 154 of 264 of all identified trains (58%). The mean number of LFNs produced in a train by Amazon River dolphins in Brazil was 2.03 sounds (SD = 1.7) and the median was 1.00. Trains ranged from 1 LFN to a series of 10 LFN sounds (Table 5).

In Peru, 159 trains of LFNs were used for sound pattern analysis. Of the suitable trains, 69 (43%) were composed of a single LFN. The mean number of LFN sounds produced in a train was 2.49 (SD = 2.09), and the median number of sounds was 2.00. Trains ranged from 1 LFN to 12 LFNs (Table 5).
Table 5. Descriptive statistics for the number of sounds per train of LFN sounds produced by Amazon River dolphins in Brazil and Peru.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Low Frequency Narrow-band Sound Trains</th>
<th>Number of Sounds per Train</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Brazil</td>
<td>264</td>
<td>2.026</td>
</tr>
<tr>
<td>Peru</td>
<td>159</td>
<td>2.491</td>
</tr>
</tbody>
</table>

**Sound Pattern Analysis (Inter-Sound Interval)**

To determine the inter-sound interval for patterns in Brazil, 89 trains of LFN sounds were analyzed. The mean inter-sound interval of sounds was 46.02 milliseconds (SD = 53.50), and the median inter-sound interval was 31.00 milliseconds. The inter-LFN interval ranged from 0-235 milliseconds (Table 6). To determine the inter-sound interval for the pattern analysis of LFN sounds produced in by dolphins at the Peru study site, 86 were analyzed. The mean inter-sound interval was 60.30 milliseconds (SD = 50.20), and the median was 60.00 milliseconds. The inter-sound interval ranged from 0.00-252.00 milliseconds (Table 6).

Table 6. Descriptive statistics for all inter-sound intervals in milliseconds of LFN sounds produced by Amazon River dolphins in Brazil and Peru.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Low Frequency Narrow-band Sound Trains</th>
<th>Inter-Sound Interval (milliseconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Brazil</td>
<td>89</td>
<td>46.02</td>
</tr>
<tr>
<td>Peru</td>
<td>86</td>
<td>60.30</td>
</tr>
</tbody>
</table>
Sound Pattern Analysis (Effective Inter-Sound Interval of Zero)

In Brazil there were 39 of the 89 trains with an effective inter-sound interval of zero milliseconds (44%). The mean for inter-sound interval of LFN sounds not including any effective inter-sound intervals of zero measured 74.77 milliseconds (SD = 49.93), and the median was 71.00 milliseconds. The range of LFN inter-sound intervals when not including 0.00 milliseconds values was 12 to 235 milliseconds (Table 7).

There were 17 of 86 trains of LFN sounds produced in Peru with an inter-sound interval of zero milliseconds (20% of trains analyzed for interval pattern). When the effective inter-sound intervals of zero ms were excluded from the sample, the mean inter-sound interval of LFN sounds rose to 79.64 milliseconds (SD = 42.23), and the median to 72.00 milliseconds, with a range of 9 to 252 milliseconds (Table 7).

Table 7. Descriptive statistics for the inter-sound interval (milliseconds) of all non-potential overlapping LFN sounds produced by Amazon River dolphins in Brazil and Peru.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Low Frequency Narrow-band Sound Trains</th>
<th>Inter-Sound Interval (milliseconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Brazil</td>
<td>50</td>
<td>74.77</td>
</tr>
<tr>
<td>Peru</td>
<td>69</td>
<td>79.64</td>
</tr>
</tbody>
</table>
**Comparison of LFN Sound Parameters in Brazil and Peru**

**Sound Peak Frequency**

When comparing the low frequency narrow-band sounds produced by Amazon River dolphins in Brazil and Peru, a significant difference was found in the peak frequencies (Hz) of the sounds (Independent Samples Mann-Whitney U: \( U = 51,170.00 \), \( p = 0.000 \), Brazil: \( n = 392 \), Peru: \( n = 325 \)) (Figure 12). The LFN sounds produced in Peru had higher peak frequencies (mean = 3712.10 Hz, SD = 2208.58) than the sounds produced in Brazil (mean = 3030.01 Hz, SD = 1951.27). There was no significant difference between the ranges of peak frequencies of the sounds produced in Brazil and Peru (Moses Test of Extreme Reaction = 705.00, \( p = 0.193 \)).

![Histograms showing the distribution of Peak frequencies (Hz) of LFN sounds produced by Amazon River dolphins in Brazil and Peru. Arrows indicate the means.](image)

**Figure 12.** Histograms showing the distribution of Peak frequencies (Hz) of LFN sounds produced by Amazon River dolphins in Brazil and Peru. Arrows indicate the means.
### Sound Duration

The duration of ‘short’ LFN sounds produced by Amazon River dolphins differed significantly between the sounds recorded in Brazil and Peru (Independent Samples Mann-Whitney U: U = 37,539.500, p= 0.001, Brazil: n= 320, Peru: n = 280) (Figure 13). The short LFN sounds had a longer duration in recordings from Peru (mean = 0.103 sec, SD= 0.027) than Brazil (mean = 0.096 sec, SD = 0.026). There was no difference in the ranges of ‘short’ sounds between Brazil and Peru (Moses Test of Extreme Reaction = 600.000, p = 0.245).

![Histograms showing the distribution of the duration (seconds) of short LFN sounds (shorter than 0.150 seconds) produced by Amazon River dolphins in Brazil and Peru. Arrows indicate the means.](image)

Figure 13. Histograms showing the distribution of the duration (seconds) of short LFN sounds (shorter than 0.150 seconds) produced by Amazon River dolphins in Brazil and Peru. Arrows indicate the means.
There was no significant difference between the duration of ‘long’ LFN sounds in Brazil and Peru (Independent Samples Mann-Whitney U: U = 5,069.000, p = 0.137, Brazil: n = 84, Peru: n = 106) (Figure 14). The mean duration of long LFN sounds in Brazil was 0.217 sec (SD = 0.095) and 0.197 sec (SD = 0.175) in Peru. In addition, the ranges of ‘long’ sound duration did not differ significantly in Brazil and Peru (Moses Test of Extreme Reaction = 187.000, p = 0.107).

Figure 14. Duration (seconds) of long LFN sounds (greater than 0.150 seconds) produced by Amazon River dolphins in Brazil and Peru. The means are indicated by an arrow.
Sound Patterns

The number of LFN sounds produced per train in Brazil was significantly different than the number of sounds produced per train in Peru (Independent Samples Mann-Whitney U: $U = 16,883.500$, $p = 0.003$, Brazil: $n = 264$, Peru: $n = 159$) (Figure 15). The trains produced by Amazon River dolphins in Peru had more LFN sounds per train with a mean of 2.491 (SD = 2.101), than in Brazil with a mean of 2.026 (SD = 1.7). The range of number of sounds per train was also significantly different between the recordings in Brazil and Peru (Moses Test of Extreme Reaction = 293.000, $p = 0.000$). In Peru, the range of number of sounds per train was more extreme (range= 1-12).

Figure 15. Histograms showing the distribution of the number of LFN sounds per train produced by Amazon River dolphins in Brazil and Peru. Arrows indicate the means.
There was a significant difference in the inter-sound interval measured for all LFN sound trains between in Brazil and Peru (Independent Samples Mann-Whitney U: U = 15,723.500, p = 0.001, Brazil: n = 89, Peru: n = 86) (Figure 16). Longer inter-sound intervals were measured in trains of LFN sounds recorded in Peru (mean = 60.30 milliseconds, SD = 50.20) than at the field site in Brazil (mean = 46.02 milliseconds, SD = 53.50). The range of inter-sound intervals of LFN sounds produced was significantly different between the trains in Brazil and Peru (Moses Test of Extreme Reaction = 328.000, p = 0.001). The LFN sounds produced in Peru had a more extreme range of inter-sound intervals (range= 0.00- 252.00 ms).

Figure 16. Histogram showing the distribution of inter-sound interval (milliseconds) for LFN sounds produced by Amazon River dolphins in Brazil and Peru. Arrows indicate the means.
When the LFN sounds with an inter-sound interval of zero milliseconds were excluded from the inter-sound interval analysis there was no significant different between Brazil and Peru (Independent Samples Mann-Whitney U: $U = 4,846.500$, $p = 0.368$, Brazil: $n = 50$, Peru: $n = 69$) (Figure 17). The mean inter-sound interval for Brazil was 74.77 milliseconds ($SD = 42.93$) and the mean for Peru was 79.64 milliseconds ($SD = 42.23$). There was no difference in the range of inter-sound intervals of LFN sounds in Brazil and Peru (Moses Test of Extreme Reaction= 225.000, $p=0.240$).

![Histogram showing the distribution of inter-sound interval (milliseconds) for LFN sounds with no apparent overlap produced by Amazon River dolphins in Brazil and Peru. An arrow indicates the means.](image)

Figure 17. Histogram showing the distribution of inter-sound interval (milliseconds) for LFN sounds with no apparent overlap produced by Amazon River dolphins in Brazil and Peru. An arrow indicates the means.
Discussion

Summary of Low Frequency Narrow-band Sounds

The recorded LFN sounds produced by Amazon River dolphins in Brazil and Peru can be characterized as being short (less than 1 second in duration), low frequency (less than 10 kHz in frequency), tonal in nature, with harmonic structure, and typically produced in trains. In this study, these LFN signals were further classified as either ‘short’ (less than 0.150 seconds) or ‘long’ (greater than 0.150 seconds) based on the duration. There were more ‘short’ LFN sounds produced than ‘long’ LFN sounds at the field sites in both Brazil and Peru.

Low frequency sounds are not unique to Amazon River dolphins. Low frequency signals have been reported in delphinid species but have been given a number of different names: “grunts” produced by Indo-Pacific humpback dolphins (*Sousa chinensis*) (Van Parijs and Corkeron 2001), and “chirps” and “grunts” in Risso’s dolphins (*Grampus griseus*) (Corkeron and Van Parijs 2001).

Most of the work on low frequency acoustic sounds has been done on bottlenose dolphins (*Tursiops truncatus*). Herzing (1996) reported ‘barks’ and ‘squawks’ in free ranging bottlenose dolphins in the Bahamas. ‘Bray calls’ were reported in groups of bottlenose dolphins in Scotland by Janik (2000a) and ‘thunks’ were recorded in captive studies by McCowan and Reiss (1995). There has been considerable confusion regarding these low frequency sounds as each study often uses its own name based on the description of the signals rather than on quantified parameters. The lack of a standardized name or analysis technique has made comparisons between studies difficult.
As previously mentioned, the present study used the nomenclature of ‘low frequency narrow-band sounds’ or LFN sounds as described by Schulz et al. (1995) and Simard et al. (2011) in bottlenose dolphins. Schultz et al. (1995) and Simard et al. (2011) characterized the LFN sounds using quantifiable elements and defined LFN sounds as described above. The results of this study on Amazon River dolphins reveal that the LFN sounds made by this species are produced at a higher mean peak frequency and have a longer duration than the LFN sounds produced by bottlenose dolphins and analyzed by Simard et al. (2011) (Olson, unpublished data).

Similar low frequency sounds have been reported in previous studies on Amazon River dolphins (Caldwell et al. 1966, Caldwell and Caldwell 1970, Best and da Silva 1989). Podos et al. (2002) and Amorim et al. (2016) recorded Amazon River dolphins in Brazil, and described pulsed, low frequency sounds called ‘vocalizations’ that were produced in a series of pulses. These sounds were observed during foraging behavior (Podos et al. 2002). Trone and colleagues (2014) referred to the low frequency sounds recorded in Peru as “whistles”, but acknowledged these are tonal low frequency sounds that do not fit the standard definition of whistles.

The results of the acoustic analysis of LFN sounds parameters reported here agree with previous research, but differences in methods and analysis may influence comparison between studies (Podos et al. 2002, Amorim et al. 2016). Amorim et al. (2016) collected data in the Japura River in Brazil, and Podos et al. (2002) collected acoustic data in the same Mamirauá lake system used in the current study. The peak frequencies of the LFN sounds recorded at my sites (Brazil mean = 3.03 kHz, Peru mean
= 3.71 kHz) are in a similar range to the previous reports on Amazon River dolphins by Podos et al. 2002 (mean dominant frequencies of vocalizations = 1.41-2.90 kHz) and three times higher than mean peak frequency of vocalizations by Amorim et al. 2016 (mean = 1.29 kHz). The range of durations, combining ‘short’ and ‘long’ LFN sounds, reported by this study (Brazil = 0.035-0.769 sec; Peru = 0.011- 0.418 sec) are also similar to the range of low frequency sounds found by Podos et al. 2002 (duration range of vocalizations from = 0.037-0.534 sec) and the top duration range of vocalizations from Amorim et al. 2016 is much longer (duration range = 0.03-2.49 sec).

Regarding pattern analysis of LFN sounds, no previous studies have reported data on the interval between low frequency sounds produced by Amazon River dolphins. The study by Podos et al. 2002 is more comparable to the current study, and found that the vocalizations ranged from 1-16 sounds (Brazil range of sounds per train = 1-10; Peru range of sounds per train = 1-12). Comparison of the number of sounds produced per train between this study and Amorim et al. 2016, was not practical due to differences in methods and sound pattern analysis.

Although mean dolphin group sizes were larger in Brazil than in Peru, LFN sounds were only produced when more than one Amazon River dolphin was present during recordings in either location, giving evidence that these signals are used in social communication. Also in Brazil, Podos et al. (2002) reported groups ranging from 1 to 14 individuals, while Amorim et al. (2016) reported groups between 1 and 4 animals during acoustic recordings. It is unclear if the previous studies recorded low frequency
calls when only one animal was present, as they did not specify the group composition when sounds were emitted (Podos et al. 2002, Amorim et al. 2016).

Although Amazon River dolphins are considered a solitary species (with no real long-term groups), sounds, such as LFNs, are produced in social settings. The function of these low frequency sounds is still unknown, and the current study gives little new insight about the information that is being transmitted, but given the plasticity of dolphin communication it is highly likely that these sounds are used for multiple functions (Griebel and Oller 2008).

Possible uses of these low frequency sounds could include communication between mothers and calves. As these relations are known to be the longest-term associations in Amazon River dolphins, communication would be crucial. Previous research on bottlenose dolphins found that mother-calf signals are important, especially when they are separated (Smolker et al. 1993). Signature whistles produced during periods of separation between mothers and their calves may function to reunite the pair by giving information about location (Smolker et al. 1993). Captive studies on bottlenose dolphins also report low frequency, short duration sounds produced by mother-calf pairs (McCowan and Reiss 1995). These sounds are believed to function as an aggressive contact call emitted by the mother when the infant is more than 1.5 meters away and are often followed by disciplinary actions from the mother towards the calf in order to maintain close proximity between the mother-calf pair (McCowan and Reiss 1995).
In recordings of Amazon River dolphins LFNs collected in Brazil, 68% of groups had mother-calf pairs present. In Peru, 55% of the recordings of groups with LFN calls present had mother-calf pairs. Podos and colleagues (2002) attempted and failed to document any responses from mother-calf pairs during playback experiments, but they acknowledged that the lack of response could be due to limitations in the quality of the playback system. Therefore, a possible function of these LFN sounds is for maintaining contact in mother-calf pairs or for the mother to aggregate the calf when it swims too far away. The LFN sounds may also be produced by mothers to serve as a warning to other adults that approach too closely, especially in the presence of aggressive males. Observational behavioral data collected in Peru indicated that mothers might produce LFN sounds as an aggressive spacing call, warning the other animals to keep their distance. When an adult Amazon River dolphin approached a mother-calf pair, the mother thrashed and slapped her fluke tail on the water, and an LFN sound was recorded at the same time (Ad lib observation).

In addition to mother-calf communication, other animals in larger aggregations may also be sharing information with one another (Nakahara 2002). This was found in Humpback whales, a species of cetaceans in which adults also do not exhibit strong bonds or occupy stable groups (Clapham 2000). These animals need to communicate as they join fluid groups during their seasonal aggregations (Clapham 2000). Amazon River dolphins are known to aggregate in certain locations, especially river confluences, during low water when animals need to leave the floodplains (Vidal et al. 1997, Martin et al. 2004, Martin and da Silva 2004b, Gomez-Salazar et al. 2011). When collecting data in
Brazil, several observations of male-male aggression were noted when recording Amazon River dolphins in large aggregations. During these recordings, LFN sounds were recorded that coincided with aggressive male behavior (Ad. Lib observation). This might indicate that in when larger fluid groups, or mother-calf pairs, LFN sounds could be used by Amazon River dolphins as either contact aggregation calls or spacing calls.

Summary of Comparison of Low Frequency Narrow-band Sounds

Few studies have focused on variation in social sounds between populations of freshwater dolphin species. When comparing acoustic parameters between LFN sounds produced by Amazon River dolphins in Brazil and Peru, I found geographic variation. The results revealed that the mean peak frequency of the LFN sounds were higher in Peru than in Brazil. The mean duration of ‘short’ LFN sounds were longer in Peru. Comparison of pattern parameters determined there was a higher mean number of sounds per train produced in Peru than in Brazil, with a more extreme range of number of sounds per train in Peru. The LFN sound trains in Peru also showed longer mean inter-sound intervals when I analyzed all trains, and a more extreme range of interval lengths than in Brazil. While no differences were found in the mean duration of ‘long’ sounds or inter-sound intervals (excluding effective zero intervals) between the two field sites. Overall, the differences in the temporal, frequency, and patterns of the LFN sounds recorded are evidence of geographic variation in Amazon River dolphins.
In evaluating geographic variation in social sounds of marine cetaceans, several studies found duration to be an important parameter. The duration of whistles of spinner dolphins vary based on location (Bazúa-Durán and Au 2004), while Rendell et al. (1999) noted that the duration of tonal calls can be distinct within several species. Another study determined that variation in frequency parameters of whistles in marine Guiana dolphins (Sotalia guianensis) are important, as there was a distinct increase in frequency from south to north (Rossi-Santos and Podos 2006).

Geographic variation between populations of Amazon River dolphins’ low frequency sounds were also reported by Amorim and colleagues (2016). Amorim et al. (2016) collected data in the Japura River in Brazil (a white-water habitat) and compared them to acoustic data collected by Podos et al. (2002) in Mamirauá (a white-water habitat). They reported pulsed sounds varied in duration and frequency, similar to the results found in the present study. The peak frequency and duration parameters of the low frequency sounds were lower in the Japura population than the Mamirauá population (Amorim et al. 2016). In the present study, the low frequency sounds recorded of Amazon River dolphins in Mamirauá (Brazil) were lower in peak frequency and duration than the sounds produced by the Peru population.

Factors for Variation

Variation in social signals of cetaceans has been suggested to be influenced by environmental conditions, group size and composition, background noise, and
evolutionary influences (Jones and Sayigh 2002, Rossi-Santos and Podos 2006, May-Collado and Wartzok 2008, Amorim et al. 2016, Leão et al. 2016). It was important to assess the role of the above factors on the geographic variation found in LFN sounds this study found between Amazon River dolphins in Brazil and Peru. Future work on social sounds need to consider all of these factors when attempting to understand geographic variation between populations.

Environmental Conditions

The acoustic signals produced by cetaceans may exhibit variation in the structure and parameters based on the physical and chemical characteristics of the water in which they are propagated. Work on Guiana dolphins in Brazil reported that physical and chemical differences in water properties may explain differences in whistle frequency in two different habitats (Leão et al. 2016). Researchers determined that differences in environmental conditions such as temperature, salinity, and water density can favor modified sounds in order to increase propagation of signals. An increase in fluvial sediments in one of the habitats may explain the increase in whistle frequency by dolphins occupying this area (Leão et al. 2016).

In this study, distinctions in the sound parameter values between Brazil and Peru could reflect different water conditions characteristic of the Amazon region. The LFN sounds recorded in Brazil were collected in a sediment-rich, white-water or várzea environment. Peruvian LFN sounds were recorded in a black-water or igapó environment. In the white-water environment, where there are high levels of suspended
sediments, sounds are subject to attenuation and scattering when propagated causing energy loss through diffraction (Stoll 1985, Kibblewhite 1989, Medwin 2005).

The shorter duration of LFN sounds in Brazil could be an adjustment for conditions in white-water, in order to prevent the signals from being degraded by the increased levels of sediments. In addition, the lower frequency LFN sounds produced in Brazil will travel farther and so may transmit information more reliably in a white-water environment (Hamilton 1980). Since there is lower scattering with fewer suspended particles in black-water, sound will propagate better (Medwin 2005). Therefore, because the risk for signal loss is diminished, Amazon River dolphins can produce longer sounds in a body of black water. This was observed in Peru, where more ‘long’ sounds were emitted, and the ‘short’ LFN sounds have longer mean duration than the sounds recorded in Brazil.

A study of Amazon River dolphins’ low frequency signals in the Japura River in Brazil repeatedly sampled the same individuals at a confluence of white and black water (Amorim et al. 2016). In accordance with my study, these researchers found a change in acoustic behavior based on abiotic conditions with lower values of parameters in white water (duration, number of harmonics, max and center frequency, bandwidth) than in black-water. In contrast with this study, I found that more sounds were produced per train in black-water (Peru) than white-water (Brazil). My results here may conflict with Amorim et al. (2016) due to other factors of sound variation, such as differences in group size.
As yet, other variables such as water temperature and the depth of rivers and the lakes occupied by Amazon River dolphins have not been studied for their possible effects on sound differences. Future research should include these factors in the evaluation of the environmental conditions assessed in freshwater habitats.

*Group Size and Group Composition*

Differences in group sizes may play an important role in the varying parameter of acoustic signals. A study on bottlenose dolphins by Jones and Sayigh (2002) found an increase in vocal activity with an increase in group size. They suggested that the number of social sounds could be correlated with the number of animals producing the sounds or that each individual produces more sounds when more animals are present (Jones and Sayigh 2002).

Amazon River dolphin group size in Brazil ranged from 2-28, and Peru ranged from 1 to 6 animals in Peru. Long-term research in the Mamirauá Reserve, Brazil, where data were collected for the current study found that high densities of Amazon River dolphins use the floodplain lake systems in central Brazil (Martin and da Silva 2004b). The resident population of Amazon River dolphins in the Mamirauá lake system is estimated to be as many as 100 animals, with 260 plus dolphins consistently using the area within ten kilometers of the lake system throughout the year (Martin and da Silva 2004b). The population of Amazon River dolphins is currently unknown in Peru, but my study site there appeared to be less densely populated than Brazil.
Seventy-three percent of the recordings from Brazil included LFN sounds present, while only thirty-six percent of recordings in Peru did. The reduced occurrence of signals emitted in Peru could be due to the smaller group sizes there. Increased communication could be necessary in Brazil groups contained a larger variation in ages and sex classes, as well as number of dolphins to associate with. In addition, there was a higher percentage of zero inter-sound intervals in the trains recorded in Brazil (44%) than in Peru (20%), which could be a result of multiple animals producing sounds as they communicate back and forth in the larger Brazilian groups.

As discussed earlier, there is a possible link between types of social bonds and the kind of communication signals (Nakahara 2002, Clapham 2000). The large numbers of animals aggregated at the Brazil study site may be producing LFN sounds more frequently as they join in the fluid groups. When Amazon River dolphins are present in these large aggregations, there may be a need for more information to be shared. My results show that the dolphins in Brazil may produce fewer LFN sounds per train. This may reduce the acoustic cluttering that occurs with increasing group sizes (Jones and Sayigh 2002). If each animal may produce trains with fewer sounds while in the large aggregations of fluid groups, this may decrease the likelihood of missing important signals.

Group composition may be an important factor in the variation of social sounds recorded in the two study sites. As previously mentioned, the presence of mother-calf pairs could have influenced the social calls emitted. Additionally, the behavior and activities of the dolphins during recordings have been reported as an important
component in the production of sounds, as groups produce significantly more sounds when socializing than when engaged in other activities (Jones and Sayigh 2002). Podos et al. (2002) reported that low frequency vocalizations were temporally associated with feeding, but also mentioned that they recorded vocalizations when no feeding occurred. I observed no feeding behaviors associated with LFN sound production during my data collections, but these aspects need to be considered and evaluated. In future work on Amazon River dolphins in Peru, we plan to further investigate LFN sound production and behaviors.

Background Noise

Biotic and abiotic background noise may influence sound production. Animals may need to modify the sounds produced based on background noise in order to avoid masking (May-Collado and Wartzok 2008). Jensen et al. (2009) found that boats at a range of 50 meters and moving at slow speeds can reduce the communication range of bottlenose dolphins. Sound-masking from boat noise or other background noise can have negative effects on social interactions if the phonating dolphins are more than ten meters away from each other (Jensen et al. 2009).

Research has shown that boat noise correlates with changes in behavior of dolphins. For example, bottlenose dolphins had longer inter-breath intervals, decreased distance between animals, increased swimming speed, and changed headings when approached by vessels, compared to control periods (Nowacek et al. 2001). In addition, specific changes in acoustic behavior have been documented in other dolphin species with increased vessel noise (Foote et al. 2004, May-Collado and Wartzok 2008).
example, increased modification of acoustic parameters was found in the presence of multiple boats when recording bottlenose dolphins (May-Collado and Wartzok 2008). Another study on bottlenose dolphins found changes in the structure of whistles in the presence of intense boat noise (Gospić and Picciulin 2016). Specifically, these dolphins produced whistles with higher frequencies, especially when foraging or socializing, probably in order to increase transmission (Gospić and Picciulin 2016). Acoustic behavior in Guiana dolphins changed with noisier conditions; higher whistle rates and shorter whistle duration were emitted with increased ambient noise (Leão et al. 2016, Bittencourt et al. 2017). The effect of sound-masking on behavior may be an issue for conservation, as animals may not be able to effectively communicate with increased abiotic noise.

The abiotic noise in the two research sites of this study was primarily from boat engines operated by the local population and visiting tourists. Boats are the primary means of transportation in these areas, and during seasonal flooding they are the only mode of transportation. At the study site in Peru, along the part of the Tahuayo River surveyed, there are six communities, one research lodge, and one tourist lodge. In the Mamirauá lake system channel where recordings were collected in Brazil there are two small communities, one tourist floating lodge, and two research floating houses. Both sites are known for seasonal fishing activities, which increased possible sound-masking due to higher boat traffic. Overall, boat traffic is presumably higher in Peru than in Brazil because the larger population of people living in and using the area. This study
found that the LFN sounds in Peru have significantly higher frequencies than those in Brazil, which could have been done to avoid masking in the presence of boats.

Natural noise may also influence the low frequency communication sounds produced by Amazon River dolphins. Large rivers and streams may have higher ambient noise, especially during high water when the flow of the rivers increases. Moreover, confluences have an increased natural noise due to the meeting of waters. This is important to consider, as confluences are a location known to be heavily used by Amazon River dolphins (Vidal et al. 1997, Martin et al. 2004). Further research on the impact of natural and anthropogenic noise on Amazon River dolphin communication is needed to increase our understanding of possible issues and to work towards more specific conservation management plans to reduce noise in both areas and throughout the range of the dolphins.

*Evolutionary Influences*

Geographic variation in acoustic behavior may be a reflection of adaptation to local environments due to selective pressures such as the environment or noise levels, as previously discussed (Foster and Cameron 1996). This may influence the need for specific acoustic parameters in order to transmit signals. Therefore, if the communication signals emitted by a sender from one population are different than a distant population, they may receive variant behavioral responses from a receiver from the other population if they come in contact (Velásquez 2014). Animals will be better adapted to communicate within the acoustic niches in their own habitats and this may affect their ability to disperse. This behavioral variation between these populations is a possible
influence on the initial stages of speciation as a result of natural and sexual selection and genetic drift (Velásquez 2014).

Very few studies have evaluated the genetics of Amazon River dolphins. Long-term research in central Brazil has shown that some dolphins will move along rivers between flood plain systems when there is no barrier, but many show site fidelity (Martin and da Silva 2004b). The same authors also have anecdotal information where one of their study animals was seen 1,000 kilometers away from where it was marked (Martin and da Silva 2004b). A study using photographic identification in Peru found the following movement patterns: the maximum range of movement was 220 km, and the mean range of movement was 60.8 km with a rate of 14.5 kilometers per day (McGuire and Henningsen 2007). Martin and da Silva (2004b) suggest that movement patterns and large ranges of Amazon River dolphins lead to potential for genetic exchange, especially with no physical barriers to prevent movement in many parts of their range. In contrast, McGuire and Henninsen (2007) suggest that Amazon River dolphins may not move between river systems as they did not identify animals in adjacent river systems during their nine-year study, despite the lack of obstruction.

The dispersal of Amazon River dolphins could be influenced by their ability to communicate in the physical properties of the water. Specifically, local habitat preferences could influence dispersal and gene flow. Adaptations for specific habitat types were reflected in the increased whistle frequencies in Guiana dolphins from south to north (Rossi-Santos and Podos 2006). Confluences of white and black water have been found to be preferred habitat for some Amazon River dolphins, but because of the
way populations are spatially structured, some dolphins (especially females) may not encounter both white and black water rivers (Vidal et al. 1997, Martin et al. 2004). When only using a specific water habitat, the dolphins may develop an acoustic repertoire that could result in low gene flow or reproductive isolation (Schmidt and Pfennig 2016). As reproductive character displacement can lead to divergence in allopatric populations, it is possible that selection for certain parameters of acoustic signals may influence reproductive encounters (Pfennig and Pfennig 2012).

In the current research, the two study sites were 1,512 km apart with no known obstructions. No current evidence suggests there is reduced gene flow between the two sites, but no genetic analysis has been done at the Peru field site. In order to assess whether the differences in these LFN sounds are influential in the genetic structure of Amazon River dolphins in these two populations, DNA sequencing will be necessary (Pfennig and Pfennig 2012).

Individual dolphins may be able to adjust their sounds based on environmental parameters if they use both water types (Leão et al. 2016). This acoustic plasticity was found in Amorim et al. (2016) when recording the same individuals in black and white water. But this does not rule out the possibility that some animals may be adapted for only certain water types, as behavioral differences can be heritable (Foster and Cameron 1996).

To summarize, when trying to understand geographic variation in sounds, it is vital to assess the role of environmental conditions, group size and composition, background noise, and evolutionary influences. It is likely that all of these elements
contribute to the variance that was found between the LFN sounds produced in Brazil and Peru, but it will take much more work to understand their individual contribution.

Research Limitations

While this study brings new insight into the multifactorial nature of geographic variation found in Amazon River dolphins, there are several limitations to the work. The study used only a single hydrophone for recording sounds produced by the Amazon River dolphins. Localization of the individual producing the sounds is not possible without a hydrophone array or multiple hydrophones (Nakahara 2002). When a hydrophone array was used to localize sounds produced by Minke whales (*Balaenoptera acutorostrata*), for which acoustic recordings are scarce, researchers were able to identify previously unknown sounds (Gedamke *et al.* 2001). When using the array for acoustic recordings, Gedamke *et al.* (2001) found that the ability to localize the animal producing the sound would vary from visual observations. Therefore, the accuracy of behavioral context increases when using a hydrophone array, especially when the animals are within close range (Gedamke *et al.* 2001). Without being able to identify the dolphin emitting the sound, behavioral context is even more difficult to document. When studying Amazon River dolphins, the visibility of the water in both field sites was extremely limited, and surface behaviors of these species can be very cryptic, further hindering behavioral data collection.
Furthermore, in recording Amazon River dolphins with a single hydrophone, the present study is also not able to decipher whether multiple dolphins were producing sounds back and forth for communication. Such interaction between animals could be responsible for the zero millisecond inter-sound intervals in trains of LFN sounds, which were recorded in both Brazil and Peru. While I was able to discriminate between the sounds for most analyses, these possible overlapping sounds may have influenced the results found for the patterns of LFN sounds. There was a higher percentage of zero millisecond inter-sound intervals in Brazil which could be due to larger group sizes with a higher potential for sound cluttering.

The effort to collect behavioral data was significantly different between study sites in Brazil and Peru. Behavioral data were collected opportunistically in Brazil, and only when obvious behaviors were observed at the surface. In Peru, an ethogram of defined surface behaviors was incorporated. In order to better understand all of the aspects that may be affecting the variation found in these LFN sounds, consistent behavioral data must be collected. Behavioral studies in black-water environments where visibility is better could bring new insight into the behavior of these dolphins and the function of the low frequency sounds they emit.

Finally, there was a difference in the length of recording effort and the season that recordings were made between my two sites. Recordings were collected in Brazil from August (as the water was receding) through the dry season until April (when flooding was almost at its peak). In Peru, acoustic recordings were collected during falling water in the months of June and July. One factor not considered by this study is the effect of
flooding seasonality on sound production of Amazon River dolphins. Such differences in data collection could influence the results, and seasonality should be considered in future work.

**Future Work and Conservation**

In order to better understand the acoustics of Amazon River dolphins, particularly the LFN sounds and the variation that was found in this current study, we need more observational and experimental work. To increase the possibility of comparative work in this field, a standardization of sound names and analysis techniques are crucial. At this time, low frequency sounds by Amazon River dolphins have been called ‘vocalizations’ and whistles (Podos *et al.* 2002, Trone *et al.* 2014, Amorim *et al.* 2016). Because the term ‘vocalizations’ is a misnomer, as dolphin sounds do not come from vocal cords or larynx but from the nasal cavity, a quantifiable name like low frequency narrow-band sounds is more appropriate (Au and Hastings 2010). It is also important for other studies to give contextual information and group composition when recording sounds, as was done in the present study. Most studies on Amazon River dolphins, including acoustic data collection, have been done in a very small portion of their possible range. For future work on the repertoire of social sounds produced by Amazon River dolphins, both tonal and pulsed, researchers need to collect data from a wider range of the species distribution.

We need further acoustic experimental studies on propagation of sounds in white and black water in the Amazon River basins. Conjointly, it is important to consider
depth, temperature, and other variables that could influence sound production and
generation (Leão et al. 2016). The depth of the rivers and lakes that Amazon River
dolphins use may play an important role, as sound propagation is different in shallow
versus deeper water (Au and Hastings 2010). The river levels in the flooded forests used
by Amazon River dolphins in this study vary depending on the annual flooding cycle.
Water levels in Brazil fluctuate between eleven and fifteen meters a year (Martin and da
Silva 2004a). While in Peru the water levels vary between eight and ten meters (Kvist
and Nebel 2001). In shallow water sound waves reflect repeatedly off the bottom and
surface of the water, which can increase scattering during acoustic propagation (Au and
Hastings 2010). Additionally, the slope of the rivers’ edge, the composition of the
bottom, and any trees or large submerged objects can affect the propagation of sounds
produced by Amazon River dolphins (Au and Hastings 2010).

In pursuance of gaining a more complete understanding of Amazon River
dolphins, we need genetic analyses to better understand gene flow and in order to
determine the level of isolation and potential for speciation. The acoustic variation found
in the Amazon River dolphins may be the first step in speciation, but since so few genetic
studies have been undertaken it is difficult to ascertain its influence at present.

The current of geographic variation in social sounds gives new insight into the
populations and the factors that may be driving local adaptations. Because variation was
found between the study populations, there may be a need for separate and specific
management plans for each, as the populations may be distinct in other ways. In other
cryptic taxa, such as blue whales, where it has been difficult to identify populations and
subspecies, acoustic characteristics were found to be a key tool for distinguishing stocks (McDonald et al. 2006). Passive acoustic studies of Amazon River dolphins could be used to better understand spatial and seasonal movements of specific populations, using the different values of the physical and pattern parameters of LFN sounds. The use of autonomous recorders could be especially important during the flooded season, when animals may enter the floodplains and boat based observation is not possible.

This study is one of the first to consider the factors that could affect the differences in the production of social sounds of Amazon River dolphins between two populations. Since Amazon River dolphins face an increasing number of threats to their populations and conservation efforts are imperative, the use of acoustic studies, such as this, to better understand the species will be crucial.


