RIVER DOLPHINS AS INDICATORS OF ECOSYSTEM DEGRADATION IN LARGE TROPICAL RIVERS

by

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"So here is my farewell to a wild river. This will be the beginning of the end of the Amazon region as we know it." - Cristina Goettsch Mittermeier concluding remarks in her article "Farewell to a wild river", referring to the beginning of dams in Amazonia.

This dissertation is dedicated to those who cannot speak and will not be able to fight for their space in the large tropical rivers basins of South America: the river dolphins.

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ABSTRACT

Freshwater ecosystem degradation in the Amazon and Orinoco river basins is increasing due to rising human population numbers, and large water development projects. Therefore, monitoring ecosystem condition in these rivers of high biodiversity is of global conservation importance. This dissertation evaluated the potential of using river dolphins as indicators of ecosystem condition in large tropical rivers of South America. First, population estimates of river dolphins were obtained by line-strip transect surveys and mark-recapture methods on photo-identifications. Using this information, I identified critical habitat, hotspots, and areas of concern for river dolphins, as well as the relationship between dolphin density and group size estimates with ecological features. Second, I evaluated the relationship between dolphin estimates and human stressors. Higher densities of dolphins occurred in rivers with low indices of overall freshwater degradation, such as rivers with high water quality and the lowest human population numbers. Thus, dolphin density estimates seem to be good indicators of freshwater ecosystem degradation in the Amazon and Orinoco basins. These top predators not only are indicator species, but also have the potential to act as flagship and sentinel species, indicating freshwater ecosystem degradation and stimulating conservation action. This dissertation highlights the large changes in the Amazon and Orinoco that are approaching fast. Indicator, flagship and sentinel species can become science-based conservation tools not to only document freshwater ecosystem degradation, but to raise awareness about broader implications of human stressors on biodiversity and river systems.

LIST OF ABBREVIATIONS USED

FGS Faculty of Graduate Studies

Dal Dalhousie University

IUCN International Union for Conservation of Nature

IWC International Whaling Comission

mo month

h hour

wk week

km kilometer

hp horsepower

SARDPAN South American River Dolphin Protected Area Network

UNEP United Nations Environment Programme

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CHAPTER 1 INTRODUCTION

Freshwater is fundamental in supporting ecosystems and biodiversity, and provides resources for food (including fisheries, irrigation, and aquaculture), hydropower, drinking, transportation and sanitation for human societies (Vörösmarty et al. 2004). Freshwater ecosystems comprise rivers, lakes, wetlands, soil water, ground-waters, coastal marshes and estuaries, and its boundaries change according with the flooding cycle (Revenga et al. 2000, Meybeck 2003). This dissertation will mainly focus on river systems.

Rivers are at risk due to multiple human stressors, including population growth, industrialization and expansion of irrigated agriculture and water development projects (Revenga et al. 2000, UNEP 2004, Alcamo et al. 2005). In most cases, we do not know the full impact of human stressors in terms of the shift of species, alterations of foodwebs, productivity and climate change, which have been generally been much more thoroughly documented in terrestrial and marine systems (Pauly et al. 1998, Myers and Worm 2003, Swartz et al. 2010). Moreover, biodiversity in freshwater ecosystems is more threatened and declining more rapidly than biodiversity in terrestrial and marine ecosystems (Revenga et al. 2000).

Measuring ecosystem degradation is usually based on the hypothesis that areas with the highest human stressors will likely be most modified and the biodiversity within them will be subjected to the highest pressure. From the literature, I identified two general approaches for assessing ecosystem degradation, which differ in the features measured:

(1) The first approach involves the direct measurement of biological, chemical and physical features of the ecosystem that change as a consequence of human stressors, including shifts in the composition of species, diversity, extinctions, and

- the reduction of productivity and habitat availability, such as for food and refuge (Swartz et al. 2010).
- (2) The second approach consists of assessing and quantifying human stressors as a proxy for ecosystem degradation and biodiversity loss, using measures such as proximity to infrastructure and human population density (Bryant et al. 1998).

These approaches are not mutually exclusive and they are often used in concert (Alcamo et al. 2005). When these two approaches are combined, the data are then analyzed using two main frameworks:

- (1) The first, temporal, framework consists of providing scientific evidence on the effects of human stressors on ecosystems and biodiversity based on past and current information (Jackson et al. 2001, Lotze et al. 2006).
- (2) The second, geographic, framework consists of providing a ranking measure of current ecosystem degradation, including indices, scores and/or risk impacts (Halpern et al. 2008). Although comparisons with previous baselines and reference points are ideal, these are often not available, thus areas may be ranked based on current values of the measures of ecosystem health and human stressors.

Typically, the first framework provides the necessary background to provide an overall view of the status of ecosystems, and the second framework provides a measure to compare it to adjacent or other ecosystems. These approaches and frameworks are not mutually exclusive, are typically used together if data are sufficient, and it is difficult to separate them. This dissertation will use both approaches, but mostly focus on the second framework.

This introductory chapter will first synthesize and analyze current global and regional approaches used to measure freshwater ecosystem degradation in the Amazon and Orinoco river basins. The Amazon, the largest river basin in the world, and the Orinoco, the third largest drainage system in the world, contain exceptional levels of biodiversity,

fish richness and endemism, thus their conservation is of high global importance (UNEP 2004, Abell et al. 2008). This dissertation includes information obtained in selected locations of Colombia, Ecuador, Peru, Bolivia and Venezuela, where the headwaters of the Amazon and Orinoco river basins are located, as well as the most productive soils, which are adjacent to the Andean foothills (Laurance et al. 2005). I will then introduce the structure and goals of this dissertation, primarily measuring freshwater ecosystem degradation in selected areas of the Amazon and Orinoco river basins.

1.1 Measuring Ecosystem Degradation: Global Assessments

I reviewed three global assessments that address freshwater ecosystem degradation in terms of human water security and biodiversity. These assessments are the Millennium Ecosystem Assessment (MEA), the Global International Water Assessment (GIWA), and the global initiative to quantify global threats to Human Water Security and Biodiversity (HWSB). These assessments are all ongoing projects that synthesize current human stressors for freshwater ecosystems in terms of human water needs and biodiversity, with the goal of raising awareness about freshwater habitat degradation. The MEA (which is the continuation of the Pilot Analysis of Global Ecosystems) focuses on providing a comprehensive evaluation of human stressors that affect human well-being and biodiversity (Alcamo et al. 2005). By summarizing information already available, MEA provided scientific evidence about the past, current and future condition of global freshwater systems, and identified some gaps in the data that is available to direct further assessments. However, it did not provide an extensive regional approach, nor an overall score or ranking of areas according to the level of freshwater degradation in the Amazon and Orinoco basins.

In contrast, the GIWA assessment provides a very detailed framework to give an overall quantitative score of habitat degradation by sub-region, based on evaluating and ranking five human stressors according to their level of importance (GIWA 2002). Hence, at a regional scale, the GIWA assessment provides a very detailed methodological framework for ranking regions based on freshwater degradation. For example, the Amazon region assessment ranked habitat modification as the human stressor of biggest concern (UNEP

2004); however, in many of the Amazon sub-regions, human stressors such as oil exploration, agriculture, mining, fisheries, increasing human population numbers and boat traffic are currently of greater concern than habitat modification.

Finally, the HWSB assessment provides quantitative indices of habitat degradation of global river systems by using biological, chemical and physical feature measurements as well as human stressors (Vörösmarty et al. 2010). As such, at a global scale, the Vörösmarty et al. (2010) HWSB assessment is the best way to rank human stressors geographically and thus provide an overall score of global freshwater habitat degradation. At a regional scale, however, the HWSB assessment has some limitations. Some results provided in the GIWA regional assessment do not seem to be considered in the HWSB global assessment. As an example, human stressors in the Amazon basin are documented to be the largest in Peru, and diminish downstream as the Amazon river makes its way through Colombia and Brazil (HWSB) (Vörösmarty et al. 2004, Palmer 2010). However, the largest city in the Amazon is located in the central Brazilian Amazon, where human stressors are expected to be as serious as in Peru, and in addition, the majority of dams have been built in Brazil (GIWA, UNEP 2004, Laurance 1998). Due to this, it is not completely clear whether the largest impacts in the Amazon are in Peru or Brazil, and considering additional local and regional information might help to clarify this.

The conclusions that can be drawn from these global assessments vary according to spatial scale and future assessments should try to incorporate global, regional and local perspectives.

1.2 Measuring Ecosystem Degradation: Regional Assessments

The majority (2/3) of the Amazon river basin, as well as the most reliable statistics (in terms of deforestation, transportation networks, and planned water development and infrastructure projects), are located in Brazil (UNEP 2004, Laurance et al. 2005). Such information has been used to estimate the degree of current and future deforestation: 15% of the Brazilian Amazon has been cleared, and under the most 'optimistic' scenario

analysis, by the year 2025, 4.9% of this region will have pristine forests and 40% will be heavily degraded (Laurance et al. 2005). This type of detailed information, however, is not available for all of the study areas considered in this dissertation in the Amazon and Orinoco regions of Colombia, Ecuador, Peru, Bolivia and Venezuela.

Given the lack of detailed information in these areas, the best approach for assessing ecosystem degradation of an area may be quantifying presence and proximity of human activity. For instance, when considering gold mining, a proportion of the mercury used for the separation of gold particles is lost in rivers and soils due to handling and volatilization (about 130 tns per year in the Brazilian Amazon) (Pfeiffer et al. 1993, Lacerda 1997). High and widespread contamination with mercury in humans and fishes has been observed, and the current rates of mercury emission are among the highest reported in the world (Pfeiffer et al. 1993). However, there are no reliable measures of the amount of mercury released, or present, in many areas. So, only the presence of gold mining can be considered as part of a degradation score assessment.

Overall, measurements of human stressors in the Amazon and Orinoco are currently the best way to provide an estimate of ecosystem degradation. This approach, however, does not include measurements of biological features of freshwater systems.

1.3 Measuring Ecosystem Degradation: Incorporating Measurements of Biological Features

Measurements of human stressors and biological features, such as population trends of indicator species, should be combined to assess ecosystem degradation. For instance, water quality can be assessed by measuring human stressors in or adjacent to the water bodies, and by measuring biological features, such as diversity, abundance and community structure of specific indicator species (Revenga et al. 2000, Karr 1999).

Indicator species can provide warning signals to document and monitor changes in ecosystems, they can represent the conservation status of a large number of species (Karr

1999, Karr 1991, Noss 1999, Dale and Beyeler 2001). To do this effectively, indicators have to be carefully selected.

Ecological indicator species in freshwater ecosystems range from macro-invertebrates (Astin 2006, Arimoro and Ikomi 2009, Moya et al. 2010), fishes (Zhu and Chang 2008, Angradi et al. 2009, de Freitas Terra and Araújo 2011), and macrophytes (Mackay et al. 2010), to organisms that live between terrestrial and aquatic ecosystems, such as odonata (Silva et al. 2010) and vascular plants (Miller et al. 2006). By collecting bottom samples, some assessments investigate the presence, absence and relative dominance of benthic foraminifera in relation to environmental stress and pollution (Albani et al. 2007). By collecting water samples other assessments obtain bacteria (Baghel et al. 2005), diatoms (Feio et al. 2009), and algae counts (Reavie et al. 2010, Schneider and Lindstrøm 2009) to inform about water quality and pollution. Other studies include multiple species with the goal of increasing the utility of ecological indicators (Griffith et al. 2005, Iliopoulou-Georgudaki et al. 2003, Zampella et al. 2006, Navarro-Llácer et al. 2010). Hence, detailed information on richness, composition, and abundance of specific species can be useful ecological indicators for assessing freshwater ecosystem degradation (Karr 1999, Moyle and Randall 1998).

The majority of ecological indicators for freshwater ecosystems have been developed for rivers in Europe and North America, where biodiversity levels are not as high as within the tropical rivers of Asia and South America. For these large tropical river basins, it has been proposed to use information on river dolphins to inform on freshwater ecosystem degradation across river basins (Smith and Reeves in press). Using top predators as indicators of ecosystem condition has been proven successful in other ecosystems (Sergio et al. 2005, Sergio et al. 2006, Sergio et al. 2008). For this reason, I propose to evaluate the potential of using river dolphins as indicators of ecosystem degradation in large tropical rivers of South America.

1.4 SUMMARY OF CHAPTER 1 AND THESIS STRUCTURE

There is lack of detailed data and access to remote places in the Amazon and Orinoco basins, as well as a lack of funding for monitoring programs. Research on these tropical river systems should be focused on creating collaborative networks for compiling existing data and for documenting and monitoring current trends. For now, quantifying human stressors as a proxy for ecosystem degradation is currently the best strategy available for evaluating the status of freshwater ecosystems. Including information on biological features, such as indicator species, will assist in this process.

Recognizing the importance of these river systems, gaps in data availability, and the urgency to improve the monitoring of increasing ecosystem degradation, in this dissertation I evaluate the potential of using river dolphins in the Amazon and Orinoco river basins as indicators of freshwater ecosystem degradation. First, abundance, sighting rates and group size estimates of river dolphins were obtained for selected areas of the Amazon and Orinoco river basins (Chapters 2, 3, 4, 5). These chapters include information of the ecology of river dolphins and their habitat, a framework to standardized methodologies to survey and to analyze data on this species, as well as an overview of the current conservation status of river dolphins in South America. Subsequently, I relate the river dolphin abundance estimates obtained in previous chapters to indices of freshwater ecosystem degradation (Chapter 6). This chapter investigates whether river dolphin abundance estimates are correlated with human stressors, and thus are good candidates as ecological indicators, flagship and sentinel species for monitoring the conservation status of large tropical rivers in South America. Finally, I provide an overview of the new information found about the ecology and abundance estimates of river dolphins in the Amazon and Orinoco basins, as well as recommendations for further studies that will monitor and raise awareness about freshwater habitat degradation in large tropical rivers (Chapter 7).

CHAPTER 2 ECOLOGICAL FACTORS INFLUENCING GROUP SIZES OF RIVER DOLPHINS

The work presented in Chapter 2 also appears in:

Gomez-Salazar, C., F. Trujillo, H. Whitehead. 2011. Ecological factors influencing groups sizes of river dolphins (*Inia* and *Sotalia*). Marine Mammal Science. DOI: 10.1111/j.1748-7692.2011.00496.x

2.1 Introduction

Group living likely evolved as a strategy to increase individual fitness by reducing the risk of predation and improving access to resources (Packer et al. 1990, Krause and Ruxton 2002, Gowans et al. 2007). Group living also has high costs. When group sizes are larger, competition for resources may increase, and this competition may be further influenced by a combination of seasonal change and other environmental factors (Elgar 1989, Fortin and Fortin 2009). This mixture of costs and benefits may lead to optimal group sizes where the net benefits to group members are maximized (Krause and Ruxton 2002).

Many cetaceans form groups, although the stability varies between species. For instance, baleen whales often live in small, unstable groups (Clapham 2000), while odontocete social structures range from the very stable groups of killer whales (Bigg et al. 1990) to the variable group sizes and short-term associations of bottlenose dolphins' fission-fusion societies (Connor et al. 2000).

Group sizes in cetaceans vary according to risk of predation and the availability of resources (Heithaus et al. 2008). Typically, smaller group sizes occur close to inshore areas where resources are more predictable and the risk of predation is lower given that there are typically more places to hide; larger group sizes occur in open water where

resources are not as predictable and the risk of predation increases (Norris and Dohl 1979, Shane 1980, Heithaus and Dill 2002, Gygax 2002, Gowans et al. 2007). Strong seasonal cycles influence the habitat use of cetaceans and potentially their grouping behavior. For instance, some populations of bottlenose dolphins (*Tursiops* spp.) modify their habitat preferences as a result of seasonal changes in the distribution of resources and the abundance of predators (Shane 1980, Heithaus and Dill 2002, Irvine et al. 1981).

While factors affecting group size have been studied in oceanic dolphins (Gowans et al. 2007), there has been less investigation of riverine species, where dolphins exist under very different environmental conditions. The Amazon and Orinoco river basins are very complex and dynamic systems with strong seasonal shifts causing fluctuations of 10–15 m in the water level over the course of a year. These changes affect dissolved oxygen concentration, fish migrations, habitat availability, productivity, and interactions between predators and their prey (Goulding 1980, Goulding 1989, Fernandes 1997, Lewis et al. 2001). For instance, during the low water season, the quality and quantity of habitat for both fish and dolphins are considerably reduced (with up to 5–10 times less area available during the low water season) (Neiff 1996). Hence, flood pulses are a major force controlling biota in riverine floodplains (Junk et al. 1989). Consequently, we would expect that the extreme and predictable seasonal changes of the Amazon and Orinoco basins would be major factors influencing the formation of groups in river dolphins.

River dolphins are top predators in these two highly complex and dynamic river basins, and have no natural predators. There are three species of river dolphins in the Amazon and Orinoco river basins: *Inia geoffrensis*, *I. boliviensis*, and *Sotalia fluviatilis*. The pink river dolphin or boto, *I. geoffrensis*, has two subspecies: *I. geoffrensis geoffrensis* in the Amazon (Brazil, Colombia, Ecuador, Peru, and French Guiana) and *I. geoffrensis humboldtiana* in the Orinoco (Colombia and Venezuela). The bugeo, *I. boliviensis*, is distributed in the Amazon and Madeira upper basins (Bolivia) (da Silva 1994, Hamilton et al. 2001, Banguera-Hinestroza et al. 2002, Ruiz-García et al. 2006, Ruiz-García et al. 2007, Ruiz-García et al. 2008, Ruiz-García 2010) while the tucuxi, *S. fluviatilis*, is distributed in the Amazon (Brazil, Colombia, Ecuador, Peru, and French Guiana)

(Caballero et al. 2007) and in the lower and middle Orinoco basin (Venezuela) (Gomez-Salazar et al. 2010).

Inia and *Sotalia* have some of the smallest group sizes of cetaceans, ranging from one to eight for *Inia* (McGuire and Winemiller 1998, Martin et al. 2004) and from one to six for *Sotalia* (Martin et al. 2004). There are few studies that have investigated variation in group sizes of river dolphins with flood pulses and habitat type, and there are even fewer studies where sampling effort was included as a weighting factor (McGuire and Aliaga-Rossel 2010) (Figure 2.1, Table 2.1).

Studies in the central Amazon (Brazil) have provided detailed results regarding habitat preferences of *Inia* and *Sotalia* over a period of 2 yr, by describing variation in density (Martin et al. 2004), as well as the seasonal movements and sex ratios of *Inia* in different habitat types over a period of 8 yr (Martin and da Silva 2004). Overall, these studies showed that *Inia* and *Sotalia* prefer confluences and areas within 150 m of the shore, and *Inia* adults are separated by sex during most of the year. Mean group sizes and SE were provided for different seasons and habitat type (see Table 2.1).

Studies in the northern Ecuadorian Amazon have been discontinuous and have been focused on distribution and encounter rates, instead of group sizes (Utreras 1996, Utreras et al. 2010). Studies in the Peruvian Amazon (Pacaya-Samiria Natural Reserve) over a period of 9 yr focused mostly on encounter rates, habitat preferences, residence, density, and abundance estimates of *Inia* and *Sotalia* (e.g., McGuire and Aliaga-Rosell 2010). Mean group sizes did not seem to vary according to season or habitat type (McGuire and Aliaga-Rossel 2010).

In the Orinoco basin, studies of the group sizes, distribution, habitat preferences, prey availability, and population structure of *Inia* dolphins were conducted in the Cinaruco River over a period of 8 mo (McGuire and Winemiller 1998). The largest group sizes were found in confluences during the rising water period; however, surveys were not conducted during the high water period.

Studies in the Bolivian Amazon over a period of 20 mo, focused on the distribution, abundance, encounter rates, and group sizes of *Inia* dolphins (Aliaga-Rossel 2002). These studies showed significant relationships between the distribution of group sizes and the habitat type and season (Aliaga-Rossel 2002) however, mean group sizes and SD were not provided. In a later study, conducted over a period of 2 mo, mean group sizes and SD were reported, which seemed to vary according to resource availability (Aliaga-Rossel et al. 2006). For instance, rivers with an influx of whitewaters in central Bolivia, which are rich in nutrients and prey, are believed to support large groups of *Inia* dolphins, while rivers with large human settlements and high boat traffic, which may disrupt the social structure of river dolphins, have smaller group sizes (Aliaga-Rossel et al. 2006).

Previous research on river dolphins has focused mainly on density estimates, encounter rates, residence patterns, and sighting frequency (mostly of *Inia*), rather than variation in group sizes and the ecological factors influencing them.

We examined group size for *Inia* and *Sotalia* in two locations within the Colombian Amazon and Orinoco river basins to determine how seasonal and environmental variation affects these species. We predicted that group sizes would change according to temporal and spatial factors, with bigger groups during the dry season when resources are concentrated and easily accessed, and in habitats that offer more availability of resources. Since areas close to riverbanks, as well as areas with aquatic macrophytes are highly important for many fish species, providing nursing areas, resources, and refuge from some predators (Henderson 1990, Winemiller and Jepsen 1998), and river dolphin densities are higher in areas closer to the riverbanks (Martin et al. 2004), we would also expect some variation in the group sizes of dolphins in relation to distance from shore and shore type.

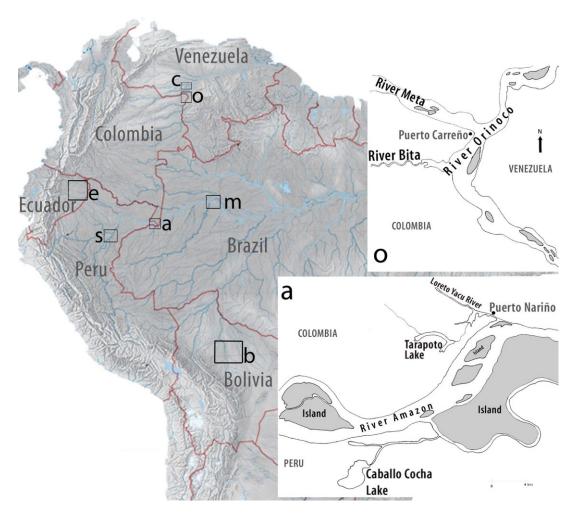


Figure 2.1 Map of study of study area showing locations where river dolphin research has been conducted.

o = Orinoco and Meta rivers (260 km², this study), a = Amazon and Loreto Yacu rivers (140 km², this study; 170.1 km², Vidal et al. 1997), c = Cinaruco River (1.67 km², McGuire and Winemiller 1998), e = Tiputini, Yasuní, Lagartococha, Cuyabeno, Napo rivers (at least 300 km, Utreras et al. 2010), s=Samiria and Marañón rivers (35.6km² and 288 km), b = Mamoré, Tijamuchi, Apere, Yacuma, Rápulo rivers (287 km, McGuire and Aliaga-Rossel 2010), m = Amazon and Japurá rivers (220.2 km², Martin et al. 2004).

Table 2.1 Estimates of group sizes of river dolphin populations in different locations of Asia and South America.

Location	Effort	# sightings	Mean group size	SD	Reference			
Yangtze River dolphin (Lipotes vexillifer)								
Yangtze River, China	November 1997 - November 1999	18	2.1	0.87	Zhang et al. 2003			
Irrawaddy dolphins (Orcaella brevirostris)								
Sundarbans Delta, Bangladesh	March 4–24 2002	89	2.3	1.36	Smith et al. 2006			
Ganges Riv	er dolphins (<i>Platanista gan</i>	getica)						
Sundarbans Delta, Bangladesh	March 4–24 2002	55	2.45	2.25	Smith et al. 2006			
Jamuna River, Bangladesh	April 22–25 1996	25	1.8	1.4	Smith et al. 1998			
Kushiyara River, Bangladesh and India	October 7–11 1995	9	3.8	2.8	Smith et al. 1998			
Pink river d	dolphin (<i>Inia</i>)							
Amazon River, Colombia	June 5-26 2002	97	2.9		Vidal et al. 1997			
Amazon and Japurá Rivers, Brazil	March 1999 - April 2001	733	1.42	0.036 (SE)	Martin et al. 2004			
Amazon and Japurá Rivers, Brazil	June 99/Oct 99/Mar00/Jul00/Apr01	92	1.3/1.6/1/1.4/1	0.14/0. 11/0/0. 12/0 (SE)	Martin and da Silva 2004			

Location	Effort	# sightings	Mean group size	SD	Reference	
Tefe Lake, Brazil	Apr01/Jun99/Oct99/Mar 00/Jul00/Apr01	63	1/1.3/1.3/1.1/1.1 /1.3	0/0.16/ 0.16/0. 06/0.14 /0.22	Martin and da Silva 2004	
Cinaruco River, Venezuela	November 1993-June 1994	489	2	1	McGuire and Winemiller 1998	
Amazon River, Colombia	December 1993 -July 2005	4419	3.4	3.1	This study	
Orinoco River, Colombia, Venezuela	July 1996 - August 2003	1725	5.3	4.5	This study	
Mamoré and Tijamuchi Rivers, Bolivia*	January 1998 - September 1999	920*			Aliaga-Rossel 2002	
Mamoré, Tijamuchi, Apere, Yacuma, Rapulo Rivers, Bolivia	August - September 1998	492	2.1/3.3/2.3/1.8/2	1.34/2.9 6/0.98/0 .75/0.81	Aliaga-Rossel et al. 2006	
Tucuxi (Soi	talia fluviatilis)					
Amazon River, Colombia	June 5-26 2003	72	3.9		Vidal et al. 1997	
Amazon and Japura Rivers, Brazil	March 1999 - April 2001	504	2.24	0.065 (SE)	Martin et al. 2004	
Amazon River, Colombia	December 1993 - July 2005	4062	3.6	2.6	This study	

^{*}Mean and SD were not reported.

2.2 METHODS

2.2.1 Field Surveys

Vessel-based surveys for river dolphins were conducted in two locations of the Colombian Amazon and Orinoco river basins (Figure 2.1) from a 6 m boat with an outboard 25 hp engine and at an observation height of about 2 m. The Amazon basin study area is located in the southern part of Colombia, and it comprises a section of about 60 linear km (approximately 140 km²) including the Loretoyacu River, a small tributary of the Amazon River that leads to El Correo and Tarapoto Lakes, a section of the Amazon River that leads to Lake Caballo Cocha along the frontier with Peru, and another section that leads to the Atacuari River, another small tributary of the Amazon River (Figure 2.1). The Amazon basin surveys were conducted between December 1993 and July 2005.

The Orinoco basin study area is located in the northeast of Colombia, and it comprises a section of about 120 linear km (approximately 260 km²), including the Orinoco River that leads to sections of two tributaries: the Meta River in the north and the Bita River in the south (Figure 2.1). Surveys of the Orinoco basin were conducted between July 1996 and August 2003. *Inia geoffrensis* occurs at both locations and *S. fluviatilis* only in the Amazon. Previous research conducted 13 yr ago in the Amazon study area estimated a population size of 346 *Inia* (CV = 0.12) and 409 *Sotalia* (CV = 0.13; Vidal et al. 1997). There are no estimates of *Inia* population sizes in the Orinoco, nor over *Inia* and *Sotalia*'s entire distribution.

Surveys followed standardized routes parallel to the riverbanks, maintaining a constant speed of 10 km/h (Table 2.2). Estimating group size is often difficult, so we conducted "closing mode" surveys in order to estimate the group size accurately (Zerbini et al. 2007). When dolphins were encountered, we recorded species, location (using a Geographic Positioning System), group size, group composition, habitat type, shore type, and distance from shore. A group of river dolphins was defined as a set of animals that

are seen together within 250 m from the boat, likely engaged in the same activities, and does not necessarily correspond to a social group (McGuire and Winemiller 1998).

Although the dark-colored waters and the shy behavior of river dolphins make it hard to track and photograph individuals within groups, the typically small group sizes and short dives (which do not last more than 2 min) allowed us to obtain group sizes accurately within the 250 m range. This range was established given that individuals within groups of *Inia* are at most a maximum distance of 50 m from each other and individuals within groups of *Sotalia* are at most a maximum distance of 30 m. This is smaller than the 250 m used to define group membership. Therefore, it is unlikely that we are under representing *Inia* and *Sotalia* group sizes by much.

Group composition was recorded as the number of adults/juveniles and calves. Calves were defined as animals <1 yr old, <1 m long, and generally dark gray. Calves are not included in further analyses. Several characteristics of the river course were used to identify six general habitat types: main river, tributary, channel, island, confluence, and lake (Table 2.3); and eight shore types (Table 2.4).

The distance of the group to the nearest shore of the river was calculated and classified into three categories: 0-50 m, 50-100 m, and $\geq 100 \text{ m}$. The distance from the boat to the dolphins, and from the group to the nearest shore, was estimated by eye, often validated using floating objects nearby whose range was determined with a laser range finder. Once group data were recorded, we resumed the survey effort immediately to avoid double counts of the same group of dolphins in each survey.

Four hydroclimatic seasons were identified for each year in the study area, according to the precipitation and water levels obtained from the environmental information system in Colombia (IDEAM; Figure 2.2).

Table 2.2 Number of group sightings for each species by year and month. Blanks indicate no effort in that month and year.

Basin/species	Mon	th											
year		_	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Amazon				_	-				_				
Inia	395	181	347	302	322	412	594	281	261	258	340	726	4419
1990)											402	402
199	1 8		122	60		70	164						424
1993	3											23	23
1994	1 75		6			33	67	10			52	50	293
1990	5			16	99	70	75	34	83	62	62	67	568
199′	7 130	31	37				15	25				60	298
1998	3		18	64	49	48	57	98	79	66	91	41	611
1999	9 24	55	35	29	27	47	8	10			6	22	263
2000	54		57	19			3						133
200	1 17	23	20	27	42	28	23		3	30	37	9	259
2002	2 21					15	13	19	6	12			86
2003	3 2	29	42	6	32	20	55	26	26	25	2	24	289
2004	1 19	43	10	81	66	81	85	59	64	63	90	28	689
200:	5 45				7		29						81
Sotalia	376	168	407	289	322	411	476	264	213	247	309	580	4062
1990)											339	339
199	1 13		167	72		106	131						489
1993	3											22	22
1994	1 80		4			14	37	10			23	37	205
1990	5			17	146	67	52	27	30	35	42	33	449
1997	7 91	2	15				20	12				44	184
1998	3		9	60	33	30	48	56	43	42	77	31	429
1999	28	57	50	27	7	31	5	12			5	11	233
2000	49		73	21			1		14	47	27	9	241
200	18	33	31	25	36	30	25	26	31	17	55	12	339
2002	2 34					13	22	65	32	43			209
2003	3 1	34	46	13	29	23	32	20	27	24	10	22	281
2004	1 17	42	12	54	61	97	74	36	36	39	70	20	558
200:	5 45				10		29						84
Orinoco													
Inia	107	86	168	108	118	117	188	176	197	185	172	103	1725
1990	5						33	84	70	69	56	50	362
199	7 28	30	19					46	57	77	99	53	409
1998	68	31	56	15	46	30	57						303
1999	9	12	58	56	13								139
2000)								51				51
200	1	5	15	10	20	30	36	11	19	39	17		202
2003	3 11	8	20	27	39	57	62	35					259
Total	878	435	922	699	762	940	1258	721	671	690	821	1409	10206

Table 2.3 Definition of habitat types.

Habitat Type	Definition	Locations surveyed			
Main River	White water rivers of Andean and Guyanese	River Amazon,			
	shield origin, typically turbid, brown-yellow in	Orinoco, Meta.			
	color with low transparency, basic pH and				
	sediment-rich (Sioli 1984). At least 400m in				
	width.				
Confluences	Intersection areas of the main channel with other	Confluence between the			
	channels or rivers. Confluences maintain	Amazon and			
	connections during all hydrologic seasons and	Loretoyacu rivers.			
	may or may not present a mix of white and black				
	waters. Approximately 250m width.				
Tributaries	Small and medium size rivers no more than 400m	Loretoyacu and Bita			
	in width. Water in tributaries are usually black	Rivers.			
	and clear, originate from the flooded forest				
Titoutaries	plains, with few suspended sediments and				
	relatively acidic, high in tannins and particulate				
	organic matter (Sioli 1984).				
Channels	Water courses no more than 300m wide and	Channels from the river			
	generally associated with island and main river	Amazon to the lake Caballo Cocha, between			
	systems. Navigability is limited depending on				
	rainy seasons.	the El Correo and			
	Turny seasons.	Tarapoto lakes.			
Islands		El Pañuelo,			
	Waters around land bodies in the water course of	Chimborazo, Ventanas,			
	main rivers with vegetation that may appear or	Bugeo, Cacao,			
	disappear due to hydrologic dynamics.	Mocagua, Patrullero,			
		San Salvador and			
		Vamos Islands.			

Table 2.4 Defintion of shore types.

Type of Shore	Characteristics
Forest	Dense vegetation, mainly represented by high trees (>6m).
Shrubs	Low vegetation (<6 m), usually in continuous patches along the shore, occasionally bordering the forest.
Flooded vegetation	Forest and shrubs swamped during the high water season.
Grass	Grass covering the shore.
Floating	Floating vegetation mainly represented by aquatic plants (i.e.
meadows	Eichornia sp., Paspallum sp., Pistia sp.).
Steep bank	Shore is a steep bank usually with low, or without, vegetation.
Beach	Sand or mud banks on the main shore or islands including those in the middle of water bodies.
Rocks	Large rocks on the shore. Occasionally related to rapids.

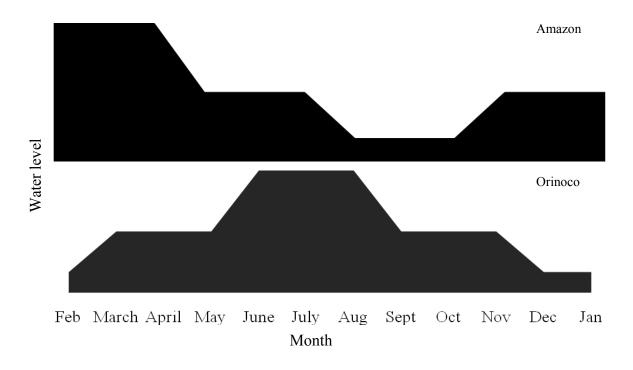


Figure 2.2 Seasons (high, low and transitional water periods) in the Amazon and Orinoco locations. Data were provided by the local Government hydroclimatic station (IDEAM).

2.2.2 Data Analysis

Statistical analyses were conducted using Systat 12. When variables did not meet assumptions of parametric tests (Lilliefors test, P < 0.05; Levene's test, P < 0.05), nonparametric tests were used. Group size was examined according to habitat type, seasonality, shore type, and distance from shore. Mann–Whitney (MW) tests were set to evaluate whether group sizes of *Inia* differed according to the location, and whether the group sizes of *Inia* and *Sotalia* in the Amazon differed. Kruskal–Wallis (KW) tests were used to evaluate whether there were differences in the group sizes of dolphins according to the habitat type, shore type, or distance from the shore.

2.3 RESULTS

Group sizes for *Inia* were larger in the Orinoco (range = 1-30, mean = 5.3, SD = 4.5) compared to the Amazon (range = 1-37, mean = 3.0, SD = 2.9; MW, P < 0.05, df = 1). In the Amazon, group sizes of *Sotalia* (range = 1-27, mean = 3.4, SD = 2.5) were larger than Inia (MW, P < 0.05, df = 1). When considering habitat types, group sizes in the Amazon for *Inia* were the largest in the island habitat (range = 1-18, mean = 3.7, SD = 2.9), lake (range = 1-37, mean = 3.2, SD = 3.4), and main river (range = 1-17, mean = 3.0, SD = 2.3; KW test, P < 0.05, df = 5). Similarly, the largest group sizes for Sotalia were found in the habitat types of main river (range = 1-23, mean = 3.6, SD = 2.4), lake (range = 1-27, mean = 3.5, SD = 2.9), and island (range = 1-19, mean = 3.5, SD = 2.5)KW test, P < 0.05, df = 5). In the Orinoco, larger mean group sizes of *Inia* were seen in the main river habitat (range = 1-30, mean = 5.5, SD = 4.7; KW test, P < 0.05, df = 2). When considering seasonality (Figure 2.3), the largest mean group sizes in the Amazon were observed in the lake habitat type during the low water season for *Inia* (range = 1– 30, mean = 4.5, SD = 4.5) and *Sotalia* (range = 1-27, mean = 5.8, SD = 5.3). In the Orinoco, larger group sizes were found in the tributary habitat during the rising water season (range = 1-19, mean = 7, SD = 4.6), and smaller group sizes in the same habitat during the falling water season (range = 1-12, mean = 3.2, SD = 2.5).

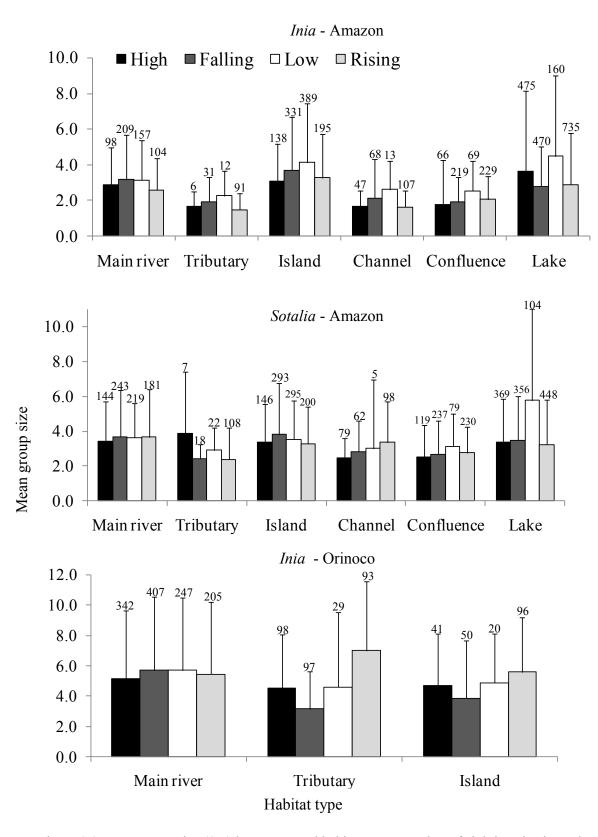


Figure 2.3 Mean group size (SD) by season and habitat type. Number of sightings is given above error bars.

Group sizes of *Inia* varied according to the shore type (Figure 2.4). In the Amazon, the largest group sizes were found off beaches (range = 1-37, mean = 4.3, SD = 4.9) and steep banks (range = 1-30, mean = 4.1, SD = 3.5; Amazon, KW test, P < 0.05, df = 7). In the Orinoco, largest group sizes of *Inia* were also found off beaches (range = 1-24, mean = 5.4, SD = 4.5), floating meadows (range = 1-26, mean = 5.2, SD = 4.3), and forest (range = 1-22, mean = 5.0, SD = 4.3; KW tests, P < 0.05, df = 7).

Group sizes of *Sotalia* did not change significantly according to the shore type (KW test, P > 0.05, df = 7). For neither *Inia* nor *Sotalia* were there significant differences in group size with distance from the shore (KW test, P > 0.05, df = 2). However, most group sightings were recorded within 50 m from the shore (40% for *Inia* in the Orinoco, 60% for *Sotalia*, and 66% for *Inia* in the Amazon).

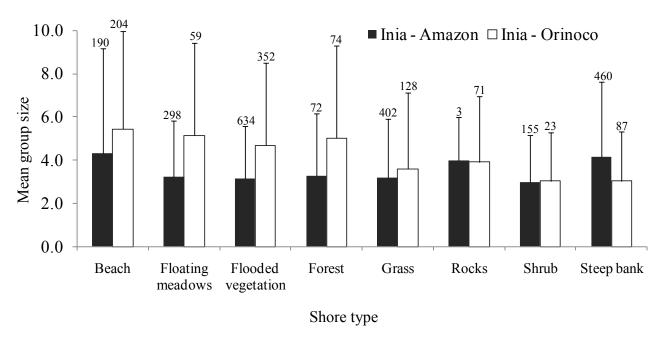


Figure 2.4 Mean group sizes (SD) by shore type. Number of sightings is given above error bars.

2.4 Discussion

Group sizes of river dolphins are often small, with two individuals being the most common group composition (Table 2.1). River dolphins have no natural predators. This lack of predation threat may partially explain why these dolphins have some of the

smallest group sizes of cetaceans. Thus, for river dolphins, the distribution of resources and habitat availability are likely more important in determining group size (Smith and Reeves 2000). Given this pattern, group sizes of river dolphins were investigated in relation to environmental variables, such as seasonality and habitat type. The largest group sizes in the Amazon were found in lakes during low water season and in the Orinoco in the tributary during the rising water season. Group sizes for *Inia* were larger in the Orinoco compared to the Amazon, and group sizes of *Sotalia* were larger than *Inia*. The largest group sizes were found off beaches and steep banks for *Inia* in the Amazon, and off beaches, floating meadows, and forest for *Inia* in the Orinoco. Group sizes did not change significantly as a function of shore type for *Sotalia*.

2.4.1 Environmental and Seasonal Variation

Group sizes of river dolphins, although typically small, can be large in habitat types with a high density of resources. The largest group sizes of river dolphins in the Amazon were found near islands, lakes, and main rivers. Fish availability is thought to drive movements of river dolphins between habitat types (Martin and da Silva 2004), and fish species concentrate in lakes, around islands, and close to the riverbanks in main rivers and tributaries, looking for resources and refuge provided by vegetation and slow currents (Goulding 1980). In the Orinoco, the largest mean group sizes were found in the main river (lakes were not surveyed in the Orinoco). Main rivers in both locations are highly productive whitewater rivers, which are very rich in nutrients and prey. Thus, productivity and availability of resources influence group sizes of river dolphins. Habitat quality also influences group sizes in carnivores (Macdonald 1983), and antelopes (*Ourebia ourebi*), which seem to form small group sizes in poor quality habitats (Arcese et al. 1995), as well as elephants (*Loxodonta africana*) in savanna habitats where smaller groups form in poor quality habitats, and larger groups form when water and food availability increase (Leuthold 1976, Moss 1988).

The most striking differences in group sizes of river dolphins were found when examining the interaction between annual seasonal changes and habitat type. The total

aquatic productivity in the Amazon and Orinoco basins is strongly affected by extreme seasonal changes. During the rising and high water seasons, areas of the main channel covering about 7,000 km² in the Orinoco and 170,000 km² in the Amazon basins are inundated with water, which forms the floodplains (Hamilton and Lewis 1990), and the aquatic productivity increases (Barthem and Goulding 1997, Lewis et al. 2000). Freshwater floods dictate the seasonal movement of fish migrating from rivers into floodplains and forests to feed and reproduce (Fernandes 1997, Henderson 1990, Barthem and Goulding 1997). During the low water season, the water from the floodplains drains into rivers; resources in rivers are therefore concentrated and easily accessed by predators, which may facilitate foraging and reduce competition between group members (Hamilton and Lewis 1990, Best and da Silva 1989a, Junk et al. 2007). By the end of the low water season, the number of accessible aquatic habitat types is limited, and the fish populations are reduced due to predation, stranding, and depletion of dissolved oxygen (Goulding 1980, Goulding 1989, Petermann 1997). Hence, we expect group sizes of dolphins to be largest when resources are concentrated and smallest when resources are dispersed.

2.4.2 Amazon

In the Amazon, as predicted, larger groups were found during the dry season when resources are concentrated and easily accessed. Similarly, larger group sizes of *Inia* dolphins in the Bolivian Amazon occurred during the falling and low water season, and groups were smallest during high water season (Aliaga-Rossel 2002).

Group sizes were largest in lakes during the low water season. Lakes in this river basin are highly productive systems that offer a significant source of organic material supporting different trophic levels (Rai and Hill 1980). Previous studies show that Amazonian lakes have higher primary productivity compared to the Amazon River (Schmidt 1973, Fisher Jr and Parsley 1979). This is due to many factors, including: (1) whitewaters from adjacent rivers, which are very high in nutrients, and penetrate lakes during the rising and high water season; (2) when the water level descends, the amount of light increases given the less-turbulent conditions, the phytoplankton and zooplankton

increase, and there is nutrient regeneration, which is translated into higher primary productivity (Lewis et al. 2001, Lewis et al. 2000, Schmidt 1973, Fisher Jr and Parsley 1979). For example, the highest diversity of phytoplankton and concentration of nutrients in lakes occurs during the low water season according to a study conducted in an Amazonian lake in Brazil, also influenced by a whitewater river, the Solimões (Rodrigues-Ibañez 1997). Thus, lakes are providing refuge and resources for many fish species, which in turn provides optimal conditions to support large group sizes of dolphins. Similarly, the largest group sizes of *Inia* in the Bolivian Amazon were recorded in lakes, which have a greater biodiversity and abundance of fish than the main rivers (Aliaga-Rossel 2002).

Large group sizes of *Inia* were also recorded beside islands during the low water season. Riverine areas beside islands are characterized by low current speeds and high availability of resources, attracting a high biomass of fish, aquatic birds, turtles, and caimans that use the beaches for nesting (McGuire and Winemiller 1998, Petermann 1997, Correa 2008). For comparison purposes, the highest biotic complexity of plant species in the Parana River Basin is usually found on islands, where the water from the main river easily reaches the soil during the transitional and high water season (Casco et al. 2005). Thus, as expected, large group sizes of river dolphins in the Amazon basin were found in habitats with high concentrations of resources and during the low water season when resources are concentrated and easily accessed.

In contrast, group sizes in channels, tributaries, and confluence waters were smaller and remained similar throughout the year. These areas are narrow in width with limited water depth and, therefore, may be used mainly for transit from lakes to rivers. Also, channels and tributaries mainly receive an influx of blackwaters, which are poorer in nutrients and sediments, and the influx of whitewaters occurs only during the rising and high water seasons.

Contrary to our results, large group sizes of *Inia* have been noted in confluences, which are considered habitats of high productivity that offer refuge, allow migrations of fish,

and appear to be preferred habitat for river dolphins (McGuire and Winemiller 1998, Martin et al. 2004, Aliaga-Rossel 2002). We suggest that the ecological importance of confluences depends on the resources that are contained within the rivers that form them, not just the presence of confluences.

2.4.3 Orinoco

Contrary to our predictions, larger groups were found in the transitional water season, and not during the dry season when resources are concentrated and easily accessed. Interestingly, other studies performed in close proximity to our location (Cinaruco River, Figure 2.1) also found larger group sizes during the transitional water season (McGuire and Winemiller 1998, Figure 2.1) when the aquatic productivity increases (Lewis et al. 2001).

Group sizes of *Inia* were the largest in the Orinoco and did not change as dynamically as in the Amazon. In fact, the largest mean group size of *Inia* dolphins ever recorded occurred during the rising water season in the tributary habitat (mean = 7, SD = 4.6). Whether this pattern is related to differences in the availability of resources is of interest.

In terms of resources, both the Amazon and Orinoco locations are under the influence of highly productive whitewater rivers, but the Orinoco receives additional nutrients from the Andean mountains transported via the whitewater Meta River. As a comparison, larger group sizes of *Inia* in the Bolivian Amazon were also recorded in areas with an influx of whitewater rivers, while smaller group sizes were found in blackwater rivers (Aliaga-Rossel et al. 2006). Hence, the presence of two whitewater rivers in the Orinoco (vs. one in the Amazon) may increase the availability of nutrients in this location, which ultimately may increase the primary productivity and potentially the availability of resources.

2.4.4 Variation Between Species

In the Amazon, variation in group sizes across seasons was greater for *Inia* than *Sotalia*. This could reflect the unique ability of *Inia* to actively search for food in restrictive

aquatic habitats. *Sotalia* do not have the same morphological adaptations as *Inia* (e.g., flexible bodies, small dorsal fins, and large pectoral fins), making them unable to exploit the flooded forest and restricting them to habitats with significant water depth to avoid stranding (Martin and da Silva 2004). Thus, large group sizes of *Inia* in lakes during the high water season and near islands during the low water season may be related to the high productivity of these systems, but mostly, to the ability of *Inia* to exploit flooded areas alongside lakes when resources are dispersed within very shallow water depths. Similarly, group sizes changed more with shore type for *Inia* than for *Sotalia*. Different shore types may not play a role in group sizes of *Sotalia* because they prefer areas that are more open with higher water flow (Martin and da Silva 2004, Martin et al. 2004).

2.4.5 Broader Implications for River Dolphin Conservation

River dolphins inhabit large areas of the Amazon and Orinoco basins (6,869,000 km² and 953,598 km², respectively) (Revenga et al. 1998, Goulding et al. 2003); however, most of the research focused on the ecology of these species has been conducted in less than 1% of this range (Figure 2.1). Due to the large size of the basin regions, it is unlikely that future surveys will be able to include the entire distribution. However, since environmental variables in this region have similar and predictable interannual patterns, our results could be useful in predicting the grouping behavior of river dolphins over the larger area.

First, we expect some differences according to characteristics of each river basin. For instance, group sizes of river dolphins in the Amazon basin increase during the low water season when resources are concentrated, and in the Orinoco group sizes increase during the transitional water period when productivity is enhanced (see also McGuire and Winemiller 1998, Aliaga-Rossel 2002, Aliaga-Rossel et al. 2006).

Second, we expect differences according to local productivity and habitat availability. For example, based on this study, we suggest that the ecological importance of

confluences will depend on the local influx of resources and nutrients, rather than just the presence of the confluences.

Third, in addition to ecological features, there are other aspects such as social structure, which influence group living. For example, male and female *Inia* have different habitat preferences (at least in central Brazil), with females and calves entering the flooded areas to look for resources and safety from male harassment (Martin and da Silva 2004). This temporal sex separation has been reported in sperm whales at sea as well as baleen whales, but not in other dolphin species (Martin and da Silva 2004). We are not aware of any social structure studies regarding *Sotalia* dolphins in freshwater systems.

The results of this study can lend insight into a number of ecological features driving the formation of groups in river dolphins, as well as direct the focus of further research and influence management actions. For instance, local communities and organizations are enhancing tourism based on dolphin watching in the Amazon and Orinoco locations that we studied. Although whale/dolphin watching has become a very valuable activity in many communities (Hoyt 2001), it may cause serious long-term problems for populations if it is not well regulated (Bejder et al. 1999, Reeves et al. 2003).

Our results may provide some guidance for identifying areas of high conservation importance for river dolphins. River margins, confluences, and lakes are areas of high densities of river dolphins (Martin and da Silva 2004, Martin et al. 2004), and which have been proposed as critical habitats for river dolphins (Gomez-Salazar et al. 2012). River margins and lakes also have the largest mean group sizes of river dolphins, and thus are preferred for dolphin watching. The critical habitats of river dolphins are primary targets for dolphin-watching activities and we know of no plans to regulate tourism in these areas.

Flood pulses are essential for maintaining productivity, and seem to be major factors governing the dolphins' behavior. If these are altered, there may be serious impacts on productivity, and other biological features such as group sizes of dolphins. Potential

threats that destroy or greatly affect the flood pulses include the construction of dams and waterways, deforestation, and climate change. Each location has a different environmental dynamic, and for this reason conservation actions should be considered at local as well as basin-wide scales.

2.5 SUMMARY OF CHAPTER 2 AND TRANSITION TO CHAPTER 3

Living in groups is usually driven by predation and competition for resources. River dolphins in South America do not have natural predators but inhabit complex and dynamic systems with strong and predictable seasonal shifts. These ecological features may provide some insight into the forces driving group formation in river dolphins and help us to answer questions such as why they have some of the smallest group sizes of cetaceans, and why group sizes vary with time and place. We analyzed observations of group size for *Inia geoffrensis* and *Sotalia fluviatilis* over a 9-year period in two locations of the Colombian Amazon and Orinoco river basins. In the Amazon, the largest group sizes occurred in main rivers and lakes, particularly during the low water season when resources are concentrated; smaller group sizes occurred in constricted waters (channels, tributaries, and confluences) which receive an influx of black waters that are poor in nutrients and sediments. In the Orinoco, the largest group sizes occurred during the transitional water season when the aquatic productivity increases. The largest group size of *Inia* ever recorded occurred in the Orinoco location which contains the influx of two highly productive whitewater rivers. Flood pulses govern productivity and major biological factors of these river basins, as well as river dolphin's ecology. Any threats to flood pulses will likely have an effect on the functionality of these ecosystems and the species living in them.

This chapter provided information on the ecology of river dolphins and their habitat at a local scale. To evaluate river dolphin's conservation status at a regional scale, the next chapter will focus on obtaining group sizes, densities and sighting rates in selected areas of the Amazon and Orinoco river basins.

CHAPTER 3 POPULATION, DENSITY ESTIMATES AND CONSERVATION OF RIVER DOLPHINS

The work presented in Chapter 3 also appears in:

Gomez-Salazar, C., F. Trujillo, M. Portocarrero-Aya, H. Whitehead. 2012. Population, density estimates and conservation of river dolphins (*Inia* and *Sotalia*) in the Amazon and Orinoco river basins. Marine Mammal Science 28 (1): 124 – 153. DOI: 10.1111/j.1748-7692.2011.00468.x

3.1 Introduction

To manage and conserve any species effectively, a good understating of population density and habitat use are critical. Riverine cetaceans generally surface inconspicuously and are highly mobile. This complicates efforts to estimate abundance, and as a result, management efforts are delayed due to a lack of knowledge. Riverine dolphins, which inhabit major river systems in Asia and South America, include some of the most endangered cetaceans (Reeves and Leatherwood 1994, IWC 2000, Reeves et al. 2000). As a result of their proximity to terrestrial habitat, major anthropogenic threats, such as the depletion of aquatic resources, water development projects, noise pollution, chemical pollution and direct capture of dolphins are likely to increase (Vidal 1993). Statistically robust and standardized density and population estimates are necessary to inform the conservation status and monitor trends of river dolphin populations worldwide (Reeves and Leatherwood 1994, IWC 2000, Reeves 2000).

Based on information obtained from rigorous surveys dedicated to estimating density and population sizes, the conservation status of many cetacean species that live close to land is of serious concern. The most dramatic case is that of the Yangtze River dolphin (*Lipotes vexillifer*) in China which is considered *Functionally Extinct* due to a lack of sightings or acoustic records during an intensive 6 wk multi-vessel survey using a line transect sampling design (Turvey et al. 2007). Similarly, the vaquita (*Phocoena sinus*) in

the northern reaches of the Gulf of California in Mexico, categorized by the IUCN as *Critically Endangered*, is in serious danger of extinction due to their isolated and localized distribution, high levels of entanglements and small population size, which was assessed using line-transect surveys covering its entire distribution (Jaramillo-Legorreta et al. 1999, Rojas-Bracho et al. 2006, Gerrodette et al. 2011). The Ganges river dolphin (*Platanista gangetica*) and the Irrawaddy dolphin (*Orcaella brevirostris*), currently classified as *Endangered* and *Vulnerable*, respectively, have also been subjects of rigorous visual vessel-based surveys (Smith et al. 2006, Braulik 2006, Smith and Braulik 2008).

River dolphins in South America are widely distributed in the Amazon and Orinoco river basins. The boto, or pink river dolphin, of the family Iniidae had three recognized subspecies: Inia geoffrensis geoffrensis in the Amazon basin, Inia geoffrensis humboldtiana in the Orinoco basin, and Inia geoffrensis boliviensis in the Bolivian Amazon basin (Best and da Silva 1989a, Best and da Silva 1989b, Pilleri and Gihr 1977). However, more recent studies have suggested that the genus *Inia* has two species: *Inia* geoffrensis in the Amazon and Orinoco basins (Brazil, Colombia, Ecuador, Peru, French Guiana, and Venezuela) and *Inia boliviensis* in the Amazon and Madeira upper basins (Bolivia) (da Silva 1994, Hamilton et al. 2001, Banguera-Hinestroza et al. 2002, Ruiz-García et al. 2006, Ruiz-García et al. 2007, Ruiz-García et al. 2008, Ruiz-García 2010). We use this taxonomy. The tucuxi, Sotalia fluviatilis, of the family Delphinidae is sympatric with *Inia* in Brazil, Colombia, Ecuador, Peru, and French Guiana in the Amazon river basin (Caballero et al. 2007, Borobia et al. 1991, Cunha et al. 2005). In the Orinoco river basin, Sotalia dolphins have been sighted along the entire Orinoco River in Venezuela below the Parguaza rapids. Further research is needed to decide whether these populations of Sotalia in the Orinoco are coastal transients making incursions into the river, or part of a riverine population that entered into the Amazon and from there made its way to the Orinoco (Borobia et al. 1991, Caballero et al. 2007).

The overall population size of river dolphins in the Amazon and Orinoco basins is currently unknown. *Inia geoffrensis* and *Sotalia fluviatilis* remain listed by the IUCN as

Data Deficient (Reeves et al. 2008) and the status of *Inia boliviensis*, if considered as a separate species, has yet to be established. Population estimates comprise one of the five scientific criteria used by the IUCN to list species into categories of threat, which are designed to draw attention to species that may be at risk of extinction (IUCN 2010). Population estimates for *Inia* and *Sotalia* in South America have been obtained sporadically from surveys conducted in small areas using varied methodologies. Previous data on river dolphins has mainly been expressed as encounter rates instead of population numbers (da Silva 1994, Pilleri and Gihr 1977, Layne 1958, Kasuya and Kajihara 1974, Meade and Koehnken 1991, Herman et al. 1996, Trujillo 2000). The first rigorous survey for South American river dolphins *Inia* and *Sotalia* using a standardized protocol of strip and line transects was conducted over 120 linear kilometers in the Amazon River, bordering Colombia, Peru, and Brazil (Vidal et al. 1997). Subsequently, these methods were used to estimate population parameters of river dolphins in the upper Peruvian Amazon basin (Leatherwood 1996), Ecuador (Utreras 1996), Peru (McGuire 2002), Bolivia (Aliaga-Rossel 2002), and Brazil (Martin and da Silva 2004, Martin et al. 2004). Results showed that *Inia* and *Sotalia* dolphins aggregate in productive environments with high fish densities and low current speeds. Densities appear to be generally higher at the river margins, confluences and lakes, and change with hydroclimatic seasons (Martin and da Silva 2004, Martin et al. 2004).

The Amazon and Orinoco basins are strongly influenced by seasonal changes in hydrology. Within the same year, variations of 11–15 m may occur in the vertical level of a river, and hundreds of kilometers in the horizontal plane (Goulding et al. 1996). These changes affect dissolved oxygen, fish migrations, habitat availability and productivity, and consequently the distribution of river dolphins (Martin et al. 2004). During the low water period, the available aquatic habitat is considerably reduced, and dolphin populations are constrained. During the high water period, more habitats become available (e.g., channels, shallow lakes, and flooded forest), and the aquatic fauna disperse. These changes are known to affect interactions between predators and their prey (Goulding 1980, Goulding 1989, Fernandes 1997).

This study comprises the largest regional initiative in South America designed to obtain detailed information on populations of river dolphins in order to evaluate their conservation status. We used standardized strip and line transect surveys in selected rivers across the Amazon and Orinoco basins in order to determine (1) what are the group sizes and densities of *Inia* and *Sotalia*, (2) which features of the environment are related to these density estimates and group sizes, and (3) what are the population sizes of river dolphins in different locations of South America. This study is part of an initiative to establish a network of Freshwater Protected Areas (FWPAs) by researchers, governments and local communities named SARDPAN (South American River Dolphin Protected Area Network).

3.2 METHODS

3.2.1 Variation Between Species

Between May 2006 and August 2007, seven surveys were conducted in large rivers in six countries of the Amazon and Orinoco basins (Table 3.1, Figure 3.1). Boats were chartered in each area in order to conduct visual surveys using standardized methods: a combination of transects running parallel (200 m strip-width transect) and at 45° (crosschannel line transects) to the shore (Vidal et al. 1997). Strip-width transects of 200 m were oriented parallel to the banks along the river margins of each river, maintaining an average distance of 100 m from the shore, as controlled by laser range finders. When the river margins were <200 m wide in some habitats such as tributaries and channels, the vessel navigated through the center of the waterway and the average strip-width was calculated by measuring distances to each shore with laser range finders (Vidal et al. 1997). Cross-channel line transect routes were conducted by selecting a starting point for the vessel to turn on a 45° angle towards the other bank, where another 200 m strip-width transect was begun. These turns were made when at least one strip transect had been completed and in places where the captain considered it safe and convenient to cross the river in order to avoid obstacles such as rocks, islands, large floating objects, and shallow areas (Vidal et al. 1997).

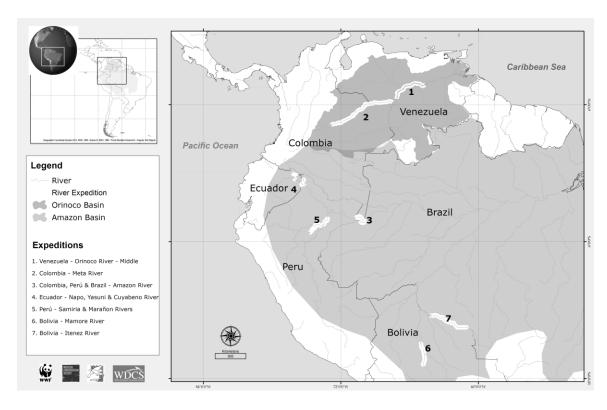


Figure 3.1 Map of the Amazon and Orinoco basins in South America showing the rivers surveyed per each country during the expeditions conducted from 2006 to 2007.

Sighting protocols were the same for both transect types. Two platforms in constant communication were installed in each ship; one at the front with at least four observers (effort data recorder, sightings data recorder, and two observers) and one at the back, with at least two observers (sightings data recorder and observer) to record sightings that were missed by observers in the front. Sightings from the forward platform were not informed to the aft platform. Positions were rotated every 2 h. Data from both the forward and aft platforms were integrated into a single data sheet at the end of each survey to confirm the number of dolphins sighted and avoid repeats. All observers had previous experience with river dolphin research. They actively searched for individuals and identified species (*Inia* and *Sotalia*) by naked eye. Observation height ranged between 3 m and 7 m above the river surface. Small ships, with low observation platforms (3 m), were used when the location was characterized by narrow or shallow habitat types. When the location surveyed consisted principally of main rivers, large ships with higher observation platforms (maximum 7 m) were preferred. Time, position, species, and number of dolphins (group size) were recorded for each sighting by both the

forward and aft platforms. Group size was the sum of the total number of dolphins seen at the surface at each sighting, and does not necessarily correspond to a social group.

Sightings started the first time that a dolphin was seen at the surface and ended 1 min after the last time a dolphin was seen at the surface or when dolphins had passed out of the observers' area of view. Estimating group size of river dolphins is often difficult and therefore the most experienced observers took the lead by giving their best estimates to the data recorder (Vidal et al. 1997). Surveys did not stop when dolphins were sighted. A compass bearing relative to the heading of the boat was used to measure the angle from the observation platform to the location of the first sighting. The distance from the platform to the dolphin was estimated by naked eye (observers were trained before the surveys to estimate the distance to inanimate floating objects in the river whose distance was validated with a laser range finder). Only the most efficient and experienced observers estimated distances during the surveys and often validated estimates using a laser range finder.

Environmental data were recorded systematically every 10 min, as well as at every dolphin sighting, and when the searching effort began and ended. A Geographic Positioning System (GPS) recorder provided information on date, time, vessel position, vessel speed (5–19 km/h), vessel direction of travel, and number of kilometers surveyed per transect. Visibility sighting conditions were ranked by the observers, at the beginning and end of each transect and when dolphins were seen. Sun glare was coded as strong (3), medium (2), low (1), and none (0); river state was coded as calm (0), small ripples (1), medium and large ripples (2), and waves and turbulence (3). Overall sighting conditions were considered excellent when sun glare and river state were both 0, good when the highest of the two condition codes was 1, moderate when the maximum code was 2, and poor when either code was 3. Five habitat types were delineated in the study areas through satellite images, and were later confirmed visually in the field (Table 3.2). Each survey was classified according to the annual flood cycle: low waters, high waters, and transitional periods (Goulding 1980, Junk et al. 1989, Sioli 1984). The survey in Ecuador was conducted during the high water season, in Peru during the low water season and all other surveys were conducted during the transitional water season.

Table 3.1Countries, dates and habitats surveyed: area, transect length, Ntrans (the number of transects performed per habitat type), and N*Inia* and N*Sotalia* (the number of dolphins seen on the surveys). S is 200m strip-width transect and L is cross-channel line transect. Data were not estimated (N/A) or not available in the field (blank spaces) for some categories.

	Country	D-4	II-bit-t	Area	(Km ²)	Lengtl	h (Km)	Ntr	ans	NIn	iia	NSot	alia
	(River)	Dates	Habitat	S	L	S	L	S	L	S	L	S	L
			Channel	51.0		30.5		10		41			
			Lake	8.0		11.7		6		40			
	Bolivia	Aug 12	Confluence			2.1		1					
	(River Itenez)	22 2007	Tributary	930.3	124.2	554.5		152		824			
		2007	m . 1	989.3						905			
			Total	111	3.5	59	8.8	1	73	90	15		
	Bolivia	June 17	Tributary	389.0		365.9		146		418			
	(River	- 22	Total										
	Mamore)	2007	Total	38	9.0	36	5.9	14	16	41	8		
п			Channel	61.0	N/A	59.6		19		39		72	
	a		Confluence	N/A	N/A	7.2		4		23		34	
s s	Colombia	Feb 21 -	Island	54.0		66.1		23		38		46	
B	(Rivers Amazon	29	Lake	9.6	N/A	12.5		8		32		36	
<u> </u>	Loretoyacu	2007	Main River	67.5	347.6	120.7	46.8	46	37	52	6	126	11
e	Javari)		Tributary	52.8	N/A	2.4		1		5		2	
			Total	245.0	347.6	268.3	46.8	101	37	189	6	316	11
\simeq			Total		2.6	31	5.2	1:	38	19	5	32	7
п			Confluence	N/A		11.1		4		7		2	
0	Ecuador		Island	N/A		2.8		1					
a z	(Rivers Napo	July 17	Channel			1.0		1					
Ξ	Cuyabeno	22 2006	Lake	7.0	N/A	16.5		15		5			
A	Yasuni		Main River	95.5	N/A	25.6	13.1	12	10				
	Lagartococha Aguarico)		Tributary	48.5		126.8		52		15		3	
	Aguarico		Total	144.0		183.7	13.1	85	10	27		5	
					4.0		6.9		5	2'	7	5	
			Channel	N/A		3.3		1		9		2	
	Peru		Confluence	N/A	37/4	6.0		3		16		36	
	(Rivers Samiria	Sep 8 -	Island	22.4	N/A	13.6		6				7	
	Marañon-	11 2006	Main River	78.4	414.7	153.3	47.0	60	46	105	25	120	30
	Amazon)	2006	Tributary	39.0		144.9		47		191		123	
			Total	139.8	414.7	321.0	47.0 8.0	117	46	321	25	288	30
			Channel	N/A	4.4 N/A	41.3	8.0	15	53	34 11	-0	31	8
			Confluence	N/A	N/A	10.0		5		16			
s i n	C-lbi-	Aug 24	Island	133.4	N/A	39.0		23		8			
ಡ	Colombia (River Meta)	29	Main River	400.5	697.2	254.2	117.2	119	109	33	10		
B	(River Meta)	2006	Ivialii Kivei	533.9	697.2	344.5		162	109	68	10		
e r			Total		31.1		117.2 1.7		71	7			
River			Channel	20.0	N/A	21.5	1./	7	/ 1	1	3		
	~		Confluence	N/A	N/A	16.1		8		20		5	
0 o	Venezuela	May 10		126.0	N/A	45.1		17		11		2	
n 0	V enezueia (River	- 18	Main River	276.2	1261.6	212.1	100.5	72	89	88	12	63	44
Orlnoco	Orinoco)	2006	Tributary	N/A	N/A	1.9	100.5	2	0)		12		
0	,			422.2	1261.6		100.5	106	89	120	12	70	44
			Total		33.8		7.2		95	13		11	
			All habitats	2863.1	2721.0	2379	325	890	291	2048		679	85
	Total Surveys		All transects		08.3		04		81	210		76	
				- , ,									

Table 3.2 Definition of habitat types surveyed during the 2006–2007 surveys in the Amazon and Orinoco river basins.

Habitat Type	Definition	Areas surveyed
	White water rivers of Andean and Guyanese	Orinoco, Meta,
	shield origin, typically turbid, brown-yellow in	Amazon – Marañón,
Main River	color with low transparency, basic pH and	Javari, Napo, Iténez,
	sediment-rich (Sioli 1984). At least 400m in	Mamoré Rivers.
	width and classified as a basin or sub-basin.	
	Intersection areas of the main channel with	Meta – Orinoco,
	other channels or rivers. Confluences maintain	Samiria – Amazon,
Confluences	connection during all hydrologic periods and	Amazon – Javari,
	may or may not present a mix of white and	Napo – Amazon.
	black waters.	
	Small and medium size rivers no more than	Samiria, Iténez,
	400m in width. Water in tributaries are usually	Mamoré, Cuyabeno,
Tributaries	black and clear, originate from the flooded	Yasuni, Lagartococha,
	forest plains and are relatively acidic and high	Aguarico and
	in tannins (Sioli 1984).	Loretoyacu.
	Water courses no more than 300m width	
Channels	generally associated with island and main river	
Chamileis	systems. Navigability is limited depending on	
	rainy seasons.	
	Waters adjacent to land bodies in the water	
Islands	course of main rivers with vegetation that may	
isianus	appear or disappear due to hydrologic	
	dynamics.	
		•

3.2.2 Group Sizes and Density Estimates

Mean group size and standard error were calculated for each species and habitat type for each country surveyed. Density estimates were calculated independently for sightings obtained within cross-channel line transects and for 200 m strip-width transects. Data

from all cross-channel transects were aggregated to estimate an overall density for each species using the software DISTANCE, version 5.0 (Buckland et al. 2001, Thomas et al. 2002, Thomas et al. 2009):

$$D = \frac{nE(i)f(0)}{2Lg(0)}$$

where n is the number of groups sighted, E(i) is the estimated mean group size for the population in habitat type i, f(0) is the sighting probability density at zero perpendicular distance (or the inverse of the effective half strip width [ESW]), L is the total transect length, and g(0) the probability of seeing a group on the transect line.

Using the data from the two sighting platforms $g(\theta)$ was estimated as follows. The detection function fitted for both species has a shoulder approximately 50 m wide (Figure 3.2).

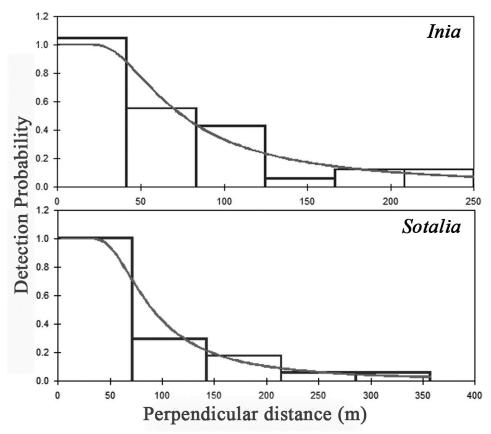


Figure 3.2 Detection probabilities for *Inia* and *Sotalia* with perpendicular distance from cross-channel line transect trackline.

Hence, data within this segment can be used to estimate $g(\theta)$. If the probabilities of missing dolphin groups that are on the transect line (in practice within 50 m) are equal from the forward and aft platforms and independent (q), then $g(\theta) = (1 - q^2)$, and the probability of a group on the transect line being missed by the first platform given it was seen at the second platform is also q. Thus $g(\theta)$ can be estimated from $g(\theta) = (1 - (n_{01}/n_{.1})^2)$, where $n_{.1}$ is the number of groups sighted from the second platform within 50 m of the transect line, and n_{01} the number of these that were missed by the first platform. An estimate of the coefficient of variation of this estimate is (from the delta method, checked using simulation):

$$CV_{g(0)} = 2(n_{01}/n_{.1})\sqrt{\frac{(n_{01}/n_{.1})}{n_{.1}(1-(n_{01}/n_{.1}))(1+(n_{01}/n_{.1}))^2}}$$

Three models were used to fit the detection function (the probability of sighting with perpendicular distance from the transect line): uniform, half-normal, and hazard-rate (Buckland et al. 2001). The best model was selected using the Akaike's Information Criterion (AIC) (Burnham and Anderson 2002). From the 200 m strip-width transects we found that river dolphins are distributed according to a gradient with higher densities closer to the shore (Figure 3.3).

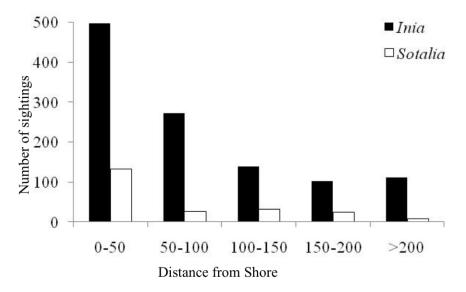


Figure 3.3 Number of sightings (*Inia* and *Sotalia*) within 50m-width strips parallel to the shore during strip transects with the vessel 100m from the shore. Sightings observed outside the 200m strip-width were excluded from further analysis.

Thus, there is a systematic variation in perceived density perpendicular to the transect line where variation in density with distance from the bank is conflated with variation in the detection probability at different distances from the vessel. In the center of the river (where cross-channel line transect surveys are performed), there does not seem to be a gradient of density relative to the transect line. Therefore, we used the detection function fitted for the cross-channel line transects to correct for undetected clusters in the 200 m strip-width transects. To do this, we estimated the mean proportion of animals detected (P_k) for each 50 m width strip (k) parallel to the track line. Thus, two P_k values for each species were calculated from the the area under the detection function (g(x)): $P_{0-50}(P_1)$ for groups detected within 50 m from the track line:

$$P_{0-50} = \frac{\int_{0}^{50} g(x)}{50}$$

and P_{50-100} (P₂) for those between 50 and 100 m from the trackline:

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

The detection probability g(x) used the detection function model selected and the parameter estimates calculated from the cross-channel line transect surveys (Buckland et al. 2001). If on the 200 m strip-width transect, n_{0-50} is the number of groups counted between 0 and 50 m from the shore (50–100 m from the boat on the shoreward side of the track line), n_{50-100} is the number of groups counted between 50 and 100 m from the shore (0–50 m from the boat on the shoreward side of the track line), $n_{100-150}$ is the number of groups counted between 100 and 150 m from the shore (0–50 m from the boat on the riverward side of the track line), and $n_{150-200}$ is the number of groups counted between 150 and 200 m from the shore (50–100 m from the boat on the riverward side of the track line), the corrected number of animals sighted was:

$$E(i) \left[\frac{n_{0-50}}{P_2} + \frac{n_{50-100}}{P_1} + \frac{n_{100-150}}{P_1} + \frac{n_{150-200}}{P_2} \right]$$

where E(i) is the estimated group size for the population in habitat type i. Densities (D_i) for 200 m strip-width transects were then calculated independently for each habitat type (i) (Buckland et al. 2001):

$$D_{i} = \frac{E(i) \left[\frac{n_{0-50}}{P_{2}} + \frac{n_{50-100}}{P_{1}} + \frac{n_{100-150}}{P_{1}} + \frac{n_{150-200}}{P_{2}} \right]}{W L_{i} g(0)}$$

where L_i is the total length of the strip transects conducted in that habitat, and W is the strip width (200 m). For areas less than 200 m wide we used the average strip-width: Bolivia, Iténez River, Channel = 166.78; Colombia, Meta River, Channel = 187.5; Ecuador, Tributaries = 64.04, Peru, Samiria River, Tributary = 105.97. Standard errors (SE) were estimated based on the coefficient of variation (CV) for the encounter rate, CV for the detection probability and the CV for $g(\theta)$:

$$SE(D) = D x \sqrt{(CV_{encounter_rate})^2 + (CV_{detection_probability})^2 + CV(g0)^2}$$

CV (encounter rate) was obtained from 200 m strip-width transects surveys by calculating the standard deviation (SD) of the sighting rates of individuals within that habitat type (per km of transect; $s_k = z_k/l_k$), where z_k is the number of individuals sighted on transect k:

$$CV_{encounter_rate} = \frac{SD(s_k)}{Mean(s_k)}$$

Based on the Jackknife procedure (Sokal and Rohlf 1981), P_k values were obtained from the detection curve by leaving out all dolphin sightings from one country (river), y, in turn $(P_{k,-y})$ and new density estimates were obtained:

$$D_{i,-y} = \frac{E(i) \left[\frac{n_{0-50}}{P_{2,-y}} + \frac{n_{50-100}}{P_{1,-y}} + \frac{n_{100-150}}{P_{1,-y}} + \frac{n_{150-200}}{P_{2,-y}} \right]}{WL_i g(0)}$$

Then "pseudo-values" (ϕ) (Sokal and Rohlf 1981) were calculated as:

$$\phi_y = mD_i - (m-1)D_{i,-y}$$

where m is the number of rivers, and the approximate SE as:

$$SE(D_i) = \frac{SD(\phi_y)}{\sqrt{n}}$$

CV (detection probability) was obtained from the cross-channel line transect correction applied by estimating the standard error (SE) of density values, D_i :

$$CV_{\text{detection_probability}} = \frac{SE(D_i)}{D_i}$$

3.2.3 Features of the Environment

We investigated whether group size and density estimates are related to the region (Amazon and Orinoco river basins), genus (*Inia* and *Sotalia*), seasons (high, low and transitional water periods), habitat type (main river, channel, island, tributary, confluence, lake) and country (Bolivia–Iténez, Bolivia–Mamoré, Colombia–Amazon, Colombia–Meta River, Venezuela, Ecuador, Peru). The variables group size and density were tested for normality and homogeneity of variances. When residuals did not meet assumptions of parametric tests (Lilliefors test, P < 0.05; Levene's test, P < 0.05), non-parametric tests were used. Analysis was conducted using SYSTAT Version 12.0.

3.2.4 Population Size

Population size (N_i) of river dolphins for each habitat i, was calculated as:

$$N_i = A_i D_i$$

The overall coefficient of variation of the total estimate for each location (country) surveyed *l* was calculated as:

$$CV(N_l) = \frac{\sqrt{\sum SE(N_i)^2}}{\sum N_i}$$

Satellite images of each study site were used to calculate the area A_i (km²) of each habitat type, using ArcView version 3.2 (ESRI, Redlands, CA). Polygons of 200 m width were created to calculate the area of the river that follows land borders, which are potentially surveyed by using 200 m strip-width transects. Areas which are potentially surveyed by using cross-channel line transects (center of main rivers) were calculated by estimating the total size of the study site (satellite images) minus the area covered by the 200 m width polygons (strip transects). Habitat-specific density estimates were extrapolated to all areas of dolphin habitat to estimate total population size within the location surveyed.

3.3 RESULTS

A total of 2,704 linear km were surveyed in selected large rivers of the Amazon and Orinoco river basins. The total size of the study area was 5,708 km². Effort varied between research areas for logistical reasons. Overall, 96% of transects were surveyed during good and moderate sighting conditions. Forty-three percent of transects were conducted with no or low sun glare, 17% with medium glare, and 1% with strong glare. Ninety-two percent were conducted with small ripples and 8% with medium and large ripples. Group size ranged from 1 to 15 for *I. geoffrensis*, 1 to 10 for *I. boliviensis*, and 1 to 26 for *Sotalia fluviatilis*. Group sizes of *Sotalia* were significantly larger than *Inia* (Mann-Whitney (MW) test, P < 0.05, 1 df), and group sizes varied with habitat type (Kruskal-Wallis (KW) test, P < 0.05, 5 df) being largest in the confluences (Table 3.3).

Table 3.3 Mean group sizes (GS) (mean number of dolphins sighted during an encounter) within region, country and habitat type for *Inia* and *Sotalia* using 200m strip-width transects. Data were not obtained in the field (blank spaces) for some habitat types in some countries. No SE was obtained (-) for some habitat types (only one sighting).

									Mair	1				
Habitat type	Chai	nnel	Confl	uence	Islan	d	Lake	;	Rive	r	Trib	utary	Tota	l
Species/Region	GS	SE	GS	SE	GS	SE	GS	SE	GS	SE	GS	SE	GS	SE
Inia (total)	1.7	0.13	2.4	0.37	1.9	0.22	1.9	0.23	1.7	0.10	1.7	0.04	1.8	0.04
Amazon														
Bolivia	1.6	0.21					1.9	0.19			1.7	0.04	1.7	0.04
Colombia	1.6	0.18	3.3	1.67	1.7	0.18	1.9	0.49	1.4	0.10	1.3	0.25	1.7	0.14
Peru	1.8	0.37	1.6	0.31					1.4	0.06	1.9	0.19	1.6	0.10
Ecuador			1.8	0.25			2.5	0.50			1.5	0.27	1.7	0.20
Orinoco	2.4	0.51	2.8	0.28	2.7	0.68			2.6	0.31			2.6	0.23
Colombia	2.8	0.48	2.7	0.33	4.0	2.00			2.0	0.50			2.4	0.35
Venezuela	1.0	-	2.9	0.46	2.2	0.58			2.9	0.39			2.8	0.30
Sotalia (total)	3.1	0.36	5.1	1.57	2.6	0.37	4.0	0.97	2.9	0.15	2.3	0.21	2.9	0.14
Amazon														<u> </u>
Colombia	3.1	0.37	11.3	7.36	2.4	0.34	4.0	0.97	2.8	0.28	2.0	-	3.1	0.29
Peru	2.0	-	4.0	0.76	7.0	-			2.7	0.21	2.4	0.22	2.7	0.16
Ecuador			2.0	-							1.0	-	1.3	0.25
Orinoco			2.5	0.50	2.0	-			3.5	0.32			3.4	0.30
Venezuela			2.5	0.50	2.0	-			3.5	0.32			3.4	0.30

Data for cross-channel line transects from all study areas were combined and imported into software DISTANCE. These data include 38 sightings for *Inia* and 27 for *Sotalia*. Densities were estimated for the cross-channel areas using the hazard-rate model for both species (Table 3.4, Table 3.5, Figure 3.2).

Table 3.4 Densities, coefficient of variation and 95% confidence interval for *Inia* and *Sotalia* obtained from cross-channel line transect surveys combining all study areas. D is density (dolphins/km²), ESW is effective half-strip width (m) (1/f(0)).

Species	Number sightings	Group size	ESW	CV [ESW]	D	CV [D]	I [95%) 6 CI]
Inia	38	1.5	97.2	0.24	0.96	0.31	0.53	1.75
Sotalia	27	3.37	113.8	0.23	1.51	0.37	0.75	3.05

Table 3.5 Results of detection function model selection by species.

	Inia				Sotalia			
Model	AIC	n. parameters	ΔΑΙϹ	ESW (CV)	AIC	n. parameters	ΔΑΙϹ	ESW (CV)
Hazard- rate	398.57	2	0	97.2 (0.24)	292.31	2	0	113.8 (0.23)
Uniform	399.64	2	1.06	107.8 (0.13)	293.43	2	1.12	139.5 (0.17)
Half- normal	399.66	2	1.09	93.7 (0.16)	293.96	2	1.64	109.4 (0.16)

The proportion of groups of dolphins sighted by the rear platform within 50 m of the transect line that were missed by the front platform was not very large, being 23% (141/611) of *Inia* sightings and 5% (7/133) of *Sotalia* sightings. Assuming the probabilities of missing dolphins from the forward and aft platforms are equal, then using the formula derived in the Methods section, we estimated $g(\theta) = 0.947$ (CV = 0.025) for *Inia*, and $g(\theta) = 0.997$ (CV = 0.003) for *Sotalia*. Thus for both species $g(\theta)$ is very close to 1, and the CVs are sufficiently small that they have no bearing on the overall precision of the density and population estimates.

The majority of *Inia* (96%) and *Sotalia* (85%) sightings were obtained while conducting 200 m strip-width transects. Sightings observed outside the 200 m strip-width on these transects were excluded from the analysis. Highest densities overall were found in the lake and confluence habitat types. Few transects were conducted in these habitat types and often transects were less than 1 km in length. When considering tributaries, highest densities were in the Samiria River in Peru (5.94 *Inia/*km², 6.08 *Sotalia/*km²) and the lowest densities in the tributaries of Ecuador (2.78 *Inia/*km², 0.28 *Sotalia/*km²). When

considering the main river habitat type, the highest density was in the Marañón River in Peru (2.72 *Inia*/km², 4.87 *Sotalia*/km²) followed by the Amazon River in Colombia (1.82 *Inia*/km², 3.35 *Sotalia*/km²), and the lowest values were in the Orinoco River in Venezuela (1.14 *Inia*/km², 1.06 *Sotalia*/km²) and the Meta River in Colombia (0.57 *Inia*/km²). Channel and island habitat types had slightly higher density estimates within each main river (Table 3.6, Table 3.7).

Based on a hypothetical plan of a river basin, we plotted overall density estimates for all surveys combined. Density in the center of the main river was obtained through midcenter line transects. Densities in the other habitat types were obtained through 200 m strip-wide transect surveys, with values presented for each 50 m strip-width. Overall, highest densities of *Inia* and *Sotalia* dolphins are within 100 m from the shore. For *Inia*, highest densities are in lakes, and for *Sotalia* highest densities are in confluences (Figure 3.4).

Highest estimated population sizes were obtained for *Inia* dolphins, the largest being in Bolivia (Iténez River) with 3,201 dolphins (CV = 0.40) and the lowest for the area surveyed in Ecuador with only 147 dolphins (CV = 1.38) (Table 3.8). The largest population of *Sotalia* was found in Colombia (Amazon River) with 1,545 dolphins (CV = 0.61), and the lowest in the areas surveyed in Ecuador with only 19 dolphins (CV = 1.37).

There were no significant differences in dolphin density across the different species (MW test, P = 0.596, 1 df) or seasons (KW test, P = 0.07, 2 df). There were significant differences in densities between regions (MW test, P < 0.05, 1 df: highest densities in the Amazon river basin), between habitat type (KW test, P < 0.05, 5 df: highest in confluences and lakes), and between country (KW test, P < 0.05, 6 df: highest in Peru and Bolivia; lowest in Ecuador and Venezuela).

Table 3.6 Mean sighting rate (n/l), estimated densities (D), standard error, coefficient of variation for the detection probability (dp), for the encounter rate (er) and estimated population sizes (N) for Inia within region, country and habitat type using 200m strip-width transects. Data were not obtained in the field (blank spaces) for some categories.

		Amazon riv	er basin				Orinoco river	basin
	Inia	Bolivia (Iténez)	Bolivia (Mamoré)	Colombia	Ecuador	Peru	Colombia	Venezuela
	n/l			0.35	0.00	0.52	0.11	0.22
L	D			1.82		2.72	0.57	1.14
īvē.	SE			2.11		3.88	3.07	4.06
n. R	CV (dp)			0.09		0.09	0.05	0.09
Main River	CV (er)			1.15		1.42	5.41	3.55
	CV			1.16		1.42	5.41	3.55
	N			123		213	228	315
	n/l	0.62	0.68	0.73	0.17	0.61	0.73	0.00
	D	3.21	3.52	3.77	2.78	5.94		
Tributary	SE	1.23	1.04		7.75	4.65		
ibu	CV (dp)	0.10	0.10	0.08	0.06	0.04		
Ţ	CV (er)	0.37	1.14	n/a	2.79	0.78		
	CV	0.38	1.14		2.79	0.78		
	N	2986	1369	199	135	288		
	n/l	0.47		0.50	0.00	0.95	0.35	0.05
	D	2.94		2.58		4.92	1.96	0.28
nel	SE	2.54		1.99		0.56	3.74	0.68
Channel	CV (dp)	0.07		0.09		0.11	0.06	0.15
0	CV (er)	0.86		0.77			1.91	2.45
	CV	0.86		0.77			1.91	2.45
	N	150		157		7	32	6
	n/l			0.37	0.00	0.00	0.12	0.26
	D			1.91			0.60	1.33
pu	SE			2.07			2.87	2.97
Island	CV (dp)			0.10			0.06	0.11
	CV (er)			1.08			4.80	2.22
	CV			1.08			4.80	2.23
	N			103			80	168
	n/l	1.56		3.56	0.11			
	D	8.10		18.48	0.56			
ke	SE	4.91		29.03	2.15			
Lake	CV (dp)	0.06		0.14	0.11			
	CV (er)	0.60		1.57	3.87			
	CV	0.61		1.57	3.87			
	N	65		177	4	0.01	1.70	1.02
	n/l	0.00		2.48	0.55	0.81	1.79	1.92
g	D			12.86	2.87	4.22	9.29	9.96
Confluence	SE			15.51	2.87	1.93	9.89	10.70
'nΩ	CV (dp)			0.06	0.06	0.04	0.04	0.06
ŏ	CV (er)			1.20	1.00	0.45	1.06	1.07
	CV			1.21	1.00	0.46	1.06	1.07
	N			22	9	11	8	84

Table 3.7 Mean sighting rate (n/l), estimated densities (D), standard error, coefficient of variation for the detection probability (dp), for the encounter rate (er) and estimated population sizes (N) for *Sotalia* within region, country and habitat type using 200m strip-width transects. Data were not obtained in the field (blank spaces) for some categories.

	Amazon ri				Orinoco river basin
	Sotalia	Colombia	Ecuador	Peru	Venezuela
	n/1	0.67	0.00	0.97	0.21
7.	D	3.35		4.87	1.06
LY.	SE	5.14		8.52	2.86
E.	CV (dp)	0.32		0.26	0.18
Main River	CV (er)	1.50		1.73	2.69
	CV	1.53		1.75	2.70
	N	226		382	292
	n/l	0.84	0.02	0.64	
	D	4.21	0.28	6.08	
Tributary	SE		1.29	4.91	
but	CV (dp)	0.09	0.09	0.10	
Ξ.	CV (er)	n/a	4.66	0.80	
	CV		4.66	0.81	
		222	13	237	
	n/l	1.02	0.00	0.62	0.00
	D	5.10		3.09	
ıel	SE	4.35		0.31	
Channel	CV (dp)	0.34		0.10	
\Box	CV (er)	0.78			
	CV	0.85		0.10	
		311		4	
	n/l	0.35	0.00	0.42	0.00
	D	1.74		2.10	
р	SE	2.53		5.14	
Island	CV (dp)	0.43		0.10	
13	CV (er)	1.39		2.45	
	CV	1.46		2.45	
		94		47	
	n/l	2.45	0.00		
	D	12.32			
0	SE	7.63			
Lake	CV (dp)	0.26			
Н	CV (er)	0.56			
	CV	0.62			
		118			
	n/l	5.61	0.39	1.73	0.28
d)	D	28.14	1.97	8.69	1.41
žnce	SE	32.75	2.80	1.43	2.01
Confluence	CV (dp)	0.09	0.10	0.10	0.10
`on	CV (er)	1.16	1.41	0.13	1.41
)	CV	1.16	1.42	0.16	1.42
	N	49	6	24	12

Table 3.8 Total areas, surveyed areas, overall population size and (CV) for *Inia* and *Sotalia* for the areas and species surveyed in the Amazon and Orinoco river basins. Some species are not distributed in some of the areas surveyed (blank spaces). Source for total areas of the Amazon and Orinoco river basins: Revenga et al. 1998, UNEP 2004.

		Are (km		Population size (CV)		
River basin	Country	Total	This study	Inia	Sotalia	
Amazon		6,547,000	2,794			
	Bolivia (Iténez)			3,201 (0.40)		
	Bolivia (Mamoré)			1,369 (1.14)		
	Colombia			1,115 (0.78)	1,545 (0.61)	
	Ecuador			147 (1.38)	19 (1.37)	
	Peru			917 (0.34)	1319 (0.43)	
Orinoco		953,598	2,915			
	Colombia			1,016 (0.85)		
	Venezuela			1,779 (0.87)	2,205 (0.89)	

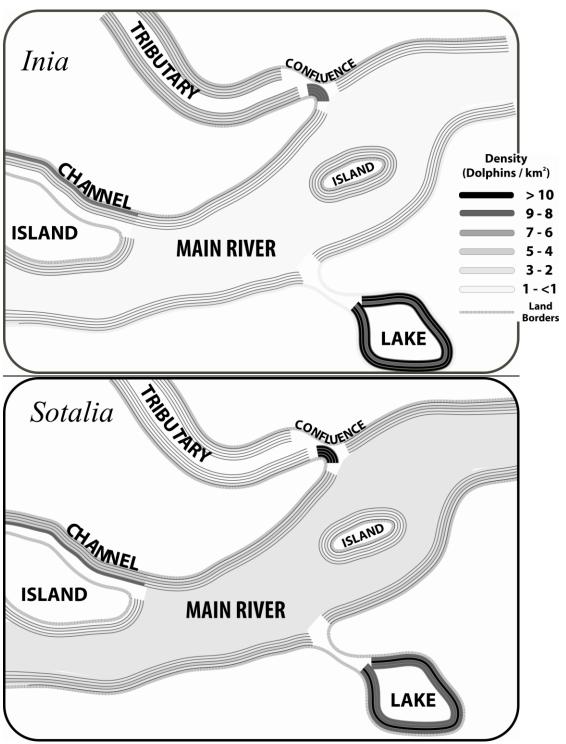


Figure 3.4 Scheme of a hypothetical section of a river basin. Results show overall density estimates for *Inia* and *Sotalia* for each habitat surveyed. Density in the center of main river was obtained through cross-channel line transects and densities in the other habitat types were obtained through 200m strip-width transect surveys, with values presented for each 50m width strip.

3.4 Discussion

This study has estimated group size, density, and population numbers for river dolphins across selected rivers of the Amazon and Orinoco river basins. The highest density of river dolphins was found in areas close to the river banks and in lakes and confluences. These results are useful in identifying critical habitat and hot spots for river dolphins, as well as locations where threats may be of special concern.

3.4.1 Methodology

The design of boat-based surveys in riverine systems is complex. We used a combination of 200 m strip-width transects and cross-channel line transects. This combination allows an adequate coverage of the different habitats within rivers with significant effort in the areas where the majority of river dolphins are found (Vidal et al. 1997, Dawson et al. 2008).

We used several techniques to perform a reliable survey with adequate detection process all along the strip width: (1) visual effort was equal on both sides of the track line; (2) short-dive intervals of river dolphins (dives generally do not last more than 2 min) and a constant low velocity of the vessels increased the probability of seeing most of the animals (Martin and da Silva 2004, Aliaga-Rossel 2002, Aliaga-Rossel et al. 2006, Herman et al. 1996, Best and da Silva 1993); (3) at least three observers with previous experience using the current method were present in all surveys; (4) members of the survey team had previous experience in river dolphin research; and (5) observers at the front and aft platforms were in communication to record sightings that were not recorded by one of the platforms. Although the proportion of animals missed by the front platform was not very large, by using two platforms $g(\theta)$ was able to be estimated and was increased to very nearly 1, and therefore we recommend that further surveys continue using both platforms.

The methods used to conduct line transect surveys for cetaceans vary with species and conditions of the survey. For instance, some studies conduct "closing mode" surveys,

leaving the transect line when a group of individuals is seen in order to estimate the group size and identify species accurately (Zerbini et al. 2007). Alternatively, "passing mode" surveys estimate the group size and species without leaving the transect line in order to avoid double-counting of individuals, as in this study. In order to reduce the bias, "passing mode" is recommended for species that have small group sizes and that are hard to track at the surface (Dawson et al. 2008). The inconspicuous behavior of river dolphins at the surface and the dark-colored waters make it hard to track multiple groups surfacing at different positions. Therefore, by maintaining the same speed without leaving the track line (passing mode), we can minimize the probability of double-counting individuals. River dolphins' small group sizes and the different surfacing behaviors of *Inia* and *Sotalia* facilitate accurate group size estimation and species identification. Using experienced observers and both aft and front platforms also aid sighting, accurate species identification, and group size estimation. Overall, for river dolphins in the Amazon and Orinoco basins, we recommend using the "passing mode" methodology when conducting line and strip transects.

Buckland et al. (2001) recommended a minimum of 60 sightings for accurate estimation of detection functions. As the number of sightings obtained during the cross-channel transects was less than this, our results should be treated cautiously. However, detection probabilities for models selected had similar density and detection function estimates. Thus, model selection may not be critical (Buckland et al. 2001).

Most of the effort was conducted during the 200 m strip-width transects, but because not all dolphins are detected within the strip, a correction factor was applied using the cross-channel line transects. This correction factor did not seem to have a large effect on the precision of the overall density estimate, as reflected by the low coefficient of variation of the detection probability.

The effective half-strip width varies according to the size and behavior of the species, weather conditions, and height of the observation platform, among other factors (Buckland et al. 2001). Given the sighting conditions, *Sotalia* dolphins are easier to see

than *Inia*. Therefore, as expected, the effective half-strip width for *Inia* in mid-center line transects (ESW = 97.16 m, 38 sightings) was smaller than for *Sotalia* (ESW = 113.78 m, 27 sightings). A previous study conducted in the Colombian Amazon reported a larger effective half-strip width of 245 m, for both *Inia* and *Sotalia* species combined (13 sightings) (Vidal et al. 1997). For further studies it would be important to conduct additional line transects in order to obtain a larger number of sightings to improve the estimation of the effective strip-width, and to examine how it varies between platforms and sighting conditions (Dawson et al. 2008). Given the differences in the surfacing behavior and group sizes of *Inia* and *Sotalia*, effective half-strip width should be calculated independently for each genus, as in this study. As a comparison, effective strip-widths of cetaceans at sea are smaller for species with shy behavior, small dorsal fins, and small group sizes and are larger for species that are easier to see because of conspicuous behavior, large group sizes, or prominent blows for large whales (Wade and Gerrodette 1993). Given the large CVs, caution should be applied when comparing results from areas and habitat types of this study. In addition, because we used different vessel types, densities in various habitat types might be biased. Our samples were not large enough to fit separate detection functions for different vessel types, but further research should take these issues into consideration.

Although lakes and confluences are the smallest areas surveyed (~1%) they contain the highest density and group sizes of dolphins. We need to monitor these areas accurately, but the current method used in these habitats must be improved. For instance, group size estimation in lakes and confluences might be biased since the large number of dolphins makes it hard to distinguish independent sightings and to estimate group sizes. Dolphins in lakes seem to aggregate with the presence of a boat, increasing the probability of double-counting individuals. Moreover, it was problematic to calculate the total area in the confluences due to their small size and proximity to the main river and tributary. Therefore, when surveying lakes and confluences, other approaches to estimating density and population are worth investigating. Mark-recapture by photo-identification of natural marks might be a suitable method for these high-density areas (Trujillo 1994, McGuire and Henningsen 2007). We therefore recommend line/strip transect surveys for most of

the habitat types (e.g., main rivers, tributaries, islands, and channels) when population and density estimates across large areas are needed, and we recommend photo-identification effort in high-density areas (e.g., confluences and lakes, see Chapter 4 and 5). The efficiency of photo-identification using natural marks has improved due to the availability of more sophisticated equipment including high-resolution digital cameras, and computer-aided software to assist in the matching of photographs.

Because surveys in each area were conducted during different seasons and because river dolphin density varies seasonally, caution should be applied when comparing results with other areas and studies. For instance, local reductions in the availability of water and resources during the dry season cause an increase in the density of river dolphins (Martin et al. 2004). In this study, results from Peru (surveyed during the dry season) may reflect the highest density values in the area. In contrast, results from Ecuador (surveyed during the rainy season) may represent the lowest values of density. Results from surveys conducted in Colombia, Brazil, Venezuela, and Bolivia (surveyed during the transitional water season) reflect the most frequent density values in these areas. Thus, repetitive surveys should be conducted in riverine areas to investigate how seasonality, habitat type, and other potential environmental variables drive variation in density. If repetitive surveys cannot be implemented, we suggest surveying areas during the transitional water periods where most of the habitat types are available (e.g., channels are not completely dry and lakes are still connected to the main rivers) to make studies more comparable.

3.4.2 Densities and Population Estimates

Densities of river dolphins were higher in the Amazon than in the Orinoco river basin. Whether this difference is due to differential anthropogenic impacts, productivity, or both in each watershed is worth investigating further.

The majority of previous river dolphin population estimates have come from sporadic studies each with a different methodology and during different hydrologic conditions. Compared with our surveys, these previous studies typically used fewer observers and only one platform in front of the boat. Field effort and data analysis were not focused on

obtaining density and population estimates. Therefore, caution needs to be applied when comparing results with this study. Here we consider results for several different survey locations, within or close to the areas surveyed in this study.

3.4.2.1 Mamoré and Iténez Rivers (Bolivia)

This study (June 2007 and August 2007, transitional water period) surveyed an area of $1,113.5 \text{ km}^2$ of the Iténez River with a large population size of 3,201 Inia (CV = 0.40) and an area of 389 km² of the Mamoré River with 1,369 *Inia* (CV = 1.14). Our encounter rates in the Mamoré River (0.68 dolphins/km) were lower than the ones previously reported in the Mamoré River (222 km, 1.6 dolphins/km, 361 dolphins \pm 32.23 SD) and some of its tributaries (65 km, 3.41 dolphins/km, 229 dolphins \pm 42.1 SD) (Aliaga-Rossel et al. 2006). However, no conclusive trends can be established because surveys were conducted using different methods and during the low water season (August–September 1998). This river basin has a low level of anthropogenic threats when compared with the other areas surveyed. The threats include entanglements of river dolphins in nets, occasional killing of river dolphins, gold mining (which leads to traces of mercury in the water), and boat traffic. The potential construction of a hydroelectric power station in the upper Madera River means that human threats could be expected to increase drastically in the near future (Tavera et al. 2010). The Amazon River basin in Bolivia contains the only population of *I. boliviensis* in the world, and although densities are higher than for *Inia* in other areas studied so far, the fact that this population is geographically isolated and has a significantly smaller range than *I. geoffrensis* needs to be taken into consideration.

3.4.2.2 Marañón and Samiria Rivers (Peru)

This study (September 2006, low water period) surveyed the Samiria River (tributary) and a stretch of the Marañón River between the city of Iquitos and the confluence with the Samiria River. Densities in the Marañón River (2.72 *Inia*/km², 4.87 *Sotalia*/km²) were lower than in the Samiria River (5.94 *Inia*/km², 6.08 *Sotalia*/km²), but both values were the highest density values of this study for the main river and tributary habitats, respectively. In an area of 554.4 km², we estimated relatively large population sizes of 917 *Inia* (CV = 0.34) and 1,319 *Sotalia* (CV = 0.43). The area surveyed is located

partially within the Pacaya-Samiria Natural Reserve, which is a well-managed Freshwater Protected Area (FWPA) with a low level of anthropogenic threats compared to the other areas surveyed. This might explain the high population estimates recorded. The high-density estimates are also a result of the surveys being conducted during the low water period, when animals and resources are concentrated. Because the anthropogenic threats in the Samiria River are minimal and are not expected to increase in the near future, this FWPA is a good candidate for conducting year-round repetitive surveys to investigate the variation of density estimates with seasonality and other environmental factors.

Previous density estimates available for the Samiria River were obtained during the falling water period, 15 yr previous to this study (0.5 *Inia*/km² and 0.4 *Sotalia*/km²) (Leatherwood 1996). Monitoring efforts in this area have been conducted from 1991 to 2000, and most estimates are given in dolphins/km. Encounter rates have ranged between 3.5 *Inia*/km during the low water period to 0.2 *Inia*/km during the rising water period; and 0.8 *Sotalia*/km during the rising water period to 0.1 *Sotalia*/km during the falling water period (McGuire and Aliaga-Rossel 2010, Leatherwood 1996). We are not aware of previous population estimates in the area surveyed. In this river, dolphins become entangled in fishing gear, are occasionally killed as a result of negative interactions with fishermen and there is substantial boat traffic (McGuire and Aliaga-Rossel 2010).

3.4.2.3 Napo, Cuyabeno, Yasuni, Lagartococha, and Aguarico Rivers (Ecuador)

This location was the smallest area surveyed in this study (144 km², July 2006, high water season) with the lowest density and population sizes estimates: 147 *Inia* (CV = 1.38) and 19 *Sotalia* (CV = 1.37). Previous research on *Inia* in tributaries of the Ecuadorian Amazon (similar to our study location) gave encounter rates ranging from 0.03 to 0.4 *Inia*/km (Utreras 1996, Utreras et al. 2010). No previous estimates were found for *Sotalia* for this area.

These estimates (although not comparable with those from this study), the low population estimates of this study and the general lack of dolphin sightings in the Napo River, are of very serious concern. The Napo River has been the site of major commercial efforts to take over land for oil and timber, oil spills have occurred periodically, and conflicts between oil companies, the military, and local communities are growing. This was the most threatened area surveyed in this study and it might be the most threatened population of river dolphins in South America studied so far.

3.4.2.4 Amazon, Loretoyacu, and Javari Rivers (Colombia)

For this location (February 2007, transitional water period), an area of 592.6 km² was surveyed, giving a total population estimate of 1,115 *Inia* (CV = 0.78) and 1,545 *Sotalia* (CV = 0.61). Density estimates in the Amazon River (main river) were the second highest of all areas for *Inia* and *Sotalia*. Lakes and confluences had the highest density estimates of the entire study. Previous research in the Colombian Amazon was conducted 13 yr ago during the low water season in a smaller area enclosed within the boundaries of this study, and it is the only study comparable in terms of methodology (Vidal et al. 1997). Overall density estimates in the Amazon River from this study (1.82 Inia/km² and 3.35 Sotalia/km²) are similar to the study in 1993 (2.02 Inia/km² and 2.78 Sotalia/km²) with the exception of lakes and confluences that have very high density estimates in our study. However, the 1993 study was conducted during the low water period (June 1993) when densities of dolphins are expected to be the highest, did not account for dolphins missed by the observers in the strip transects, did not use two platforms to account for dolphins missed and did not correct for undetected clusters during the 200 m strip-width transects given the density gradient detected with distance from the shore, making comparisons problematic. The major threats in the area surveyed are the entanglement of dolphins in nets (which have been reduced during the last 10 yr), competition with fisheries, and an increase in the number of people and motor boats in the area. Recently, there have been reports of *Inia* dolphins being killed in one of the tributaries located on the frontier of Peru and Brazil, the Javari River, to be used as bait in the "mota" (Calophysus macropterus) fishery (see below). If this continues, the population size of dolphins in this area will likely decrease in the near future.

3.4.2.5 Meta River (Colombia)

The Meta River is one of the most important tributaries of the Orinoco River. Only *Inia* dolphins are found in this area. There are no previous estimates of *Inia* densities and population sizes in this location. Our study (August 2006, transitional period) was conducted throughout the entire Meta River, from its confluence with the Orinoco to near its headwaters in the Andean mountains. Densities in the Meta River, which is one of the largest areas surveyed in this study (1,231.1 km²), were the lowest for the main river (0.57 *Inia*/km²) with a small population size of 1,016 *Inia* (CV = 0.85).

The anthropogenic threats in this area are substantial and are expected to increase. The Meta River is an important navigation link between the Orinoco region of Colombia and Venezuela, and therefore there are plans to transform it into a waterway. The economy of this region in Colombia is based on agriculture, cattle ranching, and oil extraction. New oil reservoirs have been discovered during the last years and oil extraction is expected to increase in the years to come.

3.4.2.6 Orinoco River (Venezuela)

Density estimates of *Inia* in the Orinoco River (May 2006, transitional water period) were higher than in the Meta River, but lower than surveys in the rivers of the Bolivian, Ecuadorian, Peruvian, and Colombian Amazon (1.10 $Inia/km^2$, 1.06 $Sotalia/km^2$). We estimated a population size of 1,779 Inia (CV = 0.87) and 2,205 Sotalia (CV = 0.89) in a total area surveyed of 1,684 km^2 .

The Orinoco River is considered to be one of the most threatened rivers in South America. Some of the anthropogenic threats in this area include gold mining (resulting in mercury contamination), the oil industry, water development projects, such as waterways and hydroelectric, intense fisheries, and boat traffic. In addition, there are reports of dolphins being killed for the "mota" fishery in this area. Although densities are not as low as in Ecuador, this is one of the most threatened areas in this study, and we know of no plans to mitigate these threats.

3.4.3 Hot Spots and Critical Habitat

We define hot spots of river dolphins as the locations with highest density estimates of *Inia* and *Sotalia*. In this study, all hot spots occurred in well-protected and well-managed areas that could act as examples for conservation actions in other locations. The highest density estimates in this study were observed in the Samiria River (Peru) and in the Iténez and Mamoré Rivers in Bolivia. The Samiria River is located in the Pacaya-Samiria National Reserve, which was created specifically to protect freshwater ecosystems (Saunders et al. 2002). Similarly, the highest densities that have been recorded for any cetacean worldwide were obtained for *Inia* (up to 18 dolphins/km² in floodplain channels) in the Mamiraua Sustainable Development Reserve in Brazil (Martin and da Silva 2004) which is the largest protected area of flooded forest (Pires 2006). Thus, high densities of river dolphins seem to be found in well-managed FWPAs. The rivers in the Bolivian Amazon (Mamoré and Iténez) are characterized by a low level of anthropogenic effects compared with the other areas surveyed, which may be related to the high densities of *Inia* dolphins observed. Thus, the Pacaya Samiria Natural Reserve in Peru, the Mamiraua Sustainable Development Reserve in Brazil and the Mamoré and Iténez river basins should be considered as hot spots for river dolphins in South America.

Critical habitat is defined as areas that are fundamental for daily and long-term survival for a whole species or a specific population (Hoyt 2005). At a regional scale, we suggest that the critical habitat for river dolphins is within 200 m of the river banks, and the confluence and lake habitat types, where fish species concentrate (Figure 3.4; Vidal et al. 1997, Trujillo 2000, Martin and da Silva 2004). Areas close to river banks are also favored by fishermen as in the Ganges River, where river dolphins (*Platanista gangetica*) preferentially occupy areas where gillnetting occurs (Smith et al. 2006).

3.4.4 Conservation Status

Inia and *Sotalia* inhabit larger areas than their counterparts in China (*Lipotes vexillifer*) and the vaquita in Mexico (*Phocoena sinus*) considered functionally extinct and in serious danger of extinction, respectively (Jaramillo-Legorreta et al. 1999, Turvey et al.

2007, Reeves et al. 2008). Even though the numbers of river dolphins in South America are overall higher than for these species, it is of concern that the scenario in terms of anthropogenic threats is very similar. We know of no management plans to mitigate these threats. In contrast, there are plans to increase the number of water development projects and expand the oil industry. *Inia* and *Sotalia* are currently listed by IUCN as *Data* Deficient partially because of a lack of population estimates (Reeves et al. 2008), and although the listing of species under Red List categories is not an ultimate goal, it is an important step in raising awareness within governments and institutions about the possible decline in dolphin numbers if human threats are not mitigated. For instance, rigorously obtained abundance estimates of the vaquita have demonstrated their small and likely decreasing population size, leading to the listing of this species as *Critically* Endangered by the IUCN (Jaramillo-Legorreta et al. 1999, Gerrodette et al. 2011). This, as a consequence, has raised awareness about the conservation status of the vaguita and the need to mitigate direct threats to their survival. We hope that the population and density estimates presented in this paper will contribute to such a process for the South American river dolphins.

Density estimates of river dolphins vary widely across South America (e.g., 0.28 *Sotalia*/km² in the tributaries in Ecuador and 6.08 *Sotalia*/km² in the Samiria River in Peru). Thus, in order to properly capture their conservation status, we may need independent listings for geographically distinct populations. For instance, each river basin could be considered a potentially distinct subpopulation. A river basin is defined as the area drained by a major river system or by one of its main tributaries (Revenga et al. 1998). Although this categorization only takes into account ecological characteristics of the watersheds, we believe that this approach could be the most optimal categorization given the lack of information on river dolphin populations. No genetic studies of these populations have been conducted. However, we do not expect large movements of river dolphins within these river basins given the results of studies conducted in other areas showing short-distance movements and high fidelity of individuals to areas where they are born (Trujillo 1994, Martin and da Silva 2004, Martin et al. 2004, McGuire and Henningsen 2007, Ruiz-Garcia et al. 2007).

We obtained the areas of each river basin (the area of water drained by a major river system or by one of its main tributaries) based on other studies and compared them with the areas sampled in this study (Meade and Koehnken 1991, Junk 1997, Revenga et al. 1998, Rosales-Godoy et al. 1999). We sampled about 0.04% of the Amazon river basin (surveys in the Bolivian, Peruvian, Ecuadorian, and Colombian Amazon) and 0.3% of the Orinoco river basin (surveys in the Venezuelan and Colombian Orinoco) (Table 3.8). Although these estimates correspond to a very small fraction of the entire potential range of river dolphin populations, it is the largest study done so far to estimate density and population sizes using standardized methods.

Surveying all areas in all river basins seems unlikely, and therefore, we recommend that further studies should examine methods of extrapolating densities of dolphins from surveyed to un-surveyed areas within river basins in order to obtain overall abundance estimates. These studies would extend the results in this paper by examining how well ecological conditions and measures of human threat predict dolphin abundance within basins. Any extrapolation should also take into account the actual distributions of the species, as the presence of river dolphins in all river basins is not certain, as well as the effective areas of their distribution, especially for *Sotalia* that has more distributional constraints than *Inia* (shallow waters, narrow channels and tributaries) (Borobia et al. 1991, da Silva and Best 1996, Aliaga-Rossel et al. 2006).

In terms of anthropogenic threats, entanglements of river dolphins in gillnets occur sporadically, but the main concerns should be the overexploitation of resources due to large-scale fisheries, like the mota (*Callophysys macropterus*) fishery in the Brazilian Amazon. Approximately 600 *Inia* dolphins are being killed per year to be used as bait for this fishery, which is widespread in Brazil (Loch et al. 2009). The number of dolphins killed per year in Brazil is at least half of the entire population sizes of river dolphins estimated in this study for some of the locations surveyed (Table 3.8). If the killing of river dolphins spreads from Brazil to other countries, population sizes will likely decline within a few years. Finally, caution should be applied when considering different species. For instance, the population size of *Sotalia* dolphins in the Amazon and Orinoco river

basins is smaller than that of *Inia*. *Sotalia* dolphins are restricted to open areas with significant water depth and they cannot access very shallow and narrow riverine areas. Thus, their distribution is significantly smaller. This should be taken into account when evaluating the impact of different anthropogenic threats for both species given as smaller and more localized populations are likely to be more vulnerable.

3.4.5 Conclusions

This study provides a baseline of population and density estimates of river dolphins in South America. Major variations in densities of dolphins by location suggest that caution should be applied when extrapolating results to areas that have not been surveyed. This study used density estimates to propose hot spots (e.g., Pacaya Samiria Reserve in Peru, Mamiraua Reserve in Brazil, Iténez, and Mamoré rivers) and critical habitat (area within 200 m from the shore, lakes, and confluences) for river dolphins in order to prioritize and encourage management actions at a regional scale. These surveys are part of an on-going study that represents an extensive effort to estimate the population size of river dolphins in South America. Results from surveys in other river basins will be available soon, and it is expected that efforts to evaluate the population levels will be the first step in raising awareness about the current conservation status of river dolphin populations and about the current and increasing anthropogenic threats in the Amazon and Orinoco river basins.

3.5 SUMMARY OF CHAPTER 3 AND TRANSITION TO CHAPTER 4

This study is part of an on-going effort to evaluate and monitor river dolphin populations in South America. It comprises the largest initiative to estimate population size and densities of *Inia* and *Sotalia* dolphins using statistically robust and standardized methods. From May 2006 to August 2007, seven visual surveys were conducted in selected large rivers of Bolivia, Colombia, Brazil, Ecuador, Peru and Venezuela in the Amazon and Orinoco river basins. Population sizes of *Inia* and *Sotalia* were estimated for different habitats (main river, tributary, lake, island, confluence and channel). A total of 291 line and 890 strip transects were conducted, covering a distance of 2,704 linear kilometers. 778 *Inia geoffrensis*, 1,323 *Inia boliviensis* and 764 *Sotalia fluviatilis* were observed.

High-density areas were identified (within 200m from the river banks, confluences and lakes) and we propose that these constitute critical habitat for river dolphins. High densities of river dolphins seem to coincide with well-managed freshwater protected areas and should be considered as hot spots for river dolphins in South America.

The previous chapters have provided a local and regional perspective in terms of the ecology of river dolphins, their conservation status and habitat preferences, based on observations during boat-based surveys. This type of research, however, does not allow researchers to develop long-term monitoring programs that could potentially answer questions about movement patterns, survival rates, and social structure. For this reason, the next chapter focuses on developing a protocol for photo-identification of *Inia* dolphins in the Amazon and Orinoco basins.

CHAPTER 4 PHOTO-IDENTIFICATION: A RELIABLE AND NONINVASIVE TOOL FOR STUDYING RIVER DOLPHINS

The work presented in Chapter 4 also appears in:

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4.1 Introduction

The process of recognizing individual cetaceans and tracking them through time allows researchers to answer a wide range of questions related to population size (Wilson et al. 1999), migration (Rock et al. 2006), distribution (Williams et al. 1993), critical habitat (Ingram and Rogan 2002), and social structure (Whitehead 2008).

Photo-identification is a noninvasive and relatively inexpensive method used to identify individuals (Hammond et al. 1990). Individuals are recognized from photographs of natural marks such as stripes to identify zebras (*Equus burchelli*) (Petersen 1972); nose scars to identify sea otters (*Enhydra lutris*) (Gilkinson et al. 2007); pigmented spot patterns in leatherback sea turtles (*Dermochelys coriacea*) (McDonald and Dutton 1996); and scars, skin patches, color patterns, callosities, and nicks and notches along fin edges to identify whales and dolphins (Hammond et al. 1990).

The efficiency of the photo-identification of natural marks has significantly improved during the last decade due to the availability of high-resolution digital cameras, rigorous and standardized protocols for storing and analyzing images, and the use of computer-aided software to assist in the matching of individuals (Hillman et al. 2003, Markowitz et al. 2003). Photo-identification of natural marks became a reliable and widely used tool to

help understand the ecology of wildlife populations, and thus, to help in the process of recommending conservation and management actions (Rock et al. 2006).

Photo-identification of natural marks in freshwater cetacean populations is a challenge. It is not easy to photograph species that spend most of their lives in dark and turbid waters, and, when at the surface, tend to be inconspicuous, shy, and unpredictable. Moreover, given that all cetacean populations that inhabit freshwater ecosystems live in the watersheds of developing countries, the funding and technology available to conduct research are limited. Consequently, photo-identification efforts are conducted mostly by using analog cameras and short-distance lenses, restricting the quantity and quality of information obtained and stored. For all these reasons, studying and identifying freshwater cetaceans using photography has proved challenging.

Some studies, however, have successfully photo-identified cetaceans in freshwater ecosystems using marks (Table 4.1). For instance, photo-identification studies of cetaceans in Asia use natural marks such as notches, distinctive shapes in the dorsal fin, and white marks to identify Irrawaddy dolphins (Orcaella brevirostris) in riverine and coastal areas (Smith et al. 1997, Parra and Corkeron 2001, Kreb and Rahadi 2004). Similarly, studies of river dolphins in Asia use nicks, scars, and white spots on the dorsal ridge and behind the blowhole to identify Baiji (Lipotes vexillifer) of the Yangtze River in China and Ganges susus (*Platanista gangetica*) in the Karnali River in Nepal (Hua et al. 1990, Zhou et al. 1998, Smith and Reeves 2000). Photo-identification studies of river dolphins in South America use pigmentation patterns, nicks, wounds, and scratches, to identify pink river dolphins (*Inia geoffrensis*) in some areas of the Amazon and Orinoco river basins of Colombia, Venezuela, Bolivia, and Peru (Trujillo 1994, McGuire and Winemiller 1998, Aliaga-Rossel 2002, McGuire and Henningsen 2007). As a result, numerous studies have demonstrated that, in spite of the challenges, cetacean species in freshwater ecosystems have individual-specific natural marks that can be photographed and used for identification.

Little is known about the conservation status of pink river dolphins, *Inia* spp., which are distributed in many rivers and tributaries of the Amazon and Orinoco basins. As a consequence, the *Inia* spp. is listed as *Data Deficient* by the IUCN (Reeves et al. 2008). Photo-identification has the potential to be used as a tool to answer questions associated with the criteria (e.g., population size) used by the IUCN, and other organizations, to list species into appropriate threat categories and, thus, to draw attention to their conservation status. For instance, while there are robust statistical means to estimate river dolphin population sizes and densities using boat-based surveys, photo-identification effort has been recommended in high-density areas (e.g., confluences and lakes) where boat-based surveys might provide biased estimates (Gomez-Salazar et al. 2012).

Previous photo-identification studies of river dolphins did not have the capabilities to assess the full potential of research using natural marks. For instance, analog cameras provided a significantly lower quantity and quality of pictures when compared with current digital images (see Table 4.1).

Table 4.1 Photo-identification studies of river dolphin populations. ID: Animals Identified, #: number of photographs suitable (number of photographs collected), IDR: Animals re-sigthed, MTD: Maximum time (and distance) btw re-sightings.

Country	Species	Location	Date	ID	#	IDR	MTD	Source
China	Lipotes vexillifer	Yangtze River	Mar 86 Dec 87	0	0 (1000)			Hua et al. 1990
China	Lipotes vexillifer	Yangtze River between Zhenjiang and Hukou	May 89 May 90	7	84 (1178)	3	373 days (200 km)	Zhou et al. 1998
Nepal	Platanista gangetica	Karnali River			? (1200)			Smith and Reeves 2000
Venezuela	Inia	Cinaruco River	Nov 93 June 94	6	32 (2184)	6	186 days	McGuire and Winemiller 1998

Country	Species	Location	Date	ID	#	IDR	MTD	Source
Bolivia	Inia	Tijamuchi River	Jan 98 Sept 99	2	27	2	239 days	Aliaga- Rossel 2002
Peru	Inia and Sotalia	Pacaya- Samiria Reserve	1991 2000	72	~270 (9000)	25	91 mo (220km)	McGuire and Henningsen 2007
Colombia	Inia	Amazon River and adjacent areas	1991 1993	20	400 (3600)	yes		Trujillo 1994
Colombia	Inia	Amazon and Orinoco River and adjacent areas	Feb 07 Aug 09	57 R* 40 L	795 (6855)	16	23 mo	This study

^{*}R = *Inia*'s right side identified; L = *Inia*'s left side identified.

In some cases, photographs were black and white, thus discrimination of colors and details of any natural marks were poor (e.g., Trujillo 1994, McGuire and Winemiller 1998). Also, little information was available about the permanence of natural marks as well as the reliability of these marks for identifying individuals and following them over the long term. Hence, using such marks could lead to seriously biased population estimates (Stevick et al. 2001). And finally, some studies have limited their number of analog photographs taken due to economic constraints (e.g., Aliaga-Rossel 2002). In summary, more information about the prevalence and reliability of natural marks in *Inia* populations is needed.

Most of the information available regarding *Inia* dolphins' natural marks come from studies where individuals have been captured and released (Martin and da Silva 2006). *Inia* dolphins are grey when born and immature, and grey, pink, or blotched pink when adults. Also, when *Inia* adults are physically active, their coloration can become lighter (Best and da Silva 1989a, Best and da Silva 1989b, Da Silva and Martin 2000). *Inia* dolphins' natural marks vary with gender and age. For instance, at least in Brazil, due to intense inter-male aggression, males are larger, pinker, more heavily scarred, and have

more life-threatening injuries (e.g., broken bones) than females (da Silva 1994, Martin and da Silva 2006). Juveniles are less scarred than adults, and tooth-rake scars are not present in animals during their first 2 yr of life (Parra and Corkeron 2001, Martin and da Silva 2006). Some of the most permanent marks are found around the blowhole as a result of depigmentation of the skin due to biting from conspecifics and/or contact with rough surfaces such as flooded vegetation (Martin and da Silva 2006). Other areas of the body, such as pectoral fins and flukes, also have permanent wounds (Martin and da Silva 2006), but these areas are not often photographed in the wild. In summary, *Inia* dolphins have conspicuous natural marks that, if photo-identified, could be used to develop noninvasive and long-term studies in some areas of their distribution. Invasive methods for identifying individuals, such as tagging, may have negative impacts on survival or reproduction (Saraux et al. 2011), thus non-invasive methods, such as photo-identification, are preferable, particularly for those species for which we have conservation concerns.

This study was undertaken to evaluate photo-identification methods of *Inia* dolphins by (1) describing natural marks suitable for the recognition of individuals, (2) evaluating the permanence of these natural marks, and (3) estimating the proportion of individuals identifiable within the population. As also suggested by McGuire and Henningsen (2007), we predicted that, through the use of digital cameras, the quality and efficiency of the photo-identification of *Inia* dolphins will improve compared to previous analogue studies (e.g., Trujillo, 1994). Our goal is to provide the basis of a standard operating procedure for photo-identification studies of *Inia* dolphins in the Amazon and Orinoco rivers, to suggest how this methodology might be improved, and to encourage the creation of long-term population monitoring programs using this tool.

4.2 METHODS

4.2.1 Field Methods

Surveys were carried out from February 2007 to August 2009 in two locations of the Colombian Amazon and Orinoco river basins within study areas comprising approximately 160 and 240 km², respectively (Figure 4.1, Table 4.2).

Groups of river dolphins were located visually from a 6 m boat, with a 25-hp outboard engine, at approximately 2 m observation height. A group of river dolphins was defined as animals that were seen together within 250 m of the boat, likely engaged in the same activities (Gomez-Salazar et al. 2011a).

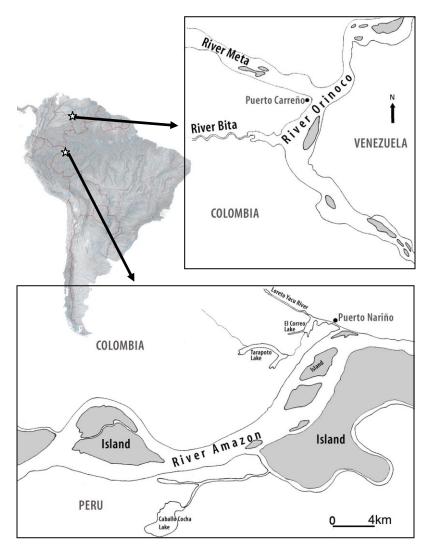


Figure 4.1 Study areas in the Colombian Amazon and Orinoco river basins

We approached groups at a distance of approximately 100 m, and photographic effort started only if individuals remained within 100 m of the boat. The effort ended after 30 min or when individuals could no longer be followed. Digital color photographs were taken using a Nikon D200 (70 to 300 mm lens) and a Nikon D80 (80 to 135 mm lens) at a

resolution of 3,872 x 2,592 pixels, which were saved in JPEG format. Photographs were taken of all individuals regardless of presence or conspicuousness of marks.

Two types of surveys were conducted. The first (December 2007 through January 2008 and December 2008 through January 2009) was specifically designed for photo-identification. These surveys consisted of encounters during which each encounter was with one group of river dolphins. For each encounter, the location (using a Geographic Positioning System [GPS]), group size, group composition (recorded as the number of adults/juveniles and calves which are dark grey and less than 1 m long), and habitat type (e.g., main river, tributary, confluence, lake) were recorded. The second type included opportunistic surveys during which encounters were not recorded because observers did not note the identity of the group being photographed.

4.2.2 Photographic Analysis

4.2.2.1 Picture Quality

Each image of *Inia* dolphins received a Quality rating (*Q*) between 1 (very poor photograph – not useful) to 5 (very good quality – ideal) (Ottensmeyer and Whitehead 2003, Auger-Méthé and Whitehead 2007, Auger-Méthé et al. 2010).

The Q rating did not depend on the markings of each individual. To do this, first, we assigned a rank from 1 to 5 on each of the following photographic criteria: focus, exposure (contrast of the body against the background), orientation of the dorsal ridge (the angle that the dorsal ridge formed in relation to a plane perpendicular to the axis of the lens), parts of the body visible above water, and size (proportion of the frame occupied by the body). Second, we assigned as the overall Q value the lowest score given to any of the criteria evaluated. For example, an image in perfect focus (5), with a good exposure (5), in which the flank and dorsal ridge of the animal are visible (5), and in which the body of the individual occupies approximately 40% of the frame (5), but in

which the dolphin is swimming towards the camera (angle = 90°) (1), would have an overall Q rating of Q1.

4.2.2.2 Photographic Matching

We identified and described major mark types of *Inia* dolphins by using all $Q \ge 3$ photographs. Each mark on each photograph was assigned a mark type (e.g., Trujillo 1994, McGuire and Henningsen 2007, Table 4.3). To facilitate the matching of individuals across surveys, we created a sketch of each distinctive individual per survey and assigned it a temporary catalog number. Best pictures and sketches of each distinctive individual were used to match individuals between surveys by eye. We selected the best picture of each distinctive individual by cropping the picture to contain only the dolphin's body and by enhancing the contrast of the picture using Adobe Photoshop. To assist the matching of individuals by eye, we recorded the mark types and the coloration of each distinctive individual on each survey on a spreadsheet and used these characteristics to find similar individuals in other surveys. We then visually compared the best image of the individuals from each of the surveys to one another, starting with the individuals that appeared to be most similar. We repeated this process separately for photographs taken from both the left and right sides of the *Inia* dolphins. Each distinct individual was assigned a final left-side and right-side catalog number. All left-side individuals were compared to all right sides to cross-verify identity when possible. For each individual, we recorded the coloration on the flank (grey, pink, or blotched pink) as well as the shape, color, and location on the body of each visible mark. To reduce the probability of errors, at least two people with experience in photoidentification matched each pair of individuals, and the matching was limited to 2 h per person per day.

4.2.3 Permanence and Prevalence of Marks

The permanence of each mark type was evaluated by using distinctive individuals with conspicuous marks such as large wounds, pigmentation patterns, and nicks that were resighted more than once. These animals were followed across resightings within and

between surveys to evaluate which mark types changed over time. For each mark type, we calculated the gain and loss rate following the methods of Auger-Méthé and Whitehead (2007). Best photographs ($Q \ge 3$) of all sightings of an individual were displayed on the screen simultaneously to record the marks that were gained, lost, and those that remained the same in subsequent sightings. For each mark type, we calculated the rate of gain (total number of marks gained/sum of the number of months between the first and last sighting of each individual) and the rate of loss (total number of marks lost/sum of the number of months between the first presence of a mark and its last presence or first absence noted). Subsequently, we calculated the prevalence of each mark type across all individuals, defined as the proportion of animals that had a specific mark (Auger-Méthé et al. 2010). Prevalence of marks was estimated for both sides of each dolphin and by using high-quality photographs ($Q \ge 4$).

4.2.4 Proportion of Individuals Identifiable

Based on the gain and loss rates, prevalence of mark types, and their location on the body, we classified these mark types into two categories: (1) reliable and (2) supplementary (see "Results"). The proportion of well-marked animals, defined as individuals with the presence of at least two reliable marks, was calculated. We examined all high-quality photographs ($Q \ge 4$) to estimate the proportion of well-marked individuals in the population.

4.3 RESULTS

A total of 6,855 photographs were taken during nine surveys conducted in the Amazon and four surveys conducted in the Orinoco. Of these photographs, *Inia* dolphins were present in 3,734 of the frames, of which 795 were suitable for analysis ($Q \ge 3$) (Table 4.2), and most of these were focused on the dorsal ridge and the flank.

Based on the definitions from previous studies, we found and described eight mark types for *Inia* dolphins: (1) pigmentation patterns, (2) nicks, (3) wounds, (4) scratches, (5) scrapes (e.g., McGuire and Henningsen 2007), (6) bends in the dorsal ridge, (7) white marks, and (8) black marks (Table 4.3, Figure 4.2).

Table 4.2 Number of days surveyed and photographs taken for each survey.

		Ama	ızon	Orin	oco
Survey Dates	# days		# of ph	otographs	
		total	<i>Q</i> ≥3	total	$Q \ge 3$
Feb-07	3	301	47		
Jul-07	1			142	46
Dec-Jan 08	9	865	125		
Feb-08	2	149	23		
May-08	1			281	96
Jun-08	3	154	37	145	17
Jul-08	1		4	186	36
Oct-08	1	213	69		
Nov-08	1	58	31		
Dec-Jan 09	8	1206	256		
Apr-09	1	31	8		
Aug-09	1	3			
Total	32	2980	600	754	195

Marks were classified as either reliable or supplementary. Reliable marks lasted for a period of at least 22 mo and were located on *Inia*'s most photographed body parts (dorsal ridge and flank). Reliable marks have low gain (≤ 0.05 per individual per month) and loss (≤ 0.07 per mark per month) rates and are generally prevalent in the population, being found in many $Q \geq 3$ images (Table 4.4).

Reliable marks included pigmentation patterns on the dorsal ridge, nicks, bends, and wounds. Pigmentation patterns on the dorsal ridge are spots of unique and irregular shapes. These have the second highest prevalence values and very low gain and loss rates. Nicks are small indentations in the dorsal ridge that allowed us to match individuals using their left and right sides. Although not as prevalent as pigmentation patterns, they have very low gain and loss rates. Even though wounds and bends were not prevalent (<

0.24), these mark types were very conspicuous, did not change during the study, and, thus, are very promising, reliable mark types. Overall, reliable marks are promising for the long-term identification of *Inia* dolphins in further studies given that they are prevalent and easy to quantify.

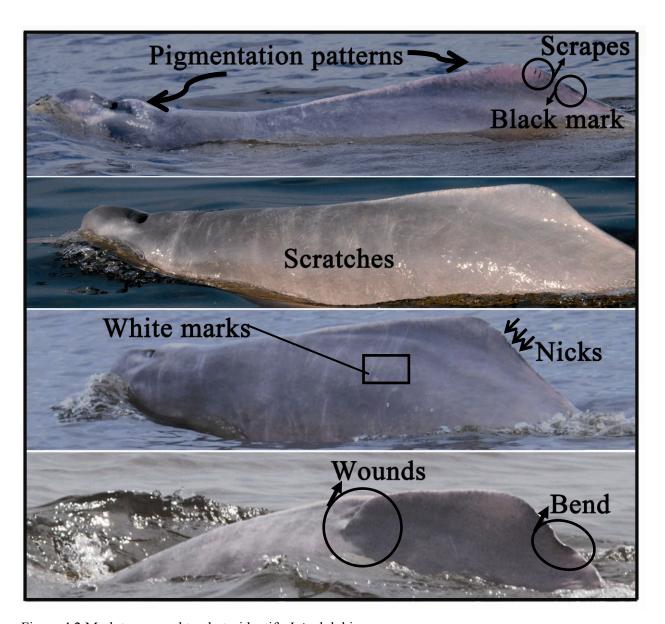


Figure 4.2 Mark types used to photo-identify *Inia* dolphins.

Table 4.3 Mark types used to photo-identify *Inia* dolphins. Bold fonts indicate reliable mark types.

Mark type	Description	Body location	Color		
Pigmentation pattern	Irregular shaped patch of color or discoloration	Head, neck, flank, back, dorsal ridge	Pink or grey		
Nick	Indentations	Dorsal ridge	n/a		
Wound	Significant losses of tissue, and/or mutilations. Wounds usually penetrate the skin, blubber and underlying muscle	Dorsal ridge, snout, peduncle or flank	Red, brown (when fresh), later skin color		
White mark	Circular or irregular patches and/or white lines, significantly thicker than scratches.	Flank	White		
Black mark	Dark coloration of uneven patches	Rear part of the ridge and flank	Black or dark grey		
Scratch	One or more straight, circular, semicircular, or "x"-shaped thin lines. These scars are mostly from tooth rakes.	Head, neck, flank, back, dorsal ridge	White		
Scrape	Singular, parallel, circular or semicircular lines or bands	Dorsal ridge and flank	Red or brown		
Bend	Bends on sections of the dorsal ridge edge	Posterior part of the dorsal ridge	Skin color		

Table 4.4 Gain and loss rates calculated for 5 dolphins re-sighted by right side photographs and 11 dolphins re-sighted using left side photographs. The prevalence of each mark type was calculated separately for left and right side photographs with qualities $Q \ge 4$ (120 pictures).

Mark type	Rate of gain* (per individual per month)	Rate of loss † (per mark per month)	(proportion	ralence of individuals mark) Right side
Bend	0.04	0.00	0.17	0.24

Mark type	Rate of gain* (per individual per month)	Rate of loss † (per mark per month)	Prevalence (proportion of individuals with mark) Left side Right side			
Black mark	0.05	0.03	0.51	0.42		
Scrape	0.36	0.14	0.41	0.46		
Nick	0.05	0.01	0.48	0.46		
Pigmentation pattern (dorsal)	0.05	0.00	0.90	0.92		
Pigmentation pattern (flank)	0.05	0.02	0.48	0.60		
Pigmentation pattern (head)	0.00	0.00	0.58	0.62		
Scratch	0.55	0.11	0.99	0.98		
White mark	0.00	0.00	0.30	0.28		
Wound	0.05	0.07	0.01	0.10		

^{* (}total number of marks gained)/(sum of the number of months between the first and last sighting of each individual)

Supplementary marks can drastically change or disappear within a few days or months and can be located anywhere on the dolphin's body. Supplementary mark types have high gain and loss rates (> 0.10 per individual per month; see Table 4.4), are not prevalent (Table 4.4), and/or are very inconspicuous. Thus, these marks were used within surveys to assist in the matching of photographs to individuals but should not be used in long-term photo-identification studies. Supplementary marks in *Inia* dolphins are scratches; scrapes; black marks; white marks; and pigmentation patterns on the head, neck, and flanks. Scrapes and scratches, although very conspicuous, have the highest loss and gain rates. Scrapes disappeared from some individuals within 1 wk, and scratches disappeared from some individuals within 1 mo. White marks are circular or irregular patches and/or

^{† (}total number of marks lost)/(sum of the number of months between the first presence of a mark and its last presence or first absence noted)

white lines that are significantly thicker than scratches, located mostly on the dolphins' flanks. Black marks were located on the lower part of the dorsal ridge, and unlike white marks and pigmentation patterns on the dorsal ridge, they did not have a defined shape. Instead, they mostly look like very dark shadows. Pigmentation patterns on the *Inia* dolphins' heads, although conspicuous, prevalent, and easy to quantify, were not often photographed and, therefore, were only used as supplementary marks when the dorsal ridge was also photographed. Pigmentation patterns on the dolphins' flanks were prevalent; however, the shape of the pigmentation patterns on the flanks was not as well-defined by nearby "anchor" points as the ones located on the dorsal ridge or the head. Thus, pigmentation patterns on the flanks are considered supplementary. Despite this, they were one of the main features used to describe body coloration (see below). In conclusion, supplementary mark types cannot be reliably used for the long-term identification of *Inia* dolphins and should solely be used to match individuals within surveys.

In this study, coloration on the *Inia* dolphins' bodies was considered a supplementary mark that assisted in the identification of individuals in the Amazon, mostly within surveys. All dolphins identified in the Orinoco were grey. In contrast, some individuals identified in the Amazon were grey (43% left side, 49% right side), some were pink (14% left side, 17% right side), and some were blotched pink (43% left side, 32% right side). We investigated the colour variation of identified *Inia* dolphins within and between surveys in the Amazon. We found that the tones in the colouration of the pink and blotched pink animals changed (Figure 4.3). These tones could change within minutes of when a photograph was taken as a result of fluctuations in the *Inia* dolphins' physical activity, or alternatively due to differences in light levels when photographs were taken. Although these tones changed, becoming lighter or darker, the colouration pattern did not alter, as also suggested in other studies (e.g., Trujillo 1994, McGuire and Henningsen 2007). Moreover, individuals seemed to retain some symmetry in the coloration patterns for left and right sides, which, in turn, acted as a supplementary feature when matching left and right sides of some individuals at least within the same survey. Therefore, given that the color variation did not affect the visualization and features of reliable marks, the

pattern of coloration on the *Inia* dolphins' bodies helped in the matching of individuals in the Amazon. Well-marked animals, with the presence of at least two reliable marks, were recorded in 66 (55%) of the 120 images ($Q \ge 4$) analyzed for this purpose. Fifty-two percent of the images had just one reliable mark type, and 3% completely lacked reliable marks.

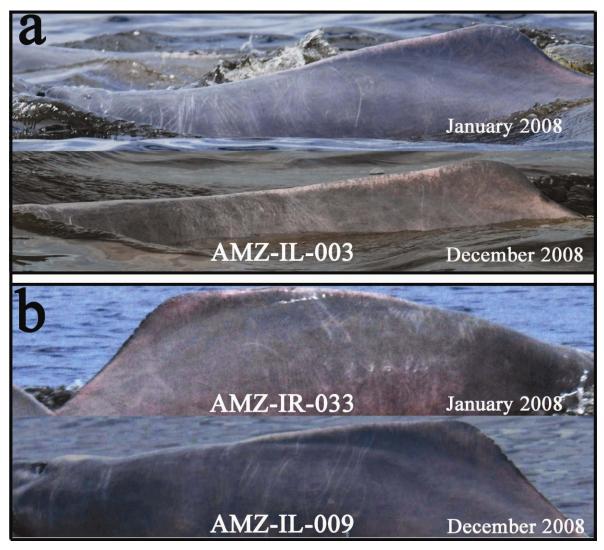


Figure 4.3 Individuals identified and re-sighted after 12 mo using a) pigmentation patterns on the dorsal ridge and b) nicks.

The photo-identification catalog consisted of information, separately for right and left side photographs, about each distinct individual: mark types present; coloration of the animal; best picture taken; *Q* values of the best picture taken; and, when possible,

pictures of the individual from the opposite side. The majority of photographs included in the catalog were Q = 3 (57), followed by Q = 4 (32) and Q = 5 (8). In the Amazon, we identified a total of 41 individuals from photographs of their right side and 28 individuals from the left. In the Orinoco, we identified a total of 16 individuals from photographs of their right side and 12 individuals from the left. We then compared the right and left sides of these individuals. As a result, a total of seven dolphins in the Amazon and two dolphins in the Orinoco were matched with corresponding left and right side photographs. In the Amazon, 15 individuals were resighted over a period of 23 mo. In the Orinoco, one individual was resighted over a period of 1 mo (Table 4.5).

Table 4.5 Re-sighting records of identified *Inia* dolphins (ID).

Date	20070223	20080102	20080105	20080513	20080612	20080622	20081004	20081102	20081203	20081207	20081211	20090106
ID												
AMZ-IR-003	CC		CC									
AMZ-IR-004	CC		CC									
AMZ-IR-030									CC	CC		
AMZ-IR-041							CC					CC
ORI-IL-009				MR	MR							
AMZ-IR-020 AMZ-IL-002	CC		CC			AR	CC	AR				
AMZ-IL-003	CC		CC				CC		CC			
AMZ-IL-005	CC									CC		
AMZ-IL-015							CC		CC			
AMZ-IL-019							CC	AR				
AMZ-IL-020								AR	CC			
AMZ-IL-023	CC	CC					CC		CC		CC	
AMZ-IL-024							CC		CC			
AMZ-IR-027								AR	CC		CC	
AMZ-IL-025								1111				
AMZ-IR-035 AMZ-IL-026										CC	CC	
AMZ-IL-009 AMZ-IR-033			CC						CC			

CC = Caballo Cocha lake (Amazon), MR = Meta River (Orinoco), AR = Amazon River.

4.4 DISCUSSION

This study, for the first time, evaluated the reliability of marks for the photo-identification of pink river dolphins (*Inia geoffrensis*) in the Colombian Amazon and Orinoco river basins. Although the brief surfacing time of *Inia* limits the possibility of visually identifying individuals in the field (da Silva and Martin 2000), *Inia* dolphins have marks that can nevertheless be photo-identified. In some locations, dolphins approach boats, and, although unpredictable when surfacing, they can show a large proportion of their bodies. Given that *Inia* dolphins surface conspicuously in some areas of their distribution, we conducted surveys to obtain high-quality digital images of individuals with the goal of describing useful marks for photo-identification.

Due to the higher quality of digital images and faster shooting capacities of new digital cameras relative to analog cameras, we obtained sufficient high-quality photographs (comparable only with the previous study conducted in Colombia; see Table 4.1) to examine the marks of *Inia* dolphins and to categorize these marks as reliable or supplementary, according to their permanence and consistency. Reliable mark types in *Inia* dolphins (pigmentation patterns on the dorsal ridge, nicks, bends, and wounds) were defined as marks lasting for a minimum period of 22 mo. These were located on *Inia*'s most photographed body parts (dorsal ridge and flank), had low gain and loss rates, and were either prevalent or very conspicuous. Relying on a single identification mark type could lead to misidentification (Karczmarski and Cockcroft 1998); therefore, we used at least two reliable marks to identify individuals.

Pigmentation patterns on the dorsal ridge of *Inia* are the most prevalent mark type and are often used as one of the principal marks for photo-identification of the species (Trujillo 1994, McGuire and Henningsen 2007). Pigmentation patterns are likely the result of discoloration of the skin, parasites, or abrasions caused by rubbing against objects or by injuries from their conspecifics. Pigmentation patterns of many species remain unchanged across multiple years such as in bottlenose whales (*Hyperoodon ampullatus*) (Gowans and Whitehead 2001) and blue whales (*Balaenoptera musculus*) (Sears et al. 2000). In contrast, pigmentation patterns in narwhals (*Monodon monoceros*) change over

time and are only useful for matching individuals within a short period of time (Silverman 1979). Pigmentation patterns were first described for *Inia* in the same Amazon location in Trujillo (1994), but it was not known whether these would be reliable marks across multiple years. Although the level of colouration of the pigmentation patterns can change over a short time scale, the pigmentation patterns on the dorsal ridge did not change over the 22 mo of the study. Long-term studies to monitor pigmentation patterns of *Inia* are required to investigate whether pigmentation patterns remain stable across multiple years.

Nicks are indentations on the dorsal fin of many cetacean species that are often used to identify individuals, from both their left and right sides, over the long term (Würsing and Jefferson 1990). The combination of nicks and pigmentation patterns is useful for the photo-identification of many species such as short-finned pilot whales (*Globicephala macrorhynchus*) and Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (Würsing and Jefferson 1990). Similarly, nicks and pigmentation patterns were the most promising features for photo-identifying *Inia* dolphins over the long term.

A reliable mark type, described for the first time in this study, is the presence of a bend in the dorsal ridge. Bends were located in the thinnest areas of the dorsal ridge (low, posterior region), possibly triggered by forcible contact with inanimate objects or injuries from conspecifics. The maximum time between resightings of a bend was 2 mo, during which time no changes to the bend were observed. It is unlikely that the original shape of the ridge could be restored after bending, thus bends are a promising feature for identifying *Inia* dolphins in the long term.

Wounds are likely to last for multiple years and are the most conspicuous mark type. Given that river dolphins do not have known natural predators, wounds are presumably caused by interactions with fishing nets or cuts produced by large knives (machete) (Trujillo 1994). Hence, these are the least prevalent mark type in our study population. Previous studies found that the majority of resightings of photo-identified *Inia* dolphins were of the most conspicuous individuals, identified by using wounds and large injuries

(McGuire and Winemiller 2007). The majority of resightings in this study, on the contrary, were of individuals identified by using the most prevalent mark type (pigmentation patterns on the dorsal ridge). In photo-identification studies, it is not the conspicuousness of the marks but their prevalence in the population, as well as their permanence, that will have the largest role in the identification of animals (Hammond et al. 1990). Therefore, wounds, although conspicuous and long lasting, have limited use because of their rarity.

Supplementary mark types (e.g., scratches; scrapes; black marks; white marks; pigmentation patterns on the flank, neck, and head) are, overall, not reliable over the long term but are useful for identifying individuals within the same survey. Scrapes and scratches can be formed from tooth rakes of conspecifics or can be single or parallel lines that may be produced by inanimate objects such as flooded vegetation. These marks cannot be used to identify *Inia* dolphins for a period longer than 1 wk and 1 mo, respectively. Scratches are highly prevalent on *Inia* dolphins; however, as has also been observed in other species, they have high gain and loss rates and, thus, limited persistence. For instance, scratches are similar to the linear marks and tooth rakes described in long-finned pilot whales (Globicephala melas), which are also not persistent in the population and therefore not useful for photo-identification studies (Ottensmeyer and Whitehead 2003, Auger-Méthé and Whitehead 2007). Black marks appear as shadows, and white marks are irregular features. It is unclear how these are formed, and although their rate of gain and loss is not as high as for scratches and scrapes, these are not prevalent in the population and are very inconspicuous. Overall, supplementary marks, such as scrapes, scratches, white marks, and black marks, should not be used to individually identify *Inia* dolphins except over the shortest term given their high loss and gain rates and/or lack of prevalence in the population.

Pigmentation patterns in the area around the blowhole are produced as a result of depigmentation of the skin caused by abrasion and biting from conspecifics (Martin and da Silva 2004). These are common and distinct among *Inia* dolphins (Martin and da Silva 2004, McGuire and Winemiller 2007); however, they are considered as supplementary

mark types in this study given that few pictures showing *Inia*'s head are available. Pigmentation patterns on the head could become a reliable mark type only if more photographic effort is employed to photograph dolphin heads with their respective flank and dorsal ridge. For instance, bottlenose whales also have unique pigmentation patterns on their heads (Gowans and Whitehead 2001). Therefore, an independent catalog of the heads was created, and individuals that were matched based on head photographs were compared to individuals matched by their fins to confirm identity and to test the reliability of other marks (Gowans and Whitehead 2001). Similarly, pigmentation patterns around the blowhole were seen on the Yangtze River dolphin (Hua et al. 1990). While the Yangtze River dolphins surfaced in a manner that allowed easy photoidentification of the head, the surfacing pattern of *Inia* made the simultaneous photographing of both head and flank a very difficult task, at least in our study. In conclusion, pigmentation patterns in the area of the blowhole could be classified as reliable marks if future studies are able to frequently photograph the head as well as each animal's dorsal ridge and flank.

Pigmentation patterns on the flank were used to describe *Inia*'s body colouration (grey, pink, and blotched pink). Because the level of colouration on the flank changes significantly, and in many instances is very inconspicuous and difficult to quantify, it is not used as a reliable mark type over the long term. However, it was useful to assist in the identification of individuals in the Amazon within surveys and, thus, to match left and right sides of some individuals given the apparent color symmetry. However, more photographs and further analysis are necessary to validate this.

In this study, it was not possible to match the left and right sides for the majority of individuals. Although increasing the photographic effort will help obtain the high-quality photographs required to obtain matches of individuals across sides, we suspect that some individuals may remain impossible to match. For instance, the most prevalent and reliable mark type used pigmentation patterns in the dorsal ridge might occur on one of the *Inia* dolphin's sides but not necessarily on its opposite side. This contrasts with nicks since the silhouette of the indentations appears the same from both sides.

The proportion of well-marked *Inia* dolphins (55%) was comparable to that of other species. For instance, 84% of narwhals, 33.6% of long-finned pilot whales, and 66% of bottlenose whales are identifiable when good quality pictures are examined (Gowans and Whitehead 2001, Auger-Méthé and Whitehead 2007, Auger-Méthé et al. 2010). The success of photo-identification studies of *Inia* dolphins is greatly influenced by the researchers' ability to obtain high-quality pictures in order to identify these reliable marks. Previous studies have suggested that it is the lack of experience and inadequate equipment that have restricted photo-identification of river dolphins (Hua et al. 1990, McGuire and Winemiller 2007). Consequently, photo-identification can be improved using digital cameras with fast shutter speeds and long lenses by researchers with considerable experience in photographing river dolphins and/or other very fast-moving subjects. Efficiency will be higher in study areas where dolphins do not avoid boats and surface conspicuously showing a significant portion of their bodies such as at Caballo Cocha Lake on the border between Peru and Colombia, the confluence of the Meta and Orinoco Rivers on the border between Colombia and Venezuela, and Tipishca del Samiria Lake in Peru.

In addition to photo-identification, natural marks have been used in other species to assess an individual's age. For instance, Risso's dolphin (*Grampus griseus*) adults become lighter with age due to loss of pigment; thus, in photo-identification studies, individuals with a moderate to very high level of scarring are considered adults (Hartman et al. 2008). Marks of *Inia* could also be used to assess the age and sex of individuals. For example, it was suggested that color, especially the pinkness of some adult males, could potentially be a proxy for *Inia's* maturity (Martin and da Silva 2006). This could be investigated by conducting photo-identification studies of marks on individuals that are already sexed, aged, and artificially marked in studies conducted in Brazil. However, these features would not appear to be useful for *Inia* dolphins in the Orinoco, where all individuals in our study were grey and without pink patches.

To summarize, this study demonstrates that pigmentation patterns on the dorsal ridge, nicks, bends, and wounds are reliable mark types for photo-identification studies of *Inia* dolphins. In addition, this study provides some direction about how to use and improve upon this methodology, and how to better evaluate other potential reliable marks such as pigmentation patterns in the area of the blowhole. Photo-identification of *Inia* dolphins in the Amazon and Orinoco using categorical mark types is a promising methodology when using digital cameras with long lenses and fast shutter speeds, as well as in areas where dolphins are more conspicuous when surfacing and, thus, more easily photographed.

Further studies should take digital images in RAW format, which require more storage but will reveal greater detail. Despite the broad distribution of *Inia* dolphins in the Amazon and Orinoco river basin, little is known about their residence patterns, population size, and social structure. Photo-identification could be an important tool in looking at these ecological data requirements and, thereby, may help to direct conservation and management actions.

4.5 SUMMARY OF CHAPTER 4 AND TRANSITION TO CHAPTER 5

Photo-identification is an important tool for studying cetacean residence patterns, population size, movements, and social structure. This knowledge directs conservation and management. We examine the reliability of photo-identification studies of pink river dolphins (*Inia geoffrensis*) with the hope of encouraging long-term population monitoring programs. From February 2007 to August 2009, 12 surveys were conducted in two locations of the Colombian Amazon and Orinoco river basins. We obtained 795 suitable digital photographs of *Inia*. We evaluated the reliability and duration of photo-identification by describing and evaluating the permanence and consistency of eight mark-types. Marks were categorized as reliable (pigmentation patterns on the dorsal ridge, nicks, bends, and wounds) or supplementary based on their prevalence in the population, and gain and loss rates. We created a catalogue of well-marked animals, defined as individuals with at least two reliable marks (55% of the images analyzed for this purpose). It contained photographs of the right side of 57 individuals, and the left side of 40 individuals. There were 16 individuals with re-sightings over a 23 mo period.

Future field surveys should use digital cameras with long lenses and fast shutter speeds in areas where dolphins are conspicuous when surfacing.

Previous chapters used line-strip transect methods to obtain abundance estimates of river dolphins. The next chapter will use another method to estimate population size of *Inia* dolphins, which is particularly appropriate over small scales: mark recapture methods on photo-identifications.

CHAPTER 5 POPULATION SIZE ESTIMATES OF PINK RIVER DOLPHINS USING MARK-RECAPTURE METHODS ON PHOTO-IDENTIFICATION

The work presented in Chapter 5 was submitted for publication:

Gomez-Salazar, C. F. Trujillo, H. Whitehead. Population size estimates of pink river dolphins (*Inia geoffrensis*) using mark-recapture methods on photo-identification. Marine Mammal Science.

5.1 Introduction

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

The size and trends, movement patterns, survival and recruitment rates of cetacean populations can be obtained by using data from photo-identification surveys (Hammond 1990, Hammond 2009, Hammond 2010). Photo-identification, using digital cameras in areas where dolphins are conspicuous when surfacing, is a reliable and non-invasive tool

to study *Inia* dolphins (Trujillo 1994, Gomez-Salazar et al. 2011b). For example, photo-identification effort is recommended when surveying *Inia* dolphins in high-density areas (e.g., lakes) (Gomez-Salazar et al. 2012). Typically, these high-density areas are also characterized by large group sizes of dolphins (Gomez-Salazar et al. 2011a), and so they attract dolphin-watching activities that so far are not regulated. Population size estimates using data on photo-identifications can be used to develop long-term monitoring programs in these critical, high-density areas, that need to be carefully monitored.

Mark-recapture methods applied to photo-identification data can provide accurate estimates of population size and trends, which are both useful in assessing the impacts of human stressors on dolphin populations over time (Wilson et al. 1999, Gowans et al. 2000, Hammond 2009). Hence, the objective of this paper is to investigate population sizes of pink river dolphins (*Inia geoffrensis*) in two locations of the Colombian Amazon and Orinoco basins by using mark-recapture methods on photo-identifications. We hope that this study will encourage further photo-identification efforts to investigate trends in population size, movement patterns, survival rates and social structure of river dolphins (e.g., Mann et al. 2000, Read et al. 2003) which can be used to develop long-term monitoring programs and ultimately to inform management and conservation actions for this species.

5.2 METHODS

5.2.1 Data Collection

Photographs of *Inia* dolphins were collected between February 2007 and August 2009 in two locations of the Amazon (Figure 5.1) and Orinoco river basins (Figure 5.2). The Amazon study area is located in the southern portion of Colombia ($3^{\circ}46'$ S $70^{\circ}22'$ W, 60 linear km of river surveyed) and the Orinoco basin study area is located in the northeast region of Colombia ($6^{\circ}11'$ N $67^{\circ}28'$ W, 120 linear km of river surveyed). Photographs were rated on quality of the image regardless of the markings of each individual. A quality rating, (Q), of 1 - 5 was given to each photograph. The rating of 1 was used for very poor photographs, which were not useful and the rating of 5 was used for very good quality – ideal for photo-identification. We selected those good quality photographs

(rated $Q \ge 3$) that showed images of *Inia* dolphins containing at least two marks of a type considered reliable.

Reliable mark types (pigmentation patterns, nicks, bends, and wounds) are those that last for a period of at least 22 mo, and are located on *Inia*'s dorsal ridge and flank (Gomez-Salazar et al. 2011b). Based on these criteria, a photo-identification catalogue was created and used in this study. More detailed information on survey design, data collection, and photographic analysis is given in Gomez-Salazar et al. (2011b).

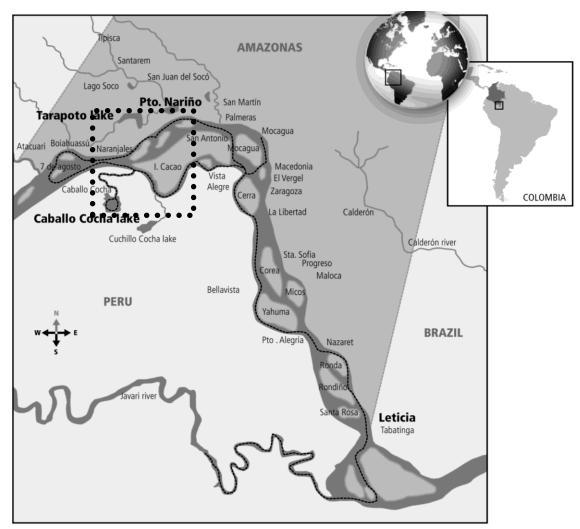


Figure 5.1 Study area in the Amazon location were population estimates of river dolphins were obtained using mark-recapture methods on photo-identification (area highlighted, Chapter 4 and 5) and line-strip transect surveys (dotted lines, Chapter 3).

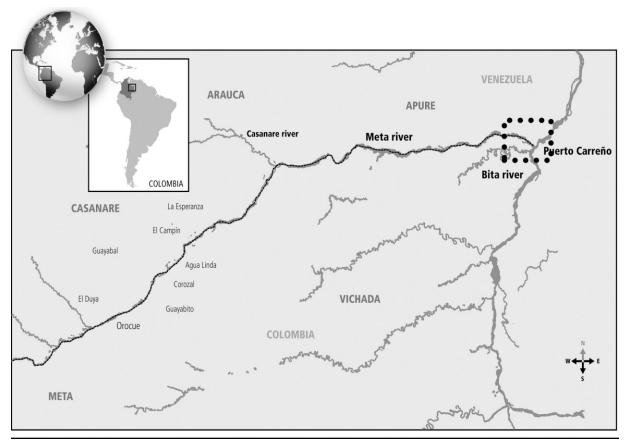


Figure 5.2 Study area in the Orinoco location were population estimates of river dolphins were obtained using mark-recapture methods on photo-identification (area highlighted, Chapter 4 and 5) and line-strip transect surveys (dotted lines, Chapter 3).

5.2.2 Data Analysis

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

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

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

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

Mark-recapture models to estimate population size make the following assumptions regarding the data collected (Hammond 2009, 2010):

- 1. The closed (Petersen) model assumes no immigration, emigration, birth or death between each pair of sampling periods.
- 2. Marks used to identify individual dolphins are unique, are not lost, and are correctly recorded.
- 3. Marking does not affect future survival or catchability.
- 4. Animals have an equal probability of being captured within each sampling occasion, (homogeneity of capture probabilities).

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

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

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

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

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

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

5.3 RESULTS

Data consisted of individuals photo-identified by Gomez-Salazar et al. (2011b): 41 individuals identified by the right side and 28 by the left side in the Amazon; 16 individuals identified by the right side and 12 by the left side in the Orinoco (Table 5.1) (see Methods in Gomez-Salazar et al. 2011b). The photo-identification was incomplete in both study areas as indicated by neither discovery curve leveling off (Figure 5.3). There were no matches between the study areas (Table 5.1). The total population size estimate for right side individuals was 129 *Inia* dolphins (CV=0.28) in the Amazon and 125 *Inia* dolphins (CV=0.77) in the Orinoco (Table 5.2). As there were no recaptures between the Orinoco samples, the upper bound of the population estimate is infinite.

Table 5.1 Number of dolphins identified by right/left sides (diagonal) and number of re-sighting records between years in two locations in the Colombian Amazon and Orinoco basins. Data were not collected in the Orinoco study area in 2007

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

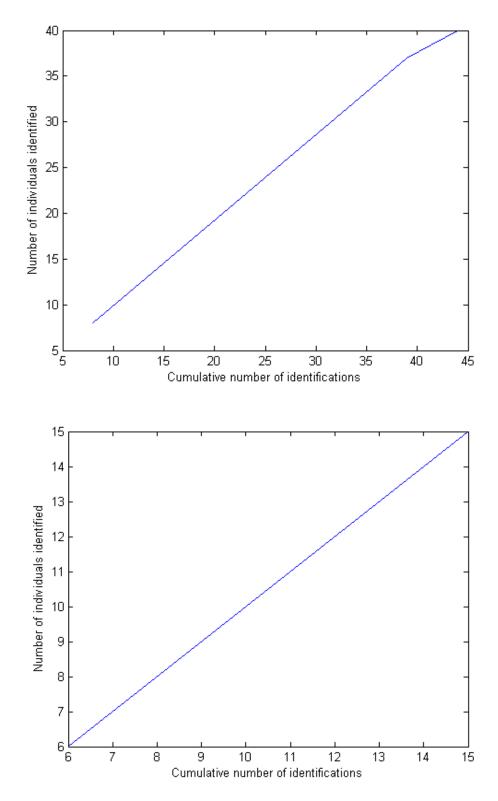


Figure 5.3 Discovery curves for *Inia* dolphins identified from the right side in the Amazon (above) and Orinoco (below). Similar curves were found for individuals indentified from the left sides.

Table 5.2 Population size (\hat{N}), total population size (N_{total}), standard error (SE), and coefficient of variation (CV) for all reliably-marked individuals using the closed (Petersen) mark-recapture model. Estimates were obtained for each location (Amazon and Orinoco), per each sample period and for the overall period of this study.

	Side of dolphin	200	07-08	200	08-09	Over	all				
Amazon		\hat{N}	SE	\hat{N}	SE	\hat{N}	SE	CV	$\hat{N}_{ ext{total}}$	SE	CV
	Right side	95	37.1	63	21.4	71	18.5	0.26	129	45.93	0.36
	Left side	39	8.2	39	11.7	39	6.7	0.17	71	24.62	0.35
Orinoco											
	Right side	69	43.2	-		69	43.2	0.63	125	96.90	0.77
	Left side	32	17.9	-		32	17.9	0.56	58	40.16	0.69

5.4 Discussion

5.4.1 Mark-Recapture Assumptions

Violations of the assumptions of mark-recapture models can result in biased abundance estimates (Hammond 2009). For this reason, this section evaluates potential violations of each assumption.

1. The closed (Petersen) model assumes no immigration, emigration, birth and/or death between each pair of consecutive sampling intervals. We expect that these populations are nearly geographically closed given the enclosed nature of the study areas (Figure 5.1 and 5.2) and that previous studies of this species have demonstrated only short-distance movements and high fidelity of individuals to areas where they are born (Trujillo 1994, Martin and da Silva 2004, Martin et al. 2004, McGuire and Henningsen 2007, Ruiz-Garcia et al. 2007). Cetaceans have low reproductive and high survival rates, with a population growth rate ranging between 2 - 10% per year (Connor et al. 2000, Barlow

and Reeves 2009). Thus, the population estimates might be biased upwards by about these amounts because of recruitment, and thus a lack of closure.

- 2. Marks used to identify individual dolphins are unique, are not lost, and are correctly recorded. A detailed protocol specifically for photo-identification of *Inia* dolphins was developed with the aim of ensuring that marks used to identify individuals are unique, are not lost, and are correctly recorded or reported (Gomez-Salazar et al. 2011b). For instance, to avoid mismatching individuals and assure that marking is unique, at least two reliable marks in *Inia*'s dorsal ridge and or flank are required for identification. To avoid marks being lost, only reliable marks were used, characterized by low rate of loss and lasting for the entire length of the study (22 mo). To reduce the probability of errors while identifying, matching and recording individuals, we followed a standardized protocol, which includes using only good quality photographs, ($Q \ge 3$), and at least two people with experience in photo-identification confirming matches (Gomez-Salazar et al. 2011b).
- 3. Marking does not affect future survival or catchability. Photo-identification is non-invasive, and will only cause short-term disturbance at the worst. It is thus unlikely to affect survival or the probability of recapture (Wilson et al. 1999, Hammond 2009). In addition, the study areas surveyed have a nearly constant presence of boats and thus we consider unlikely that the survey boats might have altered the dolphins' behaviour between sampling occasions.
- 4. Animals have an equal probability of being captured within each sampling occasion. Our field survey design was developed to minimize unequal probabilities of capture given potential differences in the behaviour of individuals. For example, to maximize the probability of photographing all individual dolphins within a group, photographic effort was ended only when individuals could no longer be followed, or after 30 minutes of taking photographs. Moreover, to minimize the probability of identifying animals with more distinctive marks, photographs were taken of all individuals regardless of presence or conspicuousness of marks. Field surveys were also designed to give adequate

coverage to all habitat types in each study area (e.g., main river, tributary, lake, confluence). However, there are several aspects of river dolphin ecology that might lead to more recaptures in certain areas. For instance, lakes and confluences contain generally higher densities estimates and larger group sizes of dolphins, and in these areas dolphin are more conspicuous when surfacing and thus more efficiently photographed (Gomez-Salazar et al. 2012, Gomez-Salazar et al. 2011a, Gomez-Salazar et al. 2011b). If particular animals use these areas preferentially, this could introduce heterogeneity. In addition, other studies have found differences in habitat preferences for male and female *Inia*, with females and calves entering the flooded areas and males remaining in the main rivers (Martin and da Silva 2004). These kinds of heterogeneity could have produced negative biases in our estimates.

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

5.4.2 Abundance Estimates: Mark-Recapture Vs. Line-Transect Methods

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

area, line-strip transects surveyed an area of 592.6km² during the transitional water period (Gomez-Salazar et al. 2012), and the mark-recapture study surveyed 140km² within that area during different months of the year (Figure 5.1). In the Orinoco study area, line-strip transects surveyed an area of 1,231.1 km² during the transitional water period (Gomez-Salazar et al. 2012), and the mark-recapture study surveyed 260km² within that area during different months of the year (Figure 5.2). Hence, the areas surveyed during this mark-recapture study are obtained during multiple water periods and are within the boundaries of significantly larger areas surveyed during the line/strip transect surveys (Figure 5.1 and 5.2; see Chapter 7, Section 2.1 for discussion on the use of the different methods to estimate population size).

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

5.5 SUMMARY OF CHAPTER 5 AND TRANSITION TO CHAPTER 6

Population size estimates of pink river dolphins (*Inia geoffrensis*) are critical to assess the conservation status of these population and the impacts of increasing human stressors in freshwater ecosystems. Photo-identifications of *Inia* dolphins were collected between February 2007 and August 2009 in two locations of the Colombian Amazon and Orinoco river basins. Population sizes of *Inia* were obtained by using the closed (Petersen) mark-recapture model on photo-identifications. The total population size estimate for right/left side individuals was 129/71 *Inia* dolphins (CV=0.36/0.35) in the Amazon and 125/58 *Inia* dolphins (CV=0.77/0.69) in the Orinoco. This study used a well-designed survey and standardized protocol for data analysis to try to meet mark-recapture assumptions.

However, photo-identification was incomplete in both study areas, and there could be recruitment and unequal probabilities of capture due to preferences of individuals for certain areas. Further effort should be focused towards expanding the photo-identification catalogue and creating long-term monitoring programs.

Chapter 5 provided another tool, in addition to line-transect methods from Chapter 3, to obtain population size of river dolphins (see Section 7.2.1. for comparison between methods). The next chapter will use results obtained in the earlier chapters to evaluate the potential of river dolphins as indicators of ecosystem degradation.

CHAPTER 6 RIVER DOLPHINS AS INDICATORS OF ECOSYSTEM DEGRADATION IN LARGE TROPICAL RIVERS

The work presented in Chapter 6 was accepted for publication: Gomez-Salazar, C., Coll, M., H. Whitehead. River dolphins as indicators of ecosystem degradation in large tropical rivers. Ecological Indicators.

6.1 Introduction

Human stressors can lead to ecosystem degradation (Alcamo et al., 2005; Foley et al., 2005). Currently, freshwater systems are at risk, with 65% of global river discharge being considered under moderate to high threat, and water security of 80% of the human population at high risk (Vörösmarty et al., 2010). In addition, biodiversity in freshwater ecosystems is in rapid decline and it is considered even more threatened compared to that of terrestrial and marine ecosystems (Revenga et al., 2000; Vörösmarty et al., 2010). Rivers are at risk due to the impact of multiple human stressors, including changes in water quantity and quality, habitat modification, exploitation of species, climate change, and introduced species (Table 6.1).

The current impacts of these stressors are dramatically increasing and are unsustainable in the long term (Alcamo et al. 2005). In this context, it is critical to measure and monitor the status of freshwater ecosystems and the extent of their degradation (Revenga et al., 2000; UNEP, 2004). Obtaining this information is critical to prioritize areas for conservation, develop global conservation strategies, provide scientific knowledge to support policy and management actions, and to restore ecosystem services (e.g., Sanderson et al. 2002, Foley et al. 2005).

Table 6.1 Principal human stressors that are responsible for freshwater ecosystem degradation and their impact on ecosystems.

Human Stressors	Impact of stressors on freshwater ecosystems
Water Quantity	Water withdrawals by domestic, industrial and agricultural needs, reservoir storage capacity
Water Quality	Source point and non-source point pollutants (e.g., organic pollutants, increased nutrients, heavy metals, microbial contamination, toxic organic compounds), suspended particles, temperature
Habitat Modification	Roads, dams, reservoirs, land transformation, land use intensity, agriculture, vegetation cover, fragmentation
Exploitation of species	Fishing pressure, destructive fishing practices (e.g., blast fishing or fishing using poison or explosives), excessive by-catch and discards, aquaculture
Climate Change	Increasing water temperature, decreasing precipitation, increasing acidification, changes in primary production
Introduced Species	Increasing the rates of species introduced in freshwater systems and the success rate of those introduced

Alcamo et al., 2005; Hoekstra et al., 2011; Revenga et al., 2000; Alkemade et al., 2009; Moyle and Randall, 1998; Alcamo et al., 2003; GIWA, 2002; Mila i Canals et al., 2009; Falkenmark, 1997; Bennett et al., 2004; Karr and Chu, 1999; Vörösmarty et al., 2010; Vörösmarty et al., 2000.

The reliability of ecosystem degradation measurements depends on our ability to accurately understand and measure the number and intensity of human stressors and their effects on biodiversity and ecosystems. Ecosystem degradation often occurs gradually, making it difficult to recognize without repeated measures of reliable indicators (Dale and Beyeler, 2001). Freshwater ecosystem degradation is sometimes measured using a suite of ecological indicators, such as macro-invertebrates, fishes, macrophytes, and organisms that live between terrestrial and aquatic ecosystems, such as odonata (e.g., Moya et al. 2011). Good, carefully-selected indicators can provide warning signals of significant but cryptic changes to ecosystems (Karr, 1999; Noss, 1999). The collection of detailed data on ecological indicators may be costly, especially in developing countries where funding is limited and where large tropical rivers of exceptionally high biodiversity are located (Revenga et al., 2000). For example, there are at least 5600 identified species of freshwater fish in the Amazon River Basin and new species are being identified at a rapid rate (Albert and Reis, 2011). As an alternative, it

has been proposed to collect data on population trends of specific indicator species

(Revenga et al., 2000; Noss, 1999). Top predators such as mammalian carnivores, sea birds and raptors are among the indicator species suggested (Furness and Camphuysen, 1997; Sergio et al., 2005; 2006; 2008; Piatt et al., 2007; Sergio et al., 2008).

Top predators tend to be concentrated in important biodiversity hotspots (Worm et al., 2003; Sergio et al., 2005; 2006). The reduction or disappearance of top predators is related to significant ecosystem transformations, including impacts on several trophic levels and changes in energy flows, marine resources' removals from fisheries, and changes in the behavior of prey (Soulé et al., 2005; Heithaus et al., 2008; Baum and Worm, 2009). Moreover, their presence or absence can indicate the extent of the footprint of human pressures; those areas with low human population and/or strong conservation and regulations tend to have the highest sighting frequencies of top predators (Baum and Worm, 2009; Sandin et al., 2008; Ward-Paige et al., 2010).

River dolphins are top predators that inhabit some of the largest tropical river basins in Asia and South America, and may be ideal candidates to serve as ecological indicators. When comparing the level of freshwater habitat degradation of these river basins, the baiji dolphins (*Lipotes vexillifer*), now functionally extinct, were distributed in the most dramatically modified river basin, the Yangtze; the blind dolphins (*Platanista gangetica gangetica* and *P. g. minor*), which are currently endangered, are distributed in the second most modified basins, the Indus and Ganges; and the pink river dolphin (*Inia geoffrensis*) and tucuxi (*Sotalia fluviatilis*), vulnerable, are distributed in the least modified of these river basins, the Amazon and Orinoco (Smith and Reeves, in press). Hence, at a global river basin scale, freshwater dolphin species are distributed across a range of human stressor levels and their status informs the global level of freshwater ecosystem degradation (Smith and Reeves in press).

In this study, we investigated whether river dolphins can indicate freshwater ecosystem degradation at a regional scale. We examined the potential for measures of river dolphin abundance to act as ecological indicators based on how well three abundance estimates (density, mean group size and sighting rate) correlated with the level of human stressors. To quantify and monitor the current and future level of human stressors, we first

developed an index of freshwater ecosystem degradation integrating four major factors: (i) water quality degradation, (ii) habitat modification, and (iii) exploitation of species, as well as (iv) human population size in the area, as a proxy for multiple human stressors (see Ward-Paige et al., 2010; Mora et al. 2011).

6.2 METHODS

6.2.1 Human Stressors and Index of Freshwater Ecosystem Degradation

For each study area (Figure 6.1), an index of the current freshwater ecosystem degradation was calculated. The degradation index was developed by listing 10 human stressors (Table 2) and by grouping them within four major categories: (I) water quality, (II) habitat modification, (III) species exploitation, and (IV) cities and human settlements. Each human stressor was coded according to four impact categories: (0) when disturbance is absent/no disturbance, (1) when disturbance is low, (2) medium and (3) high (Table 6.2). Score index of freshwater ecosystem degradation for each major category (water quality (I), habitat modification (II) and species exploitation (III), Table 2) were the average of those for the human stressors coded in that category, and an overall score index of freshwater habitat degradation was obtained by summing over these three major categories (Table 6.3), with overall value ranges from 0 to 8.5 (8.5) being the highest degradation). In addition, the information on human population size (cities and human settlements (IV), Table 6.2) for each river area surveyed was obtained using the databases of the population census for each country surveyed (Table 6.2 and 6.4, DANE, 2005; INE 2001; INE, 2001; INEI, 2007; INEC, 2010). The future trend in freshwater habitat degradation was estimated for each human stressor based on current knowledge of, for example, water development projects planned (Table 6.3). Information used to provide current and future degradation index scores consisted of observations in the field, published and unpublished reports, and personal communications with researchers from each location surveyed (see also Gomez-Salazar et al., 2012, section 3.4.2).

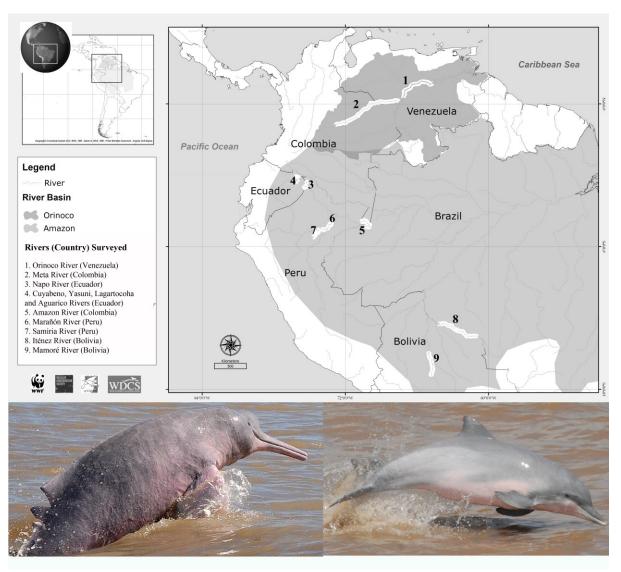


Figure 6.1 Map of the nine study areas surveyed (modified from Gomez-Salazar et al., 2012) and the river dolphin species studied in the Amazon and Orinoco (left: pink river dolphin *Inia geoffrensis*, right: tucuxi *Sotalia fluviatilis*). Photographs by Fernando Trujillo.

Table 6.2 Human stressors and definitions for each impact category. Codes for impact categories are (0) no disturbance reported, (1) low disturbance, (2) medium disturbance, (3) high

disturbance. Some human stressors do not include high impact categories (-).

Human Stressors		Impact Categories [Range distance from the study area]							
		Low (1)	Medium (2)	High (3)					
I. Water Quality									
1. Oil exploitation	Any size	100-200 km	50-100km	Within 50km					
2. Tourism	Tourist resorts	50-100 km	Within 50km	-					
3. Ship traffic	Commercial, fishing, naval or transportation	Sporadic routes	Known shipping routes	-					
4. Mining	Any	100-200km	50-100km	Within 50km					
II. Habitat Modif	fication								
5. Dams	Any size	500-1000km downstream or 100-200 km upstream	200-500km downstream or 50-100 km upstream	Within 200km downstream and/or within 50km upstream					
6. Waterways	Any size	500-1000km downstream or 100-200 km upstream	500-1000km downstream or 100-200 km upstream	Within 200km downstream and/or within 50km upstream					
III. Exploitation									
7. Entanglements/killing of dolphins to avoid competition for resources (fish)		Rare (recorded once or twice in the area)	Occasional (recorded once per year)	Frequent (recorded at least once per month)					
8. Killing of river dolphins for bait	Number of dead dolphins killed for bait	Rare (recorded once or twice in the area)	Occasional (recorded once per year)	Frequent (e.g. mota fishery established in the area)					
9. Fisheries		Subsistence	Commercial, main destination is cities within the river basin	Commercial, main destination is cities within and outside the river basin					
IV. Cities and hu	man settlements								
10. Human population size		Less than 100.000	Between 100.000 - 200.000	More than 200.000					

Note: 200km is the maximum distance surveyed of a 6 m boat with an outboard 25 hp engine (e.g. tourism boats). 100 km is the maximum distance surveyed per day on a boat/canoe (e.g. "peque-peque", mostly used by local communities) pushed by a home-made propeller or small engine. 5 km is the maximum distance surveyed in a canoe per day. Ships are larger than boats and have capacity for more than 10 people. Locations not defined under low (1), medium (2), or high (3) threat categories are ranked as not-known in the area (0).

Table 6.3 Overall score index for each impact category (High, Medium and Low, see Table 6.2) and risk trend levels of freshwater ecosystem degradation. The overall score index is the sum of the means over the four main categories of human stressors (water quality, habitat modification, exploitation of species, cities and human settlements, Table 6.2).

Overall S	Score Index	Summing over the different types of human stressors				
High	Majority of human stressors are classified as high or medium, no stressors are classified as low	≥ 4				
Medium	Majority of human stressors are classified as medium	≥ 3 and < 4				
Low	Majority of human stressors are classified as low or no known, no stressors are classified as high	< 3				

Risk Trend (within the next 10 years)



Risk is expected to increase (e.g. construction of water development projects planned, increase number of oil stations, etc.)

Risk is expected to remain similar, although some increase in human population size is expected.



Risk is expected to reduce given some conservation or management actions in the area.

Risk not recorded in the area, and not expected to appear in the next year.

6.2.2 River Dolphin Abundance Estimates

Boat-based surveys were conducted between May 2006 and August 2007 in selected large rivers of the Amazon and Orinoco river basins (see details in Gomez-Salazar et al., 2012). From that study, we obtained abundance estimates for two species of river dolphins (*I. geoffrensis* and *S. fluviatilis*) in 9 areas, which comprise 12 rivers in 5 countries (Figure 6.1). Abundance estimates were expressed as 1) river dolphin densities, or the number of dolphins per square kilometer surveyed (number · km⁻²), 2) mean group size of river dolphins, with group defined as a set of animals that are seen together within 250m from the boat (see Gomez-Salazar et al., 2011), and 3) sighting rates of river dolphins, or the number of dolphins per km surveyed (number · km⁻¹).

6.2.3 Correlation Between River Dolphin Abundances and Human Stress

To evaluate whether river dolphin abundance estimates correlated with the indices of human stress, the non-parametric Spearman rank correlations were calculated, and tested against the null hypothesis of no correlation between dolphin abundance measure (density, group sizes, sighting rates) and each degradation score index (overall index of freshwater degradation, water quality degradation, exploitation of species) as well as the score index of human population size for the river areas surveyed (Table 6.5).

6.3 RESULTS

6.3.1 Overall Score Indices as Indicators of Freshwater Ecosystem Degradation

Overall, we found that the Napo, Orinoco, and Meta rivers were currently the areas with the highest degradation index, the Amazon, Marañón and the Ecuadorian tributaries with medium values, and the Samiria, Iténez and Mamoré rivers with the lowest (Table 6.4). These indices were generally consistent across the four different categories of degradation (Table 6.4). In addition, human population size was significantly and positively correlated with overall degradation index and water quality (Table 6.5). This means that, as expected, those areas that are less populated have lower ecosystem degradation and better water quality. Density and sighting rates were significantly and positively correlated with each other (Table 6.5).

6.3.2 River Dolphin Abundance Estimates and Human Stressors

Density and sighting were significantly and negatively correlated with the overall degradation index, water quality degradation and human population size (Table 6.5). Higher density estimates of river dolphins occur when rivers have a low index of overall freshwater degradation, when rivers have better water quality, and in areas that are less populated (Figure 6.2). For instance, rivers with a high index of overall degradation had some of the lowest density/sighting rates of the pink river dolphin (0/0: Napo River in Ecuador; 0.57/0.11: Meta river in Colombia, respectively) and tucuxi (0/0: Napo River in

Ecuador; 1.06/0.21: Orinoco River in Venezuela, respectively). A similar pattern was evident for density estimates and exploitation of species (Figure 6.2), although the two indices were not significantly correlated (Table 6.5).

Group size did not appear to be closely related to the other two abundance estimates nor to any of the indices of human stress. In addition, neither group sizes nor sighting rates were significantly correlated with species exploitation (Table 6.5). Finally, for the majority of human stressors the risk of freshwater ecosystem degradation was expected to increase and no stressors were expected to decrease (Table 6.4).

Table 6.4 Score index and risk trends of freshwater ecosystem degradation, and human population size across the nine areas surveyed (Figure 6.1). The direction of the arrows indicates whether the risk of degradation is expected to increase, decrease or remain the same (see Table 6.3). Overall score index is the sum of the means over the four main categories of human stressors (water quality, habitat modification, exploitation of species, cities and human settlements, Table 6.3).

-	Countr	y (Ri	ver)															
Human Stressors	Venez (Orino		Color (Me		Ecua (Na		Ecuado (Tributa		Colon (Ama:		Peru (Marai		Peru (Sam		Boli (Iter		Boli (Mam	
Overall Score Risk	4.5		4.5		4.2		3.8		3.5		3.4		2.3		2.3		2.9	
I.Water Quality	2.5		2.5		2.5		2.5		1.5		1.8		1.0		1.0		1.3	
 Oil Tourism Ship traffic 	3 2 2	↑ ↑	3 2 2	↑ ↑	3 2 2	↑ ↑	3 2 2	↑ ↑ ↑	0 2 2	↑	2 2 2	↑ ↑	0 2 1	↑ ↑	0 2 1	↑	0 2 2	↑
4. Mining ⁽¹⁾	3	Ť	3	<u>†</u>	3	<u> </u>	3	Ť	2	<u>†</u>	1	$\stackrel{'}{\rightarrow}$	1	$\stackrel{'}{\rightarrow}$	1	$\stackrel{'}{\rightarrow}$	1	Ť
II. Habitat Modification	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
5.Dams6.Waterways	0	1	0	↑	0	↑	0	↑	0		0		0		0	↑	0	↑
III. Species exploitation	2.0		2.0		1.7		1.3		2.0		1.7		1.3		1.3		1.7	
7.Entanglements 8. Killing of	2	\rightarrow	2	\rightarrow	2	\rightarrow	2	\rightarrow	2	\rightarrow	2	\rightarrow	2	\rightarrow	2	\rightarrow	2	\rightarrow
river dolphins for bait	1	1	1	1	0	1	0	1	1	1	0	1	0	1	0	1	1	1
9. Fisheries	3	1	3	↑	3	↑	2	↑	3	↑	3	↑	2	↑	2	↑	2	1
IV. Cities and human settlements 10. Human Population Size	432,4	109	181,2	276		20	54,695		117,1	125	437,6	65	92,0	000	94,2	210	195,	844

⁽¹⁾ Particularly gold mining implies possible contamination by mercury residuals. (2) Rivers Aguarico, Cuyabeno, Yasuni and Lagartococha, (3) River Amazon – named Marañon at the location surveyed, (4) Freshwater Protected Area.

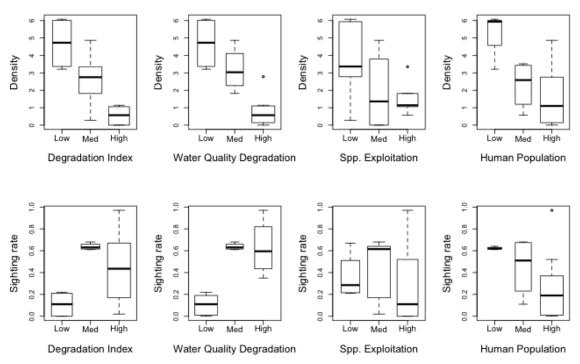


Figure 6.2 River dolphin abundance estimates (density: number · km-2 and sighting rates: number · km-1, from Gomez-Salazar et al., 2012) according to score indices of freshwater ecosystem degradation (overall score index of degradation, water quality degradation, species exploitation and human population size, Table 6.4) for the nine areas surveyed between 2006 and 2007 (Figure 6.1). Box plot includes median, lower and upper quartile, sample minimum and sample maximum.

Table 6.5 Spearman's rank test correlation results between river dolphin abundance estimates (Gomez-Salazar et al. 2012) and degradation index of freshwater ecosystems (Table 6.4) for the 9 areas surveyed between 2006 and 2007 (Figure 6.1).

	Sighting Rate	Density	Species	Degradation Index	Water Quality Degradation	Species Exploitation	Human Population
Group Size	0.49	0.42	0.23	-0.10	-0.18	0.34	-0.17
Sighting Rate	-	0.86*	0.09	-0.70*	-0.81*	-0.21	-0.48*
Density		-	0.05	-0.81*	-0.83*	-0.45	-0.64*
Species			-	0.11	0.13	0.06	0.17
Degradation Index				-	0.91	0.72	0.73*
Water Quality Degradation					-	0.49	0.81*
Species Exploitation			21.1			-	0.33

Emboldened*: correlation is significant at the 0.01 level.

6.4 Discussion

6.4.1 River Dolphins: Indicator, Sentinel and Flagship Species in Large Tropical Rivers

Our study illustrates that both density estimates and sighting rates are good ecological indicators of overall freshwater ecosystem degradation, water quality degradation and human population size, and given that they are well correlated, obtaining only one of these estimates may be sufficient to use as indicator. Density, in addition, seems to respond more linearly to the levels of overall ecosystem degradation, water quality and human population size (Figure 6.2). Also sighting rate will depend on the methodology of the survey, which may vary between studies, whereas density estimates correct for this. In summary, we recommend using density estimates of river dolphins as an indicator of freshwater ecosystem degradation. The results of this study are in line with previous studies that also found the density of top predators decreases with increasing human stressors (Sandin et al., 2008; Baum and Worm, 2009).

In contrast, there is no correlation between freshwater ecosystem degradation and the dolphins' group sizes. Group sizes vary according to habitat type and seasonality, responding to changes in the aquatic productivity and availability of resources (McGuire and Winemiller, 1998; Gomez-Salazar et al., 2011). Hence, group sizes may change mainly as a response to ecological factors, and for this reason group sizes may not to be good indicators of ecosystem degradation.

Using river dolphins as indicator species to develop monitoring programs and assessments of freshwater ecosystem degradation has multiple advantages, including the fact that (1) river dolphins are typically distributed in all habitat types of the Amazon and Orinoco river basins, with the exception of rapids and areas with very high ecosystem degradation (e.g., Napo River); (2) river dolphins are relatively easy to observe in an ecosystem where the majority of species occur under waters of high turbidity; (3) river dolphin surveys are relatively easy to conduct (see Gomez-Salazar et al., 2012) and require less sample processing compared with surveys of some other potential indicators such as invertebrates and algae; (4) river dolphins are long-lived and thus provide

responses to long-term risks of ecosystem degradation; (5) potentially, river dolphins can act as sentinel species by providing early warnings about current or future increases in ecosystem degradation, such as bioaccumulation of heavy metals or other contaminants, as previously studied in other aquatic mammals (e.g., Wells et al., 2004; Bossart, 2011); and finally (6) river dolphins are a charismatic species, thus, can be used as flagship species to raise public awareness about conservation and management issues (Sergio et al., 2008). However, abundance estimates of other taxonomic groups, such as aquatic birds or fishes, might complement, and add to the value of, dolphin-based assessments of ecosystem degradation (Karr, 1999; Piatt et al., 2007; Navarro-Llácer et al., 2010). For example, the methodology and platforms used to conduct river dolphin surveys (Gomez-Salazar et al., 2012) could be used to survey other taxonomic groups such as aquatic birds and reptiles.

6.4.2 Current and Future Freshwater Ecosystem Degradation in the Amazon and Orinoco

Due to limitations on data availability, most of the score indexes used in this study are the result of presence and proximity of human activities rather than the proximate stressors that result from these activities. For instance, when considering gold mining, mercury used for the separation of gold particles is discharged into rivers and soils where it threatens biodiversity (Pfeiffer et al., 1993). However, there are no reliable statistics in our study areas on mercury release, so we use the presence and proximity of gold mining as part of the score index. Despite these limitations, the degradation index was correlated with human population size, which suggests the scores are providing effective measures of degradation.

The majority of the human stressors considered in this study are expected to increase due to growing human population numbers and to the growing interest in economically developing the Amazon and Orinoco basins (Laurance et al., 2005, Table 4). Areas with both high and low indices of freshwater degradation were within rivers that are planned to be dramatically altered by water development projects or oil exploration in the near

future (Table 6.4). For example, waterways are being planned in the Meta, Napo, Iténez and Mamoré rivers.

6.4.2.1 Areas with Highest Overall Score Index of Freshwater Degradation

The Napo, Orinoco and Meta Rivers were identified as rivers with the highest degradation. This is the result of intense oil exploration and development in these areas, which in turn has caused multiple crude spills, increased road construction to facilitate accessibility in the area, increased ship traffic to transport people and machinery, increasing forest colonization, land speculation, commercial hunting and thus large socioeconomic changes in the regions (Laurance et al., 2005; Portocarrero-Aya et al., 2010; Trujillo et al., 2010a; Utreras et al., 2010). Industrial farming, deforestation, expansion of cattle ranching, the use of pesticides and chemical fertilizers, as well as fishing with chemicals and explosives have also been documented. There is growing interest in expanding industrial farming and oil exploration, and waterways are being planned (Trujillo et al., 2010a). For instance, the commerce and navigability in the Napo is expected to increase as a result of the construction of the Manta-Manaus transportation corridor to connect the Pacific coast and the Brazilian central Amazon (Utreras et al., 2010; Utreras, 2011).

6.4.2.2 Areas with Lowest Overall Score Index of Freshwater Degradation

SSamiria, Iténez and Mamoré Rivers were identified as rivers with lowest degradation. In fact, these areas have relatively low human stressors when compared with the other areas surveyed (McGuire and Aliaga-Rossel, 2010; Tavera et al., 2010; Gomez-Salazar et al., 2012). The Samiria River is located within the Pacaya-Samiria National Reserve, which is a well-managed freshwater protected area. However, waterways to improve navigability are planned in the Itenéz, Mamoré, and Madeira Rivers, and a hydroelectric dam is planned in the Madeira River (Tavera et al., 2010).

6.4.2.3 Additional Human Stressors

There are major human stressors that were not considered in our risk score assessment and that might have an impact on river dolphin populations, such as land-use changes, climate change, chemical pollution and aquatic noise. Major land-use changes, for instance, have a direct effect on the aquatic ecosystems by, for example, altering the flooding pattern and causing erosion and sedimentation (Laurance, 1998). Climate change will alter the biosphere on a larger scale and is expected to accelerate but its consequences in the Amazon and Orinoco have not been extensively investigated (Alcamo et al., 2005; Laurance et al., 2005). In addition, aquatic noise has been documented to affect large parts of marine ecosystems, including marine mammals, fishes and invertebrates (Weilgart, 2007) and its effects on freshwater ecosystems are unknown.

Finally, the capture of dolphins for use as bait is one of the most serious human stressors that might increase in the near future. For instance, in the central Brazilian Amazon, approximately 600 pink river dolphins are killed each year for bait (Loch et al., 2009), and if this activity spreads from Brazil to our study areas, the population sizes and densities of dolphins will likely decline within a few years (Gomez-Salazar et al., 2012).

6.5 SUMMARY OF CHAPTER 6 AND TRANSITION TO CHAPTER 7

Human stressors are currently impacting both the Amazon and Orinoco river basins and these are likely to increase. However, there is a lack of standardized monitoring programs to track these human stressors in most of the countries that overlap these basins, and no clear ecological indicators have been identified. In this study we investigated the relationships between measures of ecosystem degradation and river dolphins as potential ecological indicators. The presence of human stressors and their distance from the areas surveyed were used to provide an estimate of ecosystem degradation. We tested three ecological indicators of freshwater ecosystem degradation using river dolphins: (i) density of river dolphins, (ii) mean group size of dolphins, and (iii) dolphin sighting rates. We found a strong negative relationship between measures of habitat degradation and

river dolphin density estimates in selected locations of the Amazon and Orinoco. Therefore, we suggest that river dolphins are good candidates as ecological indicators, flagship and sentinel species for monitoring the conservation status of large tropical rivers in South America. We suggest that further effort should be directed toward collecting reliable data on human stressors, creating collaborative networks for compiling existing data, and documenting and monitoring current trends in freshwater ecosystem degradation and indicator species in the Amazon and Orinoco basins with the goal of targeting areas for recovery or sustainable management.

CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

The Amazon and Orinoco river basins are areas of high biodiversity and of global conservation importance. According to global assessments, these river basins have some of the lowest levels of ecosystem degradation among the world's rivers (Vörösmarty et al. 2010). This dissertation, however, illustrates that human stressors in these basins are increasing due to rising human population numbers, and the emergence of large water development projects. These projects will open commercialization networks, and improve navigability and road accessibility (Laurance et al. 2005, Trujillo et al. 2010). Hence, raising awareness about impending changes in these river basins, as well as developing monitoring programs to track ecosystem condition is of high conservation priority.

River dolphins are top predators that inhabit the Amazon and Orinoco basins. Top predators not only have the ability to act as flagship species to successfully motivate conservation actions, but can also act as indicators of ecosystem condition (e.g., Sergio et al. 2008). However, the efficacy of using river dolphins as indicators of ecosystem conditions was unknown. For this reason, I evaluated the potential of using river dolphins as indicators of ecosystem degradation in large tropical rivers of South America. From the onset, however, the lack of data on population estimates of rivers dolphins and information on human stressors in the Amazon and Orinoco was evident. Therefore, I developed methods using field surveys and robust data analysis to obtain population assessments of river dolphins and measurements of ecosystem degradation.

7.1.1 Key Findings on River Dolphins

Inia geoffrensis and *Sotalia fluviatilis* remain listed by the IUCN as *Data Deficient*, partially because of a lack of population data. Moreover, the status of *Inia boliviensis*, if considered a separate species, has yet to be established. This dissertation, therefore, reduced the deficiency by providing population estimates of river dolphins in South America, using line-strip transect surveys and mark-recapture methods on photo-identification (Chapters 2, 3, 4 and 5). This information will be useful as a baseline for future monitoring programs.

In addition to providing population estimates, I investigated the relationship between these dolphin population estimates and ecological features (Chapter 2). River dolphins do not have natural predators and inhabit complex and dynamic systems with strong and predictable seasonal variability. These features partially explained why river dolphins have some of the smallest group sizes among cetaceans, but also vary according to habitat type, season and river basin. In the Amazon, for instance, group sizes are largest during the low water season when resources are concentrated. In the Orinoco, group sizes are largest during the transitional water season when the aquatic productivity increases (Chapter 2).

Subsequently, by examining information on river dolphins at a regional scale, I identified critical habitat for river dolphins in South America. These critical habitats are located within 200 m of the riverbanks, confluences and lake habitat types (Chapters 2 and 3). This designation is important when proposing policy and management actions (e.g., regulation of dolphin watching activities). I also proposed hot spots of river dolphins in South America, as those locations with high-density estimates of *Inia* and *Sotalia*, which also coincide with low human stress (Chapters 3 and 6). In addition, I identified areas of concern for river dolphins in South America, as those locations with the lowest density estimates of *Inia* and *Sotalia* and with high human stress (Chapters 3 and 6).

Finally, I examined the relationship between dolphin estimates and human stressors with the ultimate goal of evaluating the potential of using river dolphins as indicators of freshwater ecosystem degradation (Chapters 3 and 6).

7.1.2 Linking Information on River Dolphins and Human Stressors to Measure Ecosystem Degradation

Originally, I planned to evaluate the relationship between human stressors and group size and abundance of river dolphins in the Colombian Amazon (Chapter 2 and 5) across multiple years. The ultimate goal of this evaluation was to develop a temporal framework (see Chapter 1) to provide information on the effects of human stressors on river dolphin estimates based on past and current information. However, due to the lack of data available on human stressors, I decided to only consider a geographic framework (see (Chapter 1) to rank current ecosystem degradation among locations. To develop this geographic framework, I calculated group sizes, sighting rates and density estimates of river dolphins in selected areas of the Amazon and Orinoco in Colombia, Bolivia, Ecuador, Peru and Venezuela (Chapter 3). For each of these areas, I developed an estimate of ecosystem degradation based on the presence of human stressors and their distance from the areas surveyed (Chapter 6). Subsequently, I investigated the relationship between river dolphin population estimates and estimates of ecosystem degradation. A significant negative relationship was found between degradation and dolphin densities. Higher densities of dolphins occurred in rivers with low indices of overall freshwater degradation, such as rivers with high water quality and the lowest human population numbers (Chapter 6).

Based on these findings, I propose that river dolphin density estimates can be good indicators of degradation. Consequently, the densities of river dolphin are worth including in ecosystem degradation assessments as direct measurements of biological features in the Amazon and Orinoco. Furthermore, river dolphins can also act as flagship and sentinel species for monitoring the conservation status of large tropical rivers in South America.

7.2 RECOMMENDATIONS FOR POTENTIAL FUTURE WORK

7.2.1 Methods to Study River Dolphins: Line-Strip Transects *vs.* Mark-Recapture Methods on Photo-Identification

I used two main methods to study river dolphins: line-strip transects and mark-recapture methods on photo-identifications. The decision regarding which method should be used will depend upon the duration and the main goals of the projects. For instance, mark-recapture methods on photo-identification are an efficient tool for long-term monitoring programs of pink river dolphins (Chapters 4 and 5). Increasing photo-identification effort and observation in the field will generally lead to an increase in the number of dolphins identified, provide information regarding the sex of individuals based on their close associations with calves (e.g., Read et al. 2003), and allow results to be compared with other similar catalogues already existing in the Amazon and Orinoco (e.g., McGuire and Henningsen 2007).

In some instances, however, long-term monitoring programs cannot be established due to constraints in logistics and funding availability. As a result, when some study areas can only be surveyed opportunistically and it is not possible to develop photo-identification, line-strip transect methods are a cost-effective tool for estimating group sizes, sighting rates, density and population sizes of river dolphins (Chapter 3).

While the population estimates obtained through mark-recapture methods correspond to the number of animals using the area, the estimates obtained through distance sampling methods correspond to the animals that were present in the area during the time of the survey (Hammond 2009, Hammond 2010). Further studies could compare both methods by conducting surveys in areas where monitoring programs are being developed (e.g., Colombian Amazon and Orinoco) with the goal of improving abundance and density estimates of river dolphins.

7.2.2 Further Studies on Rivers and Dolphins in South America

I suggested that river dolphin density estimates might be good indicators of ecosystem degradation in the Amazon and Orinoco basins. Their value as indicators can be further tested at a river basin scale. To do this, I recommend directing research to areas where previous surveys already exist to facilitate the development of monitoring programs. Research should also focus on additional *key* locations in the Amazon and Orinoco. To make comparisons between areas as valid as possible, future effort in these *key* locations should carefully select the time of the year to conduct surveys and the size of the areas surveyed. Priority of additional *key* locations should be based on the following criteria:

i) Potential Hot Spots and Areas of Concern

By using the ecosystem degradation index developed in this dissertation, areas with low risk of ecosystem degradation (potential hot spots) and areas of high risk of ecosystem degradation (areas of concern) can be identified. Subsequently, surveys could be conducted in these selected areas to continue evaluating the role of dolphins as indicators, and ultimately to continue monitoring *key* areas of the Amazon and Orinoco.

ii) Representative Sub-basins

This dissertation surveyed less that 1% of the entire Amazon and Orinoco river basins, and only included information on river dolphin populations that inhabit 3 out of the 14 sub-basins described for the Amazon (UNEP 2004). Further studies should prioritize *key* areas located in sub-basins that have not yet been studied to obtain a more representative picture of the conservation status of these freshwater ecosystems.

iii) Upcoming Water Development Projects

Surveys in areas where water development projects are proposed, or are at early stages of construction, are particularly important because of the future changes that those drainage areas will face due to the construction of dams and water ways. Dams, for instance, are built for flood control, irrigation and hydroelectric power, but the final outcome often does not meet the expected economic benefits and instead generates major environmental, social, and health impacts (WCD 2000). In terms of biodiversity and

ecological processes, the construction of dams can fragment populations, reduce river flow, affect river pulses, change the water quality, and ultimately contribute to the extinction of many species (WCD 2000), including perhaps river dolphins. Hence, further studies should survey areas of the Amazon and Orinoco basin river basins before and after the construction of water development projects to investigate their effects on top predators and on the river system itself. Such studies will ultimately raise awareness about the potential impacts of additional dams in these regions.

In relation to this final recommendation, I have proposed a study to conduct a multi-day boat-based survey, following standardized methods described in this dissertation, to determine population size, density, habitat selection and distribution of river dolphins across a significant part of the Xingu River in Brazil. This study will ascertain the current conservation status of these dolphins populations, will enable the tracking of changes in abundance population trends as well as on habitat selection, and ultimately will provide information to help establish the current conservation status of the Xingu river basin before and after the construction of what will be the third largest dam in the world: Belo Monte.

7.3 FINAL REMARKS

Large changes in the Amazon and Orinoco are approaching fast. A growing human population in these and near-by areas will increase the demand for resources, and thus will stimulate more deforestation and construction of large water development projects.

"Although the precise degree of freshwater impoverishment remains to be fully documented, there can be little doubt that the losses are already great"

- Harrison and Stiassny 1999 in Revenga et al. 2000.

To raise awareness and recommend management and policy actions, the degree of freshwater impoverishment needs to be carefully documented. For this, I strongly suggest the use of ecological indicators, flagship and sentinel species. These species can become science-based conservation tools. Studies of them not only document freshwater

ecosystem degradation, but also can raise awareness about broader implications of stressors on biodiversity and river systems. In this thesis, I suggest that river dolphins can be used in this way to direct conservation efforts in large tropical rivers of South America. In addition, I encourage further studies to test a similar approach using other wildlife species, such as aquatic birds, that have similar potential as indicator, flagship and sentinel species.

"So here is my farewell to a wild river. This will be the beginning of the end of the Amazon region as we know it."

These were Cristina Goettsch Mittermeier's concluding remarks in her article "Farewell to a Wild River", referring to the construction of the Belo Monte dam in the Amazon basin, as a symbol of change that will profoundly impact both the biodiversity and local communities that depend on these river systems.

I hope that efforts to document freshwater ecosystem degradation using river dolphins and other wildlife species will help us avoid giving a final farewell to these wild rivers.

Appendix 1 PUBLICATIONS

The work presented in Chapter 2 also appears in:

Gomez-Salazar, C., F. Trujillo, H. Whitehead. 2011a. Ecological factors influencing groups sizes of river dolphins (*Inia* and *Sotalia*). Marine Mammal Science. DOI: 10.1111/j.1748-7692.2011.00496.x

The work presented in Chapter 3 also appears in:

Gomez-Salazar, C., F. Trujillo, M. Portocarrero-Aya, H. Whitehead. 2012. Population, density estimates and conservation of river dolphins (*Inia* and *Sotalia*) in the Amazon and Orinoco river basins. Marine Mammal Science 28 (1): 124 - 153 DOI: 10.1111/j.1748-7692.2011.00468.x

The work presented in Chapter 4 also appears in:

Gomez-Salazar, C., F. Trujillo, H. Whitehead. 2011b. Photo-identification: a reliable and non-invasive tool for studying Pink River Dolphins (*Inia geoffrensis*). Aquatic Mammals. 37(4), 472-485, DOI 10.1578/AM.37.4.2011.472

The work presented in Chapter 5 was submitted for publication:

Gomez-Salazar, C., F. Trujillo, H. Whitehead. Population size estimates of pink river dolphins using mark-recapture methods on photo-identification. Latin American Journal of Aquatic Mammals.

The work presented in Chapter 6 was accepted for publication:

Gomez-Salazar, C., Coll, M., H. Whitehead. River dolphins as indicators of ecosystem degradation in large tropical rivers. Ecological Indicators.

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