CONSERVATION ECOLOGY AND PHYLOGENETICS OF THE INDUS RIVER DOLPHIN (*PLATANISTA GANGETICA MINOR*)

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Conservation Ecology and Phylogenetics of the Indus River dolphin (*Platanista gangetica minor*)

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An Indus dolphin breaks the surface in view of a barrage. Photo credit: Aftab Rana, Adventure Foundation Pakistan

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Abstract

The historical range of the Indus River dolphin has declined by 80% since the 19th century and has been fragmented into 17 river sections by construction of irrigation barrages. Dolphin sighting and interview surveys showed that river dolphins persist in six river sections, have been extirpated from ten, and are of unknown status in the remaining section. Logistic regression and survival modelling showed that low dry season river discharge was the primary factor responsible for the Indus dolphins range decline.

Abundance of the three largest Indus dolphin subpopulations was estimated using tandem vessel-based direct counts, corrected for missed animals using conditional-likelihood capture-recapture models. The entire subspecies was estimated to number between 1550-1750 in 2006. Dolphin encounter rates within the Guddu- Sukkur subpopulation (10.35/km) were the highest reported for any river dolphin and direct counts suggest that this subpopulation may have been increasing in abundance since the 1970s when hunting was banned.

The dry season habitat selection of Indus dolphins was explored using Generalised Linear Models of dolphin distribution and abundance in relation to river geomorphology, and channel geometry in cross-section. Channel cross-sectional area was shown to be the most important factor determining dolphin presence. Indus dolphins avoided channels with small cross-sectional area <700m^2, presumably due to the risk of entrapment and reduced foraging opportunities.

The phylogenetics of Indus and Ganges River dolphins was explored using Mitochondrial control region sequences. Genetic diversity was low, and all 20 Indus River dolphin samples were identical. There were no haplotypes shared by Indus and Ganges River dolphins, phylogenetic trees demonstrated reciprocal monophyletic separation and Bayesian modelling suggested that the two dolphin populations diverged approximately 0.66 million years ago.

Declining river flows threaten Indus dolphins especially at the upstream end of their range, and it is important to determine how much water is required to sustain a dolphin population through the dry season. Fisheries interactions are an increasing problem that will be best addressed through localised, community-based conservation activities.

Acknowledgements

Chapter 2 - The 2006 dolphin survey expedition covered a significant portion of Pakistan's territory, passing through remote and often insecure areas in three Provinces. As much as a scientific endeavour it was a logistical and security operation and its success is attributed to the collaboration and facilitation of numerous different organisations and government departments in Pakistan, in particular: Sindh Wildlife Department, Punjab Wildlife Department, NWFP Wildlife Department, Adventure Foundation Pakistan, WWF-Pakistan, Ministry of Environment, Sindhi tribal leaders, the Punjab, Sindh and NWFP Police Forces, Punjab and Sindh Irrigation Departments, Water and Power Development Authority (WAPDA) and the staff at Jinnah, Chashma, Taunsa, Guddu, Sukkur and Kotri Barrages. The logistical support that underpinned the success of the expedition was ably undertaken by the expert volunteers at the Adventure Foundation Pakistan: Rauf Ahmed, Mubashar Azam, Mohammad Abuhu, Aftab Rana and Imdad. Huge credit is due to the scientific teams that worked long hours in hot, remote and sometimes dangerous situations: Abdul Haleem, Abdul Razzaq, Ashfaq Khan, Albert Reichert, Babar Hussain, Khalil Kundi, Kunwer Javed, Malik Farooq Ahmad, Mohammad Saleem Chaudhry, Rafiq Rajput, Samiullah Khan, Syed Athar Hussain, Tahir Ehsan, Uzma Khan, Zafar Ali, Shabir Ahmad, Muhammad Hamid, Iqbal Khaskheli, Amir Buksh Bullo and Zahid Bhatti. Valuable statistical input was provided by Sharon Hedley.

The survey was funded by the Pakistan Wetlands Programme of WWF-Pakistan, that itself received funding from UNDP-GEF and the Embassy of the Kingdom of the Netherlands, the Whale and Dolphin Conservation Society – UK and WWF-Pakistan. ASTER Satellite images were provided by NASA specifically for this project and were prepared by Raza Shah and Urooj Saeed.

Chapter 3 - I would like to extend thanks to my colleagues Uzma Noureen, Masood Arshad and Najam ul-Huda who travelled with me for ten days around Punjab conducting interviews with numerous colourful characters. In every town visited the fishing community was located, usually a very poor settlement of grass houses and charpai's, and we requested to speak with the oldest fishermen present. Old men were wheeled out, carried out and hobbled out of their houses to speak with us. They told us fascinating stories of a bygone era. Some were sweet, some were cantankerous,

some were grandfatherly, and all were memorable. In addition to information on the decline of the Indus dolphin, we also collected information on the decline of river otters, gharial and crocodile, and the illegal trade in freshwater turtles. Ahmad Khan, Masood Arshad and Richard Garstang facilitated and encouraged the interview surveys and had faith that they would show interesting results. The initial idea for this work grew from discussions with Sam Turvey about the decline of the Yangtze River dolphin. The survey was funded by the Pakistan Wetlands Programme. The Marine Mammal Commission supported my attendance at the 19th Biennial Conference of the Society of Marine Mammalogy in Tampa, Florida, where I presented the information from this chapter, "*Causes and dynamics of Indus River dolphin range decline*" and received the J. Stephen Leatherwood Memorial Award for conservation of marine mammals in South and Southeast Asia.

Chapter 4 - The dolphin survey data used in this chapter was collected in 2001 and 2006 by a large number of individuals from numerous organisations including Sindh Wildlife Department, Punjab Wildlife Department, NWFP Wildlife Department, Zoological Survey Department and WWF-Pakistan. The cross-section study was devised and conducted by Albert Reichert with assistance from Tahir Ehsan and the participation of many members of the 2006 dolphin survey team. Technical advice and guidance on the geomorphic classification and cross-section calculations was provided by Albert Reichert, preliminary classification of river geomorphology was conducted by Samiullah Khan at PWP and Jason Alexander at the US Geological Survey provided invaluable assistance and review of the geomorphic aspects of the chapter. Vital statistical guidance was provided by Mike Lonergan at SMRU. Valuable reviews of this chapter were contributed by Mike Lonergan, Albert Reichert, Jason Alexander, Uzma Khan, Gianna Minton and Tim Collins. Satellite images were generously provided by Michael Abrams at NASA. Funding was provided by the Whale and Dolphin Conservation Society, WWF-Pakistan and the Ministry of Environment's Pakistan Wetlands Programme, which receives its support from UNDP GEF and the Royal Netherlands Embassy.

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Financial support during all my studies was provided by the British Federation of Women Graduates, the University of St. Andrews, and the US Marine Mammal Commission.

It was Brian Smith who first introduced me to river dolphins when he encouraged me to join him on a survey of the Karnaphuli River in eastern Bangladesh in early 1999. I was captivated by Bangladesh and the river dolphins and that survey started me off along this current path. My brother Rich was the one who encouraged me to go to Pakistan in the first place, having spent two years volunteering with WWF-Pakistan conducting raptor surveys in the Himalaya and Karakoram mountains. I doubt I would have ever gone to Pakistan without this introduction, and it is unfortunate that our stays never did overlap. Richard Garstang, Conservation Advisor with WWF-Pakistan answered my original letter, and over the following 12 years has facilitated, encouraged and supported my work. Nobody loves wildlife, or Pakistan, more than Richard. Randy Reeves was the one who kept me going. His quiet and constant support and encouragement gave reassurance that someone in the outside world was paying attention and that what I was doing had value outside Pakistan as well.

WWF-Pakistan gave me a home and a family in Pakistan, have been supportive of my work, and tolerant of my mistakes. I have spent months in the field with many good friends, and hope that there will be many more expeditions to come. I have so many friends and colleagues in Pakistan to thank: Samiullah Khan, Zafar Ali, Mubashar Azam, Uzma Noureen, Uzma Khan, Masood Arshad, Ahmad Khan, Ghulam Akbar, Ejaz Ahmed, Ali Habib, Mehjabeen Abidi-Habib, Hussain Bux Bhagat, Mumtaz Malik, Imtiaz Tajwer, Khalil Kundi, Tahir Ehsan, Nabila Khan, Shafaq Masood, Umer Waqas,

Babar Hussain, Rafiq Rajput, Najam ul-Huda, Abdul Haleem, Imdad, Yakoob, Khalid, Anna and Mark Pont, and Melissa Payson to name but a few.

At the beginning of this PhD project, I spent 1 torturous year waiting in Islamabad for a permit from the Home Department to allow me to travel to the field. Doug and Humera I can never thank you enough for putting me in touch with Salman who finally solved the problem in less than a week. Salman, I am forever grateful to you. Despite my permit, once in the field conducting the range wide survey described in Chapter 2, I was detained at Dera Ghazi Khan and kept under house arrest for a week with my own troop of black burka-clad female ninja guards present night and day. When we were released it was on the condition that we not survey the remaining 300 km of river in Punjab because of security concerns (specifically the 'Bosun Gang'). We used cranes to put the boats on trucks and drove with an escort of police vehicles with mounted machine guns to Sindh where the survey resumed. That is why survey coverage of the Taunsa to Guddu river section (subpopulation 3) was incomplete in 2006. In general, the Pakistani authorities have been very tolerant of the dolphin survey expeditions that we have conducted over the years, they have cooperated with us to minimize the risks in tribal and insecure areas while attempting to allow us to do our job, despite their lack of understanding about what we were doing or why we doing it.

My friends in St. Andrews: Alice, Sol, Theoni, Danielle, Becky, Sonja, Marjolaine, Rene, Inez, Aaron, Gwen, Cormac, Sanna, Tess and so many more made life in St. Andrews a great deal of fun. My friends in Rwanda: Katie and Glenn, Barbara, Katie K, Maria, Christelle, Catherine, Thierry, and Christina, gave me a support team, even though dolphins, the ocean, Pakistan, and the university were a million miles away. Gianna, thank you for your support, you and your project in Sarawak are an inspiration. Moth, those lively marine mammal discussions were thoroughly enjoyable, and your endless enthusiasm and energy for conservation keeps me on track. Thank you to my old friends GillyC, Jo Gaps, Kate G, and Victoria, who always manage to keep tabs on me wherever I am in the world and whatever I am up to. The other crazy Asian river dolphin women: Isabel, Danielle and Dipani, just knowing that you are out there, fighting the same fight, dealing with the same issues, and working for the same goals, makes it all easier somehow. I hope we meet again soon to share our stories.

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Albert Reichert, second boat captain and river hydrologist, you were my partner in the field in Pakistan, throughout the write-up and through all the numerous other things that life has presented over the last five years (not least of which was a baby!). For supporting our family by working so hard to pay the bills for most of the last 5 years, while I was a struggling student, I am eternally grateful. You endured with me the painful months of writing, and now that it is over, I am looking forward to sharing a life that is a little more carefree, creative and adventurous.

Mum and dad, without your rock steady support I never would have had the courage to do the things I have done. Albert and Bebe, your constant interest and positive attitude helped enormously.

Pakistan is a country of passion and extremes, colour and contrast that has captivated me since I first landed there in April 1999. It has taught me many lessons, made me wiser, and provided endless adventure and challenges. The Indus River is my favourite place to be. From reading this thesis one might imagine a broken, depleted, polluted stream, but in the places where the dolphins and the flow remain, the incredible river is a huge, it threads, winds and curves around sand bars and islands in an intertwined wilderness that can leave one lost and confused. A desert river, there are no trees and few plants along the river banks, instead it sits on a bed of white sand with mica that sparkles like diamonds in the endless sunny days. People are few and far between, the view is only of water and sand, and the only sounds are of skylarks and sand pipers. Across the wide shallows there are numerous spoonbills, duck, flamingo's, cranes, egrets and herons. Hard and soft shell turtles sunbath on exposed bars. Gorgeous Indian River terns fly along with the boat and lay their eggs, exposed to intense heat, on the mid-channel sand bars. My favourite place is lying in the dark in my tent pitched on the velvet sand, a few meters from the river bank, listening to the

blind river dolphins surfacing and breathing loudly in the river a few meters away as they have done for millennia.

There are numerous young Pakistani's working in difficult circumstances and against the odds to conserve the mighty Indus River and its river dolphin. I hope that the information in this thesis will provide a small helping hand to their tireless efforts.

Chapter 1
General Introduction

1.1 South Asian river dolphins

The Indus and Ganges River dolphins (*Platanista gangetica minor,* and *Platanista gangetica gangetica,* respectively) are two closely related dolphin subspecies that occur only in the freshwater river systems of the Indian subcontinent. The Indus River dolphin occurs in the Indus River system in Pakistan and India, and the Ganges River dolphin has a larger range in India, Bangladesh and Nepal occurring in the Ganges, Brahmaputra and Karnaphuli-Sangu River systems (Fig. 1.1). The species (*Platanista gangetica*) and both subspecies are classified as Endangered by the IUCN World Conservation Union (Braulik et al. 2004; Smith et al. 2004; Smith and Braulik 2008). Both South Asian river dolphins are among the world's most endangered dolphins, and are listed as mammals of very high conservation priority due to their evolutionarily distinctiveness and threatened status (Isaac et al. 2007). Although they are charismatic and endangered mammals that may act as indicators of aquatic health (Turvey et al. in press) or flagship species for aquatic conservation, very little is known even about the basic biology of these animals, the factors involved in their decline are not well understood, and their conservation is only beginning to be addressed.

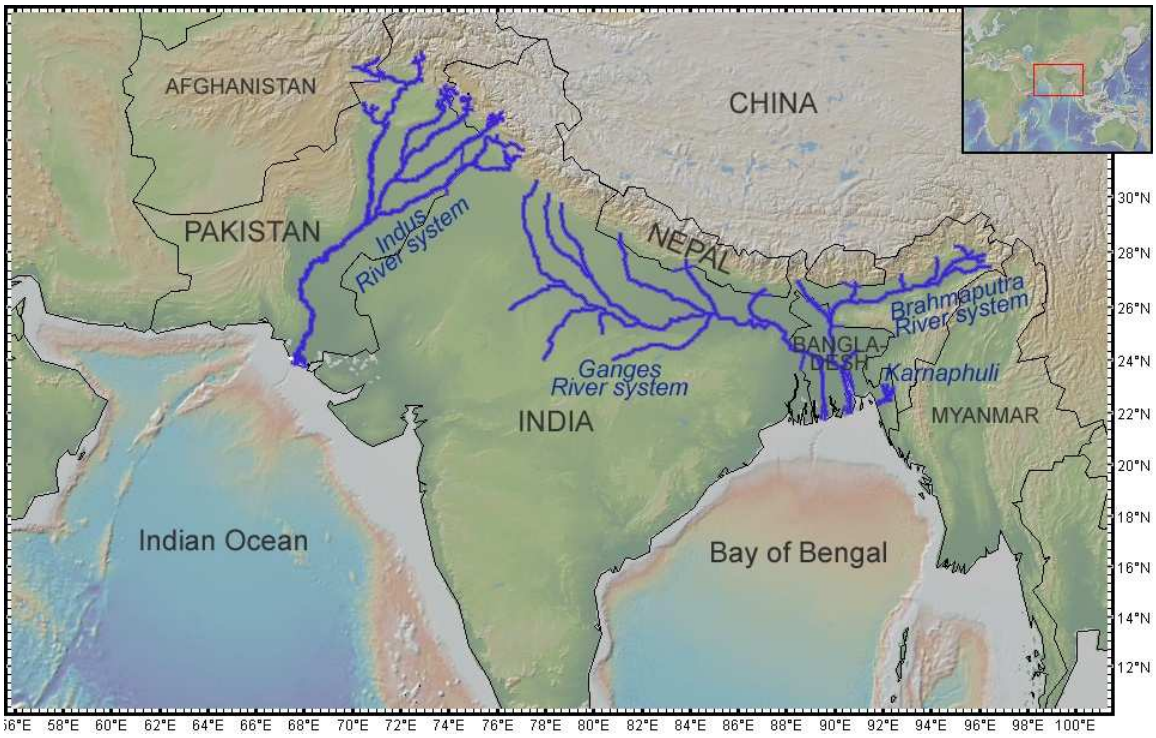

Figure 1.1 – The geography and river systems of South Asia

1.1.1 Other River Dolphin Species and Populations

River dolphins and porpoises occur only in Asia and South America. The number of recognised species and their taxonomic arrangement has changed considerably with the increasing amount and sophistication of research. In the past, because of their similar habitat and external appearance all the obligate river dolphins were classified together into a single Superfamily, the Platanistoidea. Recent genetic studies have clearly shown that they are in fact not closely related at all, each belonging to a separate family (Cassens et al. 2000; Hamilton et al. 2001; Milinkovitch and Cassens 2001). It is now believed that quite different marine cetacean ancestors colonised rivers in different geographic locations, and at greatly different times.

At present there are considered to be two species of freshwater dolphin in South America: the Amazon River dolphin *(Inia geoffrensis*) and the tucuxi (*Sotalia fluviatilis).* Current taxonomic classification considers the Amazon River dolphin to have three geographically distinct subspecies: *Inia geoffrensis geoffrensis* from most of the Amazon and the Araguaia/Tocantins River basin; *Inia geoffrensis humboldtiana* from the Orinoco River basin; and *Inia geoffrensis boliviensis* from the river systems of Bolivia, with populations in the Madeira drainage area upstream of the Teotônio rapids in Brazil (Rice 1998; Hollatz et al. 2011). It is possible that as more information becomes available additional South American river dolphin species and subspecies will be described.

In Asia the situation is more complex with several freshwater species, and then freshwater subspecies or populations of cetaceans that are otherwise marine in distribution. The baiji *(Lipotes vexillifer*) which is now extinct (Turvey et al. 2007), inhabited the lower reaches of the Yangtze River in China, which also currently hosts the Yangtze River subspecies of finless porpoise (*Neophoceana asiaeorientalis asiaeorientalis*). There are at least five freshwater populations of the otherwise coastally distributed Irrawaddy dolphin (*Orcaella brevirostris*). These are located in the Ayeyarwady River in Myanmar, the Mekong River of Cambodia, Laos and previously Vietnam, the Mahakam River of Kalimantan Province, Borneo, Indonesia, Chilika brackish water Lake, India and Songkhla brackish water lake, Thailand.

Perhaps because of their differing origins, many freshwater cetacean species have dissimilar behavioural patterns and social organisation. In addition, the types of rivers

occupied by river cetaceans encompass a wide spectrum of habitat types with substantially varying climates, geology, flow regime and surrounding terrestrial landscapes. Because of the great differences between rivers and species it can be difficult to draw meaningful comparisons between them. However, the one thing that they do have in common is that their freshwater distribution has placed them in close proximity to humans and, although the specific threats and factors driving their decline vary geographically, almost all the river dolphins are threatened with extinction (IUCN 2011).

1.2 The Indus River

The Indus River rises in Tibet, flows through NW India and enters Pakistan in the north flowing for the entire length of the country to the Arabian Sea (Fig. 1.3). It has five main tributaries; the Jhelum, Sutlej, Chenab, Ravi and Beas Rivers. These rivers merge with one another to form the Panjnad River, which then joins the Indus mainstem just downstream of Multan and Panjnad barrage. The Indus leaves the Himalayan foothills and enters the plains at Kalabagh town, 3 km upstream of Jinnah Barrage. From Kalabagh it flows at a gentle gradient (averaging 13 cm/km), primarily SSW, for approximately 1600 km to the sea.

The river runs through semi-desert and irrigated agricultural land, as well as some small remnant areas of native riverine scrub forest located between Guddu and Sukkur barrages. The river is broad, shallow and braided and naturally highly turbid. As it is sand-bedded it is constantly eroding its bed and banks, and consequently there is very little vegetation either submerged in the water, or on the banks. The configuration of channels, islands and sand bars is constantly changing, and the river channels are frequently completely re-organised during the annual flood. Temperatures in Pakistan in the summer (May to September) can rise to 50^0C and in the winter (November to February) can drop close to freezing. The vast majority of the rain falls during the monsoon between June and August. Indus River discharge is highly seasonal, with peak flows of approximately 700,000–1,000,000 cubic feet per second (cusecs)[1] (this is the unit of measure used for river discharge in Pakistan) occur between June and August when the river is fed by Himalayan melt-water and monsoon run-off, while flows as low as 12,000 cusecs[2] occur in the dry season between December and April.

[1] Approximately 20,000 to 28,000 m^3/s

[2] Approximately 340 m^3/s

The river system is highly modified and managed, and the natural flow regime has been significantly disrupted. Large-scale diversion of river water for irrigation in the dry season causes discharge to diminish as the river flows towards the Arabian Sea. For part of the dry season the river is dry downstream of Kotri barrage and no water flows through the delta (Fig. 1.2). Human habitation is sparse but increases with proximity to the delta. The only large towns along the course of the Indus River are Dera Ismail Khan, Sukkur and Hyderabad. The river is little used for commercial traffic probably because passage is blocked by barrages, and the few vessels present are oar-powered or motorized ferries and fishing boats.

Figure 1.2 – View of the Indus River looking downstream from Kotri barrage. Instead of flowing water there are only pools and sand dunes. Photo credit: Gill Braulik

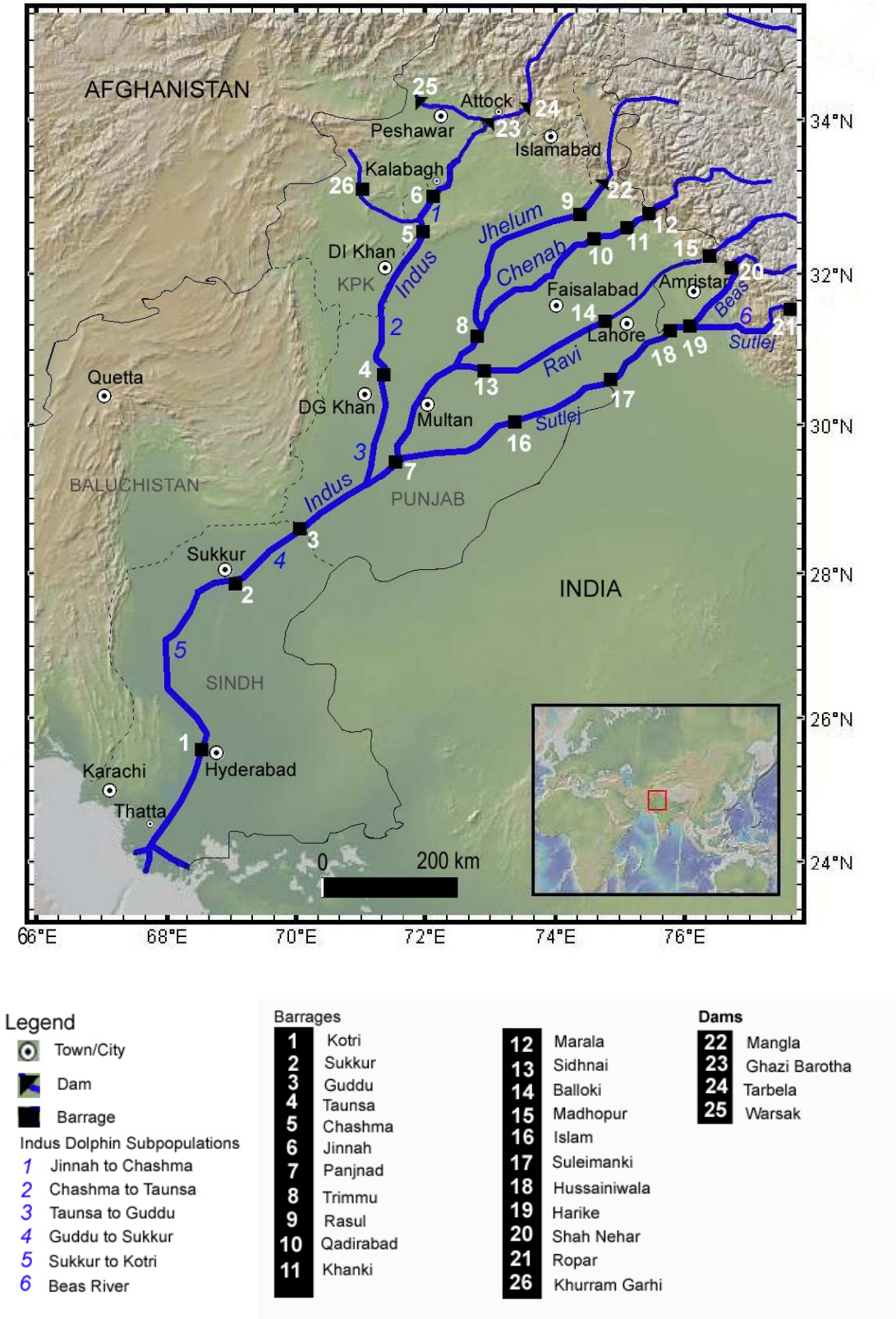

Figure 1.3 - The Indus River system, and the location of irrigation barrages and dams.

1.2.1 Indus River Mega-fauna

At present, the Indus plains are comprised of desert, semi-desert, scrub and irrigated agricultural lands. However, several centuries ago the native vegetation and fauna of the area was primarily forest and grassland inhabited by numerous large mammals including the tiger (*Panthera tigris*), leopard (*Panthera pardus)*, Asiatic cheetah (*Acinonyx jubatus venaticus)* and Indian rhino (*Rhinoceros unicornis*). All but the leopard are now locally extinct. Freshwater mega-fauna in the Indus River system previously included mugger crocodiles (*Crocodylus palustris*) which were hunted extensively and are now found in only a few isolated areas of Sindh (Ahmad 1999). The harmless, fish-eating gharial crocodilian (*Gavialis gangeticus*) once widespread but now extinct in Pakistan (Ahmad 1999). Two species of otter, the smooth otter *(Lutra perspicillata*) and the Eurasian otter *(Lutra lutra*) were once common, but these animals were decimated by hunting for their pelts and now persist in only a very few locations (WWF-Pakistan unpublished). There are eight species of turtle that inhabit the Indus River system, including four soft-shelled species, that can reach more than 1m in length, and four smaller hard-shelled species. Freshwater turtles were formerly abundant, but a new illegal trade in soft-shelled turtle parts for use in Chinese traditional medicine has resulted in massive turtle kills and greatly reduced wild turtle numbers in the last ten years (Pakistan Wetlands Programme/WWF-Pakistan 2008).

A commercially important fishery for the migratory shad (*Hilsa ilisha*) existed in the Indus River prior to construction of the barrages that blocked their migration. The fish used to enter the Indus River in great numbers each year in the middle of January, ascended the river to spawn during June, July and August, and returned to the sea in November (Islam and Talbot 1968). Before construction of Sukkur barrage in 1932, Hilsa would migrate all the way to present day Taunsa barrage. The Kotri and Sukkur barrages do contain fish ladders but these were inappropriately designed for use by Hilsa. The fishery has totally collapsed resulting in the loss of around 9000 jobs and an important source of protein for local people (Moazzam 1999).

The Indus dolphin is one of the last aquatic mega-faunal species remaining in the Indus River system.

1.3 Previous Dolphin Research

Research on the South Asian river dolphins has been sparse and sporadic, with work conducted initially in the 1870's, then 100 years later in the 1970s, and with a gradual increase in studies over the last 20 years. A large manuscript detailing the distribution, anatomy, osteology, life history and morphology of dolphins in the Indus and Ganges was produced by John Anderson (1879). Although this study was conducted almost 150 years ago, it is still one of the most relevant and detailed works on this species. In the 1970s there was a flurry of interest in South Asian river dolphins, and research was conducted into dolphin communication, behaviour and life history using captive animals (Herald 1969; Herald et al. 1969; Kasuya 1972; Pilleri 1970c; Pilleri et al. 1970), and Georgio Pilleri initiated numerous studies on dolphins in the Indus and Brahmaputra Rivers (Pilleri 1970b, 1972, 1979; Pilleri and Bhatti 1978, 1982; Pilleri and Zbinden 1973-74). From the 1990's until the present, the emphasis has been on monitoring the distribution, encounter rate and abundance of apparently declining populations, documenting threats, and suggesting conservation strategies to halt the decline (Reeves 1997, 1998; Reeves et al. 1991; Reeves and Leatherwood 1995; Reeves et al. 2000; Sinha 1997; Smith and Reeves 2000a, b; Smith et al. 2000).

1.4 Historical Information on Indus River dolphins

1.4.1 Historical Distribution

One of the most valuable pieces of research undertaken on the Indus and Ganges dolphin was a detailed map of their distribution produced by Anderson in 1879 (Fig. 1.4). It provides a baseline for comparison with the present distribution and for measuring range declines. Anderson describes how he compiled the information on distribution: "*I commenced a correspondence to render my inquiries [about the river dolphin] complete, and also drew up a series of questions to elicit all the facts regarding its distribution and habits. This schedule of queries was printed and circulated by Government among the civil and other officials resident along the courses of the greater rivers of India and Burma, and among the members of the Pilot Service. Notwithstanding that the inquiry was of a novel and rather unusual character, the replies were most complete and full of interest, and, more-over, examples of the dolphin were sent to me from the Indus, Ganges and Brahmaputra*" (Anderson 1879). In the mid-1870s the Indus and Ganges dolphins were never observed in the ocean, and in the Indus system were found throughout the year in the Indus, Jhelum, Ravi, Chenab and Sutlej Rivers from the Himalayan foothills to the estuary, a range of

around 3500 km (Reeves et al. 1991). The patrol at Kalabagh on the Indus River reported dolphins as constantly present, and they were said to be found in the Indus in April as high upstream as Attock (Fig. 1.3). The reports all confirmed that dolphins have the widest range during the flood season and that distribution decreases when the rivers flow is low (Anderson 1879).

It is difficult, almost 150 years later, to verify the information collated, but, in general, it appears to be reliable. The only exception is in Nepal which was not under British Administration, and where the upper distribution of the Ganges River dolphin was later found to be 100 km further upstream than shown on Anderson's map (Kasuya and Haque 1972). Dolphins were reported to extend their distribution into the foothills of the mountains in the Indus and Jhelum Rivers, in the Beas and Sutlej they were distributed only to the base of the foothills, and in the Ravi and Chenab their distributional limit was further downstream on the plains, apparently delimited by the Grand Trunk Road, the major transport route at the time (Fig. 1.4). These small differences in the upstream extent of distribution may be partly due to the seasonal range fluctuations being recorded differently in different rivers, or that differing habitat in each river resulted in different upstream distributional limits.

The shifting, shallow channels, and rapid velocity meant that, unlike on the Ganges, a regular steam boat service was only maintained on the Indus for a few decades in the early to mid-1800s (MacLagan 1885). Consequently, there are few accounts of travel on the Indus that can be examined for Indus dolphin sightings to verify Anderson's distribution map. Alexander Burnes was a British officer who led the first expedition on the Indus travelling from the delta to Lahore bearing gifts for Rangit Singh from the British King. He reported dolphins in the Indus from the delta up to Sukkur and also sighted several at the confluence of the Ravi and Chenab in July 1835 (Burnes 1835). A few years later, dolphins were reported to be present south of Thatta just north of the delta (Fig. 1.3) (Burnes 1842) and to be "very numerous" between Thatta and Sukkur (Hall 1848). In the 1860s dolphins were noted to ascend the Punjab rivers (Adams 1867), and a specimen collected from the Sutlej was presented to the Indian museum prior to 1879 confirming their presence in that river around that time (Anderson 1879). Evidence of their distribution at the far upstream end of their range is a report from the 1840s that 'before its junction with the Sutlej, the Beas is frequented with porpoises' (Anon. 1846). This is the same area where dolphins were recently re-discovered in

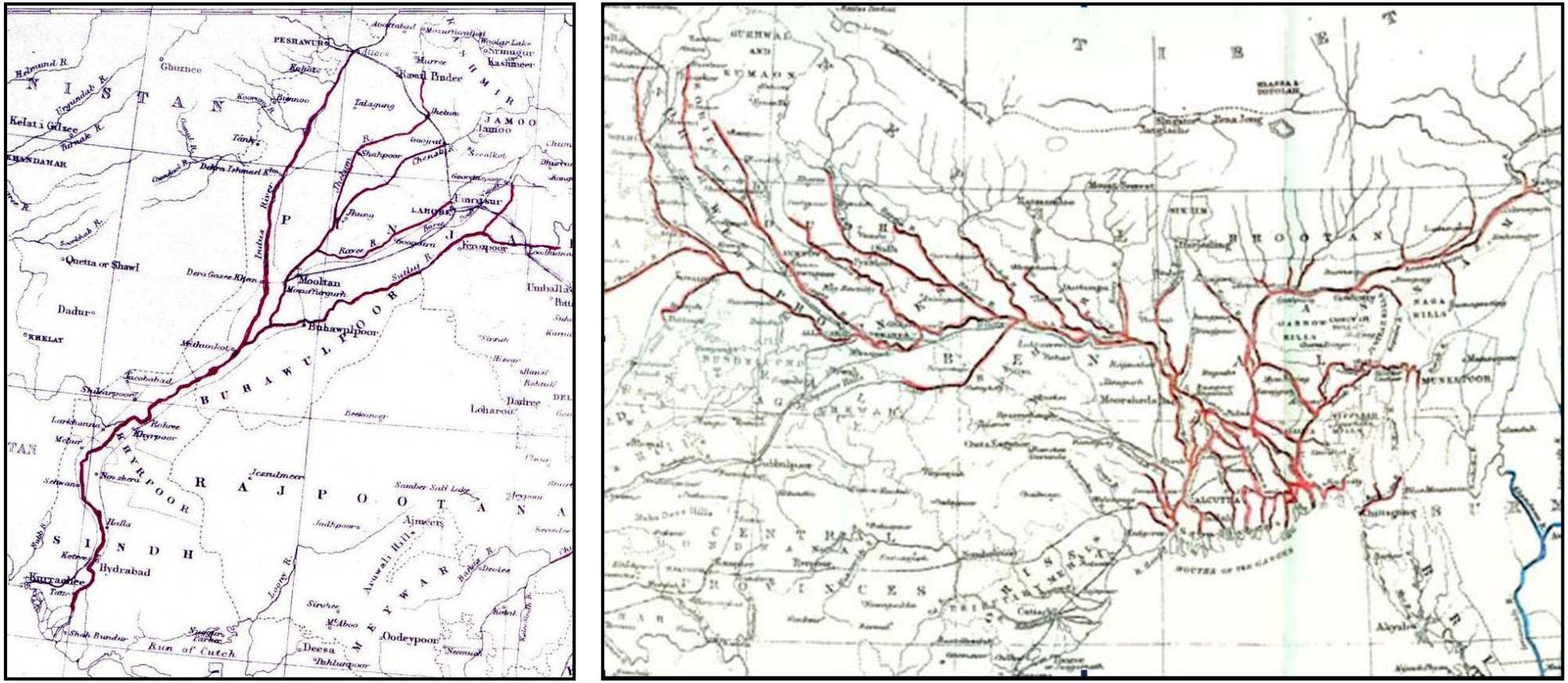

Figure 1.4 - Distribution of the Indus (above left) and Ganges (above right) River dolphins in the 1870s. Replicated from Anderson (1879).

India (Behera et al. 2008). These few records are all in agreement with the distribution described by Anderson.

1.4.2 Historical Abundance

In 1874, it was suggested that the Indus and Ganges dolphins were most abundant in the middle portion or lower third of their range (Jerdon 1874) which corresponds with the current high dolphin density area on the Indus in northern Sindh (Braulik 2006), and also with observations in the Ganges system (Sinha et al. 2000). This pattern is consistently demonstrated by most species; populations are larger and less variable near the centre of their geographic range where the environment is most suitable (Brown 1984; Channell and Lomolino 2000; Gaston 1990, 2008). Prior to large-scale water diversion, the Indus River had approximately four times the annual discharge of the Jhelum, or Chenab Rivers, six times that of the Sutlej and thirteen times the discharge of the Ravi (IUCN 2011). If discharge alone can act as a broad indicator of dolphin abundance, the Punjab tributaries may have historically supported lower dolphin densities and smaller populations than the Indus, and the Jhelum and Chenab may have had greater dolphin abundance than the smaller rivers the Ravi, Sutlej and Beas. In 1901, Blanford (1901) reported that *Platanista* sp. was not numerous and was once far more widespread, evidence that more than 100 years ago the South Asian river dolphins were already perceived to be in decline.

1.5 Development of the Indus Basin Irrigation System

1.5.1 Barrage Construction

The Indus plains are semi-arid, and the vast majority of the rain falls during the short summer monsoon with the result that for centuries agriculture has been reliant on people's ability to harvest water from the rivers. Since the 1880s, (just after Anderson produced his dolphin distribution map), 19 irrigation barrages, or gated-dams, have been constructed on the lower Indus within, or at the limits of, the former range of the dolphin (Table 1.1; Fig. 1.3). The Indus basin irrigation system is now claimed to be the largest irrigation system in the world. Barrages are low, gated diversion dams comprised of a series of gates (usually 60 to 70) used to control the elevation of an upstream 'head pond' (Fig. 1.5). The head pond is maintained not to store water, but to divert it into lateral canals (Fig. 1.6).

Figure 1.5 – Upstream view of Sukkur Barrage. Photo credit: Gill Braulik

Figure 1.6 - Aerial photo of the Indus River (flowing from right to left) at Sukkur barrage, illustrating the canals, barrage and change in flow above and below a barrage. Source unknown.

The first six barrages were commissioned at the end of the 19th century and were located on the Punjab Rivers, five at the base of the foothills, at the approximate upstream limit of dolphin distribution, and the sixth was the Sidhnai barrage on the River Ravi (completed in 1886) that was the first to fragment the dolphin population, separating the Ravi River from the rest of the Indus River system. Completion of Panjnad barrage in 1933 was significant as this split the former range of the Indus dolphin into two, separating dolphins in the Indus River from those in the five Punjab tributaries. By 1940, (~70 years ago), the Jhelum, Chenab, Ravi, Sutlej and Beas Rivers were already fragmented into at least seven different sections by barrages whereas barrage construction had only just begun on the Indus River and dolphins could move relatively unimpeded until completion of several barrages around 1960 (~50 years ago) (Fig. 1.3; Table 1.1).

Table 1.1–Chronology of barrage construction within the historical range of the Indus dolphin

#	River	Barrage	Construction Completed*	#	River	Barrage	Construction Completed*
1	Ravi	Madhopur	1879	**12**	Chenab	Panjnad	1933
2	Sutlej	Ropar	1882	**13**	Chenab	Trimmu	1939
3	Ravi	Sidhnai	1886	**14**	Indus	Jinnah	1946
4	Chenab	Marala	1887	**15**	Indus	Kotri	1955
5	Chenab	Khanki	1892	**16**	Sutlej	Harike	1955
6	Jhelum	Rasul	1901	**17**	Indus	Taunsa	1959
7	Ravi	Balloki	1917	**18**	Indus	Guddu	1962
8	Sutlej	Suleimanki	1926	**19**	Chenab	Qadirabad	1967
9	Sutlej	Hussainiwala	1927	**20**	Indus	Chashma	1971
10	Sutlej	Islam	1927	**21**	Beas	Shah Nehar	1983
11	Indus	Sukkur	1932				

*The exact date of completion quoted often varies by several years, especially for the older barrages. As these constructions typically took several years to complete this may be due to the difference between the onset of barrage construction to actual completion and commissioning. In addition, many older barrages have been improved and redesigned several times since their initial construction. The most commonly reported completion date is presented here.

The former range of the Indus dolphin became gradually more and more fragmented over time. For example, a section of the Indus River was isolated between Jinnah and Sukkur barrages in 1946; this 700km long river section existed for 13 years until it was

split into two on completion of Taunsa barrage in 1959. The Jinnah-Taunsa and Taunsa-Sukkur sections that resulted existed for 12 and 3 years respectively, until they were then further subdivided by construction of new barrages (Guddu and Chashma barrages) to reach the current configuration of four river sections. There have been 33 river sections of different lengths created since the onset of barrage construction, comprising 16 larger former fragments and 17 smaller current fragments. The longest un-fragmented portion of dolphin habitat, and the mean fragment size, has declined steadily as habitat became progressively more subdivided (Fig. 1.7).

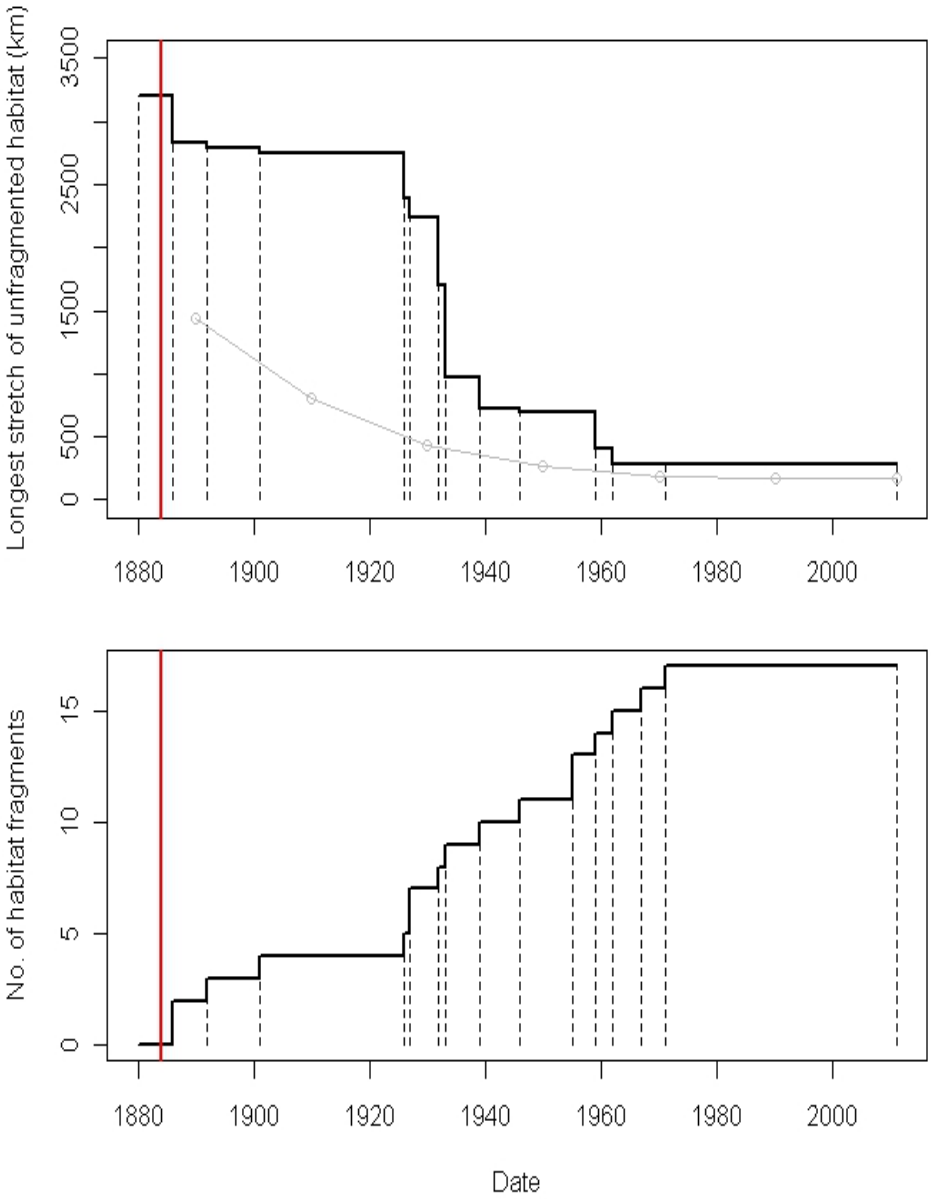

Figure 1.7 – Timing of Indus dolphin habitat subdivision, and the decline in size of the longest portion of unfragmented Indus dolphin habitat. The red line denotes the onset of barrage construction and the light grey line, the mean fragment length.

1.5.2 Water Diversion

The partition of India in 1947 saw creation of a new international border that bisected the Indus River system; all the rivers previously inhabited by dolphins now flow through India prior to entering Pakistan. In April 1948, India turned off the flow of the Ravi and Sutlej Rivers, at the beginning of the critical sowing season, by diverting all water at Madhopur and Hussainiwala barrages (Fig. 1.3) (Kazi 1999). The Indus Water Treaty was agreed in 1960 and the flows of the Indus, Jhelum and Chenab, amounting to 75% of the total, were allocated to Pakistan, and water in the Ravi, Beas and Sutlej Rivers, allocated to India. This has had two results of significance for the Indus dolphin: 1) India has the rights to the Ravi and Sutlej therefore all the water in these rivers is utilised within India, and they are now usually dry when they enter Pakistan, and 2) most of Pakistan's water resources are in the west but the greatest population and the major irrigated agricultural areas are in the east. This problem was solved by construction of massive link canals to transfer water from the western rivers to those in the east so that agricultural lands south of the Ravi and Sutlej could continue to be irrigated (Fig. 1.3). Opening of the link canals fundamentally changed the way water was managed in the Punjab tributaries. It allowed for the complete diversion of a river's flow at upstream barrages as the river could be replenished downstream by a link canal, and the flow subsequently completely diverted again, at a barrage further downstream. Prior to construction of the link canals some flow remained in each river for its entire length so that land adjacent to the furthest downstream barrage could be irrigated. The result is that since the 1970s, when the majority of the link canals opened, for several months of the year, the Ravi and Sutlej are almost completely dry and there is no water released through Khanki, Qadirabad, Trimmu and Panjnad barrages on the Chenab River, Balloki and Sidhai on the Ravi and Suleimanki and Islam on the Sutlej (Fig. 1.8) (Federal Flood Commission 2010).

Water diversion has been steadily increasing and the cultivable area expanding as new canals are built, existing canals extended and their capacity increased, and the barrages refurbished. Meanwhile, river discharge has been steadily declining (IUCN 2011).

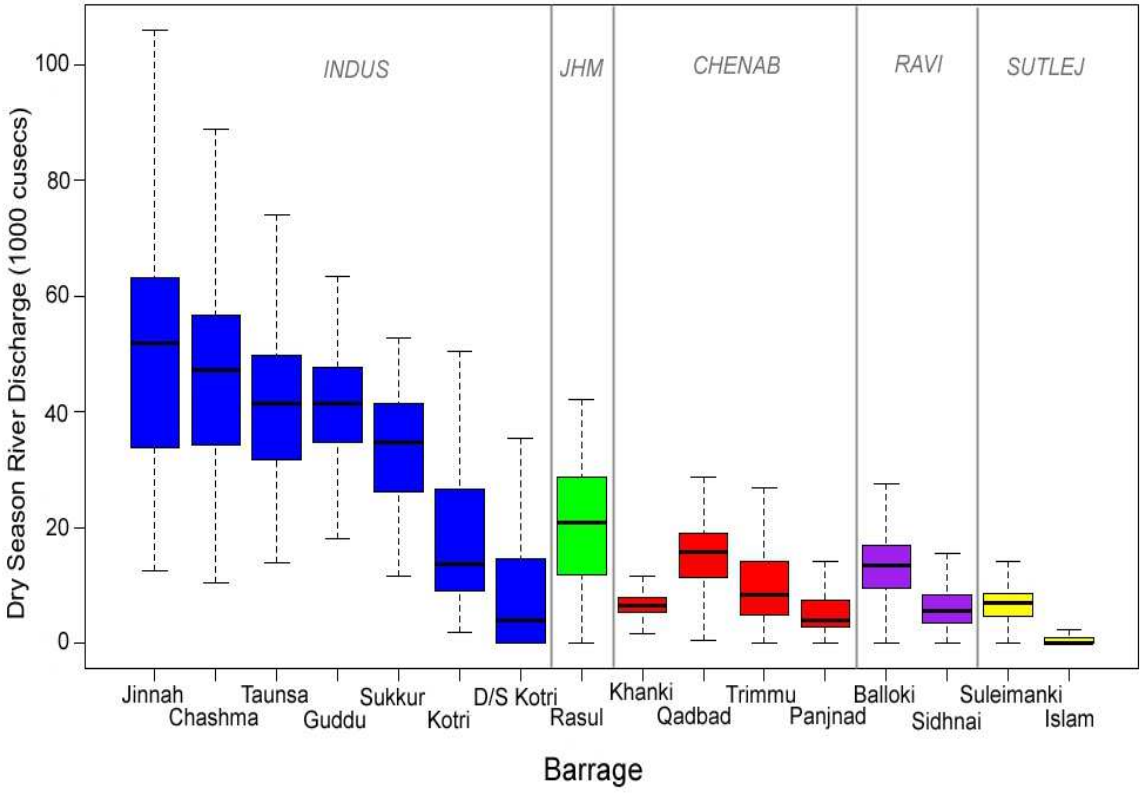

Figure 1.8 - Dry season (October to March) river discharge above each barrage (cubic feet per second) on the Indus River system in Pakistan. Note: It is only the first 5 barrages on the Indus River that did not receive zero discharge (the lower whisker) occasionally. D/S Kotri refers to the water that is released downstream of Kotri barrage to the delta. JHM=Jhelum River (Federal Flood Commission 2010).

1.6 Current Status of the Indus River Dolphin

1.6.1 Present Distribution

Today, Indus dolphins occur in five subpopulations on the Indus mainstem, bounded by Jinnah, Chashma, Taunsa, Panjnad, Guddu, Sukkur and Kotri Barrages (Fig. 1.3). A sixth Indus dolphin subpopulation occurs in the Beas River in India (Behera et al. 2008). River dolphins have been extirpated from the Indus mainstem upstream of Jinnah Barrage, downstream of Kotri barrage and from the five Indus tributaries in Pakistan. The linear extent of occurrence is now approximately 1000 km (Braulik 2006), an estimated 99% of the dolphin population occurs in only 690 km of river, which corresponds to almost an 80% reduction in effective linear range from 1870 (Reeves et al. 1991).

Irrigation barrages restrict the movement of dolphins rendering them isolated into separate subpopulations. A subpopulation is defined by IUCN as “geographically or otherwise distinct groups in the population between which there is little demographic or genetic exchange (typically one successful migrant individual or gamete per year or less)”(IUCN 2001). The term, ‘subpopulation’ was first applied to the populations of Indus dolphins that occur between barrages by Reeves (1991). It has long been suggested that dolphins may occasionally be able to traverse the barrage gates and move between subpopulations (see Section 1.9), but the only hard evidence of this was one radio-tagged dolphin that was documented moving through the gates of a barrage three times, during a brief period when the barrage gates were fully open (Toosy et al. 2009). Although it is possible that future studies will determine there is considerable movement of dolphins through some barrages and the term ‘subpopulation’ will be subsequently deemed inappropriate, at present there is no evidence that migrants are frequent, and therefore, in-line with previous authors, throughout this thesis I use the term ‘subpopulation’ for dolphins that occur between irrigation barrages in the Indus River system. Subpopulations are named according to their bounding barrages and to aid their identification are also numbered from 1 to 5 in a downstream direction (see Fig. 1.3).

After entering the plains, the river flows through Punjab province, and from Guddu barrage continues south through Sindh Province. Between Chashma and Taunsa barrages, for approximately 100km, the river forms the boundary between Khyber Phakhtunkhwa Province (KPK) (formerly known as the North Western Frontier Province) and Punjab, and therefore for 100km south of Dera Ismail Khan, KPK Province also takes responsibility for managing the river and the river dolphins.

1.6.2 Present Abundance

In 2001 a comprehensive visual direct count survey of the entire known range of the Indus dolphin was conducted (Braulik 2006). An abundance estimate of 965 Indus River dolphins was produced from the sum of the best estimates of group size. The sum of the low estimates and the high estimates of group size were 843 and 1171 animals, respectively. Encounter rates increased as the survey proceeded downstream to Sukkur barrage (Fig. 1.9 and 1.10). Only two dolphins were recorded in the furthest upstream subpopulation (number 1) between Jinnah and Chashma barrages. The sum

of best group size estimates in subpopulation 2, between Chashma and Taunsa barrages, was 84 dolphins (0.28 dolphins/km). In subpopulation 3, between Taunsa to Guddu barrages, 259 (0.74 dolphins/km) were recorded, and between Guddu and Sukkur barrages (subpopulation 4), 725 dolphins (3.60 dolphins/km) were counted. In the final downstream subpopulation (number 5), located between Sukkur and Kotri barrages, only 18 dolphins were observed. Correction of the population estimate to account for groups missed by the primary vessel generated an overall estimate of abundance for the subspecies of about 1200 (Braulik 2006).

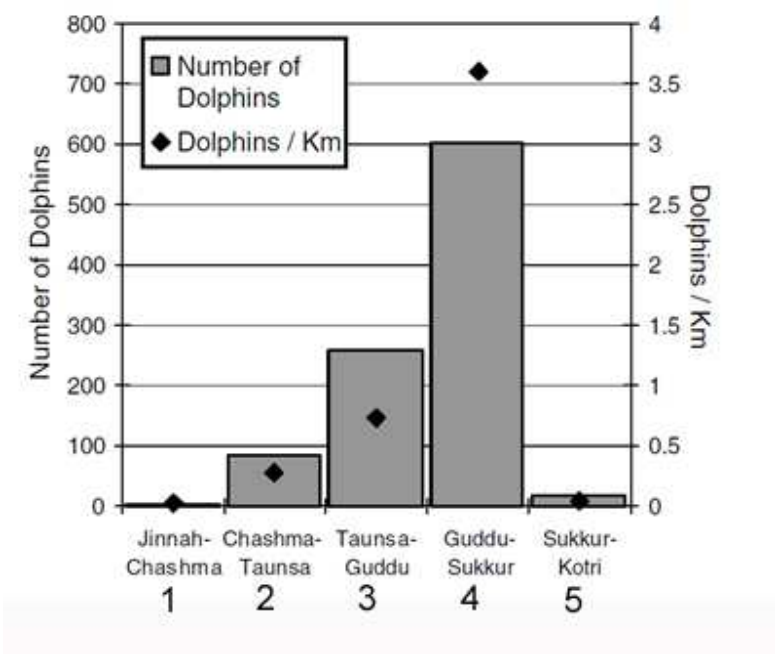

Figure 1.9 – Abundance and encounter rate of Indus River dolphins in each subpopulation in 2006 (Braulik 2006)

Abundance monitoring of the three largest dolphin subpopulations (numbers 2, 3 and 4) has been conducted principally by the Provincial Wildlife Departments since the early 1970s, using visual direct counts from vessels or counts from the river bank. The Sindh and Punjab wildlife departments used different survey methods that preclude direct comparison of counts between Provinces, nor is it possible to determine their accuracy or estimate their precision. All published counts for the Guddu–Sukkur, Taunsa–Guddu and Chashma–Taunsa subpopulations (numbers 4, 3 and 2) were compiled by Braulik (2006), and this is reproduced in Table 1.2. This table is an expansion and update to previous compilations of count data (Bhaagat 1999; Gachal

and Slater 2002; Reeves and Chaudhry 1998). Where several counts were conducted in the same year and month, only the highest count is presented.

1.6.3 Encounter Rate

In 2001 the encounter rate recorded in the Guddu–Sukkur subpopulation (number 4) was almost five times greater than in any other Indus River dolphin subpopulation (Braulik, 2006). This encounter rate (averaging 3.60 dolphins/km, peaking at 5.05 dolphins/km), was several times greater than that recorded for the Ganges River dolphin in rivers of India and Bangladesh (Bashir et al. 2010; Choudhary et al. 2006; Sinha 2000; Smith et al. 2001; Wakid 2009). It was also much greater than those recorded for other Asian River dolphins, such as Irrawaddy dolphins, *Orcaella brevirostris*, in the Ayeyarwady River, 0.09-0.47 dolphins/km (Smith and Hobbs 2002; Smith and Tun 2007), the Mahakam River, 0.142 dolphins/km (Kreb 2002) and the Mekong River, 0.197 dolphins/km (Beasley 2007).

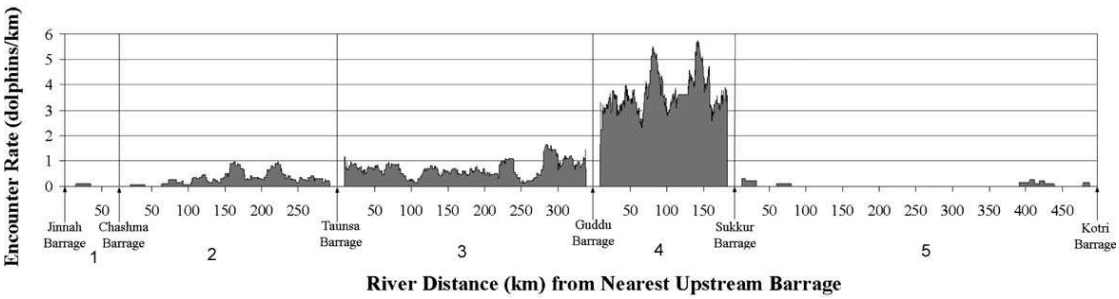

Figure 1.10 – Twenty kilometres moving average encounter rate of Indus River dolphins between Jinnah and Kotri Barrages (Braulik 2006).

1.6.4 IUCN Red List Assessment

The red list classification of Endangered for *Platanista gangetica* was based on criterion A2, a previous population decline of more than 50% in three generations. The listing of Endangered for the Ganges River dolphin subspecies was based on criteria A2, A3 and A4, previous, present and predicted future population decline of more than 50% in three generations, and that of Endangered for the Indus River dolphin subspecies on A2, B1 and C1, previous population decline of more than 50% in three generations, small extent of occurrence, severe fragmentation and a declining population estimated as less than 2500 mature individuals.

Table 1.2 – Published counts of Indus River dolphins between Guddu and Sukkur barrages (subpopulation 4), Taunsa and Guddu barrages (Subpopulation 3) and Chashma and Taunsa barrages (Subpopulation 2) reproduced from Braulik et al (2006).

Table 1 – Published counts of Indus River dolphins between Chashma, Taunsa, Guddu and Sukkur Barrages

Guddu–Sukkur subpopulation			Taunsa–Guddu subpopulation			Chashma–Taunsa subpopulation		
Date	Count	Reference	Date	Count	Reference	Date	Count	Reference
Jan 1974	138	Pilleri and Zbinden (1973–74)	Apr 1979	36	Pilleri and Bhatti (1980)	Oct–Nov 1987	39	Niazi and Azam (1988)
Dec 1974	182	Kasuya and Nishiwaki (1975)	Dec 1983	72	Chaudhry and Khalid (1989)	Winter 1987	47	Chaudhry et al. (1999)
Feb 1977	171	Pilleri and Bhatti (1978)	Apr 1985	61	Khan and Niazi (1989)	Mar 1989	15	Chaudhry and Khalid (1989)
Apr–May 1977	187	Pilleri and Bhatti (1978)	Aug 1985	71	Chaudhry and Khalid (1989)	April 1990	20	Chaudhry et al. (1999)
May 1977	198	Pilleri (1977)	Sept–Oct 1985	62	Khan and Niazi (1989)	Nov 1991	35	Chaudhry et al. (1999)
Oct 1977	168	Pilleri and Bhatti (1978)	Oct–Nov 1987	62	Niazi and Azam (1988)	Nov 1992	49	Chaudhry et al. (1999)
Feb–Mar 1978	191	Pilleri and Bhatti (1978)	Mar 1989	83	Chaudhry and Khalid (1989)	Nov 1993	51	Chaudhry et al. (1999)
May 1978	241	Pilleri and Bhatti (1978)	Apr 1990	107	Chaudhry et al. (1999)	Mar 1994	34	Chaudhry et al. (1999)
Apr 1979	240	Pilleri and Bhatti (1980)	Nov 1991	108	Chaudhry et al. (1999)	Nov 1994	62	Reeves and Chaudhry (1998)
June 1979	292	Pilleri and Bhatti (1980)	Nov 1992	124	Chaudhry et al. (1999)	Apr 1995	38	Chaudhry et al. (1999)
Sept 1979	291	Pilleri and Bhatti (1980)	Nov 1993	111	Chaudhry et al. (1999)	Apr 1996	43	Chaudhry et al. (1999)
Feb 1980	291	Bhatti and Pilleri (1982)	Mar 1994	128	Chaudhry et al. (1999)	Winter 1997	39	Chaudhry et al. (1999)
Apr 1980	346	Bhatti and Pilleri (1982)	Nov 1994	100	Reeves and Chaudhry (1998)	Winter 1998	31	Chaudhry et al. (1999)
Mar–Apr 1982	360	Bhaagat (1999)	Apr 1995	117	Chaudhry et al. (1999)			
Mar 1986	429	Khan and Niazi (1989)	Apr 1996	124	Chaudhry et al. (1999)			
March 1987	450	Reeves and Chaudhry (1998)	Dec 1996	143	Reeves and Chaudhry (1998)			
Apr–May1989	368	Bhaagat (1999)	Winter 1997	90	Chaudhry et al. (1999)			
Mar–Apr 1990	387	Bhaagat (1999)	Winter 1998	100	Chaudhry et al. (1999)			
Mar–Apr 1991	398	Bhaagat (1999)						
Mar–Apr 1992	410	Bhaagat (1999)						
1992	439	Reeves and Chaudhry (1998)						
Mar–Apr 1993	426	Bhaagat (1999)						
Mar–Apr 1994	435	Bhaagat (1999)						
Mar–Apr 1995	447	Bhaagat (1999)						
Apr–May 1996	458	Mirza and Khurshid (1996)						
May 1999	104	Gachal and Slater (2002)						
June 1999	220	Gachal and Slater (2002)						
Aug 1999	367	Gachal and Slater (2002)						

1.7 Habitat use

Almost every study conducted on river dolphins in Asia has commented on their extremely patchy distribution and preference for various river features, especially confluences, however in almost all cases this has been a qualitative observation (Bashir et al. 2010; Haque et al. 1997; Jerdon 1874; Kasuya and Haque 1972; Khan and Niazi 1989; Sinha 1997; Sinha et al. 2000; World Wide Fund for Nature - India 2001). Other river morphological or hydrological features that have been noted as areas of dolphin concentration are: downstream of shallow places, in narrow places (Kasuya and Haque 1972), narrow and deep sections of river (Pilleri 1970b), in deep locations (Bairagi et al. 1997) where the current is weak (Pilleri and Zbinden 1973-74), in deep water pools (Bashir et al. 2010), off the mouths of irrigation canals, near villages and ferry crossings (Pilleri and Bhatti 1982; Pilleri and Zbinden 1973-74; Sinha 1997), downstream of bridge pilings (Choudhary et al. 2006; Sinha 1997; Smith et al. 2001), downstream of sand bars and sharp meanders (Sinha 1997) and in channels with muddy, rocky substrates (Kelkar et al. 2010). In the Indus River, dolphins are occasionally sighted in larger secondary channels or braids, but generally encounter rates are very much lower in such places than in the main channel (Braulik 2006). In the Ganges River above Narora barrage, 14% of sightings occurred in side channels, and the encounter rate was 0.07 dolphins/km, compared to 0.18 dolphins/km in the main channel (Bashir et al. 2010). In the Patna area in Bihar, Ganges River dolphins occurred in the same locations preferred by fishermen, and sites with dolphins had a higher biomass of smaller sized fish than areas from which they were not recorded (Kelkar et al. 2010).

It is clear that South Asian river dolphins are patchily distributed according to characteristics of their habitat but there have been few studies that statistically tested which types of habitat are preferred in different seasons or locations. The two most comprehensive are summarised below:

Smith (1993) conducted detailed studies of dolphin habitat at the extreme upstream limits of Ganges dolphin distribution in Nepal. Depth and velocity were mapped in three locations where dolphins were routinely present (primary habitat) and three that were occasionally used (marginal habitat) and it was concluded that dolphins consistently used the same areas characterised by high prey availability and low

velocity. River dolphins were assumed to be exploiting the 'hydraulic refuge' provided by counter-current eddies in deep pools. At the opposite end of the range of the Ganges River dolphin, in the Sundarbans mangrove forest in Bangladesh, river dolphins showed a consistent preference for water of approximately 12m deep, from a possible range of 0 to 40m, irrespective of season (Smith et al. 2009). Generalised additive models (GAMs) showed that Ganges River dolphin distribution was dependent on low salinity, high turbidity and moderate depth during both low and high flow and with preference for wide sinuous channels with at least two small confluences or one large confluence (Smith et al. 2009). A second study conducted in the eastern Sundarbans using data collected by eco-tourism vessels recorded similar results (Smith et al. 2010).

1.8 Behaviour

Studies of *Platanista* behaviour and movement patterns are complicated by the fact that the water is very turbid preventing views of underwater behaviour. Animals never approach boats and bow ride, and it is not possible to identify individuals using photo-ID because they do not appear to have any unique features and obtaining photos is prohibitively difficult. It was only when dolphins were kept in clear water in captivity that anything of their underwater behaviour could be discerned. Three female Indus dolphins captured by Herald et al. (1969) were kept in holding pens in Karachi *en route* to the USA and this was the first time that their unique side-swimming behaviour was observed. One pectoral flipper either touched the bottom or trailed just above it, the tail was normally higher than the head, the body angled at approximately 10^0 to the bottom and the head moved continuously from side-to-side as the animal swam. The lower flipper repeatedly touched the bottom during side-swimming and it was thought to have a tactile function (Pilleri and Pilleri 1987). Pilleri (1970) suggested that side-swimming may only occur in certain situations and is an adaptation that allows dolphins to move through shallow water.

Indus and Ganges River dolphins surface alone; only mothers and very young calves have been seen surfacing in near synchrony. Animals show only the top of the head and back when surfacing, or the rostrum, head and back. Breaches are very rare, and the tail flukes are almost never visible (Sinha et al. 2010).

It has been reported that dolphins move downstream in the winter dry season when river discharge is low, and that as the flood waters rise in the monsoon season dolphins move upstream into the smaller tributaries (Anderson 1879; Kasuya and Haque 1972; Kelkar et al. 2010; Shrestha 1989; World Wide Fund for Nature - India 2001). Given the large variation in river discharge and velocity a seasonal movement seems probable. However since construction of Farakka barrage in India and the Indus basin irrigation system in Pakistan long-range seasonal movements are now blocked by dams and barrages.

1.9 Life History

Information on the life history of *Platanista* sp. is extremely limited; the little data available originates primarily from studies conducted by Anderson (1879) or Kasuya (1972).

1.9.1 Growth

Growth layers in *Platanista* teeth are present in both dentine and cement; however the dentine layers are more regular and easily counted (Kasuya 1972). Nineteen individuals from the Brahmaputra that were between 76 cm and 2 m in length were aged; individuals 76-113 cm were less than one year old and those 113-126 cm were between 1 and 2 years of age. The data indicated an approximately 65% increase in body length during the first year of life which is similar to Delphinid species. The oldest individual recorded was a 28 year old male, 199 cm in length, which, based on ankyloses of the vertebrae was not yet physically mature (Kasuya 1972). The largest female collected from the Ganges was 252 cm while the largest male was 213 cm (Anderson 1879). The largest female Indus dolphin recorded was 230 cm and the largest male was 212 cm (WWF-Pakistan unpublished). The data suggest that *Platanista* growth continues for a longer period than most other cetaceans, especially in females.

1.9.2 Sexual Dimorphism

The length of the head is larger in adult females than adult males of corresponding length due to their longer rostrum (Kasuya 1972). Sexual dimorphism is expressed after females reach about 150 cm in length; the female rostrum continues to grow after the male rostrum has stopped growing, eventually reaching approximately 20 cm

longer. The tips of longer rostrums begin to curve upwards and in rare instances downwards. Body length, position of genital aperture and umbilicus, and perhaps insertion of flipper also were thought to exhibit sexual dimorphism (Kasuya 1972; Anderson, 1879). The body weight of adult females is lower than adult males of equivalent length, which is probably accounted for by sexual dimorphism in rostrum length of females, as weight is comparable in juveniles of each sex (Kasuya 1972). Other toothed cetaceans where females are larger than males are *Pontoporia, Lipotes, Phocoena phocoena* and *Sotalia fluviatilis,* the only obvious common factor among these species that may lead to large female size, is an apparently simple social structure (Brownell 1984).

1.9.3 Sexual Maturity

Kasuya (1972) assumed that male Ganges dolphins may attain sexual maturity at about 10 years, and at body length about 170 cm or less and Harrison (1972) reported a 185 cm male that was approaching sexual maturity. The largest known immature female is 150 cm (Anderson 1879) and the smallest mature female is 200cm (Harrison 1972). Kasuya (1972) concluded that females attain sexual maturity between 170 and 200 cm and Harrison (1972) suggested sexual maturity is reached at a length of 170-180 cm. Harrison (1972) examined the *corpus lutea* of four pregnant Ganges River dolphins and found that a 200 cm female had had two previous pregnancies, a 203 cm animal had had five, 206 cm had one and 240 cm had two. Brownell (1984) estimated that if females become mature around eight years old and have a two year breeding cycle an average female will be able to reproduce for about 22 years and produce nine to 11 calves in a lifetime.

1.9.4 Calving

Body length at birth is approximately 70 cm (Kasuya 1972; Anderson 1879). The smallest recorded calf, captured in the Ganges in January was 63.1 cm long (Sinha et al. 1993) and the largest foetus was 89 cm (Kukenthal 1909). A calf 67.4 cm was captured, its teeth had not erupted and it was assumed to be still suckling, however, within one month anterior teeth erupted and examination of its stomach contents showed it was feeding on fish (Kasuya 1972). A young female 95 cm long captured in June had milk in the stomach and intestine (Sinha 1993), and a 99 cm male that died in a canal in Sindh in 2000, had a stomach full of fish and was unaccompanied by its

mother (Braulik unpublished). Kasuya (1972) concluded that calves start feeding one or two months after birth and will be weaned within one year.

1.10 Echolocation

Indus dolphin echolocation has only been studied while they were kept in captivity in the USA and Switzerland in the early 1970's. The dolphins were reported to echolocate continuously, producing between 20-50 clicks per second (Herald et al., 1969; Pilleri and Pilleri 1987). Echolocation stopped for only 3-5 second periods that coincided with drifting behaviour and reduced motor activity. The total of these pauses was about 7 hours and they were interpreted by Pilleri et al. (1976) as periods of polyphasic sleep. Click duration ranged from 40 to 70 µs, peaking at 50 to 60 µs. Click frequencies were primarily 50-80 kHz, with a secondary peak at 160-200 kHz and with the dominant frequency being 80 kHz (Pilleri et al. 1976b). Herald (1969) and Herald et al. (1969) reported maximum click energy between 15 and 60 kHz peaking at 45-50 kHz.

The acoustic emission field was found to be highly directional, extending in two relatively narrow cones dorsally and ventrally in front of the dolphin which is quite different from the single cone that extends in front of the rostrum in other Odontocetes (Pilleri and Pilleri 1987). The sonar field was strongest 15-25° from the axis of the rostrum and declined substantially below 15^{0} and beyond 60^{0} in the dorsal and ventral planes. The field was slightly larger in the ventral plane and extended further back on the left side than the right, perhaps due to the left skew of the skull and maxillary crests. Between 0 and 15^{0} from the axis of the rostrum there was a discontinuity in the emission field. Pilleri concluded that the continuous side-to-side head movement of *Platanista* while swimming was to eliminate this effective 'blind spot' in its acoustic field (Pilleri and Pilleri 1987). While in captivity, dolphins approached fish or other objects of interest at an angle of 25-30° to the rostrum axis, with the throat region and ventral acoustic field facing the object (Pilleri and Pilleri 1987; Pilleri et al. 1976b).

A brief study on the echolocation clicks of free ranging Ganges River dolphins was conducted in India in 2007. Interclick interval (ICI) averaged 24 ms (range 20-60 ms), on axis clicks were approximately 40 µs duration, were of 65 kHz frequency and source levels were between 150-180 dB re µPa (Ura et al. 2007). The study concluded that the beam width was very narrow as the array would not record clicks unless a dolphin was directly facing it.

The extensive maxillary crests of *Platanista* skulls undoubtedly play a role in directing sound, however the mechanism and their precise function is not understood. There is no indication that *Platanista* use acoustic signals for communication and no whistles or other sounds have been recorded (Pilleri and Pilleri 1987).

1.11 Diet

Information on the diet of *Platanista* is derived from stomach contents analysis of a small sample of individuals from each river system. Diet appears to vary according to location and/or season, but is generally composed of a large variety of bottom-dwelling fish and prawns. The most common items in two juvenile Indus dolphin stomachs were the Tank Gobi *Glossogobius giuris,* and freshwater prawns *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii* both of which are demersal and gregarious (Butt 1977). They have also been recorded to feed on catfishes *Wallago attu* and *Sperata aor* and the carp *Catla catla*, (Pilleri and Zbinden 1973-74) and *Cirrhina cirrhosus* (Roberts 1997). In the Ganges-Brahmaputra River, dolphins have been reported feeding on a variety of river prawns, catfish, herrings, carp, perch and eels (Sinha et al. 1993). Sinha et al. (1993) suggested that feeding may decline during the summer monsoon due to erosion of the river bottom by floodwaters and because fewer smaller fish are available prior to the summer spawning season. He suggested that post-monsoon, in the early autumn, prey availability would increase with the reduction of the flood and influx of juvenile fish and prawns. If the dolphins fast or reduce their consumption in the summer, the resulting reduction in the blubber layer would coincide with the hottest summer months when they need to expel heat. An increase in consumption following the monsoon would enable the blubber layer to thicken in time for the cool water temperatures in winter.

1.12 Threats and Management

1.12.1 Dolphin Hunting

Detailed accounts of the hunting bags of British officers, often totalling thousands of birds and mammals of numerous species, were regularly published in the Journal of the Bombay Natural History Society or Journal of the Asiatic Society of Bengal in the 1800s and early 1900s. These lists never included a freshwater porpoise (as they were referred to at that time), and Indus dolphins were apparently not targeted by colonial

hunters. Freshwater dolphins were however killed for food and oil by numerous indigenous groups over the course of several centuries. Anderson (1879) reported that at Sukkur the Dhopel people catch dolphins in shallow water with the aid of trained otters. He also reported that in Sindh the Kehuls eat dolphin, and in Punjab the Choorahs, Dhopels, Sainsees, Budous, and Burars eat dolphin flesh. The Moras, who were Muslim boatmen, also consumed dolphin. At that time, dolphin oil was reported to be used as medicine and for lighting.

Around 1900, porpoise oil was reported to be sold by low-caste people in Ghazi Ghat, near the present Taunsa barrage in Punjab (McNair 1908). In 1915, in Dera Ghazi Khan, Lowis (1915) gave a detailed explanation of how dolphins were captured by the local Kehal people. They constructed a viewing platform in shallow water, and attached a fish to a nearby stake. A tethered tame otter was released into the water and would try to reach the fish. The noise of the otter would attract a dolphin and as it approached the dolphin would attempt to catch the fish attached to the stake, at which point the fisherman would cast his net over the dolphin to capture it. A similar method was used by fishermen in Sindh to capture dolphins for Georgio Pilleri in 1969 (Pilleri 1970a).

In the early 1970s, when Georgio Pilleri visited the Indus River in Sindh, he observed several boats equipped for catching dolphins, with a large number of body parts and oil drums onboard. The oil was reported to be used both externally and internally as medicine and also fed in relatively large quantities to livestock (Pilleri 1972). He suggested that the muslim majority disdained dolphin meat (because it is considered haram or unclean), but that the non-muslim Jubber caste continued to consume it. Locals reported that there were many fewer dolphins present than in the past (Pilleri and Zbinden 1973-74) and Pilleri concluded that the Indus dolphin had been severely decimated by hunting and was in danger of disappearing completely (Pilleri 1977). In 1974 a reserve for the Indus dolphin was declared in the 190km stretch of river between Guddu and Sukkur barrages (subpopulation 4). In the early 1970s the dolphin became a protected species when the Wildlife Acts of Sindh, Punjab and KPK Provinces were passed. Within a few years, and following some prosecutions, hunting in Sindh ceased and the dolphin population began to show signs of recovery (Bhatti and Pilleri 1982; Pilleri and Bhatti 1982).

Following enforcement of the ban on dolphin hunting in Sindh, it appears that the hunters moved upstream to Punjab to avoid the strict hunting controls downstream. This is despite the fact that deliberate killing of dolphins is banned in both Punjab and Sindh. In 1977, Pilleri reported that upstream of Guddu barrage a large number of boats were equipped for catching dolphins, and that fragmentation of the habitat and reduced flows made hunting easier (Pilleri and Bhatti 1978, Knuckey, unpublished). Reeves et al. (1991) and Reeves (1991) reported that Kehul fishermen below Kalabagh were engaged in hunting dolphins, and also reported stories of dolphin hunts at Chashma, Ghazi Ghat and Taunsa in the early 1980s. After this there is no more evidence that dolphin hunting persists anywhere in Pakistan. Following the partition of India in 1947, Pakistan became increasingly Islamic and it is probable that hunting and consumption of dolphins subsequently declined because it is forbidden by Islamic law.

1.12.2 Pollution

It is estimated that only 8% of urban and industrial wastewater in Pakistan is treated; leaving more than 90% of industrial and municipal effluents to find their way into the water courses (Directorate of Land Reclamation Punjab 2007). The magnitude of surface water pollution problems in Pakistan has increased at a dramatic rate over the last ten years (Qadir et al. 2007). The plains are intensively cultivated with cotton, wheat and sugar cane. Pesticide use is increasing annually at a rate of about 6% (World Bank 2005). Pesticides, mostly insecticides, sprayed on the crops mix with the irrigation water, which leaches through the soil and enters groundwater aquifers and sometimes contaminates water supplies. This appears to be the case in the recurring problems of water-related deaths in Hyderabad (World Bank 2005). The quantity or quality of agricultural runoff has not been measured or tested at the national level.

The Punjab rivers flow through the industrial and agricultural heartland of Pakistan and as a consequence are more polluted than the Indus which passes through more remote areas (Directorate of Land Reclamation Punjab 2007; Ghaznavi 1999; Tariq et al. 1996). The River Ravi flows through Lahore, a city of approximately 10 million people, and is the most polluted river in the country and a considerable concern for human health (Ali et al. 2000). More than three quarters of all Indus dolphins occur in the Indus River below the Panjnad River confluence and are downstream of cities inhabited by more than 100 million people (Federal Bureau of Statistics of Pakistan

2003). At present, there have not been any comprehensive studies evaluating the role of pollutants on Indus dolphins or measured levels in their tissue. However, especially considering the decline in the flushing effect of abundant water, it is possible that especially at the downstream end of its range where levels are likely to be highest, pollution has the potential to affect the Indus dolphin (Reeves et al. 2003).

1.12.3 Fisheries Interactions

Mortality from accidental capture in fishing gear is the greatest threat to most cetaceans (Northridge 2009; Read 2008), however fisheries related mortalities of Indus dolphins have only been documented occasionally and previously this has not been considered one of the larger threats to this subspecies. Indus dolphins are accidentally captured in nets when they stray into irrigation canals, which, due to their narrow and shallow dimensions, are easily and heavily fished. Net entanglement is likely to be a major issue between Sukkur and Kotri barrages (subpopulation 5) where the Indus flow is so severely depleted that fixed nets span the river. However, in general, the Indus River main channel has not been intensively fished as fishing activity concentrates in side channels and adjacent pools that are reported to be warmer and have a higher fish density (Khan 1947). The low intensity of fishing in the main channel is partly because the water is too swift for easy manoeuvrability of oar-powered boats (Khan 1947). Fisheries bycatch is likely to become an increasing threat as boats become mechanised and able to negotiate the main channel. For the last twenty years, there was a fish contractor system in place in Pakistan in which the rights to fishing grounds were auctioned by the government and were purchased by powerful fish contractors. Contractors allowed fishing only on the condition that fishermen surrendered approximately 75% of the fish catch to them, and that the remainder was sold to them below market value (Jabbar 2005). In 2007, the contractor system in Sindh was abolished and now local indigenous fishermen can obtain their own licenses to fish. This action is likely soon to affect the other provinces (Anon. 2011a). The removal of the fish contractor system for allocating fishing licences within the dolphin reserve has led to larger numbers of unskilled fishermen using the river, and there has been a co-incident jump in the number of dolphin mortalities especially within the last year. In January 2011, at least 6 dolphins were killed within the Protected Area when locals supposedly used chemicals to kill fish (Anon. 2011) and between January and October

2011 there have been at least 28 carcasses discovered (Anon. 2011b) when in previous years there were seldom any carcasses found at all.

1.12.4 Canal Entrapment

In 1999 it was discovered that Indus dolphins occasionally enter irrigation canals through the flow regulator gates adjacent to irrigation barrages. Once inside a canal, it is very difficult, or impossible, for dolphins to return to the river against the high velocity, turbulent flow inside the gates. Canals run for hundreds of kilometres and are heavily used and visited by people, and dolphins in canals are at high risk. In addition, each year, all canals are drained of water for several weeks to be dredged of silt. Even if stranded dolphins survive until canal closure, they will almost certainly die when the canals are drained of water and therefore a dolphin rescue programme was initiated by the Sindh Wildlife Department and WWF-Pakistan. More than 82 dolphins have been rescued and returned to the river since 2000 (Bhaagat 2002; Khan 2005). The number of dolphins located each year varies dramatically presumably due to differences in the numbers of dolphin entering canals in the first place, but also due to differences in the number of dolphins detected. Changes in leadership in the local Sukkur office of the Sindh Wildlife Department influences staff motivation to locate trapped dolphins, and the amount of funding available to them for surveying the canals (for example, access to a motorcycle) influences how efficiently dolphins are detected. The quality of the capture operations also varies substantially and dolphins sometimes die during rescue. There have also been occasional reports of dolphins located in canals that originate from Guddu or Taunsa barrages, but these are rare, and no formal rescue programme has been initiated at these barrages.

1.12.5 Downstream Migratory Attrition

It has been suggested that dolphins sometimes move through barrage gates and between subpopulations (Reeves 1991; Reeves et al. 1991). In the past it was assumed that such movement would be primarily uni-directional, downstream through barrages, and that upstream movement would be less frequent, due to the high gradient, rapid and turbulent flow, and frequently shallow water in, and downstream of, the gates. The result would be the gradual attrition of upstream subpopulations. Even a low downstream migration rate could dramatically affect the persistence of upstream subpopulations over time. Downstream migrants would not survive below Kotri Barrage

where the Indus River is dry for much of the year. There have been no published sightings of dolphins moving through barrage gates either in Pakistan or India (Sinha 1997). However, one dolphin that was radio-tracked did move through the gates of Sukkur barrage three times during a one month period, eventually ending up in the Sukkur-Kotri (subpopulation 5) river section downstream (WWF-Pakistan, unpublished). This evidence from a single animal shows that movement is possible, at least at Sukkur barrage, but there is still no information on the magnitude, or net movement direction at different barrages which is what will influence the attrition of upstream subpopulations. There is, however, circumstantial evidence supporting the downstream migratory attrition theory (Braulik et al., 2006; Reeves et al. 1991):

1. Each subsequent downstream subpopulation, except the last, is larger than the preceding one, despite a continually diminishing river flow (Braulik, 2006). The exception to this trend is the small subpopulation furthest downstream (5: Sukkur–Kotri) that persists in severely degraded habitat. It is possible that this subpopulation is augmented by, or consists solely of, migrants from the upstream subpopulation (4: Guddu– Sukkur).
2. Each year Indus River dolphins enter irrigation canals through flow regulating gates that are very similar to barrage gates. Once dolphins enter canals they are usually unable to travel back upstream through the canal gates and return to the Indus River. The fact that dolphins are often present for many months in the canal immediately downstream of the gates, and do not pass back to the river is evidence of this. As dolphins are known to pass downstream through canal gates regularly, it seems likely that they also pass downstream through similar barrage gates.

The magnitude of downstream dolphin migration at each barrage would likely vary based upon differences in engineering design, operational cycle, diversion capacity and location as well as dolphin density in each subpopulation. Barrage permeability would determine subpopulation immigration and emigration rates, and therefore whether migration results in a net attrition or augmentation of that subpopulation. For example, if the downstream migration rate at a barrage is high, the subpopulation upstream would suffer rapid attrition. Alternatively, if the downstream migration rate at a barrage were low, the upstream subpopulation would contribute few migrants downstream and may instead exhibit its own net increase from upstream immigrants. Sukkur barrage diverts more water than other Indus barrages and its gates are therefore lowered, or closed, for a larger part of the year. High dolphin abundance

between Guddu and Sukkur barrages may therefore be the result of high immigration through Guddu barrage and low emigration through Sukkur barrage, resulting in an overall augmentation of the subpopulation by downstream migration (Braulik 2006).

1.12.6 Freshwater

Freshwater ecosystems support around 10% of all currently identified species while occupying only 1% of the earth's surface (Dudgeon et al. 2006). However, these ecosystems are experiencing declines in biodiversity far greater than those in most terrestrial environments (Strayer and Dudgeon 2010). Intensive use of freshwaters by humans has led to widespread habitat degradation, pollution, flow regulation and water extraction, fisheries overexploitation and alien species introductions that are causing declines and extinctions of freshwater species (Strayer and Dudgeon 2010). As a freshwater dependent, large mammalian top predator, resident in one of the most arid and densely populated regions of the world, South Asian river dolphins are highly vulnerable.

The primary threats to the Indus dolphin are considered to originate from the irrigation network, in the form of habitat fragmentation by barrages and degradation or removal of habitat due to extraction of water. The statement, '*In a land where it seldom rains, a river is like gold*' (Albinia 2008), could not be more appropriate to this situation. Provision of water is one of the most politically charged issues in Pakistan. The vast majority of the nation's water comes from the Indus River, and the river passes through neighbouring India prior to entering Pakistan which makes river discharge a very sensitive issue. The finite surface water resources are under great pressure from a large and rapidly growing population (177 million, growing at 2.1% p.a. in Nov-2011 (Population Census Organization 2011)) and expanding economic and agricultural sector. New dams, barrages, river linking projects, and hydropower developments are planned and many are already under construction, and there is constant demand to develop more irrigated agriculture. Per capita water availability has dropped to one of the lowest worldwide, and at present there is little culture of water conservation (Asian Development Bank 2010). Consequently, the future of the Indus dolphin is tied to much larger national issues of governance, security, poverty alleviation, and water management.

1.13 Objectives of this study

This thesis adds considerably to the limited ecological knowledge of Indus River dolphins and it was designed to answer specific questions that are important for their conservation and management. Given the great human pressure on Indus River dolphin habitat, the difficulty of working in the field in Pakistan, and limitations on funding, it was important to conduct applied research that would be of direct use to the Pakistani authorities for conservation.

The thesis Chapters are organized as follows:

Chapter 2 – Distribution, abundance and trends in abundance;
Chapter 3 – Causes and dynamics of Indus dolphin range decline;
Chapter 4 – Habitat availability and habitat use;
Chapter 5 – Phylogenetics of the *Platanistidae* family; and
Chapter 6 –General Discussion, which presents a synthesis of all the findings, places them in a wider ecological context, and lays out a framework for conservation, management and future research avenues.

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Chapter 2

Abundance of Indus River dolphins estimated using mark-recapture from tandem vessel surveys in 2006

Abstract

Robust estimates of abundance are vital for the management of threatened species but these have not previously been generated for endangered South Asian river dolphins due to challenges of survey design. An estimate of abundance for the Indus River dolphin in 2006 was generated by conducting tandem vessel-based direct counts, and conditional-likelihood capture-recapture models were used to correct for missed animals. Including group size, sighting conditions and survey vessel as covariates, the three largest Indus River dolphin subpopulations were estimated as 101 (CV=44.1%) between Chashma and Taunsa barrages, 52 (CV=14.9%) between Taunsa barrage and Ghazi Ghat, and 1289 (CV=33.4%) between Guddu and Sukkur barrages. Sighting probability was high; 75.3% of groups were seen by both independent survey teams, but single animals were almost five times more likely to be missed than groups of 3 or more. Dive time studies indicate that groups were missed primarily due to perception bias, rather than availability bias. As group size increased, there was significantly greater variability in the estimates of their size ($z=11.68$, $df=62$, $p=<0.001$), possibly due to the longer observation time required to count larger groups. Dolphin encounter rates within the Guddu- Sukkur subpopulation (10.35/km) are the highest reported for any river dolphin and direct counts suggest that this subpopulation may have been increasing in abundance since the 1970s, probably due to the cessation of hunting and possible immigration from other subpopulations.

2.1 INTRODUCTION

The ability to estimate abundance with relative accuracy and/or precision is imperative for assessing the status of endangered species and monitoring the effectiveness of conservation measures. An index of relative abundance can be used to detect population trends over time but estimates of absolute abundance are especially important for highly endangered species. Asian river dolphins are among the most threatened mammals and the two South Asian river dolphin subspecies, the Indus (*Platanista gangetica minor*) and Ganges (*Platanista gangetica gangetica*) River dolphins are listed as Endangered by the International Union for the Conservation of Nature (IUCN) due to large range declines, fragmentation by dams and barrages, and habitat degradation due to pollution and water diversion (Braulik et al. 2004; Smith et al. 2004). Future declines in their abundance and range are probable as habitat continues to deteriorate and the importance of robust estimates of absolute abundance is high. However, abundance estimation for South Asian river dolphins is challenging as the two methods commonly used to estimate cetacean abundance, distance sampling and photo-identification, are difficult or impossible to apply to this cryptic species and its environment (Dawson et al. 2008; Smith and Reeves 2000). In the absence of a robust alternative, direct counts in discrete river sections have been conducted, but these have seldom applied a correction factor for missed animals, did not include measures of precision and had unknown biases (Behera and Rao 1999; Bhaagat 1999; Braulik 2006; Sinha and Sharma 2003; Smith 1994; Smith et al. 2001; Smith and Reeves 2000). The Sub-committee on Small Cetaceans of the International Whaling Commission (IWC) noted in 2000 that few reliable abundance estimates were available for any species of freshwater cetacean and that the habitat and behaviour of these animals posed particular problems for abundance estimation (IWC 2001).

2.1.1 Challenges to Survey Design on the Indus River

Capture-recapture analysis of photo-identified animals is commonly used to estimate abundance of cetaceans (Hammond 2009) as well as many other types of organism (Amstrup et al. 2005; Borchers et al. 2002). This method relies on capturing images of uniquely marked animals from a population; the proportion of identified individuals recaptured during subsequent sampling events is then used to estimate abundance (Borchers et al. 2002). Features used for identification of cetaceans range from

permanent and semi-permanent marks on dorsal fins or tail flukes, to the shape of callosities, or unique colour patterns (Hammond 2009). This method has very limited possibilities for South Asian river dolphins because, 1) they are extremely difficult to photograph as they surface alone, unpredictably, for about 1 second and they do not approach boats, and 2) they lack a prominent dorsal fin and rarely possess any identifying features (Smith and Reeves 2000). Not a single individual could be identified from 1200 photographs of Ganges River dolphins (Smith and Reeves 2000).

Distance sampling is widely used to estimate abundance of plants and animals. It relies on the assumption that sighting probability declines with distance from the observer, and assuming no sightings are missed at zero distance allows the calculation of density or abundance within a defined area (Buckland et al. 2001). The primary challenge to the application of line or strip transect methods to South Asian river dolphins is that rivers are very shallow and survey vessels are restricted to travelling down the *thalweg* (the line that follows the deepest part of the river). This results in vessels travelling along a single curving transect that periodically approaches alternate banks as the river meanders. Indus River dolphins are seldom recorded in water less than 2 m deep (see Chapter 4) and dolphin distribution is biased towards the deep water along the survey transect. This survey design results in the unavoidable violation of two critical assumptions of distance sampling as the transect line is not placed randomly with respect to the dolphins and is neither randomly nor systematically located within the survey area resulting in unequal coverage of habitat (Buckland et al. 2001). Other less significant challenges to distance sampling in this environment include measuring perpendicular sighting distance when surveying moving objects from a sharply curving path (Hiby and Krishna 2001; Kreb 2002), frequent constrictions in the river channel that cut off the full potential detection width causing a narrowing or unusual shoulder in the detection function (Dawson et al. 2008) and the presence of a continuous downstream population density gradient (Braulik 2006) that prevents extrapolation of data from one area to another.

As river features are oriented along the longitudinal axis of a river, transects running perpendicular to the river flow, from bank to bank, such as those used in the Amazon River (Martin and da Silva 2004; Vidal et al. 1997), could be the optimal survey design for river dolphins, but navigational constraints preclude this approach on the Indus (Dawson et al. 2008; Thomas et al. 2007). A strip transect at a standard distance from

the river banks was also employed on surveys in the Amazon River (Martin and da Silva 2004; Vidal et al. 1997), and a single downstream transect used for an adapted line transect survey on the Yangtze River (Zhao et al. 2008) but these methods are also very difficult to apply on the Indus as the river varies in width rapidly and repeatedly from approximately 50 m to 1000 m and the vessel cannot maintain a standard distance from the river bank. Aerial surveys have not been attempted for South Asian river dolphins, but high water turbidity would prevent animals being detected below the surface and the extremely brief surfacing time would make detection from above unlikely.

2.1.2 Indus River Dolphin Surveys

Aerial surveys of terrestrial and marine mammals frequently obtain simultaneous counts using independent observer teams, so that mark-recapture can be used to correct abundance estimates for missed animals (Carretta et al. 1998; Crete et al. 1991; Graham and Bell 1989; Hiby and Lovell 1998; Marsh and Sinclair 1989a; Samuel and Pollock 1981). A similar method, using independent teams on a single vessel, was used by Smith et al. (2006) to estimate abundance of Ganges River dolphins and Irrawaddy dolphins (*Orcaella brevirostris*) in the Bangladesh Sundarbans. These methods have been adapted in the present study, to estimate abundance of Indus River dolphins by conducting direct counts using independent observation teams on vessels travelling in tandem along a thalweg transect, and conditional-likelihood capture-recapture models were used to correct for missed animals.

Since 1974, there have been dolphin direct counts conducted primarily by Sindh Wildlife Department (SWD) between Guddu and Sukkur Barrages (subpopulation 4) (Bhaagat 1999; Braulik 2006; Reeves and Chaudhry 1998) (Table 1.1). There is no comprehensive documentation of the methods that were used in these counts, they were not consciously standardised and do not include measures of precision. They are likely to be underestimates of the real population size as no correction was made for animals that were missed when they were underwater (availability bias) or that surfaced in view but were not recognised (perception bias) (Marsh and Sinclair 1989a; Smith et al. 2006). However, the surveys were typically conducted using visual observers on a single oar-powered vessel travelling downstream during the dry season (Gachal and Slater 2003) and there was some consistency in the staff that conducted

surveys over years. If the methods, and hence the proportion of dolphins missed, remain relatively stable over time, they may provide an indication of trends in abundance. Given that these surveys represent an unusually long time-series of counts of a very little known and endangered river dolphin, I explore the trends in abundance that they indicate.

2.2 METHODS

2.2.1 Study Site

The survey was conducted in March and April 2006 when Indus discharge was at its annual low. It was conducted from north to south, in a downstream direction, and a portion of the habitat in each of the five extant Indus River dolphin subpopulations was covered.

2.2.2 Field Survey Methods

Survey methods duplicated those employed during a baseline dolphin status survey conducted in 2001 (Braulik 2006). Observations were conducted from two oar-powered wooden boats travelling in tandem at 5-7 km/hr downstream. The boats were separated by 1-3 km (9-36 minutes), surveyed along the same track and used identical survey methods. Teams observed from a 2 m high platform using 7x50 binoculars and the naked eye. The rapid and unpredictable surfacing behaviour of Indus River dolphins, combined with the relatively narrow survey strip, meant that most sightings were made by the naked eye as that maximised the observers' field of view. Each observation team consisted of three forward observers, one rear observer, and a data recorder. All observers received training prior to the survey and each vessel had a minimum of two observers with prior dolphin survey experience. Observers switched regularly between the forward and rear vessel. Environmental conditions and river width were recorded at the beginning and end of each period of survey effort, every 30 minutes when observers rotated positions and when conditions changed. The effect of wind on the river surface was evaluated according to the following 'river state' scale: 0 = Water surface glassy; 1 = ripples without crests; 2 = small wavelets with crests but no white-caps; 3 = large wavelets with scattered white-caps; 4 = small waves with fairly frequent white-caps. When viewing conditions deteriorated to river state 3, surveying was postponed until conditions improved. A Garmin MapSounder 176 unit was used to

record the survey track and river depth at 1 m intervals and a Garmin 76S GPS recorded the location of the boat when dolphins were sighted. These devices collect horizontal geographic positions accurate to within 3 to 5 m.

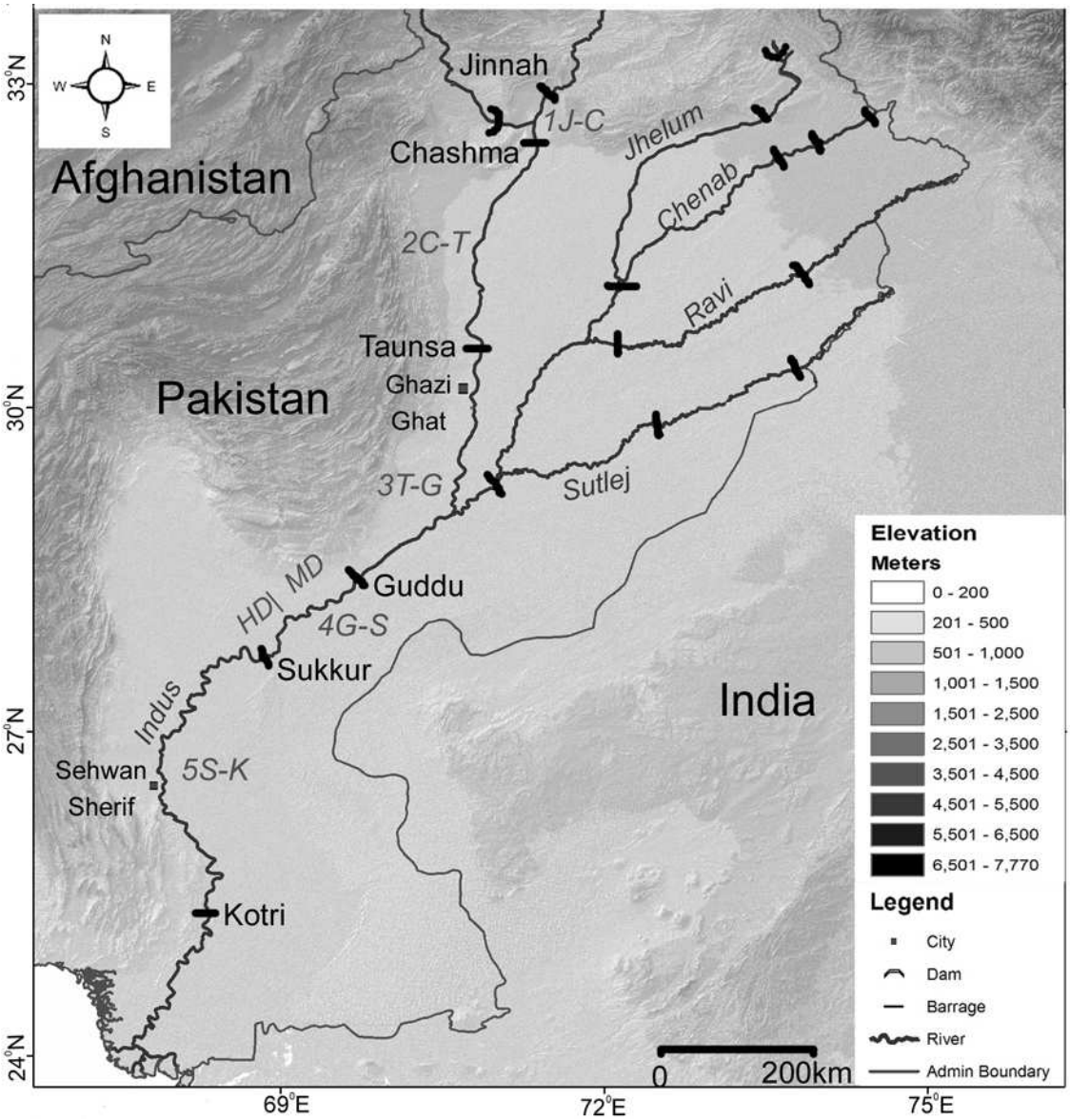

Figure 2.1 – Map of the Indus River system illustrating the barrages that form the boundaries between the five subpopulations. Each subpopulation is denoted by the following acronyms that include the sequential number of the subpopulation and the barrages it is bounded by: 1J-C = Jinnah to Chashma, 2C-T= Chashma to Taunsa, 3T-G= Taunsa to Guddu, 4G-S = Guddu to Sukkur and 5S-K = Sukkur to Kotri. MD = Moderate density portion of 4G-S, HD = High density portion of 4G-S.

Indus River dolphins typically occur in loose aggregations, rather than cohesive groups, so a group was defined as animals occurring within 500 m in similar fluvial habitat such as a meander bend, channel constriction etc. (Smith et al. 2006; Smith and Reeves 2000). In the lower half of the 4G-S river section encounter rates were very high; dolphins occurred continuously with no obvious gaps between groups and river features, such as constrictions, or mid-channel bars, were used to delineate groups and facilitate counting. The extreme turbidity of the Indus River water means that animals cannot be seen prior to breaking the surface. When a dolphin was sighted, the boat continued moving downstream while observers focused on obtaining an accurate group size estimate. At the detection location, observers estimated the distance to the animal, where possible using laser range finders to measure the distance to nearby objects, such as river banks, to improve the accuracy of distance estimations. Generally, dolphins were sighted downstream and ahead of the survey vessel and remained relatively stationary, so that the vessel approached and passed through groups on its downstream passage. The ‘detection location’ was recorded by GPS when a dolphin was first sighted and an ‘exact location’ was recorded when the estimated centre of the group was perpendicular to the vessel. The observer team worked together to reach a best estimate of group size by consensus. To account for uncertainty in group size estimates, low and high estimates were also made. Very small animals that appeared to be less than 100 cm in length were recorded as calves. In rare instances, when a group or individual appeared to be moving up or downstream, the direction of movement was recorded.

Navigation on the complex braided channels was aided by the use of satellite images. As the summer flood annually rearranges Indus River channels, only satellite imagery recorded in the same season as the survey are useful for navigation. Significant secondary channels were identified using satellite images and were surveyed by the rear boat while the forward vessel continued along the main channel in non-tandem survey effort. Groups located at confluences were assigned to the main channel and two-way radio communication between survey vessels reduced the chance of double counting. The geographic locations of all vessels encountered were recorded, along with presence or absence of a motor, the approximate length of the vessel, and the boats activity.

2.2.3 Meeting Model Assumptions

Abundance was estimated using capture-recapture methods adapted for tandem visual surveys (Marsh and Sinclair 1989a). The basis of this method is that all sightings made by the forward survey vessel are 'captured', the second vessel then surveys the same area and the distance between the exact geographic positions of each group is used to classify sightings as unique if they were 'missed' by one of the boats or as duplicates or 'matches' if they were seen by both. Closed population capture-recapture analyses includes assumptions that if violated result in biased estimates of abundance. The fundamental assumptions are: 1) that the population is geographically and demographically closed between sampling events, 2) captures are recognised correctly, 3) captures are not lost, 4) capture does not affect the probability of recapture, and 5) all groups in all circumstances have an equal likelihood of capture (no capture heterogeneity) (Borchers et al. 2002). The approach taken to comply with, or correct for violation of each of these assumptions is described below:

1. Population closure – The assumption of population closure is reasonable as the population that is being surveyed is bounded into a linear strip by the lateral river banks and up and downstream between irrigation barrages with closed gates. As the two surveys, or capture events, were separated by less than 36 minutes, significant demographic changes would not have occurred.

2. Capture recognition – To determine which sightings were matches and which were missed, a determination was made based on the distance between the 'exact' geographic positions of each group, combined with supporting information on group size and the group movement direction. Using a small threshold distance will result in recognition of more missed sightings and therefore a larger abundance estimate, conversely a wide threshold distance will result in fewer missed sightings being recognised and a smaller estimate of abundance. The threshold distance was selected based on a frequency distribution of distances between potentially matched sightings (see Section 2.2.4 below).

3. Capture loss – Capture loss would occur if the forward vessel sighted a dolphin group that then moved a considerable distance before it was sighted by the second survey vessel. In this instance instead of being identified as a group previously captured and therefore matched, it would be identified as a new group that had

been missed by the first vessel. Field experience indicates that over a short space of time Indus River dolphins typically do not move more than a few hundred meters and capture loss is not expected to contribute greatly to bias (Braulik, unpublished). However, to minimise this potential the time between the two surveys was kept as short as possible (< 35 mins) without causing interference between the boats and group movement direction was included in the matching process.

4. Capture does not affect the probability of recapture - If dolphins changed their behaviour or movement direction in response to the forward vessel they may have been either more or less visible to the rear survey vessel, and sighting probabilities would differ between the two vessels. To allow for this possibility models were designed that included i) a separate sighting probability for each vessel and ii) a single uniform sighting probability for both vessels combined.

5. Capture heterogeneity - A standard assumption of capture-recapture is that there is equal capture probability for all groups in all circumstances. During this survey, single animals were much more likely to be missed than groups of three or more, so this assumption was undoubtedly violated. Neglecting to account for this capture heterogeneity will result in abundance being underestimated. The Huggins conditional likelihood method was therefore adopted as this allows for capture heterogeneity to be modelled as a function of sighting covariates (Huggins 1989, 1991).

An additional source of potential downward bias in this survey is that animals were missed because they were too distant from the observers. To minimise this bias, geographic coverage of available habitat was maximised by surveying the entire length of the Indus main channel and deploying a separate boat to survey large side channels, behind islands and the far side of wide channel habitat. Detection probability was maximised by surveying only in excellent and good survey conditions (river state 0 to 2) and at a relatively slow speed. Perpendicular sighting distance could not be generated as the vessel was surveying moving animals from a sharply curving path, so the relationship between radial sighting distance and river width was explored to reveal whether the majority of animals were likely to have been detected within the river channel.

2.2.4 Identification of Matched Groups

The distance between the exact position of each dolphin group was measured along the centre of the river channel using ArcView 3.2. When two sightings made by the same boat were within 500m of one another (the pre-determined group definition), the sightings were deemed to be the same group and were condensed into a single sighting with a new central location. The new group size was the sum of the two subgroups. This process resulted in 12 groups being condensed into six.

The geographic positions of sightings from each survey vessel were compared and a distance threshold used to evaluate whether a sighting was matched (seen by both boats) or missed (seen by only one boat). Previous studies have selected thresholds based on knowledge of species travel speeds, sometimes combined with the time lag between surveys, and have varied substantially from 9.3 km for tandem aerial surveys of bottlenose dolphins (*Tursiops truncatus*) (Carretta et al. 1998), to 500m for double platform vessel surveys of Ganges River dolphins (Smith et al. 2006). Virtually nothing is published about the movements or swimming speeds of Indus or Ganges River dolphins on which to base the determination in this study, however, field observations indicate that in general groups do not move a great deal over a period of hours (Braulik, pers.obs.), and therefore a relatively small threshold distance is appropriate. A frequency distribution of the distance between the exact geographic positions of potentially matched dolphin groups (those within 2 km) was generated, and the obvious clumping of distances was used to guide selection of an appropriate distance threshold with which to classify groups as matched. I selected a threshold distance that allowed for some dolphin movement between detections. I assumed that matches were made without error; however, to test how robust the results were to the selection of threshold distance, a sensitivity analysis was conducted to compare the influence of six candidate thresholds (300 to 800 m) on the number of sightings identified as matched. For example, if the geographic locations of two sightings, measured along the centre of the river, differed by 450 m, they would be counted as separate groups that were each missed in the 300, and 400 m threshold scenarios, but as matched groups in the 500, 600, 700 and 800 m scenarios.

2.2.5 Abundance Estimation

2.2.5.1 Estimating Number of Groups

Abundance was estimated separately for each subpopulation. Sightings made during tandem survey effort were analysed using mark-recapture for closed populations in a Huggins conditional-likelihood model implemented by the program MARK (White and Burnham 1999). Whereas the Lincoln-Peterson mark-recapture estimator uses maximum likelihood to estimate abundance, the more complex Huggins model allows much greater flexibility as it conditions abundance out of the likelihood (Huggins 1989, 1991). The modelling was conducted using dolphin groups, rather than individual animals as the unit, in order to satisfy the assumption of independence between detections. Capture probabilities are modelled as a function of sighting covariates according to the following formula (Huggins 1989, 1991):

$$\text{logit}(p_{ik}) = Ln\left(\frac{p_{ik}}{1-p_{ik}}\right) = \beta_0 + \sum_j \beta_j x_{jk}$$

Where: logit = link function, p = the probability of capture i = forward or rear survey boat, k = dolphin group, j=covariate, β_0 = intercept and β_j = slope for covariate value x_j.

Group abundance and variance during tandem effort was estimated within MARK using a Horvitz-Thompson like estimator (Horvitz and Thompson 1952):

$$\hat{g}_t = \sum_{k=1}^{g_t} \frac{1}{\hat{p}_k} \qquad \text{var}(\hat{g}_t) = \sum_{k=1}^{g_t} \frac{1}{\hat{p}_k}(1-\hat{p}_i).$$

where $\hat{g}_t$ is the estimated number of groups present during tandem effort and $\hat{p}_k$ is the estimated probability that school k is detected by either platform.

Covariates used in the model were selected based on similar studies, combined with knowledge of the Indus River environment and of Indus River dolphin behaviour. Perception bias of Ganges River dolphins was influenced only by group size not by sighting conditions or channel width (Smith et al. 2006). Detection of harbour porpoise (*Phocoena phocoena*) was influenced by group size and sea state (Hammond et al. 2002), and the vaquita (*Phocoena sinus*) by sea state (Gerrodette et al. 2011). I included group size, river surface state and sighting vessel as covariates in models for each subpopulation. Group sizes used were those recorded in the field. Although

possible error in group size estimation is not explicitly accounted for, the effect on the abundance estimate and variance is expected to be small. When group size estimates for matched groups differed, the estimate from the forward vessel was always used as this was considered the most reliable.

In general, the more parameters that are included in a model the better it fits the data, but the lower the precision of the estimates. Akaike's Information Criterion corrected for small sample sizes (AICc) was used to select the most parsimonious model with the fewest parameters, according to the following guidelines: 1) differences of less than two in AICc values were taken to indicate that the models have approximately the same weight 2) differences of more than two but less than seven in AICc values indicate there is considerable support for a real difference between the models and 3) differences of more than seven between AICc values indicate that there is strong evidence of a difference between the two models (Burnham and Anderson 2002). To account for uncertainty in model selection if the best fitting models were separated by less than 2 AICc points they were averaged based on their normalised AICc weights. Unlike other mark-recapture models, there is no good way to test goodness-of-fit for closed capture models, however, model averaged estimates of abundance weighted according to AICc are more robust than single model estimates and if this method is used the necessity for testing goodness-of-fit is waived (Stanley and Burnham 1998).

2.2.5.2 Estimating Mean Group Size

Mean group size was estimated ignoring potential errors in recorded school sizes, but attempting to correct for smaller schools being less detectable. To produce an estimate of mean group size ($\bar{s}$) in each subpopulation the detected number of groups of each group size (n_j) were corrected by the average detection probability of a school of particular size ($\hat{p}_j$) output by MARK, and this used to estimate a group size distribution, from which the mean was determined:

$$\bar{s} = \frac{\sum_{j=1}^{s_{\max}} \frac{n_j \cdot j}{\hat{p}_j}}{\sum_{j=1}^{s_{\max}} \frac{n_j}{\hat{p}_j}}$$

The variance in estimated mean school size was generated from the sample variance of the estimated recorded school sizes, after adjusting for variability in detection probability.

2.2.5.3 Non-tandem Effort

The capture-recapture method described above was reliant on tandem survey effort, however, portions of non-tandem survey effort were conducted in all river sections. In addition, the 4G-S subpopulation was subdivided into two strata: the upper 106 km with moderate dolphin density (MD) (3.63 dolphins/linear km), and a lower 98 km with high density (HD) (9.06 dolphins/linear km). In the HD sub-section there were no obvious gaps between aggregations which made it impossible to determine whether sightings from the two vessels matched. Capture-recapture using tandem survey data was therefore not conducted on the HD sub-section and these data were treated as non-tandem. To account for groups missed in each subpopulation during non-tandem survey periods a correction factor (f_m) determined from the tandem-effort survey was applied: $f_m = \hat{g}_t / g_{ft}$ where g_{ft} is the number of groups seen by the forward vessel during tandem effort. The group size from tandem effort was applied to sightings made during non-tandem effort, except for the 4 G-S HD sub-section where group size was substantially larger than other areas, and corrected group size was calculated using the method described above, and the group size detection probabilities determined from tandem survey effort in the 4 G-S MD sub-section. Sighting conditions in side channels were very different from the main channel and it was considered inappropriate to apply the main channel group correction factor to these areas so individuals seen in side channels (*ns*) were added to main channel sightings without correction.

2.2.5.4 Estimating abundance

Abundance of dolphins in each subpopulation seen during tandem survey effort $\hat{N}_t$ was estimated as $\hat{g}_t\ \bar{s}_t$, and the CV: $CV\left(\hat{N}_t\right) \approx \sqrt{\left[CV^2\left(\hat{g}_t\right) + CV^2\left(\bar{s}_t\right)\right]}$.

Abundance of dolphins in each subpopulation seen during non-tandem survey effort $\hat{N}_{nt}$ was estimated as $\hat{g}_{nt}\ \bar{s}_{nt} + ns$, and the CV: $CV\left(\hat{N}_{nt}\right) \approx \sqrt{\left[CV^2\left(\hat{f}_m\right) + CV^2\left(\bar{s}_{nt}\right)\right]}$.

The CV of the correction factor is derived from: $se\left(\hat{f}_m\right) = \dfrac{\sqrt{\mathrm{var}\left(\hat{g}_t\right)}}{g_{ft}}$.

Total subpopulation abundance was generated by summing the tandem and non-tandem sightings, and total metapopulation abundance by totalling the abundance in each subpopulation. CV's were combined using the delta method, and if there were shared factors between strata these were factored out to account for covariance (Buckland et al. 2001; Gerrodette et al. 2011).

Log-normal confidence intervals, where the lower limit cannot be smaller than the number of unique individuals sighted (M_{t+1}), were calculated according to the following (Williams et al. 2002):

Lower CI = $M_{t+1} + \left(\dfrac{\hat{f}_0}{C}\right)$ Upper CI = $M_{t+1} + \left(\hat{f}_0 C\right)$

Where: $\hat{f}_0 = \hat{N} - M_{t+1}$, and $C = \exp\left\{1.96\left[\mathrm{Ln}\left(1 + \dfrac{\mathrm{var}(\hat{N})}{\hat{f}_0^{\,2}}\right)\right]^{1/2}\right\}$

The confidence intervals generated for the current study are skewed because the lower bounds are constrained to lie between the abundance estimates and *Mt+1*, and as sighting probabilities were high these values are similar. By contrast the upper bound of the confidence interval is unconstrained and is influenced by the precision of the abundance estimates.

2.2.6 Availability Bias

The contribution of dolphin availability to total detection bias (corrected for by the tandem surveys) was investigated using radial dolphin sighting distances, vessel speed

and the dive and surface behaviour of groups of different sizes. In the dry-season of 2008, between Chashma and Taunsa barrages (subpopulation 2), dive times of groups or individuals were recorded with a stop watch from a vantage point on the river bank. A dive time was the interval between surfacings that lasted longer than 2 seconds. Group size was recorded when dive time monitoring began and if this subsequently changed, the time and new group size were recorded. Surfacing of this species is so rapid and unpredictable that it is not possible to accurately measure surface interval in the field, therefore surfacings were recorded with digital video and surface time measured by sequentially viewing each frame. Footage was recorded at 25 frames/second resulting in surface intervals accurate to 0.08 seconds. The probability that a group was available to be seen by observers was determined according to the following (Barlow et al. 1988): $p = (st + w) / (st + d)$, where p = the number of surfacings when a survey vessel was present, st = mean surface time, d = mean dive time, and w = the time window that individuals or groups were within range of observers, determined using sighting distances and vessel speed.

2.2.7 Bias in Group Size Estimates

It is challenging to accurately estimate group sizes of Indus River dolphins because groups are dispersed and the surfacing of individuals is not synchronised. In order to determine the time required to obtain a good estimate of group size, in the dry-season of 2008, between Chashma and Taunsa barrages (subpopulation 2), timed counts of groups of different sizes were conducted from the river bank. Dolphin groups less than 200m from the bank were located and observers recorded their best estimate of group size at regular intervals for 20 minutes. The assumption was made that the actual group size did not change during this exercise, but if the group or a subgroup moved away from the observers then counting was abandoned. Estimated counts typically increased with observation time before becoming asymptotic at which point the count was considered to be stable.

2.2.8 Trends in Abundance

Dolphin direct counts of the Guddu to Sukkur subpopulation have been conducted for 34 years. Annual and total rates of population change were calculated and trends in abundance examined using linear regression. A power analysis was then conducted

using the programme TRENDS (version 3.0) to determine what level of abundance estimate precision would be necessary to allow the observed trend to be detected with different levels of confidence (Gerrodette and Brandon 2000). Power analysis was based on the general inequality equation (Gerrodette 1987):

$$r^2 n^2 \geq 12CV^2 (z_{\alpha/2} + z_{\beta})^2$$

Where *r* is the annual rate of population change, *n* is the number of surveys, CV is the co-efficient of variation of the abundance estimate, $z_{\alpha/2}$ is the probability of committing a Type I error (the probability of rejecting the null hypothesis when it is true- in this case assuming an increasing trend when there is none) and, z_{β} is the probability of committing a Type II error (the probability of accepting the null hypothesis when it is false- in this case assuming no increase when in fact there is one).

2.3 RESULTS

2.3.1 Survey Summary

The survey expedition was conducted from 23rd March to 24th April, 2006, and covered 808 km of the Indus River and 126 km of significant secondary channels coincident to the main channel. The channels between Jinnah barrage and Ghazi Ghat bridge (65 km downstream of Taunsa Barrage) (subpopulations 1,2 &3), between Guddu to Sukkur barrages (subpopulation 4) and between Sehwan Sharif and Kotri barrage (subpopulation 5) were surveyed (Fig. 2.1). Approximately 300 km (81%) of the river between Taunsa and Guddu barrages (3) were not surveyed due to the poor security situation near Rajanpur. Environmental conditions were generally excellent for viewing cetaceans, 46% of survey effort was conducted in glassy surface conditions and 92% of survey effort was conducted in river surface state two or better.

River discharge and channel width decrease in the downstream direction due to the diversion of water at each barrage (Table 2.1). There was no significant difference between the daily counts recorded by each boat (paired t-test, p = 0.704). As expected the number of sightings declined with radial distance from observer; the majority of dolphin sightings were detected at between 0 and 400m (Fig. 2.2). Mean dolphin radial sighting distance was 401 m (SD=279.1), consistently greater than half the mean river width (200-300m), and sightings often occurred at distances up to 1km (Fig. 2.2).

Although, these are radial sighting distances, not perpendicular distances, this still illustrates that the majority of surfacings within the river channel could be detected. The small number of sightings seen from 0-100 m is likely because the good environmental conditions and frequent surfacing of dolphins allowed them to be easily detected at greater distances, however it is also a possibility that dolphins were avoiding the survey vessels.

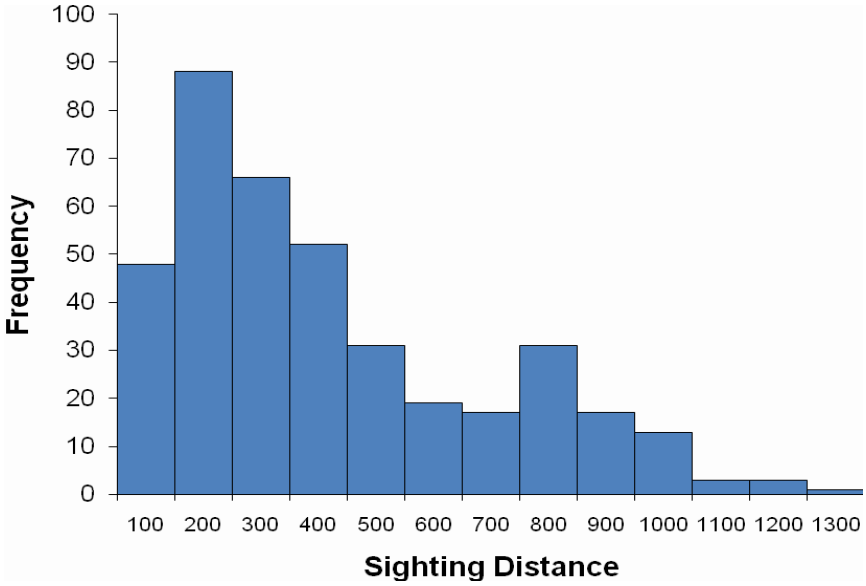

Figure 2.2 – Frequency of dolphin radial sighting distances. The available survey strip, represented by half mean river width, varies between 200 and 300 m depending on the section of river.

Dolphin encounter rate and mean group size increased from the northern extreme of the range, downstream to Sukkur barrage. As mean group size increased, the distance between groups decreased. Direct counts derived from the sum of group size estimates of the forward survey vessel plus animals sighted in secondary channels totalled: 1 in section 1J-C (Jinnah to Chashma barrages); 82 in section 2C-T (Chashma to Taunsa barrages); 44 between Taunsa barrage and Ghazi Ghat bridge (3T-GG); 1275 in section 4G-S (Guddu to Sukkur barrages); and four in section 5S-K (Sukkur to Kotri barrages). Determined from the data of both boats combined, calves accounted for approximately 14% (13 calves) of total individuals in 2C-T, 7% (4 calves) in 3T-GG and 11% (142 calves) in the 4G-S section. Dolphins were sighted in secondary channels only in the 2C-T (4 individuals) and 4G-S (5 individuals) sections, and encounter rates in these channels were very low, 0.08/km and 0.3/km, respectively,

compared to adjacent uncorrected main channel encounter rates, 0.27/km and 6.23/km, respectively (Table 2.1). Within each surveyed subpopulation, distribution was biased in the downstream direction; the centroid of distribution in the 2C-T subpopulation was 63.8% of the distance from Chashma to Taunsa barrage, and in the 4G-S subpopulation it was 55.5% of the distance downstream of Guddu barrage.

Table 2.1 – Summary of direct counts recorded in the Indus River dolphin range-wide abundance survey in 2006

River Section	*Direct Count (Low, High)*	*Dolphins/ km*	*Mean Group Size*	*Mean Distance between Groups (Km)*	*Mean River Width (m)*
Jinnah-Chashma	1	-	-	-	651 (SD=339)
Chashma-Taunsa	82 (75-103)	0.27	1.98 (SD=1.61)	3.23 (SD=2.98)	578 (SD=272)
Taunsa-Ghazi Ghat	44 (39-51)	0.68	2.63 (SD=1.46)	2.34 (SD=1.46)	637 (SD=288)
Guddu-Sukkur	1275 (1138-1469)	6.23	7.65 (SD=7.52)	1.24 (SD=1.05)	411 (SD=165)
Sehwan Sharif-Kotri	4	-	-	-	243 (SD=139)

A total of 134 boats were recorded, the vast majority being open wooden vessels between 5 and 10m in length. 53% of boats were motorised and the remainder were oar or sail powered. Most vessels were either motorised ferries that traverse the river (38%) or subsistence fishing boats (31%). The largest number and density of vessels were located in 2C-T (60 boats, 0.20/km) and 5S-K (57 boats, 0.33/km). Very few boats were recorded in the 4G-S section where dolphin encounter rate was highest.

2.3.2 Identification of Matched and Missed Dolphin Groups

Between Chashma barrage and Ghazi Ghat, the forward vessel recorded 42 groups and the rear vessel 45. Matching of sightings in most areas was unambiguous because there were long distances between detections, encounter rate was low (<1 dolphin/km),

groups can only move in two directions, up- or downstream, and most potentially matched groups were very close to one another. Of potentially matched sightings the geographic locations of 88% occurred within 600 m, and 78% were within 400 m (Fig. 2.3). 600 m was selected as the appropriate threshold distance for determining matched sightings as it encompassed the majority of probable matches, allowed for some group movement between surveys and it was greater than the 500 m distance used to define a group.

Sensitivity analysis showed that the 300 to 800 m distance thresholds resulted in less than 5% difference in the corrected number of groups estimated by the Huggins mark-recapture model (Table 2.2), but if the threshold was reduced to 300 m the number of sightings classified as missed substantially increased. The sensitivity analysis clearly demonstrates that changing the threshold distance used to define matched groups from 400 to 800m, does not exert a great influence on the resulting estimates of abundance.

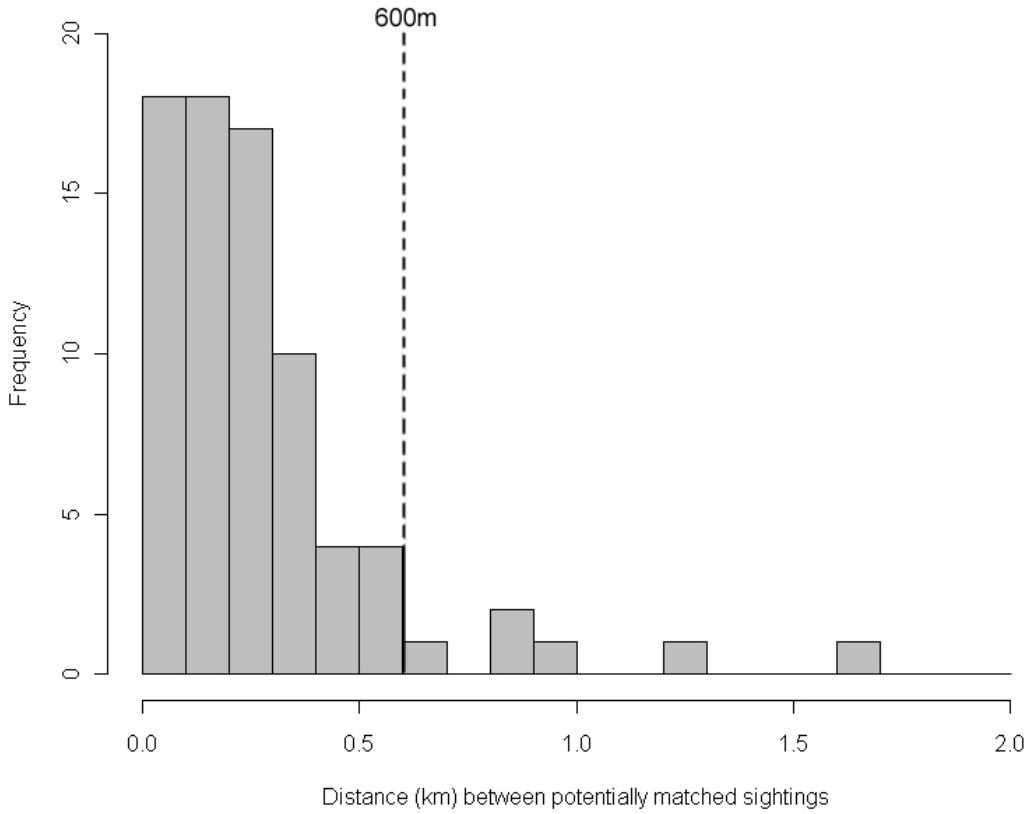

Figure 2.3 – Distance between the exact geographic positions of potentially matched dolphin groups. Vertical line indicates the 600 m distance threshold selected to classify sightings as matched.

Table 2.2 – Comparison of difference distance thresholds used to identify matched and missed sightings

Distance Threshold	***nf***	***nr***	***mfr***	$\hat{g}_t$	**se(**$\hat{g}_t$**)**	***% difference*** $\hat{g}_t$ ***from 600m***
300m	41	41	26	56.8	3.6	+4.6
400m	41	41	29	55.1	2.8	+1.6
500m	41	41	31	54.2	2.4	0
600m	**41**	**41**	**31**	**54.2**	**2.4**	**0**
700m	41	41	32	53.8	2.2	-0.7
800m	41	41	32	53.8	2.2	-0.7

nf, nr, mfr = number of groups seen by the forward, rear and by both vessels during tandem survey effort between Chashma and Ghazi Ghat; $\hat{g}_t$ = Correct number of groups calculated using the Huggins model. se($\hat{g}_t$) = standard error of the correct number of groups.

2.3.3 Estimation of Abundance

A binomial Generalised Linear Model (GLM) was used to test the relationship between the time lag between tandem vessels and the probability that a dolphin group was missed. Four sightings that occurred when there was more than 35 minutes separating the vessels were reclassified as occurring during non-tandem effort, and in the remaining dataset the probability that a dolphin group was missed was independent of survey time lag (GLM, z=-1.56, p=0.12). In 50% of tandem survey effort the vessels were separated by less than 10 mins, and in 75% the boats were less than 20 mins apart. Sighting probability was high; 75.3% of groups were seen by both independent survey teams, but single animals were almost five times more likely to be missed than groups of 3 or more. Mark-recapture analysis was conducted on the tandem survey data from each of the three largest dolphin subpopulations, but was not conducted on the sightings that occurred between 1J-C (1 animal) and 5S-K (4 animals), due to the small sample size.

2.3.3.1 Chashma to Taunsa

In the 2C-T section, during tandem survey effort, the forward boat recorded 27 groups, the rear 26, 18 sightings were matches and 17 were unique. Nine groups were sighted

during non-tandem main channel effort, and four individuals were recorded in side channels. Missed groups were significantly smaller than matched groups (Mann-Whitney test, W = 258.5, p < 0.0001) (Fig. 2.4). Of the 35 sightings, 48.6% were missed by one team, including 77.8% of single animals, and 37.5% of groups of two. All groups of three or more were sighted by both vessels. There was no significant effect of river state on the proportion of sightings that were missed (Mann-Whitney test, W = 170.5, p = 0.76). The model with the lowest AIC_c included a single capture probability influenced only by the covariate group size. The mean group size observed in the field, 1.98, was corrected to 1.50 (CV=8%) based on group size sighting probabilities generated by the model. The final abundance estimate for this subpopulation was 101 (95% CI = 74-317, CV = 44.1%) (Table 2.3).

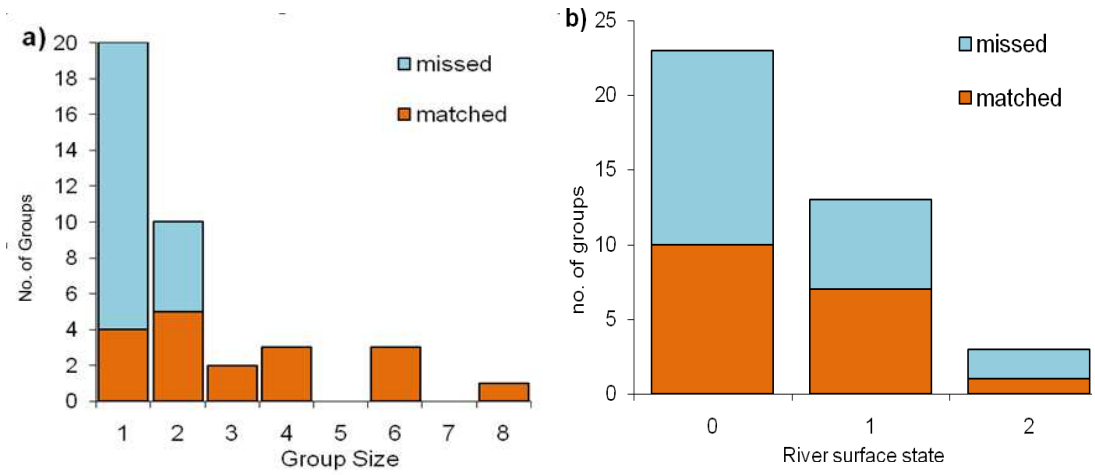

Figure 2.4 - Frequency of missed and matched sightings by a) groups size and b) river state between Chashma and Taunsa barrages.

2.3.3.2 Taunsa to Ghazi Ghat

Between Taunsa barrage and Ghazi Ghat (subpopulation 3) 14 groups were seen by the forward vessel and 15 by the rear vessel. Thirteen were classified as matched, and only 3 were missed. There were 3 non-tandem sightings and no groups recorded in side-channels in this section. The top three candidate models that were averaged included the influence of river surface state on sighting probability. As the final models did not include the covariate group size, the mean group size recorded in the field 2.63

(CV = 12.7%) was assumed to be unbiased. Abundance for this portion of the 3T-G subpopulation was estimated to be 52 (95% CI = 50-102, CV = 14.9%) (Table 2.3).

2.3.3.3 Guddu to Sukkur

In the MD sub-section of the 4G-S subpopulation 43 groups were seen by the forward vessel, 35 by the rear vessel and 33 were classified as matched. 27 groups were seen during non-tandem effort and 5 individuals in a side channel. All groups of six or more individuals were seen by both vessels, but 50% of single animals were missed. Matched groups were significantly larger than those that were missed (Mann-Whitney test, $W = 88$, $p < 0.01$) and there was no obvious effect on sighting probability attributable to river state (Mann-Whitney test, $W = 254$, $p = 0.13$) (Fig. 2.5). The model with the lowest AICc included the covariate group size and a different capture probability for each vessel. Corrected group size estimates were 4.73 (CV=11.0%) in the MD section and 9.26 (CV=9.1%) in the HD section. The group correction factor of 1.09 was applied to the sightings in the HD sub-section to give a final abundance estimate of 1289 (95% CI =1192-4120, CV = 33.4%) for this subpopulation (Table 2.3).

2.3.3.4 Metapopulation Abundance

The sum of the above three abundance estimates and the animals sighted between Jinnah and Chashma (subpopulation 1), and Sukkur and Kotri (subpopulation 5) was 1447 (CV = 57.2%). Three hundred km of dolphin habitat, between Ghazi Ghat and Guddu barrage (within subpopulation 3), was not covered by the present survey, therefore, to provide an approximate estimate of subspecies abundance, I include data from previous surveys. In 2001, 200 dolphins were recorded in the 300km section that was missed in 2006 (Braulik 2006). The direct counts recorded in the surveyed portion of this subpopulation in 2001 and 2006 (45 versus 44, respectively) were very similar indicating that no large changes have occurred (Braulik 2006). However, conservatively allowing abundance in that area to have changed ±50% in the intervening five years means that there may have been between 100 and 300 individuals in the unsurveyed stretch in 2006, and I therefore suggest that the subspecies numbered approximately 1550-1750.

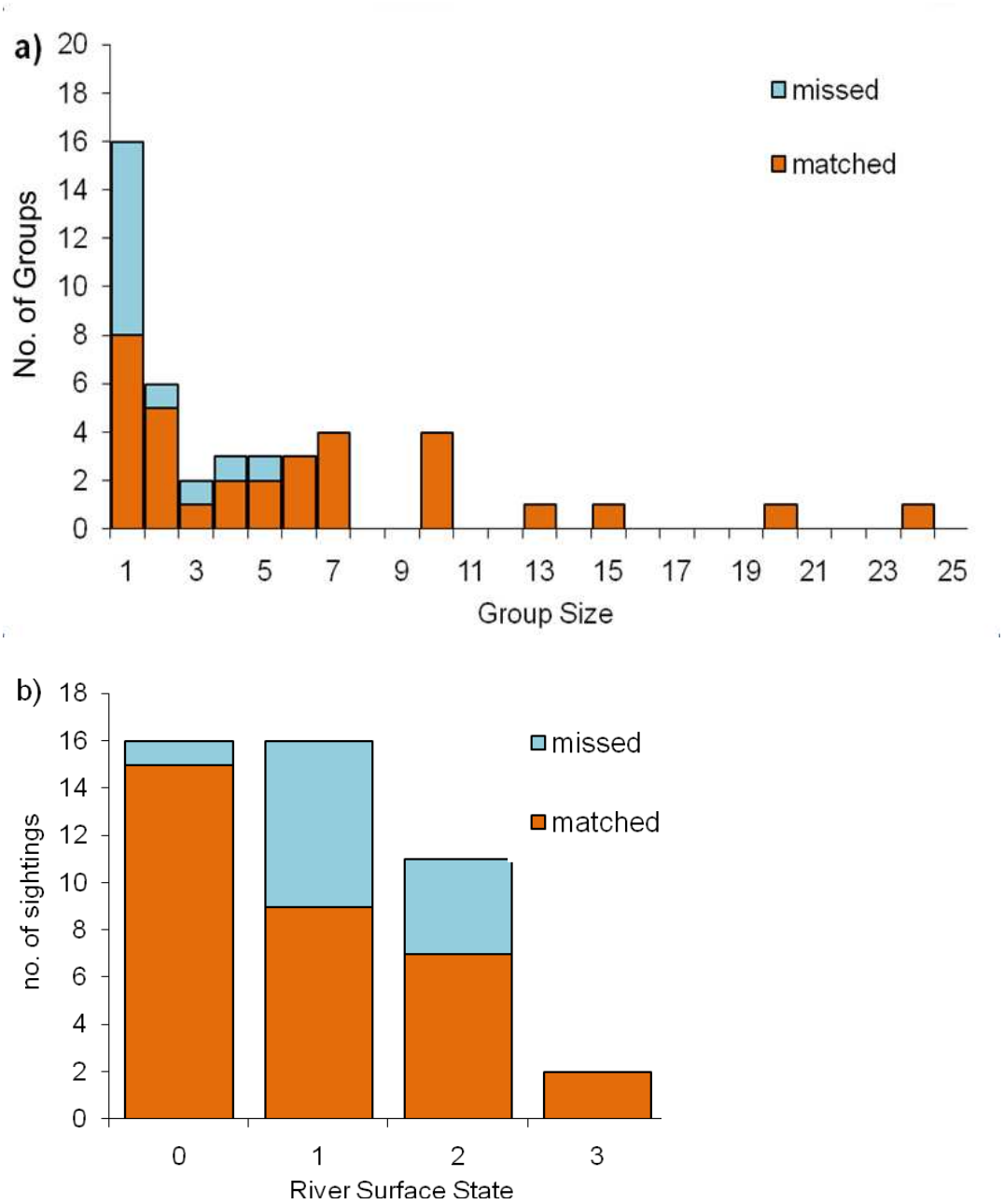

Figure 2.5 - Frequency of missed and matched sightings by a) groups size and b) river state between Guddu and Sukkur barrages.

Table 2.3 – Summary of Indus River dolphin subpopulation abundance estimation.

River Section	***Strata***	***nf***	***nr***	***mfr***	***Mt+1***	$\hat{p}$	f_m	f_m ***CV***	$\hat{g}$	$\hat{g}$ ***CV***	***ns***	$\bar{s}$	$\bar{s}$ ***CV***	***Ň***	***Ň CV***	***95% CI***	**er**
2 C-T	tandem	27	26	18		0.864			48.5	21.1		1.50	8.0	73	22.5		
	non-tandem	9					1.80	37.9	16.2	-	4	1.50	8.0	28	38.7		
	Total				70									**101**	**44.1**	**74-317**	**0.34**
3 T-GG	tandem	14	15	13		0.999			16.4	5.0		2.63	12.7	43	13.7		
	non-tandem	3					1.17	5.80	3.5	-	0	2.63	12.7	9	14.0		
	Total				50									**52**	**14.9**	**50-102**	**0.80**
4 G-S MD	tandem	43	35	33		0.879			47.0	18.0		4.73	11.0	223	21.1		
	non-tandem	27					1.09	19.7	29.5	-	5	4.73	11.0	145	22.5		
4 G-S HD	non-tandem	91					1.09	19.7	99.5	-	0	9.26	9.1	922	21.7		
	Total				1189									**1289**	**33.4**	**1192-4120**	**6.30**
	Grand Total				**1309**									**1442**	**57.2**	**1312-7014**	

2 C-T=Chashma to Taunsa, 3 T-GG=Taunsa to Ghazi Ghat, 4 G-S=Guddu to Sukkur. MD=Moderate density sub-section of the Guddu to Sukkur subpopulation. HD=High density sub-section of the Guddu to Sukkur subpopulation. *nf, nr, mfr* = number of sightings seen by the forward, rear and by both vessels during tandem survey effort. *Mt+1*= number of unique individuals sighted during the survey. $\hat{p}$ = sighting probability. f_m = group correction for non-tandem effort. $\hat{g}$ the corrected number of tandem or non-tandem effort sightings. n_s = number of individuals recorded in side channels, $\bar{s}$ = corrected mean group size. ***Ň*** = Abundance estimate. er= encounter rate.

2.3.4 Availability Bias

A total of 1156 dive times were collected from 33 groups ranging in size from 1 to 5 individuals. Mean group dive time decreased as group size increased because there is no synchronisation in surfacing behaviour in this species (Table 2.4). Dolphin surfacings lasted from 0.60 to 1.76 secs, averaging 1.01 secs (SD=0.28; CV=27.3%; n=103), and 61% of surfacings lasted for less than 1 second. Although not specifically investigated, surface interval appears to be unaffected by group size and individuals seldom surface at the same time even in large groups. Consequently, the proportion of time spent at the surface increased with group size and ranged from 1.3% to 4.7% (Table 2.4). A frequency distribution of dolphin radial sighting distances (Fig. 2.2) indicated that detection probability was high to 400m and then slowly declined. It would take 4.81 mins, travelling at the mean survey speed of 5 km/hr to cover 400m and this was used as the time window within which animals could be detected. All of the dive-surface cycles recorded were considerably shorter than 4.81 mins, and it was therefore concluded that the contribution of availability bias to total detection bias was negligible. These data illustrate that there are many more opportunities to detect larger groups than single animals, however, even single animals would typically surface several times in view of observers.

Table 2.4 – Sighting availability of Indus River dolphin groups

Group Size	Dive Time (n; 95%CI)		% time at surface	Surfacings within sighting time window (4.81mins/288s)*
1	78s	(181; 60-97s)	1.3	3.7
2	56s	(282; 28-83s)	1.8	5.1
3	51s	(337; 29-72s)	2.0	5.6
4 & 5	22s	(356; 6-37s)	4.7	12.8

*calculated from the mean sighting distance of 401m which would take 4.8 minutes to traverse at the target survey speed of 5km/hr.

2.3.5 Group Size Bias

Biased estimates of group size contribute to biased estimates of abundance and such biases can be difficult to evaluate and correct. Comparison of the group size estimates for matched sightings (excluding data in the high density part of the 4G-S section where group size was arbitrarily determined) demonstrates the variability and uncertainty associated with group size estimation. For 30% of matched groups, group size estimates were identical and 75% of estimates were within two individuals despite the time delay between surveys (Fig. 2.6a). The difference between group size estimates were on average greater and more variable as group size increased. This relationship was modelled using a Poisson Generalised Linear Model (GLM) to allow for the non-constant variance observed in the data (Breusch-Pagan test, χ^2=90.84, p<0.001). With the y-intercept constrained to zero, a highly significant relationship between group size and variability in the estimates of their size was observed (z=11.68, df=62, p=<0.001) (Fig. 2.6b).

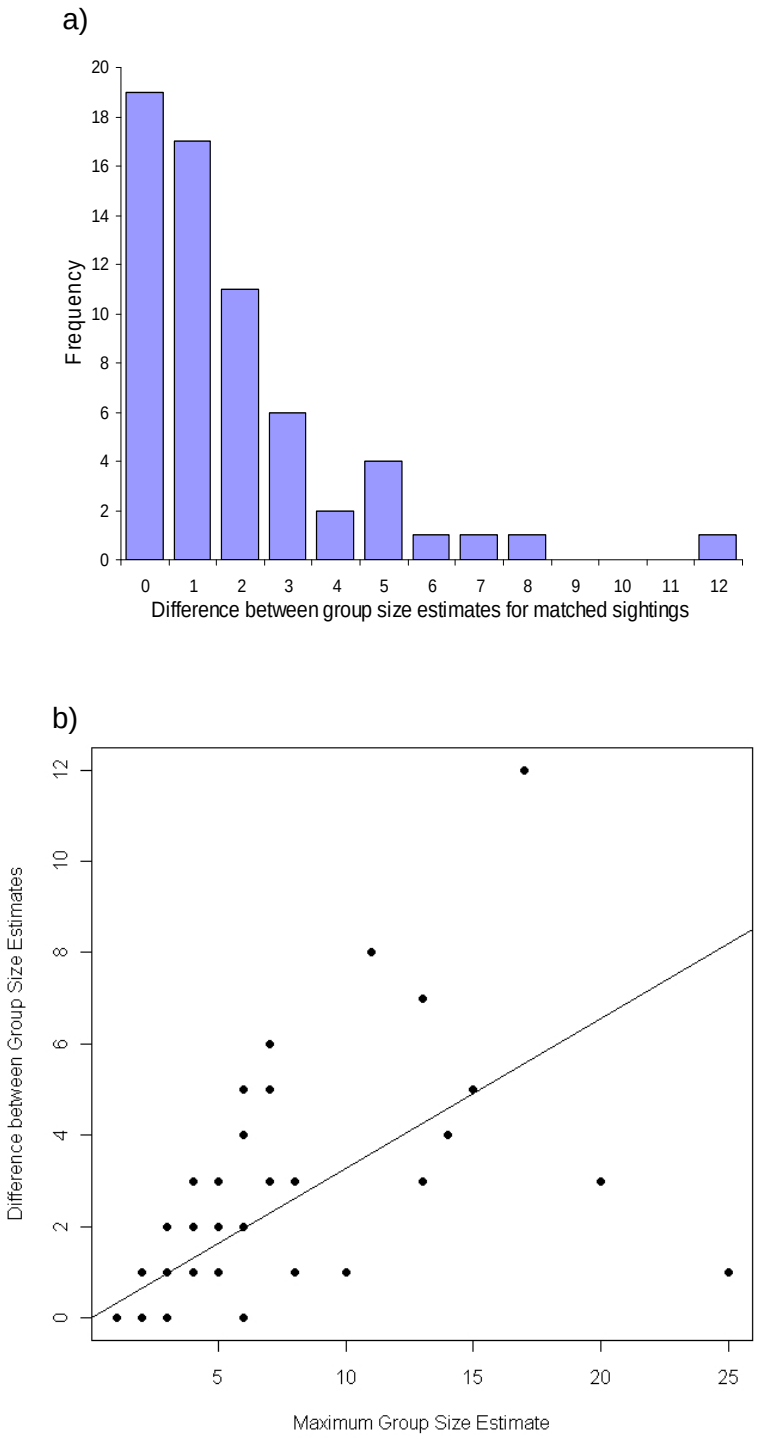

Figure 2.6 – Difference in group size estimates for matched sightings

One hypothesis to explain the decrease in precision of group size estimates as group sizes increase is that the unsynchronised surfacing behaviour and lack of group cohesion means a longer observation time is required to estimate the size of large groups. On average, the larger the group size, the longer observation time was

required to reach a stable group size estimate. The time required varied significantly by group size (ANOVA, F=8.4603, p<0.001). For groups of two on average 4 min 21 sec (SD=3 min 53 sec, n=14) was required to obtain a stable size estimate, for groups of three, four or five, the average time required was 10 min 13 sec (SD=6 min 3 sec, n=16) and for groups of six, seven and eight, 13 mins 42 secs (SD=7 mins 3 secs, n=10) of observation time was needed (Fig. 2.7). Counts for groups of two were obtained significantly quicker than groups of 3, 4 and 5 (Tukeys test; p<0.05) and groups of 6, 7 and 8 (Tukeys test; p<0.001), but there was no significant difference between the latter two categories, which is likely to be due to the increased variability in time taken to estimate the size of larger groups.

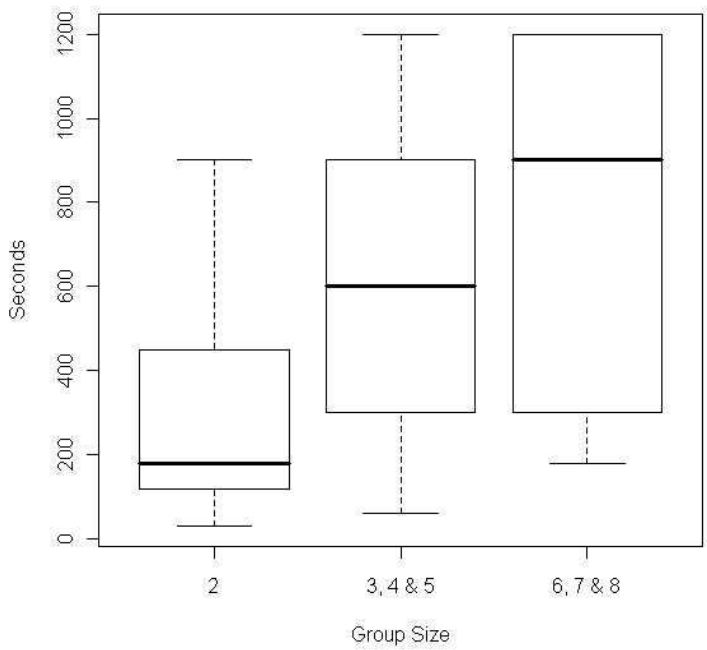

Figure 2.7 – The time taken to estimate the number of dolphins in a group according to group size

At an average survey speed of 5km/hr it would take 5 minutes to cover 400 meters (the average sighting distance) and estimate group size before the boat passed the group. This would allow sufficient time to count groups of two, but groups of three or more may have been underestimated. According to this experiment, groups of 3, 4 or 5 would need to be detected at 850m, and groups of 6, 7 or 8 at more than 1100m, to allow sufficient time to obtain an accurate estimate of their size. Although larger

groups are likely to be seen at greater distances, these data indicate that large group sizes may be underestimated because there is insufficient time available to estimate their size with accuracy. However, during these vessel surveys the rear observer was also involved in counting after the boat had passed the group, thereby increasing the time that each group is observed and presumably also reducing underestimation in counts.

2.3.6 Trends in Abundance

The direct counts generated by the present survey and those from 2001 (Braulik, 2006) used identical field methods and recorded very similar counts in every subpopulation except for between Guddu and Sukkur (subpopulation 4) (Table 2.5) where the 2006 count was 64.5% greater than 2001. Direct count surveys were also conducted by Sindh Wildlife Department (SWD) during the same time period. They reported 500 dolphins between Guddu and Sukkur barrages in 2001, and 807 in 2006 (SWD, unpublished data); an increase of 61.4% over the same 5 year period. The absolute counts recorded by the two groups were different, due to different methods, but both recorded a similar increase.

Table 2.5 – Comparison of direct counts of Indus River dolphin subpopulations recorded in 2001 and 2006 using identical survey methods

Subpopulation	***2001 (Braulik, 2006)***	***2006 (this study)***
1 J-C	2	1
2-C-T	84	82
3-T-GG	45	44
4-G-S	775*	1275
5 S-K	18	4^

*In 2001, 602 dolphins were counted, and after extrapolation of a conservative mean encounter rate (3.6/km) to an unsurveyed 33.3 km segment, 725 estimated (Braulik 2006). As the unsurveyed segment was in a very high density area application of the encounter rate from adjacent channels (5.0/km) is more realistic and this was therefore applied to generate a revised estimate of 775 animals in 2001 in this subpopulation. ^ the whole 5S-K subpopulation was not surveyed in 2006, so figures cannot be directly compared between years

Counts increase from 138 in 1974 (Pilleri and Zbinden 1973-74) to 902 in 2008 (SWD, unpublished) and show an exponential growth rate. The natural logarithm of counts against time demonstrates a steady, statistically significant, increase (Linear Regression: F=135, p=<0.001) equivalent to approximately 5.75% per year (Fig. 2.8).

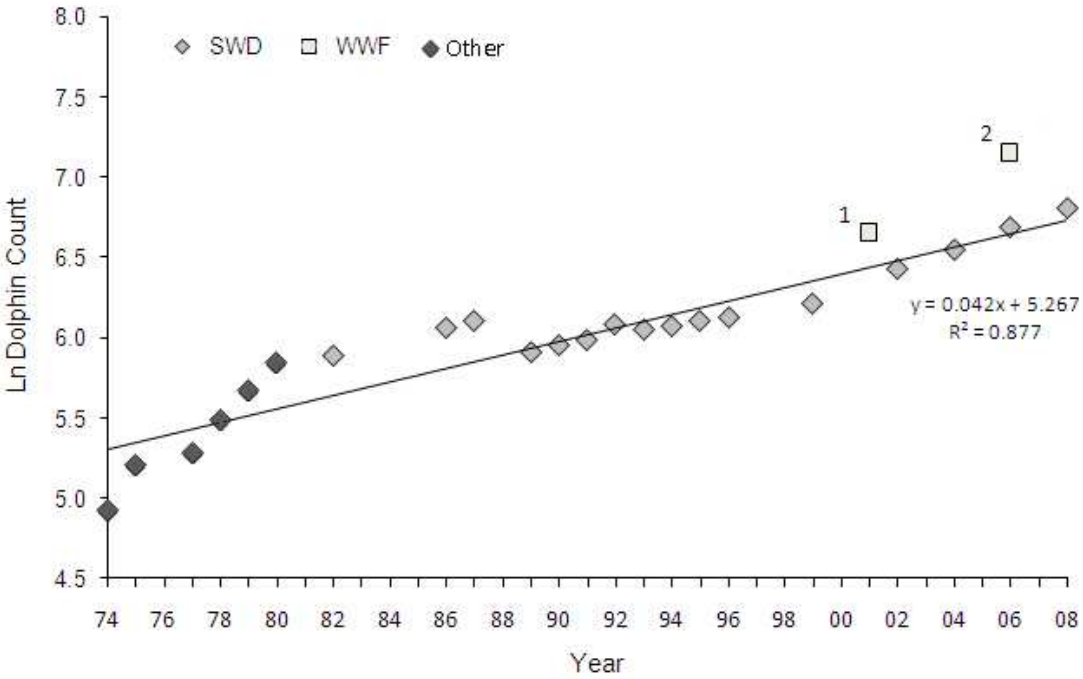

Figure 2.8 – Natural logarithm of Indus River dolphin (*Platanista gangetica minor*) direct counts recorded between Guddu and Sukkur barrages between 1974 and 2008. The natural logarithm was applied to the counts to transform the exponential increase into a linear increase that could be examined using linear regression. 1= Braulik 2006, 2=the direct count recorded in this study. SWD=Counts conducted by Sindh Wildlife Department. WWF/PWP=World Wildlife Fund-Pakistan and Ministry of Environment's Pakistan Wetlands Programme. Other=counts conducted by authors other than SWD or WWF, for details see Braulik 2006 and Bhaagat 1999.

A power analysis indicated that at a confidence level of 0.05 a CV as large as 54% would allow the trend to be correctly detected. When the probability of committing Type I or Type II error is further reduced to 0.01, a CV up to 32% would allow the trend to be correctly identified. Both these CV's are similar to those computed for the present survey. The large population increase and the frequent surveys mean that estimates can be relatively imprecise and the trend can still be detected with a high level of confidence.

2.4 DISCUSSION

2.4.1 Survey Design

In medium and low density areas the survey method was effective, but it was not possible to apply at very high densities (> 6 dolphins/linear km). Such densities, however, are exceptional and have not been reported elsewhere in Asia. Tandem boats were used because there were no suitable vessels for placing independent teams on a single boat. The advantages of using tandem vessels are that both perception and availability bias can be evaluated simultaneously, that both observer teams are viewing from the same height platform so data are comparable and that the presence of a second boat provides flexibility for surveying side channels. However, tandem vessels require that there are two trained observer teams, which is often not the case, they require additional cost and greater logistical coordination and data analysis is more challenging due to the time lag between surveys. Whether using tandem vessels or two platforms on a single vessel, the direct count capture-recapture survey method following a thalweg transect shows great potential for abundance estimation of dolphins in confined areas, or shallow rivers such as the Indus, Ganges, Brahmaputra and Ayeyarwady where dolphin densities are generally low and traditional methods for estimating abundance cannot be easily applied.

2.4.2 Potential for Bias in Abundance Estimate

2.4.2.1 Meeting Model Assumptions

The greatest uncertainty involved in this study is the ability to correctly recognise captures. Individuals within Indus River dolphin groups are often quite dispersed, therefore the group locations used in the matching process are inherently inexact. In addition, the exact position was recorded when a group was judged to be perpendicular to the vessel, and therefore does not necessarily represent the centre of the group. Both of these factors may have contributed to errors in recognizing matched sightings. However, the frequency distribution of distances between potentially matched sightings demonstrates that there is little ambiguity in identifying matches, the sensitivity analysis showed little change in the number of matches even when quite different distance thresholds were used and the great majority of sightings could be readily determined as matched or missed.

Groups moving and therefore not being recognized as matched (analogous to capture loss) may have occurred on occasion, but the high proportion of matched sightings (75.3%) and that matched sightings were on average only 200 m from one another indicates that this was not a significant source of bias. To reduce the potential for capture loss the time between the two surveys was kept as short as possible without causing interference between the boats and group movement direction was included in the matching process.

The best-fitting models for the 4G-S MD section included a lower capture probability for the rear survey vessel, which might suggest that in the high density area dolphins avoided the vessels. In all other areas, there was a uniform capture probability across vessels which indicates no vessel attraction or avoidance behaviour.

Small groups are more likely to be missed during a visual survey than large groups (Barlow 1988; Barlow and Forney 2007; Carretta et al. 1998; Chen 1999; Innes et al. 2002; Pollock and Kendall 1987). Sighting probability of Indus River dolphins was generally high, which is the best way to minimize the bias caused by individual heterogeneity (Borchers et al. 2002) and as data were modelled with covariates, bias due to sighting heterogeneity should be low. A larger sample size would have allowed inclusion of more covariates, which may have further improved the models.

As the surveys covered the majority of the subspecies habitat, it was not necessary to estimate detection probability as a function of perpendicular distance or to extrapolate animal densities to a larger area. Perpendicular sighting distance could not be generated, however, mean sighting distance was consistently greater than half the mean river width, so the assumption that the majority of dolphins within the river channel could be detected is not unreasonable. However, in especially wide sections of the river it is still probable that dolphins were missed.

2.4.2.2 Availability and Perception Bias

The data presented here demonstrate that Indus River dolphin groups of all sizes surface frequently and were consistently available to be detected by observers. The failure of observers to detect or recognise surfacing (perception bias) was therefore primarily responsible for missed groups. This result is similar to that from a study of

Ganges River dolphins in Bangladesh (Smith et al. 2006). It was suggested by Dawson (2008) that perception bias is potentially largest for species (such as the Indus River dolphin) that occur as single animals or in small groups and do not show much of their body when surfacing. Perception bias is often highest for inexperienced observers (Barlow et al. 1988; Laake et al. 1997) and it is likely that observer inexperience contributed to the higher proportion of missed groups between Chashma and Taunsa barrage (subpopulation 2) which was the first to be surveyed. This reinforces the importance of training and observer experience in future surveys, however even in excellent survey conditions and with experienced observers it is inevitable that some groups will still be missed (Barlow et al. 1988).

2.4.2.3 Bias in Group Size Estimation

Estimation of *Platanista* group size is challenging because individuals do not surface at the same time and because groups have poorly delineated boundaries. The lack of synchronisation in group surfacing may have developed because in such shallow habitat a group can easily maintain acoustic contact while individuals surface independently. The data indicate that there is considerable variability in the estimates of large group sizes and that large groups may be underestimated. However there is no way to document over-counting if it occurred. Increased variability and bias in group size estimates with increasing size has been documented in surveys of marine dolphins (Barlow and Forney 2007; Carretta et al. 1998; Gerrodette et al. 2002; Marsh and Sinclair 1989b; Rugh et al. 2008). Comparison of vessel-based group size estimates of schooling marine dolphins, with true counts from aerial photos showed that there was considerable variation in estimates, but generally observers tended to underestimate on average by about 25% (Gerrodette et al. 2002). Averaging the independent group size estimates of each observer has also been shown to be a reliable way to estimate true group size of marine dolphins (Barlow 1995; Forney and Barlow 1998).

Future surveys on the Indus River will need to incorporate methods to further evaluate and calibrate group size estimates (Gerrodette and Forcada 2005; Gerrodette et al. 2002). Use of aerial photos will not be feasible given the inability to see dolphins through the turbid water. Two possibilities that hold promise are developing a correction factor derived from bank-based counts, and averaging observers'

independent estimates. However, errors in group size estimation, are potentially a smaller problem for abundance estimation of Indus River dolphins than for marine dolphins that often form schools of several hundreds and where sighting conditions are often poor (Barlow et al. 1988).

2.4.3 Abundance and Encounter Rate

At least ten Indus River dolphin subpopulations have been extirpated in the last century (Reeves et al. 1991). The farthest upstream (1J-C) and downstream (5S-K) extant Indus subpopulations, and the one in the Beas River, are each estimated to number ten or fewer individuals and all are unlikely to persist in the long-term, leaving only three that are potentially viable. Conserving the 2C-T subpopulation is of high priority, because it is the smallest of these three and due to its small size is highly vulnerable to extirpation. Its loss would mean Indus River dolphins remain in only approximately 550km of river, dramatically increasing the vulnerability of the subspecies. Small populations are susceptible to random demographic stochasticity, environmental catastrophes, inbreeding depression and loss of genetic diversity that can all contribute to increased extinction risk (Lande 1988; Purvis et al. 2000; Rosel and Reeves 2000).

Encounter rate in the 4G-S subpopulation was 6.30 dolphins/linear km, peaking at 10.35 dolphins/linear km in an 80 km section. These are the highest encounter rates reported for any river cetacean. High densities of Amazon River dolphins (18 km^2 and 4.2 per linear/km) are also observed in specific favourable habitat (Martin and da Silva 2004), but the encounter rates reported here are more than double those in the Amazon and occur over a much wider area. Encounter rates in the 2C-T subpopulation are similar to those reported for other Asian river dolphins but those in 4G-S are several times higher (Table 2.6).

Given the degree of disturbance to the natural flow and sediment transport regime of the Indus River system (Alam et al. 2007) and that this subpopulation is subjected to pollution from upstream, it is hard to understand how the environment can support such an unusually high density of dolphins. However, the present survey was conducted when animals were concentrated by dry-season flow levels, the high observed density is presumably ephemeral because for much of the year river discharge is higher, and density and competition for resources is reduced. At present, few quantitative data are available as a basis for comparing habitat quality, prey availability and dolphin mortality

rates in the 4G-S subpopulation with other parts of the Indus River to show why this area is so important for Indus River dolphins. However, some superficial differences can be noted: 1) upstream the river is wide, braided and shallow but between Guddu and Sukkur barrages it changes character due to a decrease in slope, and is primarily a single, sinuous channel of greater mean depth (albeit lower discharge in the dry season) 2) the 4G-S subpopulation has been legally protected in the Sindh Dolphin Reserve since 1974, and local communities are aware of the importance of dolphins due to awareness raising by the provincial wildlife department, 3) human activity in this section is lower than elsewhere, in general because it is an insecure tribal region where people do not move freely, and specifically as the river corridor is remote and forested and sometimes provides refuge for bandits. A future study to compare the fish resources and habitat quantity and quality in each dolphin subpopulation is the logical next step and will shed light on why the Guddu to Sukkur area is so important for Indus River dolphins. This is addressed partially in Chapter 4.

Table 2.6 – Comparison of dolphin encounter rates on the Indus River with those recorded for other river dolphins. Where multiple encounter rates were published only the highest is noted.

River	***Encounter Rate***	***Citation***	***River***	***Encounter Rate***	***Citation***
Yangtze	0.71/km^2	(Zhao et al. 2008)	Indus 2C-T	0.27/km	This study
Mekong	0.02/km	(Beasley 2007)	Indus 3T-G	0.74/km	(Braulik 2006)
Mahakam	0.13/km	(Kreb 2005)	Indus 4G-S	3.60/km	(Braulik 2006)
Ayeyarwady	0.47/km	(Smith and Tun 2007)	Indus 4G-S	6.23/km	This study, subpopulation mean
Brahmaputra	0.23/km	(Wakid 2009)	Indus 4G-S	10.35/km	This study, mean over 80km highest density section
Ganges: Bihar	1.80/km	(Choudhary et al. 2006)	Amazon: Boto, Brazil	4.20/km	(Martin and da Silva 2004)
Ganges: Sundarbans	0.47/km	(Smith et al. 2006)	Amazon: Boto, Columbia	0.60/km	(Vidal et al. 1997)
Ganges: Karnaphuli	1.36/km	(Smith et al. 2001)	Amazon, *Sotalia*, Columbia	1.62/km	(Vidal et al. 1997).

2.4.4 Trends in Abundance

Until the early 1970's, dolphins between Guddu and Sukkur barrages (subpopulation 4) were hunted for food and oil by minority tribes that lived along the river (Pilleri and Zbinden 1973-74). Active hunting ceased probably by the mid to late 1970's, however there are no other legal controls on human activities inside the dolphin reserve and fishing is permitted. The observed increase in abundance between Guddu and Sukkur barrages is likely to be the direct result of the removal of hunting pressure on the subpopulation. Although there is no quantitative historical information on the fish fauna or habitat of the Indus River, the increase in dolphin abundance is unlikely to be due to improvements in habitat or prey availability, as new dams and barrages have been constructed in the last 35 years, the Indus seasonal flood cycle has been greatly disrupted, dry season discharge has declined and levels of pollution have increased dramatically as the country becomes industrialised (Tariq et al. 1996; World Bank 2005). The river corridor in upper Sindh is a tribal area subject to banditry and lawlessness, resulting in low levels of human activity compared to other parts of the river that may contribute to low dolphin mortality rates.

Another factor to consider that could theoretically contribute to the increase in abundance between Guddu and Sukkur is the role of immigration and emigration from other subpopulations. It is probable that dolphins can traverse irrigation barrages and move between subpopulations during the few weeks of the year when gates are fully open (Braulik 2006; Reeves et al. 1991). Many factors are likely to influence whether animals move through a particular barrage including its design, river discharge, hydrology, adjacent dolphin density and most importantly how the barrage is operated and how frequently the gates are opened. It is likely that dolphins never traverse some barrages, but frequently traverse others. If the predominant movement of migrants is downstream, this 'downstream migratory attrition' would result in the decline of all upstream subpopulations and the increase of the subpopulation at the downstream end (Reeves et al. 1991). At present there is no information on how many dolphins may move through barrages, but for this to contribute to the increase in abundance between Guddu and Sukkur barrages immigration through Guddu barrage would need to be greater than emigration through Sukkur barrage. However, as the increase in counts from 2001 to 2006 between Guddu and Sukkur exceeded the total number of animals recorded in all other subpopulations, immigration cannot be solely responsible for the increase in this area. The large proportion of calves and juveniles observed (11%)

suggests that this subpopulation is reproducing rapidly and if immigration does occur, it is likely supplementing the increase, rather than being solely responsible for it. It is essential that there is continued monitoring of the Indus subpopulations, using standard survey methods to provide more robust data for determining trends in abundance. Radio or satellite tracking of dolphins in different locations and seasons will also help to shed light on dolphin movement between subpopulations.

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Chapter 3

Causes and dynamics of Indus River dolphin range collapse

Abstract

The historical range of the Indus River dolphin has declined by 80% since the 19th century and has been fragmented into 17 river sections by irrigation barrages. Understanding the spatial and temporal pattern, and factors responsible for this decline are essential to address the conservation of the remaining animals. Dolphin sighting and interview surveys show that river dolphins persist in six river sections, have been extirpated from ten, and are of unknown status in the remaining section. Last dolphin sighting dates were established for each extirpated dolphin subpopulation. The mean time from final habitat fragmentation, by barrages, to subpopulation extirpation was 49 years (SD=23; range=9-74). Seven potential drivers (river slope, river size, fragmentation date, length of river section, dry season river discharge, distance from former range limit and number of river confluences) were included in three sets of regression models to select those which best explained 1) the spatial pattern of range decline, 2) the temporal pattern of subpopulation extirpation, and 3) the time to extirpation after habitat fragmentation. Low dry-season river discharge, due to water extraction for human use, was found to be the principal factor that explained the dolphin's range decline. The probability that a dolphin subpopulation has been extirpated increased with decreasing dry season discharge and increasing proximity to the range limit. Subpopulations were extirpated earlier and more quickly at the periphery of the historical range and where dry season river discharge was low. There is predicted to be only a 5.2% chance that Indus dolphins remain in the Sutlej River near the India-Pakistan border which has not been surveyed. The dolphin subpopulations that are most likely to disappear in the future are predicted to be those above Harike headworks and downstream of Sukkur barrage. Comprehensive environmental flow assessments that consider the habitat requirements of river dolphins and fish, as well as human requirements for irrigation water, are essential for a sustainably managed river system, and for the future of the remaining Indus dolphins.

3.1 INTRODUCTION

Indus River dolphins have been extirpated from 2500 km of river in the last 150 years, equivalent to an approximate 80% decline in their range (Braulik et al. 2004; Reeves et al. 1991). They are believed to no longer occur in the majority of the five largest Indus tributaries, the Jhelum, Chenab, Ravi, Sutlej and Beas Rivers (hereafter termed 'the Punjab rivers'), and are confined now to only the Indus mainstem. Details of when dolphins disappeared from the Punjab rivers are vague, and the causes not clearly understood. Contributing factors are likely to include the construction of twenty irrigation barrages that fragmented the former range of the dolphin into 17 sections, and depleted dry-season river flows as water is extracted for irrigation and other human uses (Braulik 2006; Pilleri and Pilleri 1979; Reeves et al. 1991; Reeves and Leatherwood 1994). Unexpectedly, in 2006 an isolated remnant subpopulation of dolphins was discovered more than 600 km from all others, at the periphery of the former range, in the Beas River in India (Behera et al. 2008) (Fig. 3.1). What factors have allowed river dolphins to persist in the Beas when they have disappeared from numerous other apparently similar areas is unknown, and the discovery raised the possibility that overlooked dolphin populations may persist in areas from which they were previously believed to have been extirpated.

Contraction of geographic range is one of the principal characteristics exhibited by declining or threatened species (IUCN 2001), and answering basic questions about how ranges and populations decline can suggest how these species might be better conserved (Channell and Lomolino 2000b; Simberloff 1986). However, a fundamental problem in understanding patterns of range decline is that rare and declining species become progressively harder to detect and it is therefore extremely challenging to obtain sufficient reliable data to analyse both the causes and patterns of such declines (Turvey et al. 2010a). In general, at the periphery of a species geographic range, populations occupy less favourable habitat and occur at lower and more variable densities. Therefore, as a species becomes endangered it is expected that its geographic range will contract inwards, and that populations will persist in the range core until the final stages of decline (Lomolino and Channell 1995). This theory does not often hold in reality, and for most endangered mammals the pattern of range decline is dictated by the spread of factors driving the decline, with those populations last impacted, regardless of their location, persisting longer than those that were historically large (Channell and Lomolino 2000a, b; Lomolino and Channell 1995). In

contrast, the decline and eventual extinction of the Yangtze River dolphin (*Lipotes vexillifer*) demonstrated a different pattern, as there was no reduction in distributional range only a steady decline in abundance (Turvey et al. 2010a).

The causes of a species decline can be numerous and complex, often interact, and are frequently difficult to identify with certainty (Allan and Flecker 1993). Habitat loss and degradation, spread of exotic species, overexploitation, secondary extinctions, chemical and organic pollution, and climate change have been identified as the major threats to biodiversity in running waters (Allan and Flecker 1993). Declines of freshwater species have been much less comprehensively studied than those of terrestrial species and the driving factors and processes may be quite different. For example, habitat fragmentation, consistently associated with increasing extinction risk, has different and possibly even more severe consequences in rivers, than in two-dimensional terrestrial habitats (Fagan 2002; Fagan et al. 2002). However, the presence of populations in several rivers can spread the risk of longitudinally correlated environmental catastrophes, such as flooding or pollution that might wipe out an entire species if present only in a single river (Quinn and Hastings 1987). Dispersal among subpopulations can also partially alleviate the effect of fragmentation on extinction rates (Reed 2004), but in contrast to two-dimensional landscapes where multiple routes of movement among subpopulations may be possible, re-colonisation in rivers has to originate from one of the neighbouring subpopulations. In addition, pollution or other habitat degradation at specific points in a riverine landscape can reduce connectivity of the system preventing dispersal (Fagan 2002).

In this Chapter I document the spatial and temporal dynamics of the Indus River dolphin range decline. Regression models are then applied to select which of a number of potential explanatory variables best explain the observed geographical pattern of decline, the timing of subpopulation extirpation, and the speed of disappearance of subpopulations after habitat fragmentation. Greater understanding of the Indus dolphin range decline will help suggest refugia where dolphins may persist within their historical range, identify extant subpopulations at greatest risk of extirpation and suggest what needs to be addressed to conserve the remaining animals.

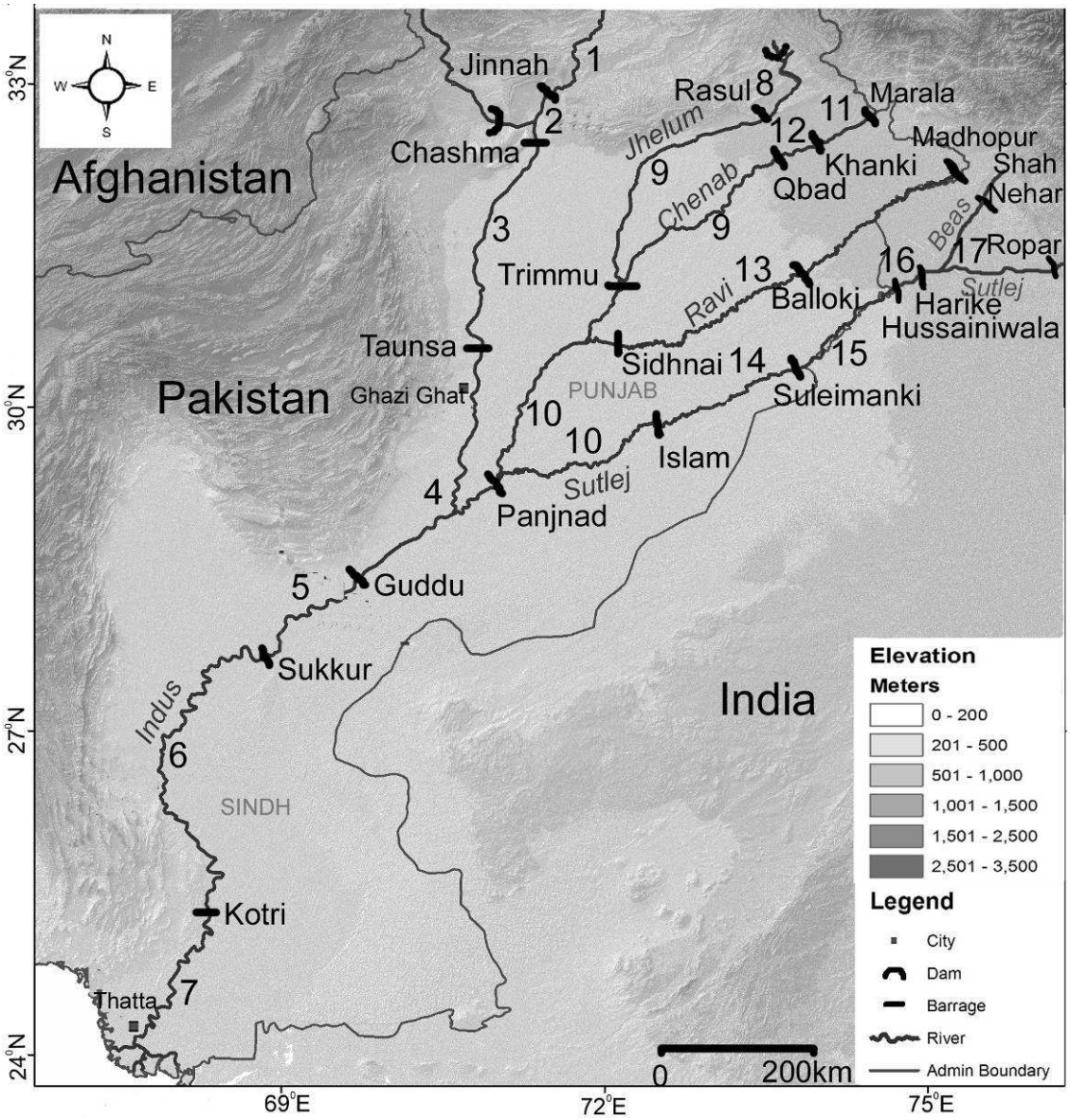

Figure 3.1 – Map of the lower Indus River system, with rivers and barrages named, and each extant or extirpated Indus dolphin subpopulation numbered. Dolphins are extant in subpopulations numbered 2 to 6, and 17, and have been extirpated from all others.

3.2 METHODS

In this chapter, the portion of river between two barrages is referred to either as a 'river section' or as a 'fragment' of dolphin habitat, and dolphins located between barrages as a 'subpopulation'. The status and abundance of the six extant dolphin subpopulations are relatively well understood (Chapter 2), but there is little information on the timing or

causes of decline from the 11 subpopulations where dolphins are presumed extirpated, which include nine in the Punjab rivers, and those above and below the current area of dolphin occupancy between Jinnah and Kotri barrages on the Indus River. This study consists of two components 1) establishment of a last sighting date of dolphins in each river section, and 2) generalised linear (GLM) and survival modelling to identify the principal causes of the dolphin range decline. These are described in Section's 3.2.1 and 3.2.2 below.

3.2.1 Last Dolphin Sighting Date

Extinction is seldom actually witnessed and it therefore must be inferred (Diamond 1987), however this is complicated by the fact that as animals become increasingly rare they are much more difficult to detect and their decline more difficult to document. Dolphins were extirpated from the Punjab rivers in Pakistan during the last century; this study is especially challenging because the declines occurred a considerable time in the past. The last sighting dates of dolphins in each river section were established by a) conducting interviews with elderly fishermen, riverside inhabitants and employees of the irrigation department, and b) conducting a detailed review of historical literature to identify dolphin sightings. Due to the great distance between the Punjab rivers and the Indus delta (approximately 1500km), interviews were not conducted downstream of Kotri barrage on the Indus River. In addition, the Harike-Hussainiwala section (Fig. 3.1, subpopulation 16) of the Sutlej River in India was not surveyed because it is a sensitive international border area. These are the only former dolphin subpopulations for which no dolphin sighting data were generated.

3.2.1.1 Fishermen Interviews

There are very few sightings of Indus dolphins in the Punjab rivers published in the scientific literature and, particularly when little scientific background information exists, local knowledge can provide the only information on historical species distributions. Local inhabitants often have detailed knowledge about their environments and the resources on which they depend (Beasley 2007). Interview surveys can provide a cost-effective way of obtaining information on the basic occurrence and distribution of species but it is important that the information obtained is carefully verified and interpreted (Aragones et al. 1997; Tregenza 1992).

Interview surveys of fishermen and riverside residents along the Jhelum, Chenab, Ravi and Sutlej Rivers in Pakistan were conducted from 1st to 10th October 2007. The objectives were to verify the former distribution of the Indus dolphin described by Anderson (1879) (Fig. 1.4), and to collect reliable dates of dolphin sightings in each river section. All barrages, headworks, large towns and bridges along the four rivers in Pakistan were visited by road vehicle. This mode of transportation was selected in preference to boat because many sections of river had insufficient water to allow vessel travel. Road access to the river banks is rare, so the use of a vehicle meant that fishers who reside in riverside villages were targeted. Fishermen who reside on boats (such as some *Mohannas*) could not be interviewed. However, given that river flow is severely depleted, the vast majority of fishers now reside in villages. Interview data from China regarding the decline of the recently extinct Yangtze River dolphin showed that as soon as baiji ceased to be encountered on a regular basis they immediately started to be forgotten by a community (Turvey et al. 2010b). The specific memories of informants who have encountered a species are unlikely to change significantly (Papworth et al. 2009), but often knowledge is not passed down across generations and over time the proportion of people in a community who have directly encountered, or remember, the species decreases (Turvey et al. 2010b). In the Punjab Rivers, dolphins were thought to have been extirpated between 20 and 80 years ago, depending on the location. To find informants that had actually witnessed dolphins first-hand our interviews by necessity targeted elderly people. All interviews were conducted with men, because in Pakistan, most rural women remain in the home and are less likely than men to have encountered dolphins.

Interviews must be carefully structured to avoid obtaining misleading results, therefore care was taken to maximise data quality and address common errors (Gill 1994; McKelvey et al. 2008). Relatively short (<30 min) closed-question surveys have generally been recommended for collecting quantifiable or factual information (Moore et al. 2010). A simple questionnaire was developed consisting of 26 straightforward questions asked during semi-structured interviews. Interviews were conducted by myself and local biologists, with individuals in their local language, either Urdu, Punjabi or Suraiki. Identification cards showing clear photographs and diagnostic features of the Indus dolphin were shown to participants, along with photos of mugger crocodile (*Crocodilus palustris*) and gharial (*Gavialis gangeticus*) that previously occurred in Punjab and may be confused with dolphins. Many rural people in Pakistan do not have

birth certificates and do not know their precise age, so respondent age was recorded in ten year categories e.g. 60-70 years. A calendar of significant local events was compiled to assist informants in identifying correct dates (Appendix II). Events that proved most useful in refining and validating dates were those of the major floods on each river and the dates of the previous rulers of the country. Attempts were made to obtain precise dates, but this was not always possible. Where precise dates were not given, sighting dates were recorded as early, mid or late in each decade.

Questions were focussed on the following topics (see Appendix I for full questionnaire):

1. Background information: name, age, cast/tribe, number of children/ grand children (5 questions);
2. Location information: number of years fishing in present location/other locations (2 questions);
3. Fishing information: gear type, target species, season, and habitat fished (9 questions);
4. General Indus dolphin information (2 questions);
5. Dolphin conservation: causes of decline, causes of mortality (2 questions); and
6. Dolphin specific sightings: date, location, behaviour, season, number of individuals (6 questions);

At each new site the fisher community was located, the objective of the survey was explained to the senior male community member and I requested to interview the oldest fishermen present.

3.2.1.2 Literature Reviews

A search was conducted through the entire collection of the Journal of the Bombay Natural History Society (1886-present) and the Journal of the Asiatic Society of Bengal (1832-1905) for references to river dolphins or porpoises. More recent local resources such as the Records of the Zoological Survey of Pakistan, Pakistan Journal of Biological Sciences, Natura (the newsletter of WWF-Pakistan) and the Pakistan Journal of Zoology were also comprehensively examined. The journal Investigations on Cetacea, which published many articles about Indus dolphins in the 1970s was also searched for information on dolphin sighting records in Punjab.

3.2.1.3 Last Dolphin Sighting Date

The sightings reported in the literature and during interviews were compiled for each river section, and the most recent taken as the estimated last sighting date. Optimal linear estimation can be used to identify extinction date based on a sighting record of at least five sightings (Collen et al. 2010; Solow 1993); however, in this case, there were an insufficient number of reliable sightings to apply the optimal linear estimation method and I therefore did not attempt to determine precisely when subpopulations were extirpated, but instead used the last sighting date as an indicator of when each subpopulation disappeared (Butchart et al. 2006). Recognising that it is difficult to precisely date extirpation, the spatial and temporal analysis, described below, was conducted by assigning inexact sighting dates to 5 year intervals (Butchart et al. 2006). If the last sighting date was, for example, early in the 1970s, for the purposes of this investigation a date of 1972 was assigned, if it was mid 1970s, 1975 was assigned, and if it was late 1970s the date used was 1978.

3.2.2 Identifying the Causes of Range Decline

3.2.2.1 Data Set

There have been 33 river sections of different lengths created since the onset of barrage construction, comprising 16 larger former fragments and 17 smaller current fragments (Appendix III). For example, a section of the Indus River was isolated between Jinnah and Sukkur barrages in 1946; this 693 km river section existed for 13 years until it was split into two parts (403 & 190 km in length) on completion of Taunsa barrage in 1959. The historical progression of habitat fragmentation has obviously played a role in the spatial and temporal pattern of Indus dolphin range decline. However, the interview data show that dolphins were present in all 16 former habitat fragments from the date of their creation until they were superseded by new smaller fragments. The extirpation of Indus dolphins occurred exclusively within the 17 river sections that are present today and that have existed in their present configuration for approximately 50 years. For this reason, and because historical environmental data are lacking, it is appropriate to focus the following analysis of the causes of Indus dolphin range decline exclusively on the 17 current sections of the Indus dolphin's former range.

3.2.2.2 Potential Drivers of Range Decline

The following seven explanatory variables that may have contributed to the Indus dolphin range decline were determined for all 17 current river sections.

1. *Fragmentation date*- The year that each of the current dolphin subpopulations was created was assigned as the date that the second of the two bounding barrages became operational. For the dolphin subpopulation upstream of Harike barrage in India the isolation date was assigned as the completion date of Hussainiwala barrage which is located only 30km downstream of Harike, and was completed 28 years earlier, isolating dolphins upstream.
2. *River length* - The number of river kilometres between two barrages.
3. *Proximity to range edge* – Satellite images projected in ArcView 3.2 were used to determine the proximity of each river section from the historical distribution limit recorded by Anderson (1879). This was determined by measuring the distance along the river's course from the former range limit to the barrage located closest to the range core. The barrage closest to the range core was selected because dolphin habitat was assumed to improve closer to the range core and that dolphins may persist at this end of a subpopulation. This variable accounts for increasing extinction risk at the periphery of the subspecies range.
4. *Size of river* - The mean annual discharge in Million Acre Feet (MAF) reported for each river prior to implementation of the Indus Water Treaty (IUCN 2011). This single figure does not account for changes in discharge along the course of a river, or over time, but provides an indicator of comparative river size.
5. *Confluences* - River dolphins are patchily distributed in the Indus River, and occur with higher frequency at confluences (Chapter 4). Hotspots of Ganges dolphin distribution also occur at the confluence of tributaries with the Ganges mainstem (Sinha et al. 2011; Sinha et al. 2010). The number of river confluences within each subpopulation was included as an indicator of the presence of favourable habitat.
6. *River slope* – The elevation of each barrage was obtained from a digital elevation model of Pakistan and India, and the average slope within each river section was calculated as the drop in elevation between the up and downstream barrages, or upstream range limit in the case of peripheral segments, divided by the length of

river. Slope exerts a direct effect on flow velocity and sediment transport and therefore influences dolphin habitat.

7. *Dry season river discharge* – River discharge data were obtained for all twelve barrages and two dams north of Guddu on the Indus River system in Pakistan for the period July 2008 to October 2011 (3 ¼ years). The daily discharge below Guddu and Kotri barrages were obtained from October 2010 to October 2011 (~1 year), and discharge below Sukkur barrage was obtained from April 1994 to January 2000, and October 2010 to October 2011 (6 ¾ years). For each dolphin subpopulation two discharges were available: a) discharge released from the upstream barrage, and b) the discharge received at the downstream barrage. As almost no water is extracted between barrages (it is extracted at barrages), these figures were almost identical, and therefore only discharge from the upstream barrage was used in the models. Discharge was often not recorded on Sundays or holidays, so the missing data were interpolated. It is very low flows that are likely to adversely impact dolphins, and as the average discharge was heavily influenced by occasional flood pulses, the median daily discharge during the dry season (1st October to 31st March) was determined using only years for which there was an entire dry season's data. Discharge of Indus tributaries in India is classified information and could not be obtained, therefore discharge above and below Harike barrage was estimated from historical published flows, by examining satellite images and aerial photos of the river at different times of year, and from information on the diversion capacity of canals. The number of years of data available differed according to barrage but the temporal discharge pattern was predictable and similar across years in each location. There were however large differences in discharge among different barrages in the Indus system (ANOVA, $F=1658.9$. $df=14$, $p=<0.0001$).

3.2.2.3 Modelling Causes of Range Decline

To explain, and attempt to identify the causes of the spatial and temporal pattern in Indus dolphin subpopulation extirpation, GLMs and a survival analysis were used with the seven explanatory variables described above as predictors of continued presence of river dolphins. Generalised Additive Models (GAMs) were used in the initial data exploration to visually investigate whether the relationship between the predictor and explanatory variables was linear and if not which type of transformation could be used

to best account for non-linearity (Redfern et al. 2006). Three sets of models were developed, with objectives summarised below:

Models of the spatial pattern of dolphin persistence: The objective of the first set of models was to identify which factors best explained the observed geographic pattern of range decline and dolphin presence. All putative Indus dolphin subpopulations (except for Harike-Hussainiwala that has not been surveyed for dolphins) (n=16) were included. The presence or absence of a dolphin population was modelled using a binomial error distribution, with presently extant populations coded as 1 and extirpated populations coded as 0. The best fitting models were then used to predict the probability that dolphins are still present in the Harike-Hussainiwala subpopulation.

Models of the temporal pattern of decline: The objective of the second model set was to identify which factors were most strongly related to when subpopulations were extirpated. Only those dolphin subpopulations that have already been extirpated and for which there was a last dolphin sighting date (n=9) were included. The number of years before the present date (PD=2011) of the last dolphin sighting (PD-LDS) was the response variable. As the response variable was a count of years that was over-dispersed relative to the Poisson distribution, the data were modelled using a GLM with a quasi-Poisson error distribution.

Models of time to extirpation: The third model set was designed to investigate which factors influenced the speed with which subpopulations were extirpated following their isolation between barrages. The time to extirpation was calculated as the number of years that elapsed from the isolation date to the last dolphin sighting date. The time to extirpation only for subpopulations already extirpated could have been modelled fairly simply using a GLM with Gamma errors, however, subpopulations that are still extant also have information to contribute to understanding the speed of extirpation. To allow inclusion of this additional information, extant subpopulations were also included in a survival regression model, which is specifically designed to model time to death or time to failure data and allows for censoring (Crawley 2007). The last sighting date for subpopulations that are still extant was calculated by subtracting the isolation date from the last sighting date assigned as the current year, 2011. Thirteen subpopulations (subpopulations 2, 10, 12 and 16 were excluded because of missing data), were included in the model, and each was qualified with a status assignment, where 1=extirpated, and 0=extant. Time to extirpation and status form the Kaplan–Meier survivorship object, which was the predictor variable modelled using the survreg

function in the survival library of the programme R (R Development Core Team 2010). Both an exponential error distribution associated with a constant risk of extirpation following isolation, and a Weibull error distribution, that fits data where the risk of extirpation rises with subpopulation age, were fitted to the data (Crawley 2007). The Weibull distribution was selected as it provided a significantly better fit (ANOVA, p=0.01 and delta AIC 5.447).

All models were implemented using the programme R 2.12.1 (R Development Core Team 2010). The datasets were small, 16 subpopulations in the binomial models, 9 for the last sighting date models, and 13 for the time to extirpation models, so considering the rule-of-thumb suggested by Crawley (2007) the number of parameters estimated was constrained to no more than one third of the total data points. Logit, probit and cloglog link functions were included in global models and the logit function which resulted in the best fit applied. Variance Inflation Factors (VIFs) that demonstrate the degree of collinearity between variables were generated from the maximal models and collinear variables removed until variance inflation factor scores were less than five (Crawley 2005; Zuur et al. 2009). Retaining collinear variables can inflate sampling variances and create unstable parameter estimates potentially affecting which variables are retained in final models (Crawley 2005). Three two-way interactions that described potentially meaningful relationships between variables (isolation date and dry season discharge, isolation date and river length, and river length and proximity to range edge) were included in model sets, as well as second and third order polynomials of significant variables. The binomial and survival models were simplified using backwards stepwise selection based on Akaike's Information Criteria (AIC) (Akaike 1973). Quasi-Poisson models were selected on the basis of quasi-AIC (QAIC) scores that incorporated the dispersion parameter from the full model, and non-significant terms were sequentially dropped based on their levels of significance. Models separated by at least two AIC/QAIC points were assumed to be significantly different (Burnham and Anderson 2002). If removal of a variable resulted in an increase in AIC of two points or more it was returned to the candidate models because it contributed important information. Goodness of fit for the GLMs was measured by determining the proportion of the total deviance explained by the final model, and by each of the significant explanatory variables. Model plots were examined for non-normality of errors, heteroscedasticity and influential points (Crawley 2007; Fox 2008).

3.3 RESULTS

3.3.1 Interview Surveys

A total of 57 interviews were conducted: 23 on the Chenab River, 11 on the Jhelum, 9 on the Ravi, 9 on the Sutlej, and 5 on the Indus above Jinnah barrage. Fishermen interviewed ranged in age from 30 to more than 90 years, however most respondents were aged between 50 and 60 (13 individuals), 40 to 50 years (12 individuals), and 70 to 80 years (10 individuals). Our focus on retired fishermen, and on areas from which dolphins have already been extirpated meant that the communities were forth-coming with information because it was not regarded by them as sensitive. However, there were very few elderly members of each fishing community and our pool of available informants was consequently small.

The majority of fishing communities interviewed on the rivers in Punjab were from the *Jebail, Malah* or *Mohanna* tribes and had been involved in fishing for many generations. These fishing communities are one of the poorer sections of society in an already impoverished region. The fishing tribes are closely related and some claim to have moved from Sindh several generations ago. Members of some of these communities may have been involved in dolphin hunting in the past, but there is no evidence that this practice continues today. On the Jhelum and Chenab most fishermen spoke *Punjabi* but near Panjnad and on the Sutlej *Suraiki* was the predominant local language. The name of the Indus dolphin in both languages is *bhulan* which is reminiscent of the sound dolphins make as they take a breath.

Of the people interviewed, 79% were full-time commercial fishermen or fish contractors and the remainder were part-time fishermen who fished for subsistence or recreation. Of those who specified, all fished in the rivers, although a few also fished in the lakes adjacent to barrages and in canals. All fishermen agreed that June to August (the flood season) is the spawning season for most river fish and that fishing is banned by the Government for those three months. 100% of fishermen reported that there were less fish than in the past, and this was due to either reduced flows and/or pollution. 82.7% of informants had heard of Indus dolphins and 65.4% reported that they, or a close family member, had seen one. Many were familiar with dolphins from sightings on the Indus River at Taunsa barrage or in Sindh but were much less likely to have seen dolphins in the Punjab Rivers. Young informants,

especially those under the age of 40, were less likely to have encountered dolphins than older individuals (Fig. 3.2).

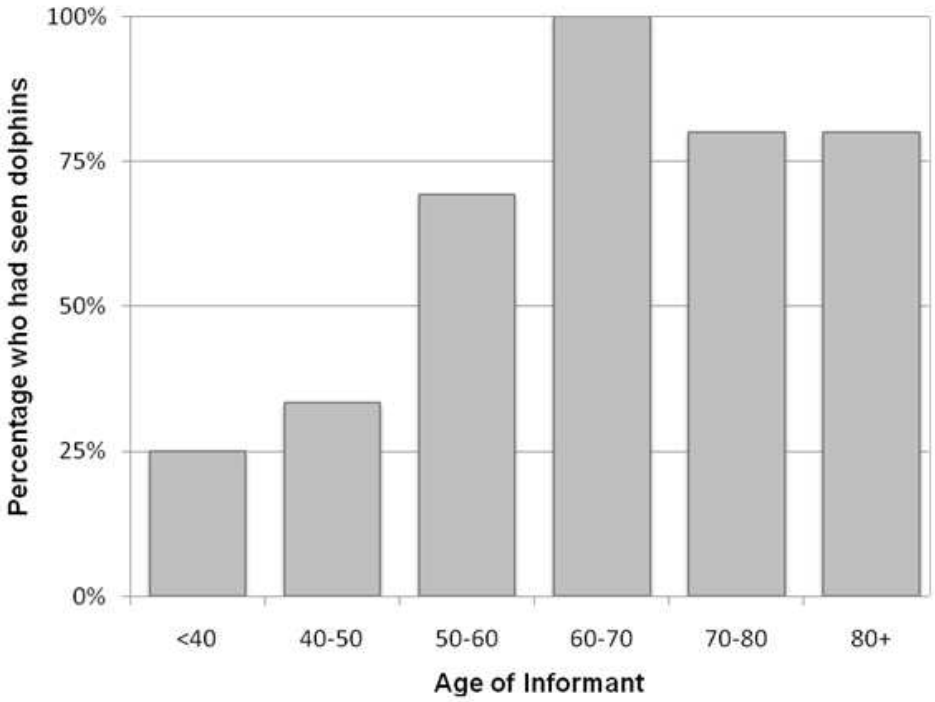

Figure 3.2 – Proportion of respondents that had seen Indus dolphins according to age group

3.3.2 Last Dolphin Sighting Date

The interview surveys did not generate any evidence that dolphins persist even in small numbers in any of the Punjab rivers in Pakistan. The following is a description of the historical Indus dolphin sightings, compiled from interviews and literature; these are also compiled in Table 3.1.

3.3.2.1 Jhelum River

Only 18% of informants (2 of 11) could recall dolphins in the Jhelum River. Dolphins were reported to have been present near to Rasul barrage in the mid-1960s by one informant and in the mid-1950s by a second. Reeves et al. (1991) reported that dolphins were in the Jhelum River in the 1970s. Fishermen reported seeing dolphins near Jhelum town, upstream of Rasul barrage, in 1975 but these animals were subsequently noted to have disappeared after the major flood the following year (Pilleri

and Bhatti 1978). A dolphin sighting in the Jhelum River prior to the floods in 1976 (Arain 1978) was cited by Reeves et al. (1991).

3.3.2.2 Chenab River

There are no records of dolphins near Khanki or Qadirabad barrages on the Chenab River since Anderson created his map in 1879. The oldest fishermen interviewed recalled seeing a group of dolphins at the Jhelum-Chenab confluence in the 1930s as he was swimming across the river. At Panjnad barrage, virtually all people interviewed had seen dolphins downstream of the barrage during the summer when dolphins move upstream from the Indus. Above Panjnad barrage in the Chenab River, fishermen reported that dolphins were present in the 1940s prior to the partition of India. There was a sighting in the 1950s in the Chenab River near to Muzaffaragarh (approximately 80km upstream of Panjnad barrage), sightings in the early 1970s between Panjnad and Trimmu barrages were reported by Roberts (1997) and a sighting just downstream of Trimmu barrage in 1981 was documented (Reeves et al. 1991). No dolphins were recorded in the Chenab in the winter of 1963/64 (Taber et al. 1967) and Khan and Niazi (1989) suggested that the Chenab dolphin population had disappeared in the 1970s.

3.3.2.3 Ravi River

The only record of dolphins in the Ravi River, was one person who reported sighting dolphins from Sidhnai Barrage in the early 1960s. There were no dolphin records from upstream of Balloki barrage, which concurs with the upstream dolphin distribution limit of Lahore depicted by Anderson (1879).

3.3.2.4 Sutlej River

In contrast to interviews conducted on the other Punjab rivers, all respondents could recall dolphins being present in the Sutlej River. In the winter of 1977-78 three dolphins were reported in the Sutlej River below the Islam headworks (Pelletier and Pelletier 1980). They were reported to be present above Islam barrage until 1966 by two informants, present until the mid-1960s by another and the early 1970s by a fourth.

Upstream of Suleimanki barrage all informants, and also some that were resident on other Punjab rivers, reported that dolphins were reliably present until the late 1980s. Two informants said that the dolphin population above the barrage disappeared in 1985, and another two thought that the population was wiped out by the large flood in

1988. In 1989, Khan and Niazi (1989) noted that the area upstream of Suleimanki barrage had supported dolphins until recently. T.J. Roberts reported that dolphins were present upstream of Suleimanki in 1972 (Roberts 1977), and subsequently that they were still present in the 1980s (Roberts 1997). Dolphins were also reported upstream of Suleimanki barrage in 1989 by experienced biologist Z.B. Mirza (pers. comm.). Irrigation department employees at Suleimanki barrage believed that dolphins were extirpated from the Sutlej because the Balloki-Suleimanki link canal was bringing extremely polluted water from the Ravi River to the Sutlej.

Table 3.1 - Indus dolphin sighting records in the Punjab rivers since the 1920s. Subpopulations numbers refer to those depicted in Fig. 3.1 and 3.3.

River	Subpopulation	#	Sighting Date	Source
Indus	1. Upstream Jinnah	1.	Kalabagh: mid 1950s	Fisher interview, this study
		2.	Kalabagh: 1950s	Fisher interview, this study
Jhelum	8. Upstream Rasul	1.	1975	(Pilleri and Bhatti 1978)
Jhelum-Chenab	9. Trimmu-Rasul-Qadirabad	1.	Jhelum: Mid-1960s	Fisher interview, this study
		2.	Jhelum: Mid-1950s	Fisher interview, this study
		3.	Confluence: 1930s	Fisher interview, this study
		4.	Jhelum: 1970s	Reeves et al. (1991)
		5.	Jhelum: 1976	(Arain 1978)
Chenab	11. Khanki-Marala	-	No sightings	-
Chenab	12. Qadirabad-Khanki	-	No sightings	-
Chenab-Sutlej-Ravi	10. Panjnad-Islam-Sidhnai-Trimmu	1.	1950s	Fisher interview, this study
		2.	1940s	Fisher interview, this study
		3.	1981	(Reeves et al. 1991).
		4.	Early-1970s	(Roberts 1997)
		5.	Winter 1977-78	(Pelletier and Pelletier 1980)
Ravi	13. Sidhnai-Balloki	1.	Early-1960s	Fisher interview, this study
Sutlej-Beas	17. Harike-Ropar-ShahNehar	1.	Extant	(Behera et al. 2008)
Sutlej	16. Hussainiwala-Harike	-	Un-surveyed	-
Sutlej	15. Suleimanki-Hussainiwala	1.	1988	Fisher Interview, this study
		2.	1988	Fisher Interview, this study

		3.	1985	Fisher Interview, this study
		4.	1985	Fisher Interview, this study
		5.	1989	Z.B. Mirza, pers. comm.
		6.	1972	(Roberts 1977)
		7.	1980s	(Khan and Niazi 1989)
		8.	1980s	(Roberts 1997)
Sutlej	14. Islam-Suleimanki	1.	1966	Fisher interview, this study
		2.	1966	Fisher interview, this study
		3.	Mid-1960s	Fisher interview, this study
		4.	Early-1970s	Fisher interview, this study

3.3.3 Dynamics of Range Decline

Fig. 3.3 illustrates the approximate last dolphin sighting date in each subpopulation, visually demonstrating the spatial and temporal pattern of Indus dolphin range decline in the Punjab rivers. The memories of informants were less exact as sighting dates were further in the past. There are also fewer sightings records for subpopulations that were extirpated longer ago. Despite this, the data should still indicate the general pattern. Records indicate that dolphins were extirpated first from the upper Chenab River, then from above Jinnah barrage on the Indus, the Ravi River, the Jhelum, and finally the Sutlej. The last subpopulations to disappear were those upstream of Panjnad barrage and upstream of Suleimanki barrage, both of which persisted until the 1980s. In some rivers upstream populations were extirpated prior to those downstream, but this pattern is disrupted by the continued presence of dolphins at the upstream periphery of their former range in the Beas River in India.

Figure 3.3 - Spatial and temporal dynamics of the Indus dolphin range decline. Numbers refer to dolphin subpopulations listed in Table 3.1 & Fig. 3.1. Grey boxes indicate barrages.

3.3.4 Causes of Range Decline

Of 17 sections of river, dolphins are extant in six, are presumed extirpated from ten, and in one dolphin presence or absence is unknown. Each Indus dolphin subpopulation, its status, estimated last dolphin sighting date, and physical characteristics are listed in Table 3.2. These data were included in each of the range decline models described below. Subpopulations 11 and 12, on the upper Chenab River were presumably extirpated first, the last sighting date is that reported by Anderson (1879). Due to the lack of reliable sighting data time to extirpation was not calculated for these two subpopulations.

Table 3.2 – Details of extant and extirpated Indus dolphin subpopulations

#	Subpopulation	River	Date [a] Isolated	Extant (1) Extirpated (0)	Last Sighting Date[b]	Time to [c] Extirpation	Length (km)[d]	River Size[e]	Conf[f]	Median [g] Discharge	Dist (km)[h]	Slope m/km
13	Balloki-Sidhnai	Ravi	1886	0	1962	74	175	7	0	0	175	0.29
11	Marala-Khanki	Chenab	1892	0	1879	N/K	35	26	0	4140	0	0.54
8	Upstream Rasul	Jhelum	1901	0	1975	74	50	23	0	27000	71	0.72
15	Hussainiwala-Suleimanki	Sutlej	1926	0	1988	62	110	14	0	7000	287	0.18
14	Suleimanki-Islam	Sutlej	1927	0	1972	43	145	14	0	0	438	0.26
17	Ropar,ShahNehar-Harike	Sutlej-Beas	1927[i]	1	N/A	N/A	220	14	1	15000[k]	148	0.38
10	Islam,Sidhnai,Trimmu-Panjnad	Chenab, Sutlej, Ravi	1933	0	1981	48	435	26	2	1231	610	0.11
9	Rasul,Qadirabad-Trimmu	Chenab, Jhelum	1939	0	1975	36	490	26	1	6975	327	0.13
1	Upstream Jinnah	Indus	1946	0	1955	9	35	93	0	40000	35	0.89
16	Harike-Hussainiwala	Sutlej	1955	N/K	N/K	N/K	30	14	0	5000[k]	184	0.33
6	Sukkur-Kotri	Indus	1955	1	N/A	N/A	318	93	0	6400	520	0.09
7	Downstream Kotri	Indus	1955	0	N/K	N/K	222	93	0	3922	205	0.09
3	Chashma-Taunsa	Indus	1959	1	N/A	N/A	230	93	0	43000	351	0.23
4	Taunsa-Guddu	Indus	1962	1	N/A	N/A	277	93	1	37262	636	0.21
5	Guddu-Sukkur	Indus	1962	1	N/A	N/A	126	93	0	34916	645	0.17
12	Khanki-Qadirabad	Chenab	1967	0	1879	N/K	45	26	0	578	0	0.44
2	Jinnah-Chashma	Indus	1971	1	N/A	N/A	60	93	0	47127	122	0.45

Dolphin subpopulation number, also shown in Fig 3.1 and 3.3 and Table 3.1, [a] Date that dolphins were confined between two barrages, taken as the completion date of the downstream barrage, [b] Where sighting dates were imprecise they were rounded, for example to 1972, 1975 or 1978. [C] The time that elapsed from the isolation date to the last sighting date [d] Length of river between barrages [e] Annual average flows calculated by averaging daily flows for the period 1922 to 1961 (IUCN 2011). [f] Number of river confluences occurring within subpopulation [g] Median daily discharge between October 1st and March 31st (the dry season) from the upstream barrage in cubic feet per second. [h]Distance to former range limit described by Anderson (1879)(Fig. 1.2). [i] Harike isolation date taken from completion date of Hussainiwala barrage nearby. [k] Estimated. N/K = not known. N/A = not applicable

3.3.4.1 Causes of the Spatial Pattern of Indus dolphin Range Decline

Where dolphins are still extant the mean dry season river discharge was 30,618 cusecs (SD=16,241) compared to a mean of 9,044 cusecs (SD=13,489) in locations from which they have been extirpated. Characteristics of river sections where dolphin subpopulations are extant or extirpated were compared using t-tests. After Holm correction for 6 tests no results were significant, although for subpopulations that are still extant mean river size (t-test, t=2.731, df=10.4, p=0.08) and median dry season discharge (t-test, t=2.736, df=9.1, p=0.08) were greater, and mean date of subpopulation isolation later (t-test, t=2.739, df=14.0, p=0.08) (Fig. 3.4). In general, sections of river where dolphins are still present were fragmented by barrages later, are further from the range periphery, are of larger size, have a shallower slope and have greater dry season discharge than river sections where dolphins are no longer found.

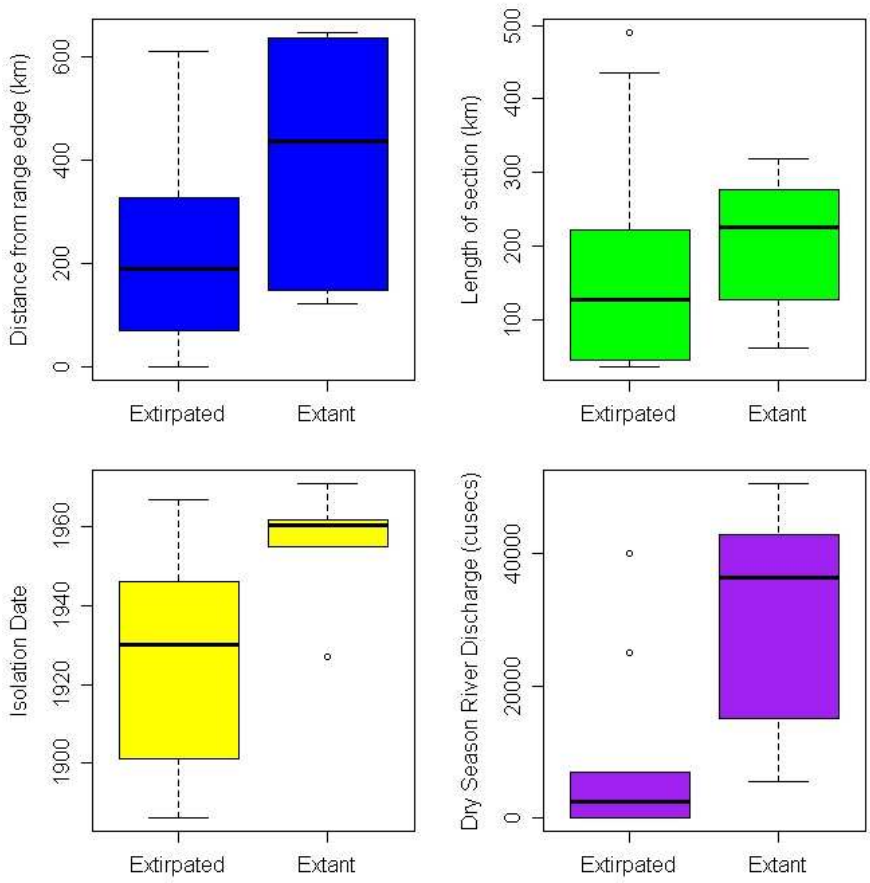

Figure 3.4 –Characteristics of river sections where river dolphins are present and where they have been extirpated.

16 subpopulations, six where dolphins are extant and ten where they have been extirpated were included in GLMs for binary data. Explanatory variables were not significantly correlated (Spearman's rank correlation test) however, the variance inflation factors generated from the full model indicated that river discharge and slope were collinear. Slope was considered to be less important than discharge in explaining dolphin distribution and range decline and was therefore removed from further candidate models. No interactions or polynomials were retained. The final model which best explained the observed spatial pattern in Indus dolphin range decline retained the explanatory variables 'distance from range edge' and 'dry season river discharge'. The probability that an Indus dolphin subpopulation is still extant increases with increasing distance from the range edge, and with increasing dry season river discharge (Fig. 3.5; Table 3.3).

Table 3.3 – Summary of spatial range decline model output

Model	***AIC***	***Δ AIC***	***% explained Deviance***	***n***	***Deviance***					
					Q	**Range**	**Is. Date**	**L**	**Conf**	**Size**
1	**17.26**	**-**	**46.8**	**2**	**7.13**	**2.77**	**-**	**-**	**-**	**-**
2	18.39	1.67	50.9	3	7.13	2.77	0.87	-	-	-
3	20.26	3.00	51.5	4	7.13	2.77	0.79	0.21	-	-
4	22.24	4.98	51.6	5	7.13	2.77	0.51	0.49	0.03	-
5	23.92	6.66	53.1	6	7.13	2.77	0.82	0.49	0.03	0.002

n = number of parameters, Is. Date = Isolation Date, L=Length of river section, Range = Distance from range edge, Size = River size, Conf. = confluences, Q=River discharge. Model in bold was the final selected model.

The best fitting model was used to predict the probability that dolphins are still extant in the Harike-Hussainiwala river section in India that has not been surveyed for dolphins. The probability that dolphins are still present was predicted to be only 5.2%. This is not unexpected given that this section has very low winter discharge and is near the periphery of the dolphin's range, both factors that increase the likelihood of subpopulation extirpation.

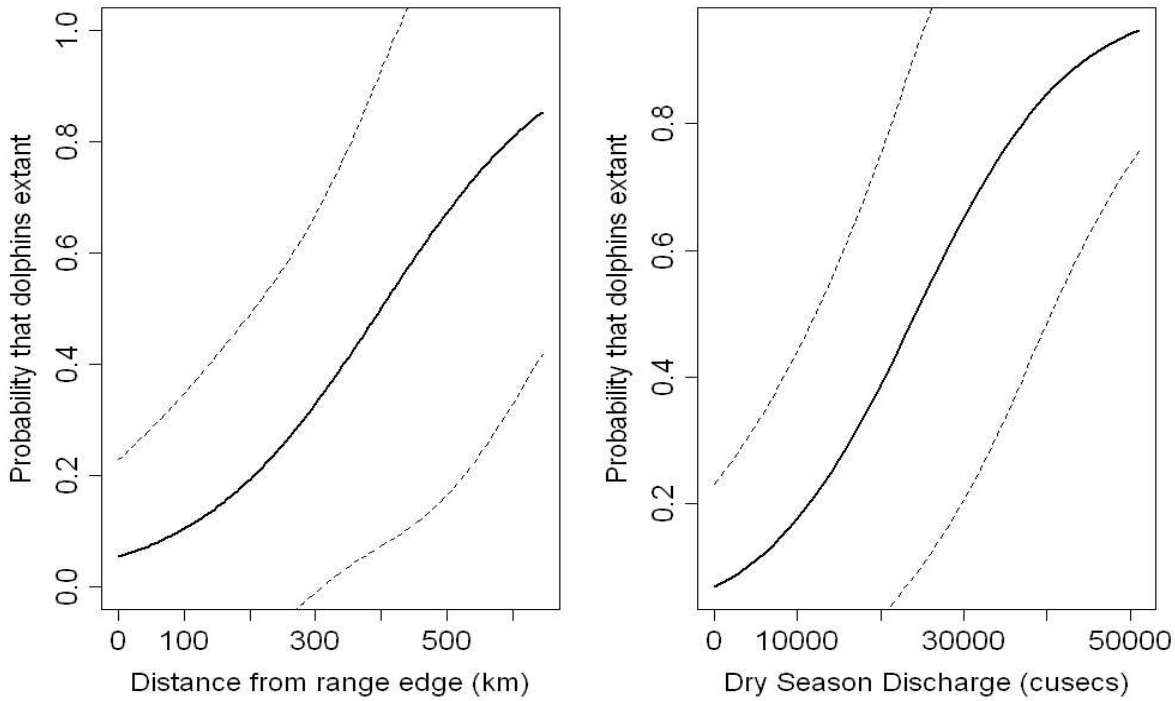

Figure 3.5 – Probability that an Indus dolphin subpopulation is extant according to proximity to the edge of the former range and the median dry season discharge (cubic feet per second).

3.3.4.2 Causes of the Temporal Pattern of Indus Dolphin Range Decline

Only those subpopulations that have already been extirpated, and for which there was a reliable last sighting date, were included in this component of the analysis (n=9). Spearman's rank correlations of the last dolphin sighting date and each of the potential drivers of decline showed no significant relationships except between last sighting date and distance from the former range edge (Spearman's rank, S=206.1, p=0.030). The final model with the lowest QAIC retained distance from former range limit, dry season discharge and river size as the three variables that best describe the temporal pattern of Indus dolphin subpopulation extirpation (Table 3.4). No interactions between variables or polynomials were retained in the final models. The relationship between discharge and last sighting date was negative indicating that dolphin subpopulations were extirpated earlier in locations where dry season discharge was lower. Earlier extirpation also occurred in subpopulations located near the periphery of the subspecies range (Fig. 3.6). River size was not strongly significant and explained a

very small proportion of deviance but removing it from the final model increased the AIC by 6 points and it was therefore retained.

Table 3.4 – Summary of temporal range decline model outputs

Model	*AIC*	*Δ AIC*	*% explained Deviance*	*n*	*Deviance*					
					Q	**Range**	**Is. Date**	**L**	**Conf**	**Size**
1	29.44		78.6	2	39.7	123.3	-	-	-	-
2	**24.68**		**93.1**	**3**	**66.9**	**123.3**	**-**	**-**	**-**	**3.1**
3	26.40		93.8	4	60.1	123.3	3.8	-	-	7.5
4	28.25		94.1	5	60.1	123.3	3.8	-	0.7	7.5
5	31.58		95.5	6	60.1	123.3	3.8	0.4	3.2	7.5

n = number of parameters, Is. Date = Isolation Date, L=Length of river section, Range = Distance from range edge, Size = River size, Conf. = confluences, Q=River discharge. Model in bold was the final selected model.

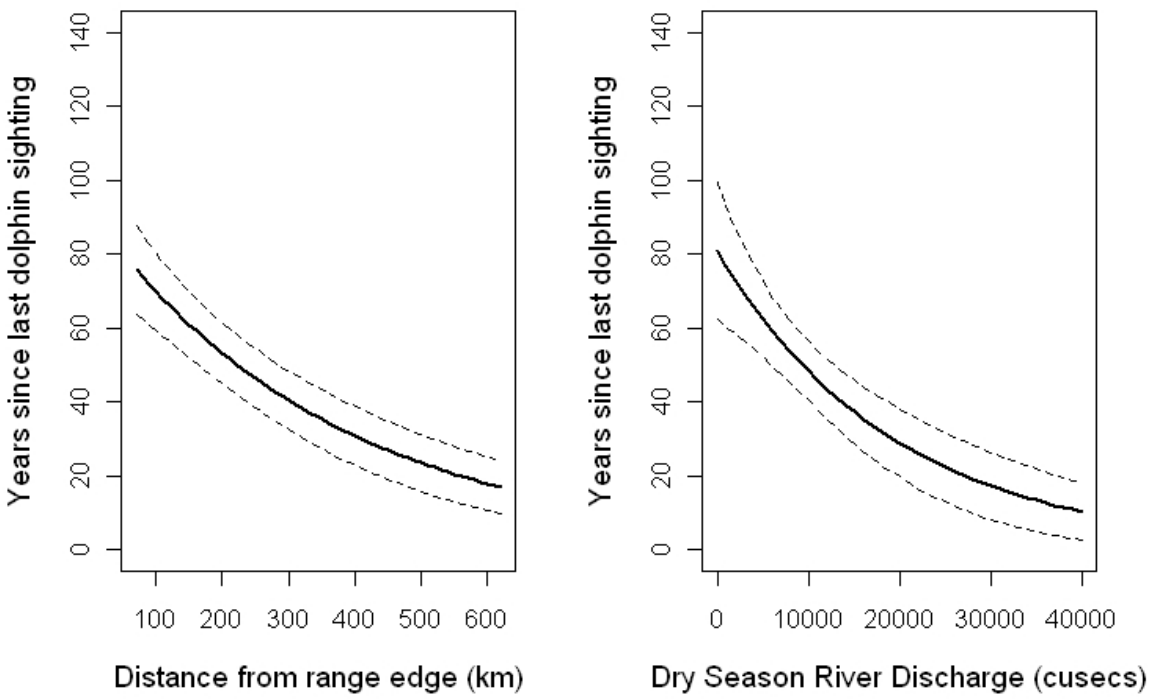

Figure 3.6 – Relationship between the number of years since a dolphin was sighted and a) subpopulation distance from historical range edge, and b) median dry season discharge, the two significant explanatory variables retained in quasi-Poisson GLM models of the temporal pattern of Indus dolphin range decline.

The best fitting model was then used to predict the last sighting date for subpopulations that are still extant. Results were as expected, with very recent (0-20 years before present) sighting dates generated for all extant subpopulations. The only anomalous result was for the subpopulation between Sukkur and Kotri barrages (subpopulation 5) (Fig. 2.1) on the Indus River. Recent surveys indicate that dolphins are present in small numbers in this river section (Chapter 2), but the model predicted that dolphins were extirpated 91 years (SE=43) ago from this river section.

3.3.4.3 Time to Subpopulation Extirpation

The mean time from subpopulation creation to dolphin extirpation was 49 years (SD=23, range=9-74), and for populations that are still extant, the mean time from subpopulation isolation to present was 55 years (SD=15, range=40-84). The slope parameter was included in this model set, and the final survival model retained three variables: median dry season river discharge (p=0.043), isolation date (p=0.054) and slope (p=0.040). Following isolation between barrages a dolphin subpopulation was extirpated more quickly as dry season river discharge decreased. Subpopulations persisted longer where the river slope is more gentle (e.g. in the lower reaches) and those created earliest persisted for longer than those in more recently subdivided river sections. The subpopulation survival curve (Fig. 3.7) indicates that after 50 years of isolation there is a less than a 50% chance that an Indus dolphin subpopulation will still be extant, and after 100 years this probability dropped to 38%. Of the six subpopulations that are still extant, the model fit the shortest time to extirpation for the subpopulation upstream of Harike headworks, and that between Sukkur-Kotri barrages (65 and 64 years, respectively), and the longest time to extirpation for the Chashma-Taunsa, Taunsa-Guddu and Guddu-Sukkur subpopulations (463, 305 and 309 years, respectively).

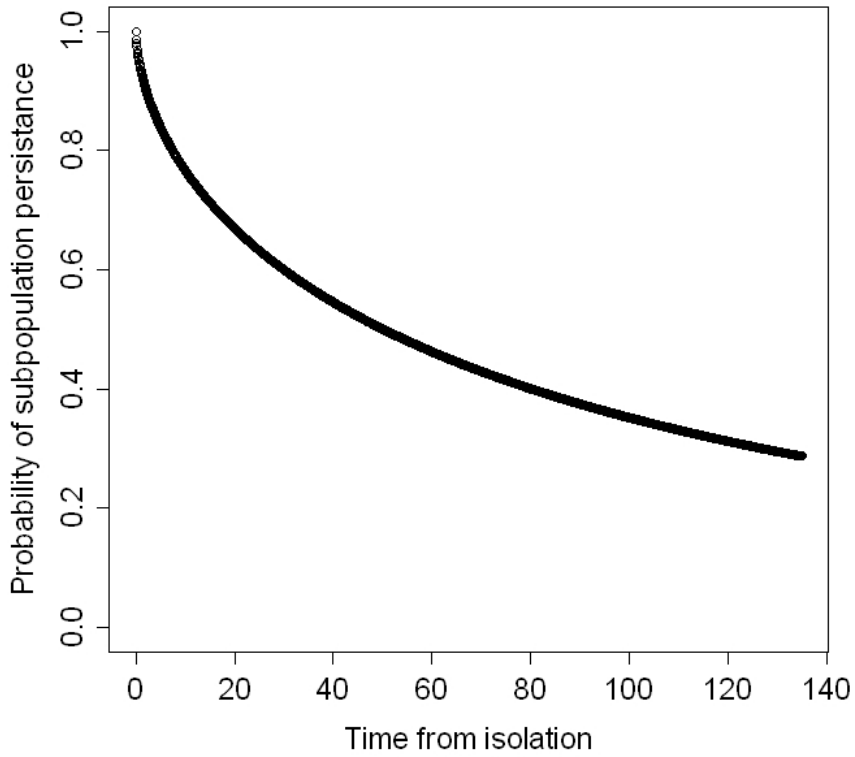

Figure 3.7 – Probability that an Indus dolphin subpopulation is extant with increasing time of isolation

3.4 DISCUSSION

3.4.1 Dynamics of Range Decline

Care must be taken when evaluating anecdotal evidence on the occurrence of rare species. There are cases where anecdotal evidence has led to vast overestimations of endangered species range and abundance (McKelvey et al. 2008) and also, where locals were unaware that a rare species occurred nearby (Hajjar 2011). The former distribution of dolphins indicated by interviews in this study concurs closely with the distribution recorded by Anderson in 1879. The only ambiguous area was the upper reaches of the Chenab River, where dolphins were reported to be present in the 1870s but interviews generated no records. It may be that the map of Anderson was inaccurate and that dolphins were never regularly present in those areas or that dolphins disappeared from the upper Chenab so long ago that they are no longer remembered by local inhabitants. As a species becomes rarer the number of false positive sightings is likely to increase. However, despite the rarity (or absence) of

Indus dolphins in the Punjab rivers, our interview data resulted in no positive recent sighting records. Species that have not been recorded for decades are more likely to have become extinct because of the length of time without positive sightings (Butchart et al. 2006). Butchart et al. (2006) defined 'possibly extinct' species as those species with recent records where:

1. The decline has been well documented;
2. Severe threatening processes are known to have occurred (e.g. extensive habitat modification);
3. The species possess attributes known to predispose taxa to extinction; and
4. Recent surveys have been adequate but have failed to detect the species.

The data collected from the 10 river sections with no recent dolphin sightings meet all of the above criteria, and I therefore conclude that there is sufficient evidence to assume that Indus dolphins have been extirpated from the Jhelum, Chenab, Ravi and Sutlej Rivers in Pakistan, as well as upstream of Jinnah Barrage and downstream of Kotri barrages. Indus dolphins remain in only six sections of river (five on the Indus and one on the Beas) bounded by irrigation barrages.

The spatial pattern of Indus River dolphin decline is very different from the gradual decline in abundance described for the Yangtze River dolphin (Turvey et al. 2010a). Indus dolphin range decline has been dictated by the contagion-like spread of extinction factors, in this case water extraction. Dolphins continue to persist in the range core primarily because the greatest threats are concentrated in the periphery of the subspecies range (Lomolino and Channell 1995).

The persistence of dolphins in the Beas River, upstream of Harike Barrage in India is likely to be due to the presence of constant water supplies that have been little depleted by diversions (Behera et al. 2008). This demonstrates that in the presence of sufficient water, and an absence of other threats, river dolphins can persist for decades even in relatively small fragments of habitat. Dolphins in the Beas River occur in what is effectively an island as the river downstream is virtually dry, and there is only connectivity with the rest of the river system for a few weeks each year during the monsoon floods. This subpopulation is of high conservation importance, as all other dolphin subpopulations occur in a single river and are therefore at risk of extirpation from environmentally correlated catastrophes (Gilpin 1990; Reed 2004; Soulé and Simberloff 1986).

3.4.2 Causes of Range Decline

3.4.2.1 Water Extraction

The river discharge data used in these models were from approximately the last ten years but they explained well the pattern of dolphin decline that occurred decades ago. There is an implicit assumption that present patterns of discharge reflect those in the past. Although discharge fluctuates annually, and has generally declined, the relative discharge among barrages (e.g. the spatial relationship) has remained relatively constant with the same locations consistently reporting high (e.g. the upper Indus) and low discharge (e.g. Punjab Rivers and downstream Sukkur) now and in the past.

The clear result of this study was the relationship between low dry season river discharge and the decline of the Indus dolphin. Reduced flows directly impact dolphins by reducing the physical space available to them, reducing average water velocity and depth and increasing water temperatures. Large-scale water extraction has rendered some tributaries of the Indus River trickles in many places and in these conditions, river dolphins cannot persist. It is obvious that if rivers become dry, species such as river dolphins, that rely entirely on freshwater will be extirpated. It is probable that if discharge falls to zero for a short period of time (e.g. 1 day) that some dolphins will be able to survive in pools until higher flows return. Consecutive days of zero flow, greater than the flow lag time between barrages (approximately 5-10 days depending on section), would render an entire river section virtually dry and quickly extirpate resident dolphins as well as other aquatic life. It is unclear whether it is the long-term reduced flows, or a period of zero flows, that extirpated dolphins from the Punjab rivers. However, it is certain that the flow required to maintain sufficient suitable habitat for a healthy population of dolphins is considerably greater than zero.

Reduced flow also impacts river dolphins through numerous indirect mechanisms, as the flow regime is a key driver of the ecology of rivers. It determines river habitat, and habitat in turn influences the distribution and abundance of aquatic organisms (Bunn and Arthington 2002; Nilsson et al. 2005). Flow regulation and water diversion results in declines in fish diversity, the dominance of generalist fish species, and increased success of invasive species (Bunn and Arthington 2002; Copp 1990; Gehrke et al. 1995; Pusey et al. 1993). All these factors are likely to have adversely affected Indus dolphins.

Dams and diversions typically dampen flood peaks, reducing the frequency, extent and duration of floodplain inundation that determines how long fish can gain access to nursery habitat and food. Reduced discharge has almost certainly affected recruitment of many Indus River species, and may have resulted in declines in dolphin prey. Floods are also important spawning cues for fish, benthic microorganisms, phytoplankton, zooplankton and possibly also Indus dolphins (Bunn and Arthington 2002). In the past Indus dolphins were reported to shift their distribution in response to the annual flood (Anderson 1879), but these movements are now blocked by irrigation barrages. Given the predictable annual flood that initiates spawning for many fish species, it seems likely that dolphin calving is also seasonal, in which case habitat fragmentation and water extraction may have negative impacts on dolphin recruitment. Water extraction contributes to, or interacts with, numerous other factors that may have played a role in the decline of Indus dolphins, including increased water temperature, higher concentrations of nutrients and pollutants, and an increase in exotic species (Allan and Flecker 1993; Xenopoulos and Lodge 2006).

3.4.2.2 Fragmentation

Interestingly, the date of habitat fragmentation was not selected by models as a significant factor driving the dolphin's decline, however, depleted river discharge and fragmentation by barrages are inextricably intertwined as barrages are responsible for water extraction, and they are a physical barrier that prevents the dispersal of dolphins out of impacted river reaches (Nilsson et al. 2005). Desert fish in fragmented habitats were more than five times as likely to have suffered local extirpations than similar species with more continuous habitat, and fragmentation of the Indus dolphin habitat has undoubtedly been the major factor involved in its decline (Fagan et al. 2002). Habitat fragmentation will also reduce the resilience of river dolphins to future threats, such as climate change. In free-flowing rivers, many organisms are likely to adapt to climate change by concomitant shifts in distribution; however, in fragmented and regulated rivers, dispersal is strongly limited thereby exacerbating the future impacts of climate change on them (Nilsson et al. 2005).

3.4.2.3 Small Populations

The decline of Indus dolphin subpopulations at the range periphery prior to those at the core generally supports the theory that where animals are least abundant they are likely to be extirpated first (Nilsson et al. 2005). Although it is water extraction combined with habitat fragmentation that have driven the decline of the dolphin, these factors generally resulted in the earlier and more rapid extirpation of the smaller populations at the range periphery. It is important to note, however, that the factors that reduce a species range are frequently quite different from those that eliminate the last individuals (Burbidge and McKenzie 1989). The factors identified above may be reducing the Indus dolphin distribution and abundance to a point where the effects of small population size (inbreeding, natural catastrophes, demographic variation etc) can cause their final extirpation (Gilpin and Soule 1986; Reed 2004; Shaffer 1981).

Smaller populations would be expected in smaller habitat fragments, and therefore a relationship between subpopulation extirpation and river section length might have been expected. In fact, length of river section was one of the first variables to be excluded in candidate models. This may be because I used only the current configuration of 17 comparatively small habitat fragments, and did not consider the progression of escalating habitat fragmentation and concomitant diminishing fragment size over time. To investigate this, I constructed an additional model considering all 33 river sections (Appendix III) in a GLM with a binomial error distribution, with dolphins recorded as extirpated (0) or still extant (1) in each river section at the point it was further subdivided. Explanatory variables offered to the model were 1) length of river section, 2) Isolation Date, and 3) End Date, taken as the year a new barrage was completed resulting in its further subdivision and creation of two, new, smaller sections. Models were constructed, evaluated and selected using the methods described in Section 3.2.2.3. When considering the entire history of habitat fragmentation, the models showed that dolphins were significantly more likely to be extirpated in smaller fragments ($p<0.05$), and that this relationship was independent of fragment creation date or duration.

This pattern of increased likelihood of extirpation in short river sections, and more rapid extirpation at the range periphery raises concerns over the future of small Ganges River dolphin populations in the upper reaches of the Ganges and Brahmaputra River systems in Nepal and India.

3.4.2.4 Other Potential Factors

It is uncommon to find the range of a mammal species fragmented into as many as 17 sections, however, for the purposes of statistical modelling this is a small sample that can present difficulties detecting patterns, and precludes the testing of large numbers of explanatory variables. River discharge and distance from range periphery provided an excellent fit to the temporal pattern of decline, explaining more than 93% of the deviance. However, the final spatial model explained approximately 46% of the deviance, and therefore other factors may have played a role in the spatial pattern of decline. Three potentially important aspects that were not included as explanatory variables are: a) water quality, b) incidental capture in fishing gear and c) hunting. These are discussed below:

a) The magnitude of surface water pollution problems in Pakistan have increased at a dramatic rate over the last ten years (Directorate of Land Reclamation Punjab 2007; Qadir et al. 2007; World Bank 2005). It is estimated that only 8% of urban and industrial wastewater in Pakistan is treated; leaving more than 90% of industrial and municipal effluents to find their way into the water courses (Directorate of Land Reclamation Punjab 2007). Water quality monitoring studies in Pakistan focus on drinking and irrigation water and there is no current or historical systematic monitoring of rivers that could provide data for this analysis. The Punjab rivers flow through the industrial and agricultural heartland of Pakistan and as a consequence are more polluted than the Indus River which passes through more remote areas and has a greater dilution capacity. However, most dolphin subpopulations had already been extirpated prior to significant declines in water quality in the 1980s and 90s, and the asynchronous timing of events indicates that water quality was not primarily responsible. Declining water quality may have contributed to the extirpation of dolphins upstream of Suleimanki barrage in the Sutlej River that persisted into the late 1980s.

b) Mortality from accidental capture in fishing gear is considered to be the greatest threat to most cetacean populations (Northridge 2009; Read 2008). However, fisheries related mortalities of the Indus dolphin have only occasionally been documented from Sindh, and this has not been considered one of the larger threats to this subspecies. In the past, the Indus River main channel was not intensively fished because the water was too swift for easy manoeuvrability of oar-powered boats, and instead fishing centred on side channels and adjacent pools

that were warmer and had higher fish density (Khan 1947). There is no evidence of historical incidental capture of dolphins in fishing gear, and although it probably did occur when the rivers were free-flowing, this is not likely to be a large factor in the decline of dolphins from the Punjab rivers. However, stranding data indicate that fisheries bycatch is becoming an increasing threat to Indus dolphins as boats become mechanised and better able to negotiate the main channel (see Section 1.9.3).

c) Indus dolphins were killed for food, oil and medicine until the late 1970s when the animal became legally protected throughout the country (Anderson 1879; Lowis 1915; McNair 1908; Pilleri 1972). Information on dolphin hunting is sparse and un-quantified and records refer only to hunting in the Indus River, where dolphins are still extant. Although it is possible that dolphins were hunted throughout the river system, there is no evidence that this was so, and the fact that dolphins persist in the places that hunting is reported to have been intense, and have disappeared from places where hunting was not reported, suggests that this was unlikely to have been the main cause of the subspecies decline. However, the timing coincides and unless more historical information on dolphin hunting in the 1800s and early 1900s becomes available, it will not be possible to completely discount the role of hunting in the subspecies decline.

3.4.3 Implications for the Future

Although the purpose of this study was to identify the cause of historical subpopulation extirpation, not to predict future declines, the conclusions can shed some light on which dolphin subpopulations are most at risk. Based on the historical pattern of decline, dolphins are most likely to disappear in the future from locations with low river discharge located closer to the range periphery. This suggests that subpopulations upstream of Harike Barrage (close to range periphery with moderate discharge) and between Sukkur and Kotri Barrages (with low discharge located a moderate distance from the former range edge) are most at risk. The Sukkur-Kotri river section (subpopulation 5, Fig. 2.1) did not conform to the speed of extirpation pattern illustrated by the other subpopulations, having persisted longer than predicted. This provides evidence to support the theory that, due to high levels of water extraction, habitat in this river section is marginal for dolphins and that a population persists only because it is supplemented by occasional migrants passing through Sukkur barrage from upstream.

One of the greatest challenges in conservation biology involves disentangling the relative contributions of multiple factors in the decline of species, especially when causes interact or vary spatially and temporally with importance (Johnson et al. 2010). Nevertheless, the primary factors identified in these models (i.e. low dry season discharge, habitat fragmentation) are the most salient for informing current management of Indus dolphins. It is clear that construction of barrages and dams within the range of freshwater dolphins will result in severe impacts resulting from habitat fragmentation, and concomitant water extraction and disruption of the natural flow regime. The levels of water withdrawals from the rivers in Pakistan are extreme, negatively affecting human communities, eroding the delta, destroying freshwater fisheries and concentrating pollutants. Comprehensive environmental flow assessments that consider the habitat requirements of river dolphins and fish, as well as human requirements for irrigation water are essential for a sustainably managed, equitable system and for the future of the remaining Indus dolphins.

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Chapter 4

Habitat use by Indus River dolphins in the low water season

Abstract

Indus River dolphins are expected to be most vulnerable during the low-water season when river flow is greatly diminished and habitat is correspondingly limited. Indus River dolphin habitat selection in the dry-season was investigated using Generalised Linear Models of dolphin distribution and abundance in relation to physical features of river geomorphology, and channel geometry in cross-section. Dolphins were more frequently encountered at locations in the river with significantly greater mean depth, maximum depth, cross-sectional area and hydraulic radius, narrower river width and a lower degree of braiding than areas where they were absent. They were also recorded with higher frequency at river constrictions and at confluences. Channel cross-sectional area was consistently selected by the statistical models as the most important factor determining dolphin presence and abundance, with the area of water below 1m in depth exerting the greatest influence. Indus dolphins avoided channels with small cross-sectional area <700m^2, presumably due to the risk of entrapment and reduced foraging opportunities. Channel geometry had a greater ability to explain dolphin distribution than river geomorphology, however, both analyses indicate similar types of habitat selection. The dolphin-habitat relationships identified in the river geomorphology analysis were scale dependent, indicating that dolphin distribution is driven by the occurrence of discrete small-scale features, such as confluences and constrictions, as well as by broader-scale habitat complexes. There are numerous plans to impound or extract more water from the Indus River system. If low-water season flows are allowed to decrease further the amount of deeper habitat will decline, and there may be insufficient patches of suitable habitat to support the dolphin population through the low-water season. Dolphins may also become isolated into deeper river sections, unable or unwilling to traverse through shallows between favourable habitat patches.

4.1 INTRODUCTION

Pakistan has been ranked as the most water stressed country in Asia, and it has one of the lowest rates of per capita water availability worldwide (Asian Development Bank 2003; Kugelman and Hathaway 2009). An estimated 90% of the country's freshwater supply is provided by surface water from the Indus River system, and the majority of the river's flow is diverted for human use, resulting in widespread environmental degradation of the river's delta, and depleted flows throughout the river system. Depleted flow and fragmentation of habitat by irrigation dams have already led to an 80% reduction in the range of the Indus dolphin (Chapter 3) and are likely to be the greatest threats to the long-term survival of this dolphin subspecies (International Whaling Commission (IWC) 2001). River dolphins are expected to be most vulnerable during the low-water season when habitat is limited and it is therefore important to determine which habitats are preferentially used at this time, so that conservation efforts can be focussed in these locations.

The spatial and temporal habitat selection of animals is a complex and dynamic function of the species' requirements for food, mates, avoidance of predators and competitors and the ability to move between habitat patches (Azzellino et al. 2008; Davis et al. 2002; Schofield 2003). Fluvial habitat within river networks is often described as a mosaic of habitat patches of different sizes that are formed principally by hydro-geomorphic forces (Crook et al. 2001; Frissell et al. 1986; Thorp et al. 2006). Consequently, fluvial aquatic species are patchily distributed and variations in hydrology, geomorphology and flow patterns play a critical role in determining their distribution (Gormon and Karr 1978; Mérigoux et al. 1999; Poff and Allan 1995; Poff et al. 1997; Power et al. 1995; Statzner and Higler 1986; Thorp et al. 2006). The distribution of prey is likely to be one of the most important factors influencing the distribution of river dolphins. However, habitat selection is frequently assessed in terms of physical habitat characteristics as these are the primary determinants of prey distribution and are more easily measured (Baumgartner 1997; Bearzi et al. 2008; Caňadas et al. 2002; Davis et al. 2002; Gregr and Trites 2001). Most riverine fish prefer specific types of habitat, and water depth is widely considered the most important variable driving their distribution (Arunachalam 2000; Bain et al. 1988; Baird and Beasley 2005; Crook et al. 2001; Sarkar and Bain 2007). For example, small or young fish often prefer shallow and slow water, whereas, larger, or older fish inhabit deeper

areas often with faster flows (Bain et al. 1988). The great physical variability of river systems, and the absence of any river dolphin predators, means that physical habitat, in particular flow, depth and velocity, likely plays a more important role in habitat selection for freshwater cetaceans than it does for cetaceans in a marine environment.

This chapter uses statistical modelling to explain low-water season Indus dolphin distribution and abundance with respect to physical characteristics of channel geometry and river geomorphology, two aspects central to conservation as they provide the template upon which all fluvial ecological structure and function is built (Vaughan et al. 2010). Multi-scale studies are well established in river systems as a consequence of their hierarchical organisation (Vaughan et al. 2010), therefore I also compare the performance of river geomorphology models at different spatial resolutions. Given the widespread changes that have occurred to the Indus River system and the increasing pressures on the remaining dolphin habitat, the ultimate objective of this study is to provide information on dolphin habitat requirements in the low-water season so that efforts can be made to maintain or protect this habitat.

4.2 METHODS

Two complimentary methods were used to investigate how Indus dolphins utilise low-water season habitat. The first examined dolphin distribution relative to empirically collected data on cross-sectional geometry in the Indus River main channel (hereafter termed the ‘channel geometry’ analysis). This analysis used data on dolphin sightings collected from a concurrent vessel-based visual dolphin survey conducted in spring 2006 (Chapter 2). The second analysis investigated dolphin distribution relative to remotely-sensed hydrogeomorphic characteristics of the Indus River (hereafter termed the ‘river geomorphology’ study). This study used data on dolphin sightings collected from a vessel-based visual dolphin survey conducted in spring 2001 (Braulik 2006).

4.2.1 Data Sets

4.2.1.1 Channel Cross-sectional Geometry

Data on channel geometry and dolphin occurrence were collected from all five dolphin subpopulations, with complete spatial coverage of subpopulations located between Jinnah-Chashma, Chashma-Taunsa, and Guddu-Sukkur barrages (subpopulations 1, 2 and 4) and partial coverage of the remaining two river sections (subpopulations 3 and

5) (Fig. 2.1) (Chapter 2). A hydrological survey vessel followed approximately 2km behind the dolphin survey vessels, and periodically recorded channel depth along cross-sections. Cross-sections were measured at 2 to 5 km intervals, and their locations were deliberately placed to include a variety of different habitat types. At each cross-section, a GPS integrated depth-sounder simultaneously recorded water depth and geographic position along a transect running from bank-to-bank, perpendicular to the river flow. Near the river banks where depths were less than 0.40 m (the limit of the sonar unit), laser range-finders were used to measure the distance from the last depth point measured with the sonar to the water edge. The depth of the near bank zone too shallow to measure with the sonar was later interpolated. Small-scale changes in depths shallower than 0.4 m would not have been captured, but these typically constituted only a negligible fraction of the total cross-sectional area.

Points along each cross-section were examined visually using a Geographic Information System (GIS) and geographic outliers, resulting from the boat briefly deviating from the straight cross-section, were removed. The following six characteristics that described the shape of the river channel were calculated for each cross-section: channel top width (*T*), mean cross-section depth ($\overline{D}$), maximum cross-section depth (d_{max}), hydraulic radius (R_h), cross-sectional area (*A*) and cross-sectional area below specific depths ($A < d_x$) (Fig. 4.1).

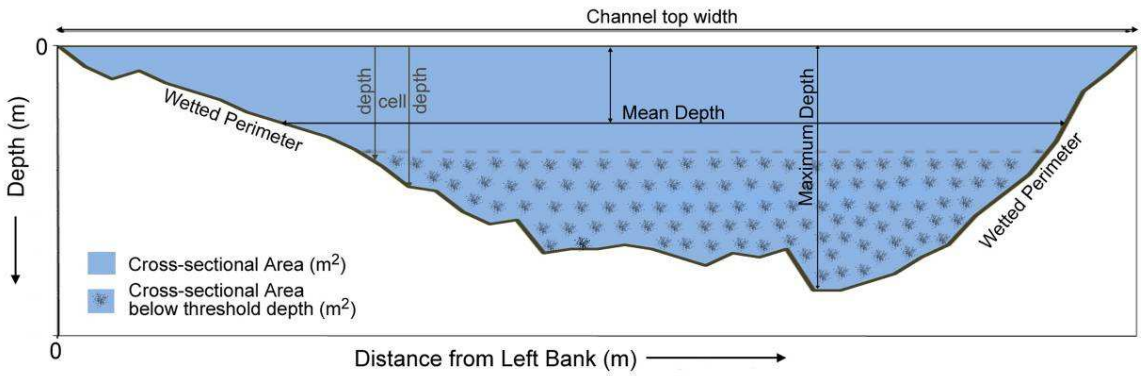

Figure 4.1 – Illustration of a typical river cross-section and summary data calculated.

The Universal Transverse Mercator (UTM) geographic coordinates of each sonar point were used to calculate the Euclidean distance from the left edge of water (as viewed looking downstream) using the distance formula:

$$s = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

where:

s = distance (m)

x_1 = the east/west Universal Transverse Mercator (UTM) position (easting) of the point at which depth is zero on the left bank (m);

x_2 = the east/west UTM position (easting) of each depth point along the cross-section (m);

y_1 = the north/south UTM position (northing) of the point at which depth is zero on the left bank (m);

y_2 = the north/south UTM position (northing) of each depth point along the cross-section (m).

The cross-sectional area between each depth point, referred to as a 'cell' (Fig. 4.1), was calculated according to the Trapezium Rule:

$$a_x = \left|(s_i - s_{i+1})\right| \times \frac{d_i + d_{i+1}}{2}$$

Where:

a_i = cell cross-sectional area.

S_i = the distance from the left bank of point i;

S_{i+1} = the distance from the left bank of point i+1;

D_i = the depth of point i;

D_{i+1} = the depth of point i+1

The component ($s_i - s_{i+1}$) represents the horizontal width of the cell, and the component [(d_i + d_{i+1}) / 2] represents the average depth of the cell. The areas of all the cells in a cross-section were summed to give total cross-sectional area. Mean cross-section depth was determined by dividing the total cross-sectional area by the channel top width. Wetted perimeter was determined by summing the distances between successive measured channel bottom points determined using trigonometry. Hydraulic radius is the ratio of cross-sectional area to wetted perimeter and represents shape-adjusted channel depth; a large hydraulic radius corresponds to narrow-deep channels, and small values to wide-shallow channels.

To further explore the importance of cross-sectional area, I calculated the cross-sectional area below a series of systematic 'threshold depths', 0.75m, 1.00m, 1.25m, 1.50m, 1.75m, 2.00m, 3.00m and 4.00m (Fig. 4.1). Cross-sectional area below each threshold depth was determined by re-calculating area for each cell but with threshold depth subtracted from each sonar depth measurement.

Sampling intervals in studies of river morphology are often scaled according to the mean channel width; because in large rivers longitudinal habitat within one channel width is considered to be generally similar (Elliott and Jacobson 2006). The average width of channel cross-sections was 343m (SD=151m, range=61-823m), therefore allocating dolphin groups to cross-sections that are within this distance could yield valid habitat associations. However, based on examination of river habitat features on satellite images, and experience in the field I believe that the habitat variables important to river dolphins vary over smaller geographic scales than this (±700m). I therefore selected the smaller distance of 200m, which ensured that dolphins were close enough to cross-sections that they be closely representative of the habitat being utilised. Using a distance smaller than this reduced the sample size of cross-sections allocated as having dolphins present, and using a distance larger than this reduced the power of models to detect habitat associations; the same variables were selected but with lower significance. Dolphin occurrence (1=present, 0=absent) and abundance (N) were allocated to each cross-section if the recorded location of a dolphin group was estimated to be within 200m of the cross section, otherwise dolphins were considered to have been absent.

4.2.1.2 Hydrogeomorphic Characteristics

Hydrogeomorphic characteristics were determined from satellite images collected in spring 2001, approximately a month before a dolphin survey was conducted (Braulik, 2006). The dolphin survey data, and satellite images provided almost complete spatial coverage of the Indus River from Jinnah to Kotri barrages; only a 30km segment between Guddu and Sukkur barrages (subpopulation 4) was not surveyed for dolphins due to civil insecurity. Satellite image resolution was 30m, which provided sufficient detail of river channels, bars and islands for the analysis, but prevented identification of smaller-scale features. I assumed the satellite images representative of the hydrogeomorphic conditions during the dolphin survey. GPS plots of the boat survey

track and dolphin sightings fell within the river channel in the remotely sensed images, thus supporting this assumption.

The scale of analysis in riverine fish-habitat models can influence the explanatory power of habitat variables to an even greater extent than in marine cetacean-habitat models due to the greater variability of the environment (Crook et al. 2001; Jaquet and Whitehead 1996; Redfern et al. 2006). Smith et al. (2009) explored the habitat selection of Ganges River dolphins in the Sundarbans, Bangladesh using 2km segments of survey track. In their analysis, Generalised Additive Models (GAMs) selected habitat parameters measured empirically in the field, over those derived from satellite images, which was attributed to the differential scales between the two datasets. Because of the fine-scale variability of the habitat, I assume that, similar to riverine fish, habitat selection by Indus dolphins occurs at relatively small scales, in the order of tens or hundreds of meters to several kilometres (Bridge 2003; Crook et al. 2001). Therefore, river segments of 1km, 2km and 5km in length, were analysed to explore how the explanatory power of habitat variables changed with resolution.

River distance was measured from up to downstream, along the centreline of the widest river channel, and segment boundaries demarcated at regular 1, 2, and 5km intervals on satellite images using GIS. Segments did not cross barrages, and the barrages formed the downstream boundary of the final segment in each section resulting in slightly shorter or longer segments than the standard in those locations. The geographic positions of dolphin groups, recorded when they were perpendicular to the vessel, were plotted onto the satellite images and each segment assigned dolphin presence (1) or absence (0) and total estimated number of dolphins (N). Using classifications of river habitat (Bridge 2003; Kondolf et al. 2003; Rosgen 1994), 12 habitat characteristics were measured for each segment at each scale. Details of how these variables were defined and measured is described in detail in Table 4.1, and illustrated in Fig. 4.2.

Table 4.1 – Hydrogeomorphic and spatial characteristics described for each river segment and included in Generalised Linear Models.

	Parameter	Acronym	Description
1	Greatest Main Channel Width	GCW	The main channel is defined as the widest river channel, and was measured perpendicular to the direction of flow at the widest point.
2	Smallest Main Channel Width	SCW	As above, measured at the narrowest point.
3	River Width	RW	The narrowest width of the entire river, including the main channel, all side channels, islands and bars, measured perpendicular to the direction of flow. Chutes were excluded.
4	Sinuosity	S	Sinuosity was recorded as the in-channel length measured along the centreline of the widest channel divided by the straight line distance (Friend and Sinha 1993; Sinha and Friend 1994). Sinuosity changes according to the scale over which it is measured, and it is therefore useful to present at several different scales (Alexander et al. 2010; Elliott and Jacobson 2006). Sinuosity was determined over three and five times the segment length including equal portions of river up and downstream: a. 1km segments: sinuosity determined over 3km (S3) and 5km (S5); b. 2km segments: sinuosity determined over 6km (S6) and 10km (S10); c. 5km segments: sinuosity determined over 15km (S15) and 25km (S25).
5	Number of Backwaters	BW	Backwaters are appended to the main channel but are not connected to the main channel via an upstream distributary and are therefore not flowing. Recorded if greater than 100m in length or width.
6	Braiding Index	BI	A count of the total number of islands or mid-channel bars within a segment that were greater than 100m in length, divided by the segment length.
7	Length of Primary Channel	PC	Primary channels are single channels that include the entire river (with no bars or islands) whose

			margin is defined by the outermost river bank (Sarma 2005).
8	Number of Revetments	Rev	Number of man-made embankments or revetments designed to control the river flow.
9	Number of Significant Confluences	Conf	Number of confluences involving two channels of approximately equal size.
10	Latitude	Lat	The latitude of the upstream segment boundary.
11	Percentage distance between barrages	Per	Calculated from the in-channel distance from the upstream barrage to the start of the segment, divided by the total in-channel distance between the nearest up- and downstream barrages.
12	Dolphin subpopulation	SP	Numbered from up to downstream. 1= Jinnah to Chashma barrage, 2=Chashma to Taunsa barrage, 3=Taunsa to Guddu barrage, 4=Guddu to Sukkur barrage and 5=Sukkur to Kotri barrage (Fig. 2.1). Hereafter, these are described as 'river sections'.

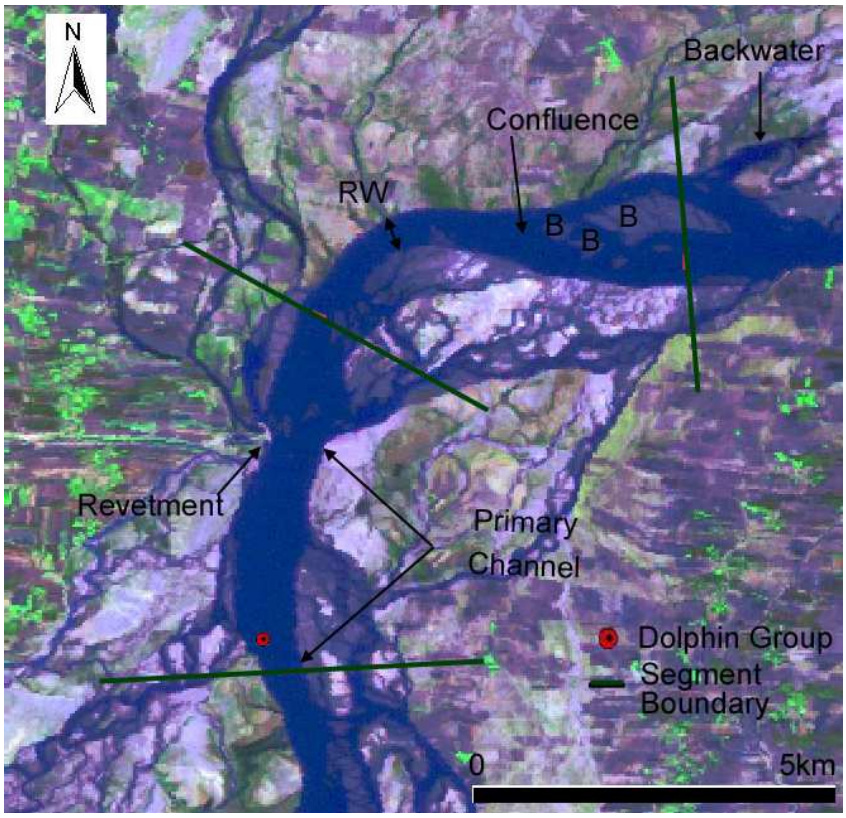

Figure 4.2 – Illustration of two 5km segments of the Indus River and the river hydrogeomorphic features recorded. RW= narrowest width of river in a segment, B = Mid-channel Bar

4.2.2 Habitat Models

Logistic regression of dolphin occurrence, and quasi-Poisson generalised linear models (GLMs) of dolphin abundance were fitted for both the channel geometry and river geomorphology analyses, using the software R (version 2.8.1) (R Development Core Team 2008). These models are an extension of simple regression that allow non-Gaussian error terms and use a link function to transform a linear function of predictor variables onto the scale of the response values (Fox 2008). The practical benefits are that they incorporate non-constant error variances and constrain their estimates to an appropriate range. Equal effort was applied at each cross-section and in each river segment so no effort modification was required in the models. Dolphin surveys were conducted during consistently excellent sighting conditions (river state 0 to 2) therefore all survey effort was included in the analysis, and no adjustment was made for variable sighting conditions (Braulik 2006; Chapter 2). There are vast differences in dolphin encounter rate with respect to each Indus dolphin subpopulation (Braulik 2006), so to prevent higher density subpopulations from dominating the analyses, dolphin subpopulation (SP) was either included as a factor in the geomorphology models of the entire river and each subpopulation was modelled separately in the channel geometry modelling. As many cetacean-habitat relationships are non-linear (Redfern et al. 2006), second and third order polynomial terms were added for variables that were significant in initial models. Some models that included many three and four-way variable interactions had slightly lower Akaike's Information Criteria (AIC) values (Akaike 1973), but as they are very difficult to interpret, only two-way interactions were included. The fit of all final models was assessed by examining residual plots, linearity of the response, and Cook's distance for influential points (Fox 2008).

Collinearity of explanatory variables was examined visually using paired variable plots and by generating variance inflation factors (VIF) from the global models. VIF's are determined by regressing covariates against one another; a high VIF indicates that a covariate is well predicted by another. There is no clear and generally accepted threshold for considering collinearity to be problematic, but VIF's of greater than ten indicate that correlation between variables may be sufficient to inflate sampling variances and create unstable parameter estimates potentially affecting which variables are retained in the model and increasing type II errors (Crawley 2005; Zuur et al. 2009). Parameters with high VIF values in the maximal model were removed until the VIF values of all variables were below ten.

4.2.2.1 Logistic regression of dolphin presence

Dolphin presence was modelled using a binomial logistic regression with presence/absence as the response variable. Logit, probit and cloglog link functions were included in global models and as resulting AIC values were almost identical (within 1 point), the canonical logit was selected. The logit transformed sighting probability, *p*, was modelled as the sum of the linear functions of *k* explanatory variables X_{ij}:

$$\text{logit}(p_i) = \ln\left(\frac{p_i}{1-p_i}\right) = \alpha + \sum_{j=1}^{k} \beta_j X_{ij}$$

The best fitting binomial model was identified via automated backwards step-wise selection using AIC values. Models with a difference in AIC of less than two were considered to have equivalent support (Burnham and Anderson 2002). The ability of the final model to correctly classify the data into presences and absences was assessed in a confusion matrix (Pearce and Ferrier 2000). This is a 2 by 2 matrix containing the numbers of data points for which the model, and the original data, indicated the presence or absence of animals; the correct classification rate, expressed as a percentage, represents how successfully a model classifies the data (Laran and Gannier 2008). The model was considered to indicate "presence" where the predicted probability of dolphin presence was greater than the observed mean probability.

4.2.2.2 Quasi-poisson GLM's of dolphin abundance

Dolphin abundance was modelled with 'number of dolphins' as the response variable in a quasi-Poisson regression model with a log link. The quasi-Poisson model was used because the count data was over-dispersed relative to the Poisson distribution. The dispersion parameter $\tilde{\phi}$ is given by (Fox 2008):

$$\tilde{\phi} = \frac{1}{n-k-1} \sum \frac{(Y_i - p_i)^2}{p_i}$$

Where *Y* = response variable, *n* = number of observations, *k* = number of explanatory variables, *p* = sighting probability

Quasi-likelihood is not a true likelihood, therefore likelihood ratio tests and AIC could not be used for inference in the quasi-Poisson models. Instead, a quasi-AIC (QAIC) that incorporated the common dispersion parameter value from the full model was calculated (Burnham and Anderson 2002):

$$QAIC = \frac{2LogLik}{\tilde{\phi}} + 2m$$

m= number of model coefficients, including slopes, intercept and dispersion parameter.

Parameters were sequentially dropped based on their *p*-values and QAIC used to evaluate whether removing a parameter resulted in a decrease in model fit (Burnham and Anderson 2002). If the QAIC increased by at least two points when a parameter was removed, its contribution to explaining the data was considered significant and it was retained. The percentage deviance explained by the final model over the null model was used as a measure of goodness-of-fit.

4.2.2.3 Channel geometry habitat models

Explanatory variables included in the channel geometry models were mean cross-section depth ($\overline{D}$), maximum cross-section depth (D_{max}), channel top width (*T*), channel cross-sectional area (*A*), subpopulation (SP) and hydraulic radius (R_h). Second and third order polynomial responses were added for *A* and *T* that were significant in initial models. Dolphin presence and abundance modelling was conducted on all cross-sections pooled for the entire surveyed length of the river, and separately on cross-sections from only the low density Chashma-Taunsa subpopulation (2), and the high density Guddu-Sukkur subpopulation (4) which were the only two subpopulations where there was complete survey coverage.

$\overline{D}$ and R_h were highly correlated, each with VIF's of 92. R_h was therefore dropped from all further models as $\overline{D}$ was considered to be the more important factor, and much of the information on channel shape provided by R_h could be captured through inclusion of two-way interaction terms of the remaining variables. Once R_h was removed the VIFs of the five remaining variables indicated acceptably low levels of collinearity.

The second group of channel geometry models included the explanatory variables total cross-sectional area, and cross-sectional area below eight threshold depths. As these were all collinear, they could not be placed together in a single model and step-wise selection employed for model selection. Instead separate models were constructed for each variable and selection was based on comparing AIC/QAIC values.

4.2.2.4 River geomorphology habitat models

Only those segments within the primary extent of occurrence of the dolphins (subpopulations 2, 3, and 4 (see Fig. 2.1)) were used in the river geomorphology habitat models. The 30km of river between Guddu and Sukkur barrages (subpopulation 4) that were not surveyed were included in the characterisation of available habitat, but excluded from the habitat models. Twelve explanatory variables (Table 4.1) were included in both the logistic presence and quasi-Poisson abundance river geomorphology models at each of the three scales. The Guddu to Sukkur dolphin subpopulation (4) occurs at high density, and at the largest segment size (5km) dolphins were present in 100% of the segments. It was therefore not possible to conduct modelling of dolphin presence/absence on data from this river section as there were no absences on which to base a habitat preference. Therefore, this subpopulation was removed from consideration at the 5km scale, and only data from Chashma to Guddu barrages (subpopulations 2 and 3) were included in the presence models. All three river sections were included in the abundance models.

Paired plots and VIF values from the global model indicated that latitude (Lat), percentage distance between barrages (Per), and river width (RW) were collinear. Removal of Lat and Per from the analyses, resulted in acceptably low collinearity of the remaining ten explanatory variables. Dolphin density and available habitat differed in each river section, therefore to include the possibility that dolphin-habitat relationships varied by area an interaction between dolphin subpopulation and each explanatory variable was included. Due to the small dataset and large number of explanatory variables, no further interactions were considered.

4.2.3 Habitat Preference

To explore differences in the habitat present within each dolphin subpopulation, the fluvial habitat characteristics in each of the five sections were summarized and compared statistically using ANOVA and Tukey's test. River and channel widths were

described at the 1km segment scale as this showed the finest resolution, whereas braiding index and sinuosity exhibited the highest variance, and were therefore analysed, at the 5km segment scale.

The habitat parameters that were consistently selected by models as exerting the greatest influence on dolphin sighting probability, were cross-sectional area and channel top width in the channel geometry study, and river width in the river geomorphology analysis. To examine whether dolphins actively selected or avoided any of these three characteristics, river width, channel cross-sectional area and channel top width were each split into bins of equal size, and the observed versus expected proportion with dolphins present in each bin tested with Fisher's exact test. Fisher's exact test was employed, rather than Pearson's Chi square or a G-test, as it is more accurate for small sample sizes (McDonald 2009). Each dataset was stratified into two at the median value, to allow detection of different relationships at large and small values, e.g. avoidance of small cross-sectional areas and concurrent selection of large cross-sectional areas. Data were truncated to remove outliers that occurred at very low frequencies.

4.3 RESULTS

4.3.1 Data Summary

4.3.1.1 Channel Geometry

Channel geometry was collected at 226 cross-sections: 85 where dolphins were present and 141 where they were absent. Seventeen cross-sections were located in the 1J-C river section, 71 in the 2C-T section, 24 between Taunsa barrage and Ghazi Ghat (3T-GG), 90 in the 4G-S section and 24 between Sehwan Sherif and Kotri barrage (5S-K). The number of dolphins recorded at cross-sections ranged from 1 to 35 individuals (mean=6.32, SD=7.17, n=85).

4.3.1.2 River Geomorphology

A total of 1351 segments of 1km length, 674 2km segments and 270 segments 5km long were defined along the mainstem of the Indus River between the Jinnah and Kotri barrages. Dolphins were recorded in 19.7% of the 1km segments, 30.3% of 2km

segments and 46.7% of the 5km segments. The number of 1km segments for each subpopulation were as follows: 1J-C = 65, 2C-T = 276, 3T-G=333, 4G-S = 183, 5S-K = 494.

4.3.2 Available Habitat

4.3.2.1 Channel Geometry

Maximum cross-section depth ranged from only 1.4 m at a location between Sehwan Sharif and Kotri barrage (subpopulation 5), to 12.3 m between Guddu and Sukkur barrages (subpopulation 4). There was no significant difference in maximum depth at measured cross-sections between each dolphin subpopulation (ANOVA, df=4, F=2.153, p=0.075), however mean cross-section depth was significantly greater between Guddu and Sukkur barrages (ANOVA, df=4, F=4.877, p<0.001) than upstream sections, Chashma to Taunsa (Tukey's test, p<0.01), and Taunsa to Ghazi Ghat (Tukey's test, p<0.05) (subpopulations 2 and 3). Similarly, hydraulic radius was significantly greater between Guddu and Sukkur (ANOVA, df=4, F=4.801, p<0.001) barrages than Chashma to Taunsa (Tukey's test, p<0.01), and Taunsa to Ghazi Ghat (Tukey's test, p<0.05) signifying a narrower deeper channel in this area (Table 4.2; Fig. 4.3). Mean cross-sectional area between Sukkur and Kotri (subpopulation 5) was almost half that recorded in other river sections due to the extremely reduced dry season discharge in this section of river. There was no significant difference in cross-sectional area between the remaining four sections (Table 4.2; Fig. 4.3).

4.3.2.2 River Geomorphology

River widths ranged from 0.10 km to 12.17 km (mean = 1.05 km, SD = 1.33), and were greatest downstream of the Indus-Panjnad River confluence, and between Guddu and Sukkur where the river splits into two channels around a large island. River width was significantly smaller in the furthest downstream subpopulation, 5S-K than for all other areas (Tukey's test, 1J-C p<0.0001; 2C-T p<0.0001; 3T-G p<0.0001; 4G-S p<0.0001) as almost all water is diverted for irrigation at Sukkur barrage (Fig. 4.3; Table 4.2). The three upstream river sections were wide and braided, while downstream of Guddu barrage the river and main channel were narrower, and there was a lower degree of braiding and higher sinuosity. The greatest main channel width within a 1km segment showed a constant and statistically significant reduction in a downstream direction (ANOVA, df=4, F=268.25, p<0.0001) (Fig. 4.3) each section significantly different from

all others (Tukey's test, all=p<0.0001 except 2C-T:3T-G p<0.005). Sinuosity recorded over 25km was significantly greater downstream of Guddu barrage, than in the three upstream river sections (ANOVA, df=4, F=37.9, p<0.0001). In contrast, the degree of braiding was greatest upstream of Guddu barrage, and declined downstream (ANOVA, df=4, F= 59.6, p<0.0001, Tukey's test all significantly different except 2C-T:3T-G). The average of 1.18 bars/km between Jinnah and Chashma barrages (subpopulation 1) (SD=0.61) declined to 0.29 bars/km (SD=0.26) between Sukkur and Kotri (subpopulation 5).

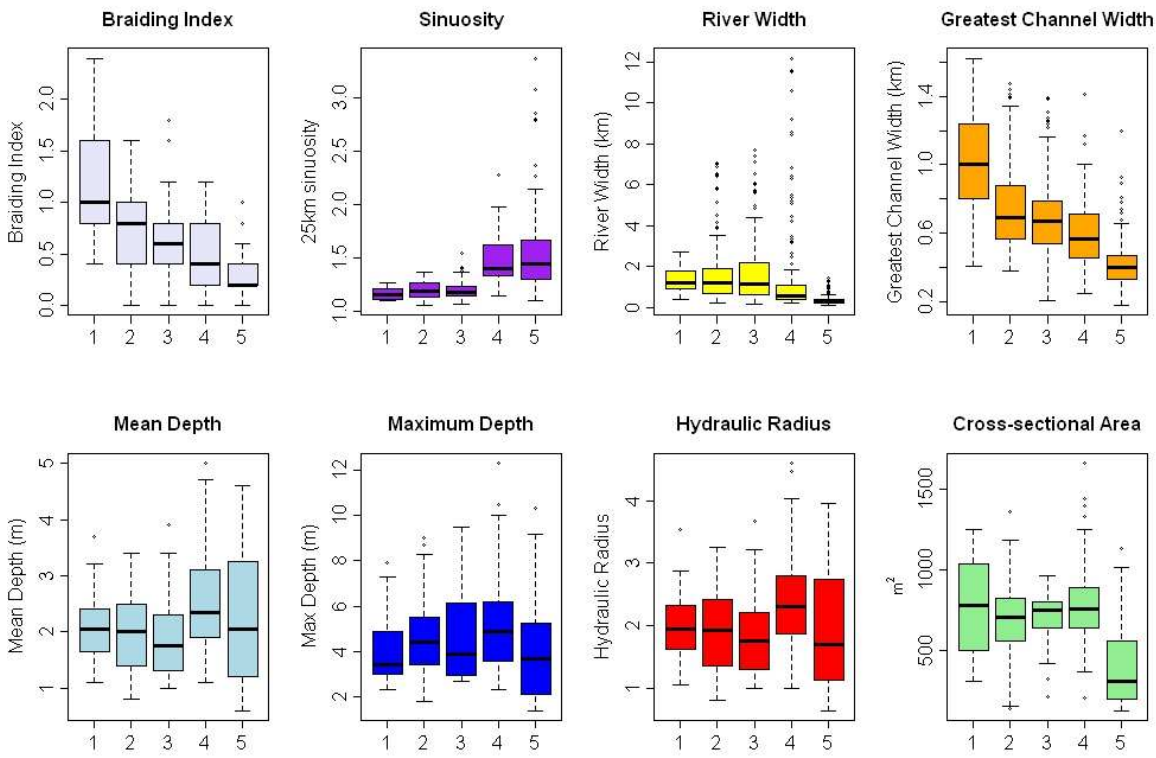

Figure 4.3 – Boxplots of channel geometry and river geomorphology characteristics in different sections of the Indus River. Numbers on the x-axis refer to subpopulation illustrated in Fig. 2.1. Subpopulation 1 is the furthest upstream, and 5 the furthest downstream.

Table 4.2 – Summary of channel geometry recorded at cross-sections, and river geomorphology characteristics in different sections of the Indus River.

	Channel Geometry				*River Geomorphology*			
River Section	*Mean Depth (m) (SD)*	*Max Depth (m) (SD)*	*Cross-sectional Area (m^2) (SD)*	*Hydraulic Radius (SD)*	*River Width (Km) (SD)*	*Greatest Channel Width (m) (SD)*	*25km Sinuosity (SD)*	*Braiding Index (SD)*
1	2.14 (0.70)	4.04 (1.67)	771 (300)	2.04 (0.63)	1.34 (0.53)	1001 (268)	1.16 (0.06)	1.18 (0.61)
2[a]	2.02 (0.68)	4.58 (1.77)	686 (226)	1.95 (0.63)	1.50 (1.24)	732 (225)	1.19 (0.08)	0.79 (0.42)
3	1.98 (0.84)	4.75 (2.04)	693 (182)	1.91 (0.76)	1.52 (1.28)	680 (196)	1.19 (0.09)	0.64 (0.41)
4	2.53 (0.83)	5.11 (1.91)	779 (233)	2.40 (0.73)	1.40 (2.29)	598 (192)	1.48 (0.25)	0.49 (0.35)
5[a]	2.25 (1.24)	4.09 (2.39)	424 (290)	1.97 (1.08)	0.31 (0.18)	409 (119)	1.55 (0.42)	0.29 (0.26)
Whole River	2.25 (0.86)	4.72 (1.94)	701 (258)	2.13 (0.77)	1.05 (1.33)	596 (245)	1.36 (0.32)	0.55 (0.44)

[a] in the channel geometry study these river sections were not covered completely, in section 2 data were collected only from Taunsa barrage to Ghazi Ghat, and in section 5 cross-sections were collected from Sehwan Sharif to Kotri barrage. SD = standard deviation

4.3.3 Dolphin Presence

Mean depth (t-test, t=3.4997, p<0.001), maximum depth (t-test, t=3.5445, p<0.001), cross-sectional area (t-test, t=2.780, p<0.01), hydraulic radius (t-test, t=3.698, p<0.002) and number of backwaters (t-test, t=2.84, p<0.01) were all significantly greater at locations in the river where dolphins were present compared to locations where the were absent. Latitude (t-test, t=-6.511, p<0.0001), braiding index (t-test, t=-3.51, p<0.001) and river width (t-test, t=-2.66, p<0.01) were all significantly smaller where dolphins were present. There was no significant difference in the remaining variables.

4.3.4 Habitat Models

4.3.4.1 Channel Geometry

Channel geometry models consistently selected channel cross-sectional area as the most important variable explaining dolphin presence and abundance (Table 4.3). The probability of dolphin presence increased with increasing channel cross-sectional area up to approximately 600 m^2, levelled off from 600m^2 to 1050m^2 and then continued to increase above 1050 m^2 (Fig. 4.4). This relationship was observed in models that included cross-sections from the entire river, those where polynomial functions were excluded, and in models that only considered the low density Chashma to Taunsa (subpopulation 2) or high density Guddu to Sukkur (subpopulation 4) subpopulations. The cross-sectional area response curve is non-linear, however a linear response is possible within the confidence intervals. Cross-sectional area accounted for 90% of the explained deviance in models of dolphin presence. The model of dolphin presence for the entire river correctly classified 65% of the responses, and was more accurate at classifying presence (80% correct classifications) than absence (57% correct classifications). Quasi-Poisson GLM models of dolphin abundance, showed that, similar to presence, abundance increased with increasing cross-sectional area and also with mean depth (Table 4.3; Fig. 4.5). All final models were separated by more than two AIC units from other candidate models indicating that results were robust to model selection. In all cases examination of residuals revealed no unacceptable patterns and Cook's distance calculations indicated that no points exerted excessive influence on results.

Table 4.3 – Channel geometry characteristics retained in final GLM models of dry-season Indus dolphin presence and abundance listed in order of significance.

Dolphin presence			*Dolphin abundance*		
Entire River	Chashma-Taunsa	Guddu-Sukkur	Entire River	Chashma-Taunsa	Guddu-Sukkur
+ A***	- A^2 *	+ A *	+A ***	+ A *	+ A **
- A^2**	+ A •		+SP ***	- A^2 *	- A^2 **
+ A^3**	- T^3 •		- A: $\overline{D}$ ***	- T *	- T^2 *
- T**			- A:T ***	- $\overline{D}$ ^2 *	
+ SP*			+ $\overline{D}$ •		
-A: $\overline{D}$ *					
Correct Presence | Absence Classifications			*% explained deviance*		
80% | 57%	87% | 57%	58% | 68%	36%	25%	23%

Variables: SP (Subpopulation), D_{max} (Maximum Depth), $\overline{D}$ (Mean Depth), T (Channel top width), A (cross-sectional area), ^ (polynomial function). +/- = Direction of relationship Significance: •=p<0.1, *=p<0.01, **=p<0.001, ***=p<0.0001. Interactions between variables denoted by **':'**

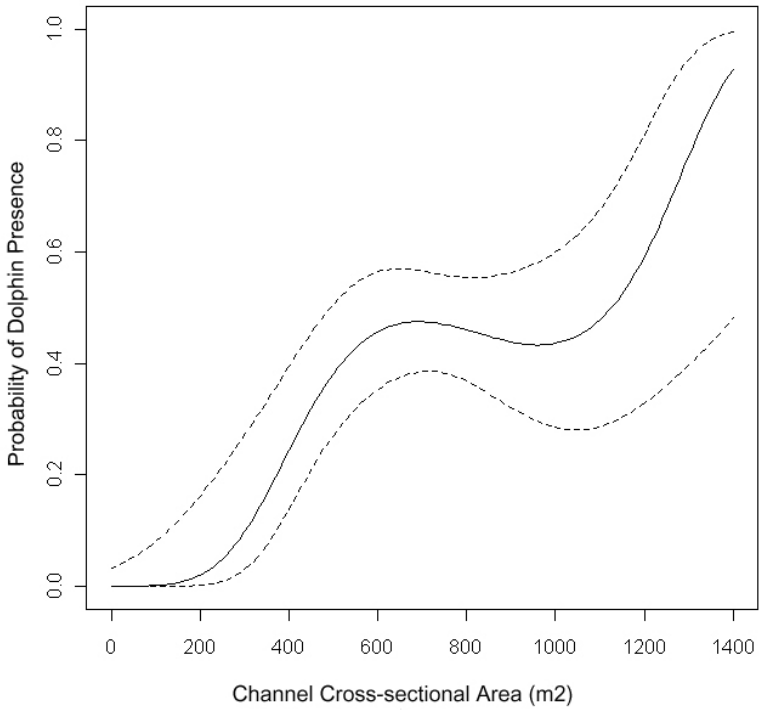

Figure 4.4 – Probability of Indus dolphin presence in relation to total channel cross-sectional area (m^2). Dotted lines represent standard error.

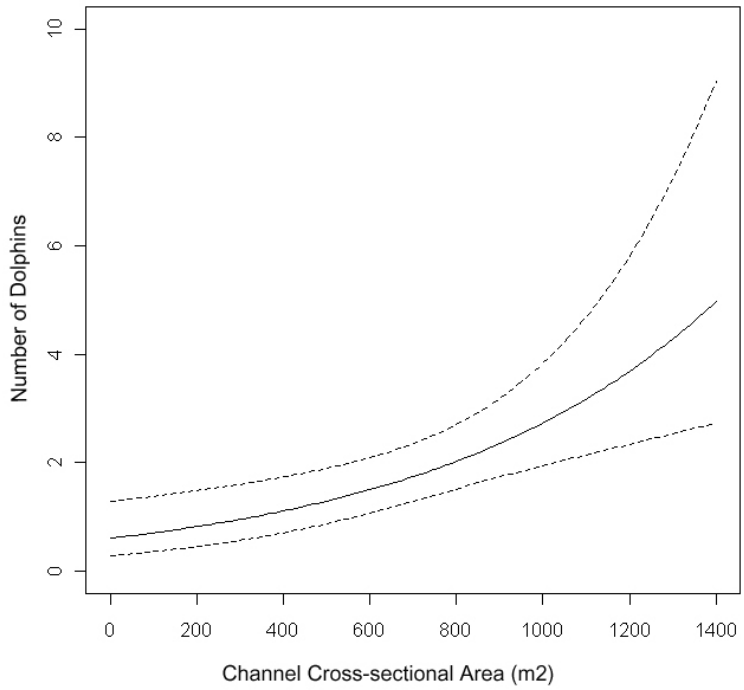

Figure 4.5 – Predicted Indus dolphin abundance in relation to channel cross-sectional area (m^2). Dotted lines represent standard error.

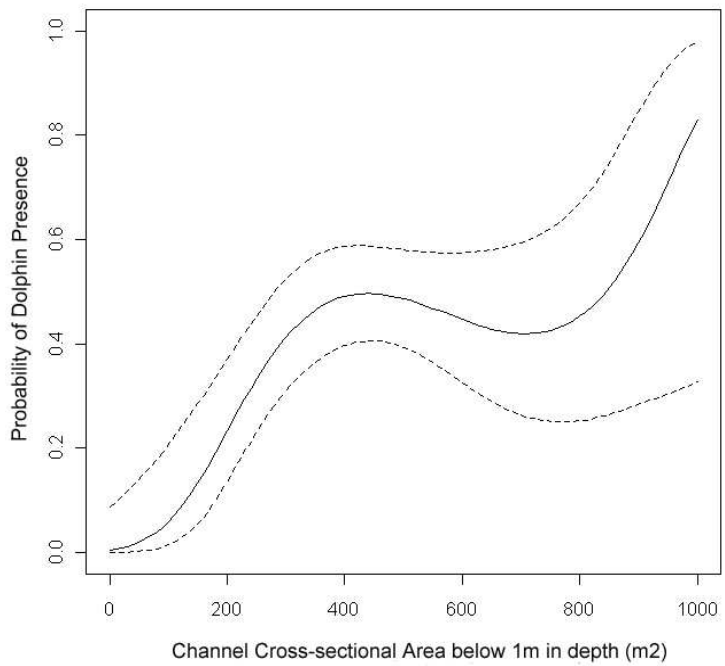

Figure 4.6 - Probability of Indus dolphin presence in relation to channel cross-sectional area below 1m in depth. Dotted lines represent standard error.

The second group of channel shape models examined the influence of channel cross-sectional area below a range of threshold depths, and indicated that dolphin presence and abundance were best explained by cross-sectional area below threshold depths between 1m and 2m, each model with approximately equal weight (Fig. 4.7). Model fit was greatly improved by excluding the cross-sectional area between 0 and 1m deep, which indicates that a large area of deep water is an important factor influencing habitat selection by Indus dolphins. The relationship between dolphin presence and total cross-sectional area (Fig. 4.4) shows the same shape as that for dolphin presence and cross-sectional area below 1m depth (Fig. 4.6).

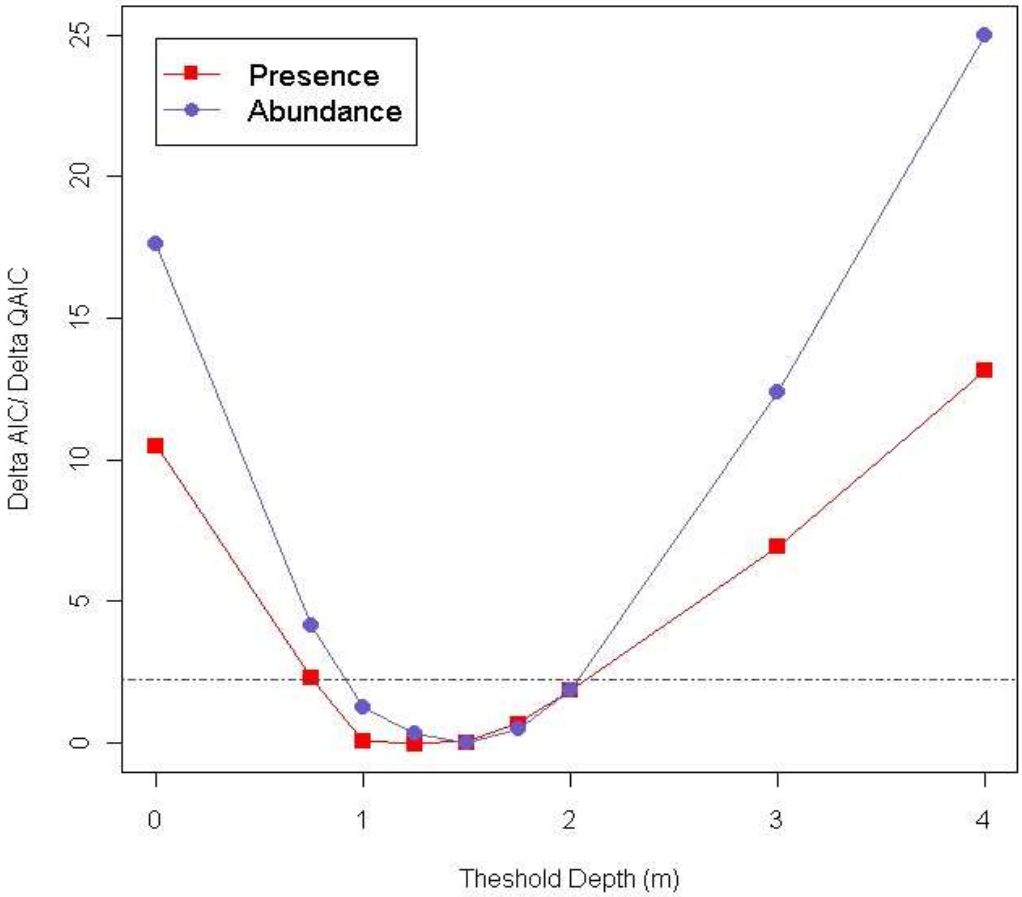

Figure 4.7 – Delta AIC (presence) and Delta QAIC (abundance) scores for models that explain dolphin presence and abundance based on channel cross-sectional area below different threshold depths. The grey horizontal line separates models that are within two AIC units of the minimum.

4.3.4.2 River Geomorphology

Examination of Cook's distance plots from the initial quasi-Poisson dolphin abundance models indicated that the two segments immediately adjacent to Guddu barrage, which had high abundance, exerted a strong influence on results. When these segments were included in models an association between dolphins and revetments which occur in the vicinity of barrages was identified. This association was not present when these two segments were omitted, and they were removed from the analysis, following which no unusual patterns in residuals or highly influential points were observed and results more closely reflected the habitat associations of dolphins in the majority of their range

Final models of dolphin abundance explained 34, 36 and 66% of residual deviance over the null model for the 1km, 2km and 5km segment lengths, respectively. Models showed the greatest classification accuracy at the coarsest resolution of the analysis. Dolphin subpopulation and river width were the primary factors that influenced Indus dolphin presence and abundance (Table 4.4). Dolphin subpopulation was the only variable included in candidate models that did not directly describe river geomorphology, and it was consistently the most important explanatory variable, accounting for 72% of the explained deviance in the 1km presence model and 88% in the 1km abundance model. An association between dolphin presence and confluences, and increasing probability of dolphin presence and abundance with declining river width (Fig. 4.8) was detected across all subpopulations. In addition to these range-wide habitat associations, the probability of dolphin presence in the low density 2C-T subpopulation was weakly related to the length of primary channel, and sinuosity. In the 3T-G subpopulation sighting probability and dolphin abundance was highest at river constrictions and at confluences. In the high density 4G-S subpopulation, dolphin abundance increased as the degree of braiding decreased (Table 4.4). Polynomial variables were fitted in all candidate models but were retained in only one case (RW in the 2km abundance model) indicating that relationships between the geomorphology variables and Indus dolphin occurrence and abundance are approximately linear.

Results of the 1km and 2km segment scale analyses were similar, but different explanatory variables were selected at the larger 5km scale; confluences were not retained, sinuosity was selected and the importance of subpopulation was reduced.

Table 4.4 – Characteristics of river hydrogeomorphology measured over three scales that were retained in final GLM models of dry season Indus dolphin presence and abundance listed in order of significance.

Dolphin Presence			*Dolphin Abundance*		
1km	2km	5km @	1km	2km	5km
+ SP (3) ***	+ SP (3) ***	- RW(3)**	+ SP (3) ***	+ SP (3) ***	- RW (3) **
+ SP (4) ***	+ SP (4) ***	- S25 (2) **	+ SP (4) ***	+ SP (4) ***	- S25 (2) *
+ Conf (3) **	+ Conf *	+ RW (2) *	- BI (4) ***	- RW^2,^3 **	- SP (4) *
- RW (3) **	- RW (3) *	+ PC (2) *	- RW (3) **		- SP (3)) •
+ RW (2) •	+ RW (2) •	- SP (3) •			
	+ SCW •				
Correct Presence | Absence Classifications			*% Deviance Explained*		
69% | 70%	74% | 64%	75% | 76%	34	36	66

Variables: SP (Subpopulation), (2)=Chashma to Taunsa barrage, (3)= Taunsa to Guddu barrage, (4)=Guddu to Sukkur barrage, Conf = Confluence, GCW=Greatest Channel Width, SCW=Narrowest Channel Width, RW=River Width, PC=Length of Primary Channel, BI=Braiding Index, S25= Sinuosity determined over 25km. ^ = polynomial function. Significance: •=p<0.1, *=p<0.01, **=p<0.001, ***=p<0.0001 +/- = Direction of relationship. @ 5km dolphin presence analysis was conducted only on data from Chashma to Guddu barrages, subpopulations 2 and 3.

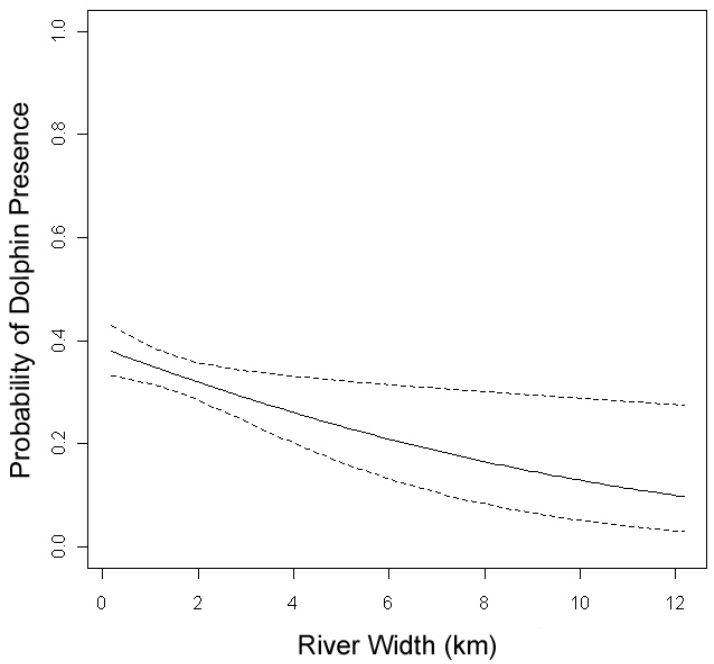

Figure 4.8 – Probability of Indus dolphin presence according to river width measured in 1km river segments. Dotted lines represent the standard error.

4.3.5 Habitat Preference

There was no significant difference between the observed and expected number of cross-sections with dolphins present when cross-sectional area was large (700 to 1300 m^2) (Fisher's exact test, p=0.938), but dolphins were encountered significantly less frequently than expected at locations with small cross-sectional area (<700 m^3) (Fisher's exact test, p=<0.05) (Fig. 4.9), and were never encountered in channels with cross-sectional areas less than 300 m^2. Dolphins occurred more frequently than expected in narrow channels that were less than 100 m wide (Fisher's exact test, p=<0.05) (Fig. 4.9). There was no significant difference in the observed and expected number of cross-sections with dolphins present according to river width (Fisher's exact test, p=0.666).

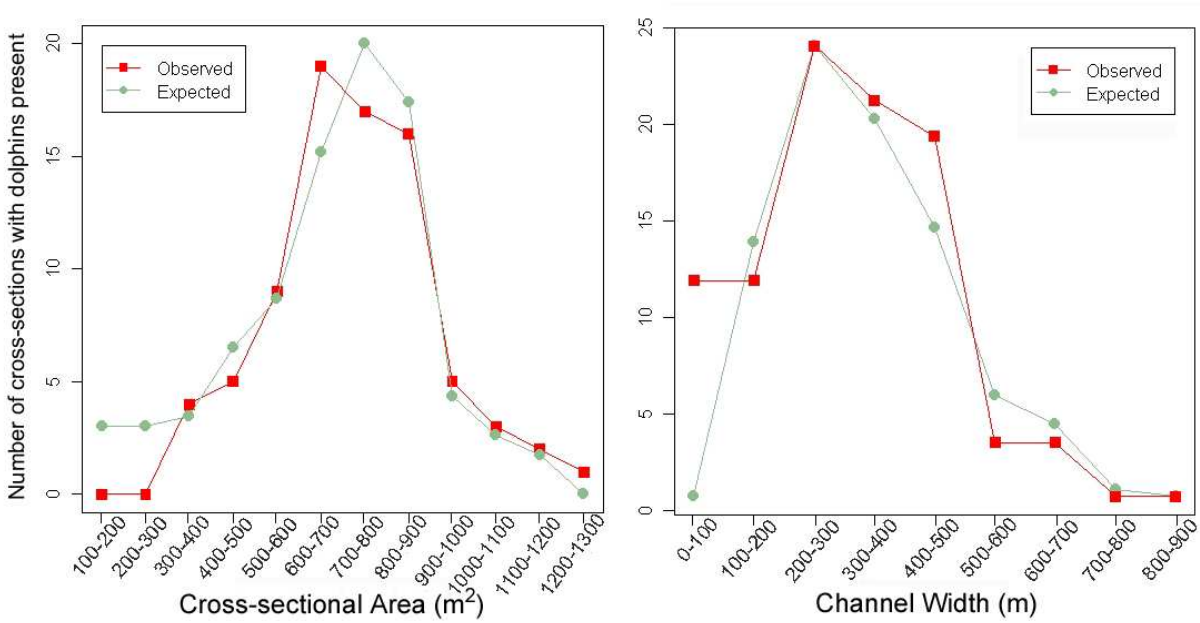

Figure 4.9 – Observed and expected number of cross-sections with dolphins present according to channel cross-sectional area and channel top width

4.4 DISCUSSION

4.4.1 Available Habitat

The differing habitat characteristics in each river section are consistent with the well documented progression in river geomorphology that occurs naturally along the course of most rivers (Bridge 2003). The Indus flows out of the Himalayan foothills onto the plains just upstream of Jinnah barrage, and the sudden reduction in gradient and water velocity causes suspended sediment to be deposited and the river to spread out across the floodplain in multiple braids. Downstream of Guddu barrage the river becomes primarily a single sinuous channel with narrower width and greater mean depth. Study of Indus River habitat is complicated by the fact that, contrary to most natural rivers, flow decreases in a downstream direction, due to irrigation diversions. Indus floodplain channels are formed by the annual flood, which is relatively little reduced by diversions for irrigation; however, prolonged periods of greatly depleted flow downstream of Sukkur barrage have created a narrower channel with smaller cross-sectional area than other sections despite expectations to the contrary based on its position near the downstream end of the river's course.

The 4G-S dolphin subpopulation occurs at the highest density recorded for any river dolphin, and the present study shows that between Guddu and Sukkur the Indus River is narrower and less braided, and the main channel has greater average cross-sectional area and depth than areas upstream. While the high dolphin density is likely to be due to a combination of many factors, the Guddu-Sukkur area provides a larger quantity of suitable habitat for river dolphins than upstream areas.

4.4.2 Habitat Selection

Cross-sectional area was consistently selected as the most important factor influencing dolphin distribution and abundance. The relationship was present in analyses of the entire river and separately within each subpopulation despite dramatic differences in dolphin density, and available habitat. This reinforces confidence in the robustness of the results, and in particular that cross-sectional area is the main spatial predictor of Indus dolphin habitat. The data show that dolphins avoid channels with less than 700m^2 cross-sectional area, and that locations with greater than 1050m^2 cross-sectional area are high-use habitat. The second set of channel geometry models demonstrate that the relationship between dolphins and channel cross-sectional area is

complex and dependent on a large area of water greater than 1m deep. Two channels can have identical total cross-sectional area, but great differences in channel shape, therefore presenting quite different dolphin habitats. This is demonstrated by Fig. 4.10 where two actual cross-sections have identical total cross-sectional area, but a very different area of deep water, resulting in almost double the probability of dolphin presence in the deeper cross-section. Larger cross-sectional area with a deep, well defined thalweg (the location of maximum depth) (Bridge 2003) is an important factor determining low-water season dolphin distribution.

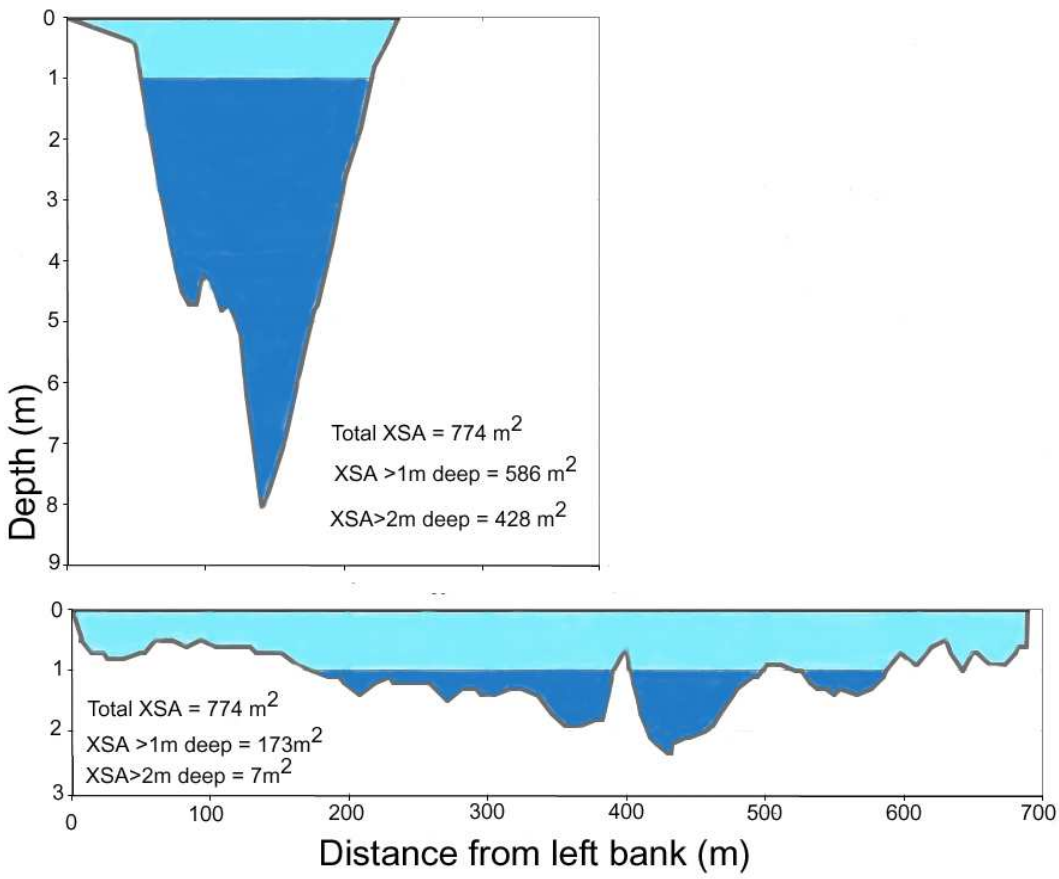

Figure 4.10 – Illustrations of two actual channel cross-sections with the same total cross-sectional area but great differences in the area below 1m in depth. Actual channel proportions are distorted due to different axis scales.

Cross-sectional area is a two-dimensional representation of three-dimensional habitat, suggesting that in the dry-season when water levels in the river are low dolphins avoid channels with small volume, selecting locations with large volume. In Nepal, Ganges River dolphins were also reported to select deep pool habitat in preference to shallows

(Smith 1993), and previous surveys on the Indus have shown very low dolphin encounter rates in secondary channels with low depth (Braulik, 2006). This is in contrast to Amazon river dolphins that do not appear to avoid shallows, often entering shallow flooded forest areas when water levels are high, and occurring with highest frequency near to the river banks and in small channels (Gomez-Salazar et al., 2012). South Asian floodplain rivers are shallow and multi-threaded, and are subject to rapid changes in discharge. River dolphins are therefore likely to have evolved the habit of avoiding channels with low depth or small cross-sectional area, due to the risk of stranding or entrapment. Although South Asian river dolphins can move through water less than 1m in depth (Smith 1993) it provides little physical space in which to swim, potentially offers reduced foraging opportunities, and is likely utilised only intermittently for transit. In the Mekong River, the freshwater population of Irrawaddy dolphins (*Orcaella brevirostris*) is reported to be resident in deep, low velocity pools during the dry season (Baird and Beasley 2005; Beasley 2007). This association is also likely to be due to a greater availability of prey, because pools typically have higher abundance and diversity of fish than other areas (Gilliam et al. 1993). In a study in the Ganges River basin most fish species specialised in specific habitat conditions, with the most numerous and diverse group associated with deep pool habitats and sandy substrate (Sarkar and Bain 2007).

Models showed that dolphins are recorded with higher frequency in narrow channels, at river constrictions and at confluences. A relationship between river dolphins and confluences has also been demonstrated for Ganges River dolphins in the Sundarbans of Bangladesh (Smith et al. 2009), Amazon River dolphins (*Inia geoffrensis*) and tucuxi (*Sotalia fluviatilis*) in the Brazilian Amazon (Martin et al. 2004), and Irrawaddy dolphins in the Mahakam River, East Kalimantan (Kreb and Budiono. 2005). The confluences recorded in this study are the confluence of large channels in multi-threaded reaches, rather than confluences of a tributary and the mainstem, however, irrespective of their origin, confluences are known to be biological hotspots (e.g. Benda et al. 2004). They typically comprise an avalanche face and a deep scour up to four times the average depth of the incoming channels, along with increased turbulence and velocity (Ashmore and Parker 1983; Best 1988; Best and Ashworth 1997). River constrictions are locations that typically have large cross-sectional area, high mean depth and velocity, and are sometimes are coincident with confluences, hence the consistent selection of similar types of habitat by the dolphins reflected in the statistical models.

4.4.3 Model Performance

Channel geometry had a greater ability to explain dolphin distribution than river geomorphology, although the analyses generated complimentary results. The river geomorphology analyses retained only several weakly significant habitat variables, with dolphin subpopulation, a spatial variable, demonstrating the greatest explanatory power. In contrast, the channel geometry models consistently selected the same parameters (i.e. cross-sectional area and channel top width), with high levels of significance. The most likely explanation for this is that dolphin distribution is driven primarily by small-scale hydrological features that were better captured by the channel geometry study. This conclusion is supported by the results of the river geomorphology study, where at the smaller scales discrete features such as confluences that exert a local influence were selected. At the largest scale (5km) sinuosity measured over 25km, a parameter linked to habitat complexity, was identified. This indicates that dolphin distribution may be driven primarily by occurrence of small-scale features, but is also influenced by larger-scale habitat complexes, a conclusion that is consistent with the widely held view of riverine ecosystems as being arranged in nested hierarchies of habitat patches (Crook et al. 2001).

Cetacean habitat models never perfectly predict cetacean distributions (Redfern et al. 2006). The correct classification rate of dolphin presence in the channel geometry models was slightly better than for the river geomorphology models, but both performed relatively well and typical of other similar studies that examine cetacean distribution in relation to habitat characteristics (e.g. Bearzi et al. 2008; Bräger et al. 2003; Gregr and Trites 2001; Laran and Gannier 2008). The river geomorphology models classified presence and absence with approximately equal ability, but the channel geometry models for the entire river, and the low density 2C-T subpopulation classified presence substantially better than absence. A possible explanation for this is that the data on dolphin distribution was derived from a single comprehensive survey, so that while dolphin presence indicates suitable habitat, especially in low density habitats not all suitable habitat will be occupied at any one time, and therefore absence does not necessarily imply unsuitable habitat. Additionally, if dolphins were missed during the survey (Chapter 2) this would result in false absences being recorded.

In general, channel geometry models for the high density 4G-S subpopulation performed less well (explained less deviance, or had a lower classification rate) than

those of intermediate and low density subpopulations or those that modelled the entire surveyed river stretch. In the lower density areas dolphins are patchily distributed allowing for detection of clear and convincing dolphin-habitat relationships. However in the 4G-S subpopulation dolphins occur almost continuously along the river channel, the majority of available habitat appears to be utilised, and associations with specific habitat features are less distinct. At higher densities and closer to the carrying capacity it is likely that dolphins are forced to exploit a wider range of habitats including those that are sub-optimal.

4.4.4 Potential Biases or Improvements to Habitat Models

Correlation between dolphin survey sighting probability and any of the habitat variables in the models may introduce bias into the results (MacKenzie 2006). The parameters most likely to be linked to dolphin detection are channel width and river width, as the probability of missing a dolphin increases with distance from an observer. When the river is very wide, it is also braided, and there is potential for animals to disperse into unsurveyed channels decreasing detection probability. Although this cannot be entirely ruled out as a potential bias, the main channel and all significant secondary channels were surveyed and the small channels that were unsurveyed are considered unlikely to be suitable dolphin habitat (Braulik 2006).

Channel geometry models identified an increasing probability of dolphin presence in narrower channels that could be interpreted as dolphins actively selecting narrow channel habitat, or alternatively that there is decreased sighting probability in wide channels. Correlation between channel width and dolphin sighting probability is unlikely to be large as 95% of cross-sections were less than 650 m wide, 92% of survey effort was conducted in excellent survey conditions, and the mean radial dolphin sighting distance was 401 m, greater than half the river width (Chapter 2). As the geomorphology models did not select channel width but instead selected other features that often occur in narrower channels, such as confluences and river constrictions, the selection of channel width by the channel geometry analysis is likely to be due to it being favourable habitat, however it is not possible to rule out a contribution from sighting biases.

Choice of explanatory variables is crucial to constructing meaningful habitat models that can adequately explain species-habitat relationships. After depth, velocity is the second most important physical variable influencing the distribution of river fauna (Sarkar and Bain 2007) and it has been reported to influence the use of habitat by dolphins in Nepal (Smith 1993). South Asian river dolphins are undoubtedly heavily influenced by water velocity and inclusion of this parameter in future habitat studies will enhance the understanding of river dolphins' fine-scale habitat use.

4.4.5 Conservation Implications

Habitat selection models can provide a biological rationale for determining which areas should be given highest conservation priority (Day 2002). However, South Asian rivers are constantly migrating and moving laterally across their floodplain and even in the dry season important dolphin habitats will gradually move. As a result, the definition of fixed spatial boundaries for river dolphin protected areas may not be an effective conservation measure. Protected areas for river dolphins will therefore need to either be large enough to encompass multiple high-use areas, or designed so that the boundaries of small-scale conservation zones can be adjusted regularly to account for migration of favoured habitats.

The habitat models presented here provide a basis for determining which areas should be given highest conservation priority. In the dry season, channel constrictions, confluences, and channels with high cross-sectional area are all high-use dolphin habitats that could benefit from management as discrete dolphin conservation zones. Conservation of small core areas that incorporate favored deep water sites have been implemented for river dolphins in the Mekong (Baird, 2001) and Mahakam Rivers (Kreb and Budiono, 2005) and the Sundarbans, Bangladesh (Smith et al., 2010). Within these zones, human activities that pose a direct threat to Indus dolphins, such as gillnet fishing and intense motorized vessel traffic, can be managed in collaboration with local communities who rely on the river for their livelihoods.

Pakistan's semi-arid climate, large and rapidly expanding human population (167 million growing at 2.09% p.a. in 2006), and dependence on irrigated agriculture is exerting substantial and unsustainable stress on existing water resources. The projected demand for water is predicted to outstrip availability before 2025 (Siddiqi and

Tahir-Kheli 2004). To mitigate this looming crisis, numerous plans to further exploit surface water supplies through the construction of storage dams and irrigation projects are being considered, that will further disrupt the natural flow regime. The importance of channel cross-sectional area to Indus dolphin habitat selection may be due to depleted dry-season flows causing dolphins to concentrate in the limited habitat remaining with sufficient volume. Such highly mobile aquatic animals would be expected to exploit numerous habitat patches (MacArthur and Pianka 1966), therefore sufficient river discharge to maintain connectivity of low-water season habitat patches is vitally important. If low-water season flows are allowed to decrease further the amount of deeper habitat will decline, there may be insufficient patches of suitable habitat to support the population through the low-water season, and dolphins may become isolated into deeper river sections, unable or unwilling to traverse through shallows between favourable patches of habitat.

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Chapter 5

High lineage divergence and low genetic diversity in geographically isolated populations of South Asian river dolphin

Abstract

Despite their endangered status, the taxonomic relationship between the two geographically isolated South Asian river dolphin populations has never been comprehensively assessed. I present the first evaluation of the phylogenetic relationship between the Indus (*Platanista gangetica minor*) and Ganges (*Platanista gangetica gangetica*) River dolphins, the population structure within each population, and their time of divergence, based on mitochondrial control region sequences extracted from museum specimens and cytochrome b sequences from GenBank. The 458bp partial control region sequences from all 20 Indus River dolphin samples were identical; there was only one haplotype shared by all individuals. Only four haplotypes were identified within the entire Platanistidae family and none were shared between populations. Limited numbers of complete 858 bp control region sequences (Indus n=1; Ganges n=13) revealed 8 variable sites, and 6 fixed differences between Indus and Ganges River dolphins, comprising 3 transitions, 1 transversion, and 2 insertion-deletions. A similar pattern of low genetic diversity was observed in a 541bp section of the cytochrome b gene, where in 19 *Platanista* sp. sequences there were 4 haplotypes each separated by a single transition. One haplotype was unique to the Indus, two were found only in the Ganges and one was shared. 75% of the genetic variation within *Platanista* was due to differences between the two populations and phylogenetic trees demonstrated a well supported reciprocal monophyletic separation. Very high F_{ST} scores, 0.921 ($p<0.001$) indicated the long-term absence of gene flow and the clear genetic differentiation of each geographically isolated population. Using a molecular clock with the divergence between the Platanistidae and Ziphiidae as a calibration point, the two dolphin populations diverged an estimated 0.66 million years ago, (95%PP 0.17-1.20 MY), possibly when dolphins from the Ganges dispersed into the Indus during river capture.

5.1 INTRODUCTION

The Indus and Ganges River dolphins are relict species, the only extant members of one of the most ancient cetacean families, the Platanistidae (Fordyce 2002; Whitmore Jr. 1994). The Platanistids radiated in the Oligocene (34-24 MY (million years) ago) and were once diverse, and widely distributed in the ocean (Cassens et al. 2000; de Muizon 2002). Despite the physical dissimilarity, molecular analyses demonstrate that the Platanistidae are more closely related to beaked whales (Family Ziphiidae) and sperm whales (Family Physeteridae), than they are to any other dolphins or porpoises (Arnason et al. 2004; Cassens et al. 2000; May-Collado and Agnarsson 2006; Nikaido et al. 2001; Verma et al. 2004). All river dolphins were previously placed together in a single family due to similarities in their morphology and habitat, however molecular studies have now definitively shown that the river dolphins are not monophyletic and the Indus and Ganges dolphins are now classified in their own family the Platanistidae (Arnason and Gullberg 2004; Messenger and McGuire 1998; Yang et al. 1992). Indus and Ganges River dolphins evolved from a common ancestor, but now are geographically isolated, the Indus River dolphin inhabiting the Indus River system of Pakistan and India, and the Ganges River dolphin the Ganges, Brahmaputra and Karnaphuli River systems of India, Bangladesh and Nepal (Fig. 5.1). Despite their endangered status, the taxonomic relationship between the two South Asian river dolphin populations has never been comprehensively evaluated, although it has been the subject of conjecture (Pilleri et al. 1982; Reeves and Brownell 1989; Reeves et al. 2004; Rice 1998).

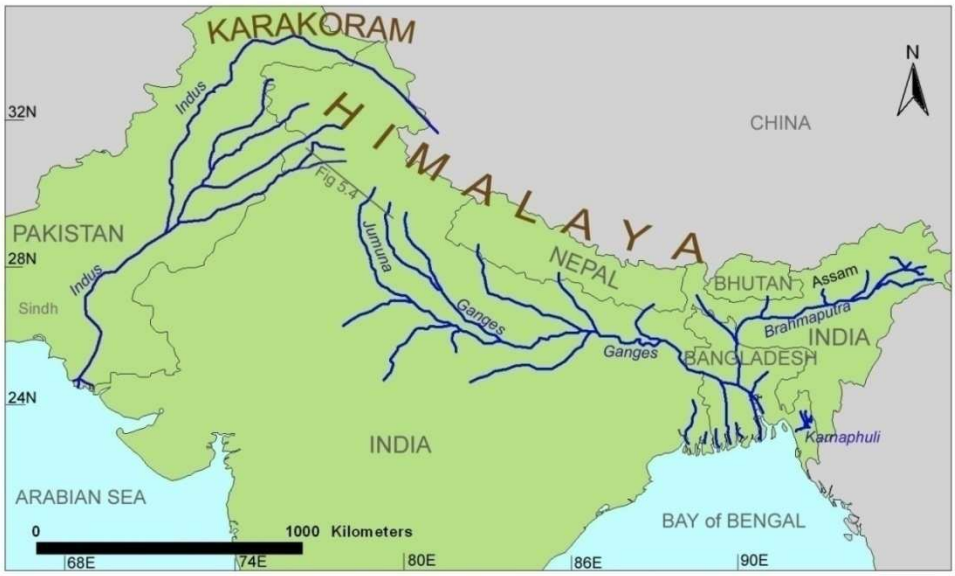

Figure 5.1 – The Indus, Ganges, Brahmaputra and Karnaphuli River systems

5.1.1 *Platanista* Nomenclature and Taxonomic Studies

The Ganges River dolphin, *Platanista gangetica*, was described in two separate accounts both written in 1801, one by a British botanist Roxburgh, the other by a Dutch missionary Lebeck (Lebeck 1801; Roxburgh 1801). There is uncertainty as to which account was published first, but most authors attribute the prior description to Lebeck (Committee on Taxonomy 2011; Kinze 2000). The Indus River dolphin was described much later, and named *Platanista indi* by Blyth (1859). It was not until 1976, that an earlier very brief mention of an Indus River dolphin *variety* was discovered by Van Bree (1976) and the senior synonym, *Platanista minor* (Owen 1853) was adopted for Indus River dolphins from this point forward.

In the 1870s, Anderson (1879) compared the external morphology, skull morphology and skeletons of Indus and Ganges River dolphins and concluded that evidence did not allow identification of different species, only that males are considerably smaller than females and that individuals show considerable size variation depending upon location. Based on these conclusions, from the 1880s until the 1970s Indus and Ganges River dolphins were considered to be subspecies. In the 1970s a number of comparative studies were conducted concluding that Indus and Ganges River dolphins had significantly different nasal crests on the skull (Pilleri and Gihr 1971), differences between the sixth and seventh cervical vertebrae (Pilleri and Gihr 1976), appreciable differences in the composition of blood proteins (De Monte and Pilleri 1979), in the ratio of free:esterified cholesterin lipids in the blubber (Pilleri 1971) and in the length of the tail (Kasuya 1972). These factors were presented as evidence of the specific status of the two populations (Pilleri et al. 1982) and from the mid-1970s until the late 1990s they were referred to as separate species. Reeves and Brownell (1989) concluded that as the sample sizes and number of adult specimens used in all earlier studies were extremely small and no statistical analyses were conducted on the data none of the arguments were adequate to recognise two species, leading Rice (1998) to subsequently change their taxonomic status from species to subspecies, *P. gangetica minor* and *P. gangetica gangetica.* This classification has now been adopted by most authors and scientific organisations (Committee on Taxonomy 2011; International Whaling Commission (IWC) 2001; Reeves et al. 2003; Smith and Braulik 2008a). The numerous changes in taxonomy imply that new information was available on which to base these decisions, in fact the comparative studies conducted so far have been very

limited and do not provide sufficient weight of evidence to defend any taxonomic classification.

5.1.2 Formation of the Indus and Ganges River Systems

The theory of the 'Indobrahm River', a single ancient river in the South Asian subcontinent that supposedly ran along the base of the Himalayan mountain range to the mouth of the present Indus (Pascoe 1919; Pilgrim 1919) was referred to by Pilleri et al. (1982) and was used by Rice (1998) to support his assertion that Indus and Ganges River dolphins did not show specific differences. However, the existence of the Indobrahm River has long been dismissed with the advent of modern methods of studying ancient river courses (Geddes 1960; Hora 1953; Ripley 1949; Ripley and Beehler 1990; Shroder Jr and Bishop 1999). Paleo-drainage and erosion patterns of the Himalaya have been described by analysing the stratigraphic record in the Indus and Bengal deep-sea depositional fans, and there is now overwhelming evidence that the Indus, Brahmaputra and Ganges Rivers, existed soon after the original India-Asia collision (~55-45 MY ago) and even before large-scale elevation of the Himalayan mountains (Brandis 2001; Clift et al. 2002; Clift and Blusztajn 2005; Clift et al. 2001; Qayyum et al. 1997; Searle and Owen 1999; Shroder Jr. 1993b; Uddin and Lundberg 1999). The Indus fan, the second-largest deep-sea fan in the world, has been accumulating since at least the middle Eocene (~45 MY ago), and the depositional record indicates that patterns of drainage and erosion have not changed greatly since the initiation of the Indus River in the Eocene (Clift and Gaedicke 2002; Clift et al. 2001; Searle and Owen 1999). Fluvial stratigraphy and paleo-current data show that a large sandy river debouched from the mountains at the same longitude as the modern Indus River and flowed south towards the Arabian Sea from at least 13.5–11.5 MY ago (Beck and Burbank 1990) and also between 8.5 to 5.5 MY ago (Friend et al. 1999). The Bengal fan, the largest deep-sea fan in the world, reaches a depth of more than 16 km and shows that sediment deposition in the Bengal basin began in the Miocene, and that there has been no major shift in either the area being drained or the location of the river mouth since that time (Uddin and Lundberg 1999). Furthermore, regional climate patterns and the seasonal monsoon have resembled their modern form since at least 22 MY ago (Clift et al. 2008; Garzione et al. 2005; Guo et al. 2002). Although the evidence clearly shows that the Indus and Ganges-Brahmaputra have been separate rivers for many millions of years, 30 MY of seismic reflection data from drill core samples and neodymium isotopes in the Indus fan were used to demonstrate that the

five major tributaries of the Indus (Fig. 5.1) were progressively captured from the Ganges sometime after 5 MY ago (Clift and Blusztajn 2005). This major drainage reorganisation was also supported by paleo-current data (Beck and Burbank 1990; Friend et al. 1999).

5.1.3 Phylogenetic Study and Conservation

For effective conservation of endangered species, such as the South Asian river dolphins, it is imperative to determine which taxa are different species, as well as to identify evolutionarily significant units within species so that conservation actions may be directed to preserve important groups (Chen et al. 2010). It is likely that the recent change to subspecific status for the South Asian river dolphins has reduced their conservation priority (Reeves et al. 2004), and as incorrect taxonomic classifications often have serious consequences for wildlife conservation (Daugherty et al. 1990) clarification of the systematics of *Platanista* was listed as highest priority by a recent cetacean taxonomic workshop (Reeves et al. 2004).

In this Chapter, I present the first evaluation of the phylogenetic relationship between the Indus and Ganges River dolphins, examine population structure within each population and estimate their time of divergence. Studies of mitochondrial DNA (mtDNA) are at the forefront of advancing understanding of cetacean and mammalian phylogenetics for several reasons: mitochondrial DNA is relatively easy to amplify and sequence, it is mostly free of problems with intermolecular genetic recombination, and there is extensive intraspecific variation that thus offers information at various phylogenetic levels (Avise 2000; May-Collado and Agnarsson 2006). The control region of mtDNA is a portion that evolves particularly rapidly as it is free of functional constraints and it thus allows fine-scale resolution of population structure and micro-evolutionary differences. Although mitochondrial molecular markers only contain information on female lineages, their use in population delimitation has been considered biologically sound (Allendorf and Luikart 2007) and a 458bp section of mtDNA control region was the focus of the current study.

A very small number of *Platanista* tissue samples collected from dead or live stranded animals is available worldwide. It is not possible to biopsy live dolphins to obtain additional samples because South Asian river dolphin's surface unpredictably and very rapidly, and they do not approach boats. Therefore, to provide sufficient samples for

this study, ancient DNA was extracted from *Platanista* skeletal specimens stored in museums.

5.2 METHODS

5.2.1 Sample Origin

Bone and/or dried tissue was collected from a total of 63 *Platanista* museum specimens, comprising 37 Indus River dolphins, 18 Ganges River dolphins, and 8 of unknown origin (see Table 5.1 for details of year, location and museum). The museum specimens were dated from two time periods, either the 1800s, or the 1970s, therefore DNA was either approximately 30 years old (43 samples), or was between 110 and 160 years old (15 samples). The five samples for which there was no information on collection date are likely to be pre-1900 because newer specimens tend to have more associated information recorded. Prior to the independence and partition of the South Asian subcontinent in 1947 the Indus and Ganges River dolphins both occurred in British India. Museum specimens collected prior to 1947, with an origin described only as India could therefore have originated from any of the Subcontinent's rivers and these samples are classified as of unknown provenance. All Indus River specimens, except one, originated from the Guddu-Sukkur dolphin subpopulation (no. 4) in Sindh. Ganges River dolphin specimens originated from Assam in north-east India, the Calcutta area, or the Ganges mainstem (Fig. 5.1).

5.2.2 Sample Collection

Prior to sampling, the work-area and tools were cleaned with 70% bleach to remove contaminating particles. Generally, bone was collected from skulls, however, if other bones were also present, I sampled these preferentially to avoid damaging the skull. A cordless electric Dremel drill and 3mm bit was used to extract bone powder from the densest part of the bone. The drill was operated at low speed to minimize heat production which further degrades DNA. Bone powder was collected on aluminium foil and then double-bagged in sealable plastic bags. A new drill bit was used on each specimen to prevent cross-contamination. Some skulls had patches of attached dried tissue, and many of the animals stored at the Stuttgart museum included entire pectoral flippers preserved in salt. If present, I also collected dried tissue using a sterile scalpel, and the sample was then double-bagged and labelled.

The majority of samples were collected from the UK and Europe and therefore did not require CITES permits for importation to the UK for analysis. Sample 1-PM (Table 5.1) was imported to the UK from Pakistan under CITES export permit (P.05/2008), CITES import permit (307866/02) and DEFRA Animal Health Permit (POAO/2008/360) (Appendix IV).

Ancient DNA extraction, amplification and sequencing poses special problems, particularly over contamination because the target DNA is in very small quantities and usually fragmented. For this study, the laboratory work, specifically the extraction, amplification and sequencing, was outsourced to the Ancient DNA Laboratory at the University of Durham. This unit has specialist facilities for extraction of ancient DNA (aDNA) including procedures for minimizing contamination with non-target DNA, and experienced personnel. I was responsible for determining the objectives and scope of the study, for locating, collecting, and transporting all samples, and finally for analysing the sequences, interpreting the results and creating this final report. The laboratory protocols are provided in Appendix V.

Table 5.1 – Platanista samples collected for this study with details of the year and location of origin, museum and record number.

Study ID	Sub-species	Tissue	Country of Origin	Location of Origin	Year Collected	Museum/ Source	Museum No.
1-PM	minor	bone	Pakistan	Punjab	1970s?	Pakistan MNH	-
18-PM	minor	dried skin & bone	Pakistan	Begari, Sindh	1978	Stuttgart SMN	42497
19-PM	minor	dried skin & bone	Pakistan	Begari, Sindh	1978	Stuttgart SMN	42498
20-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	Nov-1969	Stuttgart SMN	45631
21-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	Nov-1969	Stuttgart SMN	45632
22-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	Nov-1969	Stuttgart SMN	45633
23-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	Nov-1969	Stuttgart SMN	45634
24-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	Nov-1969	Stuttgart SMN	45635
25-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	Nov-1969	Stuttgart SMN	45636
26-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	45637
27-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	45638
28-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1974	Stuttgart SMN	45639
29-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1974	Stuttgart SMN	45640
30-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1976	Stuttgart SMN	45641
31-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1976	Stuttgart SMN	45642
32-PM	minor	dried skin & bone	Pakistan	Chak, Guddu-Sukkur	1976	Stuttgart SMN	45643
33-PM	minor	dried skin & bone	Pakistan	Guddu-Sukkur	Apr-1977	Stuttgart SMN	45644
34-PM	minor	dried skin & bone	Pakistan	Guddu-Sukkur	Apr-1977	Stuttgart SMN	45645
35-PM	minor	dried skin & bone	Pakistan	Guddu-Sukkur	Apr-1977	Stuttgart SMN	45646
36-PM	minor	dried skin & bone	Pakistan	Guddu-Sukkur	Apr-1977	Stuttgart SMN	45647
37-PM	minor	dried skin & bone	Pakistan	Tappu Island, Sukkur	1969	Stuttgart SMN	46802
38-PM	minor	bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	46833

Study ID	Sub-species	Tissue	Country of Origin	Location of Origin	Year Collected	Museum/ Source	Museum No.
39-PM	minor	bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	46834
40-PM	minor	bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	46835
41-PM	minor	bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	46836
42-PM	minor	bone	Pakistan	Chak, Guddu-Sukkur	1972	Stuttgart SMN	46837
43-PM	minor	bone	Pakistan	Chak, Guddu-Sukkur	1957	Stuttgart SMN	46844
49-PM	minor	bone	Pakistan	Guddu-Sukkur	1969 or 1970	NM Scotland	1991.43.4
50-PM	minor	bone	Pakistan	Guddu-Sukkur	1969 or 1970	NM Scotland	1991.43.5
51-PM	minor	bone	Pakistan	Guddu-Sukkur	1969 or 1970	NM Scotland	1991.43.6
52-PM	minor	bone	Pakistan	Guddu-Sukkur	1969 or 1970	NM Scotland	1991.43.7
53-PM	minor	bone	Pakistan	Guddu-Sukkur	1969 or 1970	NM Scotland	1991.43.8
54-PM	minor	bone	Pakistan	Guddu-Sukkur	1969 or 1970	NM Scotland	1991.43.9
55-PM	minor	bone	Pakistan	Guddu-Sukkur	1975	NM Scotland	1991.43.2
56-PM	minor	bone	Pakistan	Guddu-Sukkur	1975	NM Scotland	1991.43.3
57-PM	minor	bone	Pakistan	Guddu-Sukkur	1975	NM Scotland	1991.43.1
71-PM	minor	bone	Pakistan	Indus River	late 1800s	NHM, London	1874.4.13.4
10-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45648
11-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45649
12-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45650
13-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45651
14-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45652
15-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45653
16-PG	gangetica	dried skin & bone	India	Gela bil, Assam	Dec-1969	Stuttgart SMN	45654
17-PG	gangetica	dried skin & bone	India	Ganges River	N/K	Stuttgart SMN	26397
44-PG	gangetica	bone	India	Hooghly River	1866	NM Scotland	1948.53

Study ID	Sub-species	Tissue	Country of Origin	Location of Origin	Year Collected	Museum/ Source	Museum No.
45-PG	gangetica	bone	India	Hooghly River	1860s	NM Scotland	1991.44.1
46-PG	gangetica	bone	India	Hooghly River	1860s	NM Scotland	1991.44.2
47-PG	gangetica	bone	India	Uttar Pradesh	1880	NM Scotland	1991.44.4
65-PG	gangetica	bone	India	River Hooghly, Calcutta	28-Dec-1865	NHM, London	1874.6.1.1
66-PG	gangetica	dried tissue & bone	India	Calcutta	late 1800s	NHM, London	1884.3.29.1
67-PG	gangetica	dried tissue & bone	India	Benares, Ganges River	13-Dec-1895	NHM, London	1895.5.20.2
68-PG	gangetica	bone	India	Ganges River	1843	NHM, London	1843.8.18.5
69-PG	gangetica	bone	India	Benares, Ganges River	N/K	NHM, London	GERM 334A
70-PG	gangetica	dried tissue & bone	India	River Jumna, Kiola near Mattra	14-Apr-1897	NHM, London	1897.6.30.1
48-U	unknown	bone	N/K	N/K	N/K	NM Scotland	1981.57.510
58-U	unknown	bone	N/K	N/K	N/K	NM Scotland	1991.49
59-U	unknown	bone	N/K	N/K	N/K	NM Scotland	-
60-U	unknown	bone	N/K	N/K	1800s?	University of St. Andrews	1
61-U	unknown	bone	N/K	N/K	1800s?	University of St. Andrews	2
62-U	unknown	dried tissue & tooth	N/K	N/K	Late 1800s	Cambridge, UMZ	C.64.B
63-U	unknown	dried tissue & tooth	N/K	N/K	1881	Cambridge, UMZ	C.62.A
64-U	unknown	dried tissue	N/K	N/K	Late 1800s	Cambridge, UMZ	C.63.A

Museums: Staatliches Museum für Naturkunde Stuttgart, Bell-Pettigrew Museum of Natural History University of St. Andrews, Cambridge University Museum of Zoology, National Museums of Scotland (Edinburgh), Pakistan Natural History Museum, N/K = not known

5.2.3 Genetic Diversity

A 458 bp section of the mtDNA control region was successfully extracted and sequenced from 29 samples (24 Indus and 5 Ganges). These sequences were then combined with an additional 14 sequences with this section of the mtDNA control region available on GenBank (Table 5.2), comprising 1 Indus River dolphin (GenBank Accession Number: AJ554058) (Arnason et al. 2004) and 13 Ganges River dolphins (GenBank Accession Number: AY102527-39) (Verma et al. 2004) to give a total sample of 43 (25 Indus and 18 Ganges). Sequences were aligned using the software ClustalX 2.1 (Larkin et al. 2007), were compared by eye to ensure optimal alignment, and then trimmed to a 458 bp continuous section using software MEGA version 5.05 (Tamura et al. 2011).

Table 5.2 – *Platanista* sp. complete mtDNA control region sequences from GenBank used in this study.

Accession Number	***Subspecies***	***River***	***Location***	***Reference***
AJ554058	minor	Indus	Not Known	(Arnason et al. 2004)
AY102527	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102528	gangetica	Ganges	Kaptai Lake, Karnaphuli River, Bangladesh	(Verma et al. 2004)
AY102529	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102530	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102531	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102532	gangetica	Ganges	Kaptai Lake, Karnaphuli River, Bangladesh	(Verma et al. 2004)
AY102533	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102534	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102535	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102536	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)
AY102537	gangetica	Ganges	Ganges River, Patna, Bihar	(Verma et al. 2004)

The number of haplotypes, haplotype (*h*) and nucleotide diversity (π) (Nei 1987), and the number and type of single nucleotide polymorphisms were assessed using software ARLEQUIN ver 3.5 (Excoffier and Lischer 2010). To infer relationships among the haplotypes a haplotype network was constructed using a median-joining algorithm

implemented in the software NETWORK version 4.6 (Bandelt et al. 1999). Selective neutrality was examined for each population using Fu's F (Fu 1997) and Tajima's D (Tajima 1989) tests as implemented in ARLEQUIN.

Nineteen mtDNA cytochrome b sequences for *Platanista* sp. are available on GenBank, and four Indus River dolphin sequences, three of which were extracted from samples stored in formalin and are of unknown reliability, were obtained as unpublished data from a PhD thesis (Table 5.3). The same methods and analyses of genetic diversity used for the mtDNA control region were conducted on these samples to provide an additional gene for comparison. Due to the small number of Indus river dolphin sequences long enough to include in the analysis (n=2), haplotypic and molecular diversity were examined in these samples, but no further analyses were conducted on the cytochrome b data.

Table 5.3 – *Platanista* sp. Cytochrome b sequences used in the current study

Accession Number	***Subspecies***	***Location***	***Bp's***	***Reference***
AJ554058	minor	Not Known	1140	(Arnason et al. 2004)
Gachal-7	minor	Sindh	814	(Gachal 2001)
Gachal-6	minor	Sindh	425	(Gachal 2001)
Gachal-5	minor	Sindh	412	(Gachal 2001)
Gachal-2	minor	Sindh	404	(Gachal 2001)
AY102512	gangetica	Kurzi, Patna, Ganges River	786	(Verma et al. 2004)
AY102513	gangetica	Digha, Patna, Ganges River	786	(Verma et al. 2004)
AY102514	gangetica	Durja, Patna, Ganges River	786	(Verma et al. 2004)
AY102515	gangetica	Patna, Ganges River	786	(Verma et al. 2004)
AY102516	gangetica	Patna, Ganges River	786	(Verma et al. 2004)
AY102517	gangetica	Durja, Patna, Ganges River	786	(Verma et al. 2004)
AY102518	gangetica	Balughat, Patna, Ganges River	786	(Verma et al. 2004)
AY102519	gangetica	Patna, Ganges River	786	(Verma et al. 2004)
AY102520	gangetica	Kasmar, Pahleza, Patna, Ganges River	786	(Verma et al. 2004)
AY102521	gangetica	Ghagha Ghat, Patna, Ganges River	786	(Verma et al. 2004)
AY102522	gangetica	Bangladesh	786	(Verma et al. 2004)

AY102523	gangetica	Durja, Patna, Ganges River	786	(Verma et al. 2004)
AY102524	gangetica	Digha, Patna, Ganges River	786	(Verma et al. 2004)
AY102525	gangetica	Pahleza ghat, Patna, Ganges River	786	(Verma et al. 2004)
AY102526	gangetica	Bangladesh	786	(Verma et al. 2004)
AF158376	gangetica	Dhaka, Bangladesh	1140	(Yang et al. 2002)
AF334483	gangetica	Stored in National Science Museum Tokyo, Japan, probably originated from Bangladesh.	683	(Hamilton et al. 2001)
AF304070	gangetica	100km upstream Patna, Ganges River, India	1140	(Cassens et al. 2000)

5.2.4 Population Differentiation

The pairwise comparison of genetic differentiation between Indus and Ganges River dolphins was evaluated using F_{ST} scores (Wright 1965) generated in ARLEQUIN 3.5 and 1000 permutations were then used to create p-values. High F_{ST} scores close to one, indicate that there is large genetic divergence between populations and is also referred to as the fixation index. Nei's pairwise distances were compared between and within populations (Nei and Li 1979), and an exact test of population differentiation was performed with 10,000 Markov chain steps (Raymond and Rousset 1995). Gene flow between the two populations was not estimated because they are separated by geographic barriers, and it is therefore almost certainly zero.

5.2.5 Phylogeographic Patterns

Phylogenetic trees were constructed using Neighbour-Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods in the programme MEGA 5.05. The four haplotypes identified from the complete *Platanista* MtDNA control region were used to create the trees, and 4 allied species used as out groups: Baird's beaked whale (*Berardius bairdii*) NC_005274 (Arnason et al. 2004), northern bottlenose whale (*Hyperoodon ampullatus*) NC_005273 (Arnason et al. 2004), dwarf sperm whale (*Kogia sima*) NC_005272 (Arnason et al. 2004), and sperm whale (*Physeter macrocephalus*) NC_002503 (Arnason et al. 2000). If the average pairwise Jukes-Cantor distance is >1 the data are not suitable for creating Neighbour-Joining trees (Hall 2008; Nei and

Kumar 2000). The Jukes-Cantor distance was considerably less than 1, and construction of a Neighbour-Joining tree was considered appropriate for this data set (Hall 2008).

The model of nucleotide substitution was tested in the software jModelTest version 0.1 (Posada 2008), which compared 88 possible substitution models including those with equal or unequal base frequencies, fixed or variable mutation rates, and the proportion of invariable sites. The Hasegawa-Kishino-Yano (HKY) model of nucleotide substitution (Hasegawa et al. 1985) was selected by jModelTest; this represents variable base frequencies and different rates of transition compared to transversion. A Maximum Likelihood topology was optimized using each model and they were then compared using Akaike's Information Criterion (AIC). The model with the lowest AIC score was used to create the ML phylogenetic tree.

The settings for creation of the phylogenetic trees were as follows:

1. NJ-The evolutionary distances were computed using the Maximum Composite Likelihood method, a uniform mutation rate among sites was applied, and a homogenous substitution pattern among lineages was assumed. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option).
2. MP- The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions with less than 95% site coverage were eliminated.
3. ML- The ML tree was constructed using the HKY substitution model selected by jModeltest. Initial tree(s) for the heuristic search were obtained automatically, when the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise the BIONJ method with a MCL distance matrix was used. All positions with less than 95% site coverage were eliminated.

The 50% majority-rule bootstrap consensus trees inferred from 2000 replicates were generated for each of the above three trees and the resulting topology and bootstrap values compared.

5.2.6 Divergence Time Estimation

The time of divergence between lineages was estimated using a strict molecular clock and an HKY model of substitution in the Bayesian phylogenetic software BEAST version 1.6.1 (Drummond and Rambaut 2007). One complete mtDNA control region sequence was included from each putative South Asian river dolphin species, and a sperm whale (*Physeter macrocephalus*) sequence used as the out group (GenBank: NC002503). Substitution rate variation among sites was estimated by the model. Coalescent tree priors are most suitable for describing the relationships between individuals in the same population/species whereas a Yule tree prior is most appropriate for between-species comparisons (Drummond et al. 2007). Given that I am exploring whether there are potentially species-level differences between two populations, either tree prior could be deemed appropriate, and I therefore compared the results generated when using each prior. Divergence times can be calibrated either by specifying a substitution rate, or by calibrating one of the internal nodes of the tree based on fossil evidence or other information.

The Indus and Ganges River dolphins are the only surviving representatives of a once diverse superfamily Platanistoidae. The long-beaked dolphins *Zarhachis* and *Pomatodelphis,* are likely to be the closest extinct relatives of *Platanista*, and are known from late Early Miocene sediment approximately 16 MY ago in the north and south Atlantic coast of North America, and from Europe (Barnes et al. 1985; Bohaska 1998; de Muizon 1987, 2002). Platanistids split from other cetaceans very early, the affinity to or with earlier fossils is not clear, and the fossil record is patchy. For this reason, I did not use fossil dates as calibration for the divergence date between the two South Asian river dolphins, and instead used the divergence time between the *Platanistidae* and a clade including *Ziphiidae* (Clade G: 28.77 MY ago, log-normal SD=0.08), that was estimated with high confidence (1.0 95% highest posterior density (HPD)) in a time-calibrated phylogeny of whales produced by Xiong et al. (2009). The *Ziphiidae* (AJ554056) and *Platanista* (AJ55408) sequences used by Xiong et al. in their phylogeny were also used to calibrate the current model. The models were run for 50,000,000 Markov Chain Monte Carlo (MCMC) steps, and were sampled every 1000 steps which was sufficient to ensure convergence on a stationary distribution for each parameter. Visual inspection of traces in Tracer version 1.5 (Rambaut and Drummond 2007) supported the removal of the first 10% of the MCMC chains as burn-in. Convergence statistics were monitored by effective samples sizes (ESS). Resulting

phylogenetic trees were created using the software TreeAnnotator v1.6.1 and plotted using Fig Tree version 1.3.1 to visually check the model outputs.

5.3 RESULTS

DNA was successfully extracted from 46.0% of the samples which is typical for ancient samples. There was a higher success rate for Indus River dolphin samples (64.9% success rate), than for the Ganges River dolphin (27.8% success rate). Despite numerous attempts, no usable DNA was extracted from any specimen collected in the 19th century. Most of the older samples were Ganges River dolphins, which explains the differences in extraction success between the two populations.

5.3.1 Molecular Diversity

5.3.1.1 Partial mtDNA Control Region Sequences

Of the 43 sequences obtained, seven were incomplete (2 missing 288 bp, 1 missing 195 bp, and 4 missing 155 bp). Incomplete sequences were removed from the analysis leaving 36, comprising 20 from the Indus and 16 from the Ganges River. Nucleotide frequencies in the Indus River samples were: Cytosine=24.45%, Thymine=32.75%, Adenosine=22.27%, and Guanine=20.52%. Nucleotide frequencies in the Ganges River dolphin samples were almost identical to the Indus, but the amount of Cytosine was slightly higher (24.51%) and Thymine was slightly lower (32.70%).

All 20 partial control region sequences from the Indus River dolphin were the same haplotype (HAP-1) with no polymorphic sites, nucleotide or gene diversity (Table 5.4 & Table 5.5). Within the Ganges River dolphin sample (n=16), there were two haplotypes, separated by a single transition, the only polymorphic site. HAP-2 was present in 75% of individuals and HAP-3 in the remaining 25% (Table 5.5). Based on the partial sequences, there were two fixed transitional differences between Indus and Ganges River dolphins and no shared haplotypes. Although higher than the Indus River dolphin, Ganges River dolphin nucleotide and gene diversity were both very low (Table 5.4). Fu's F test (F=0.872, p=0.539) and Tajima's D test (D=0.650, p=0.846) returned no significant results, supporting the hypothesis of selective neutrality in the Ganges River dolphin. The analysis showed very low genetic variability and revealed only three

haplotypes, so to investigate whether the analysed 458 bp portion of the control region was more highly conserved than other parts of the control region, the above analyses were repeated on the 14 samples from GenBank for which the entire 858 bp control region had been sequenced and the results compared.

5.3.1.2 Complete mtDNA Control Region Sequences

Examination of the entire 858 bp control region provided double the number of MtDNA base pairs for analysis, but contributed only 1 additional haplotype (HAP-4), and similar molecular diversity indices to the partial sequences for each putative species. One additional Ganges River dolphin haplotype (HAP-4) was identified that differed from HAP-2 by a single transition and from HAP-3 by two transitions. In the entire mtDNA control region, there were only two polymorphic sites (0.23% variable sites) in the Ganges River dolphin samples. Haplotype 2 was shared by animals that originated from the Ganges River at Patna, and also by two specimens collected from the Karnaphuli River system approximately 1000km away in eastern Bangladesh.

When comparing genetic differences between the two geographically separated populations considerably more fixed differences were discovered when examining the longer section of mtDNA. Although the sample size was small and included only a single sample from the Indus, 8 variable loci, and 6 fixed differences between Indus and Ganges River dolphin samples were present, comprising 3 transitions, 1 transversion, and 2 insertion-deletions (Table 5.5). 75% of the genetic variation within the Platanistidae family is accounted for by fixed differences between Indus and Ganges River dolphins. The median joining haplotype network from the samples is very simple, and reflects the low variability and few haplotypes found within each population (Fig. 5.2). It also clearly demonstrates the substantially greater genetic distance between the Indus and Ganges River dolphin populations than those recorded within each population.

Table 5.4 – Nucleotide and haplotype diversity in MtDNA control region samples from the Indus and Ganges-Brahmaputra rivers. Partial sequences, Indus: n=20, Ganges: n=16. Complete control region sequences, Indus: n=1, Ganges: n=13.

	Nucleotide Diversity		***Haplotype Diversity***	
	458bp control region portion	Entire 858bp control region	458bp control region portion	Entire 858bp control region
Indus dolphin	0.00 ±0.00	N/A[1]	0.00 ± 0.00	N/A[1]
Ganges dolphin	0.0009 ±0.0010	0.0012 ±0.0009	0.400 ± 0.114	0.641 ± 0.097
Both Combined	0.0027 ±0.0019	0.0019 ±0.0014	0.584 ± 0.054	0.692 ± 0.093

[1]Not calculated because there was only 1 Indus sample with the entire control region sequenced.

Table 5.5- Haplotypes identified in a 458bp portion (shaded grey), and in the entire (858bp) Mitochondrial control region in two geographically isolated populations of *Platanista*. The position in the sequence where the transition occurred is numbered in the header. Partial control region sample size n=36: comprising n=20 Indus, n=16 Ganges, the entire control region was obtained from sequences available on GenBank n=14: comprising n=1 Indus, n=13 Ganges. Frequency refers to haplotype frequency recorded in the complete control region sequences.

Position	***41***	***71***	***123***	***140***	***297***	***418***	***633***	***704***	***Population***	***Frequency***
Hap-1	**A**	C	**Indel**	**T**	**C**	**T**	T	**C**	Indus	100%
Hap-2	G	C	C	A	T	C	T	Indel	Ganges	53.9%
Hap-3	G	T	C	A	T	C	C	Indel	Ganges	30.8%
Hap-4	G	T	C	A	T	C	T	Indel	Ganges	15.4%

Nucleotides in bold represent fixed differences between the Indus and Ganges River dolphins.

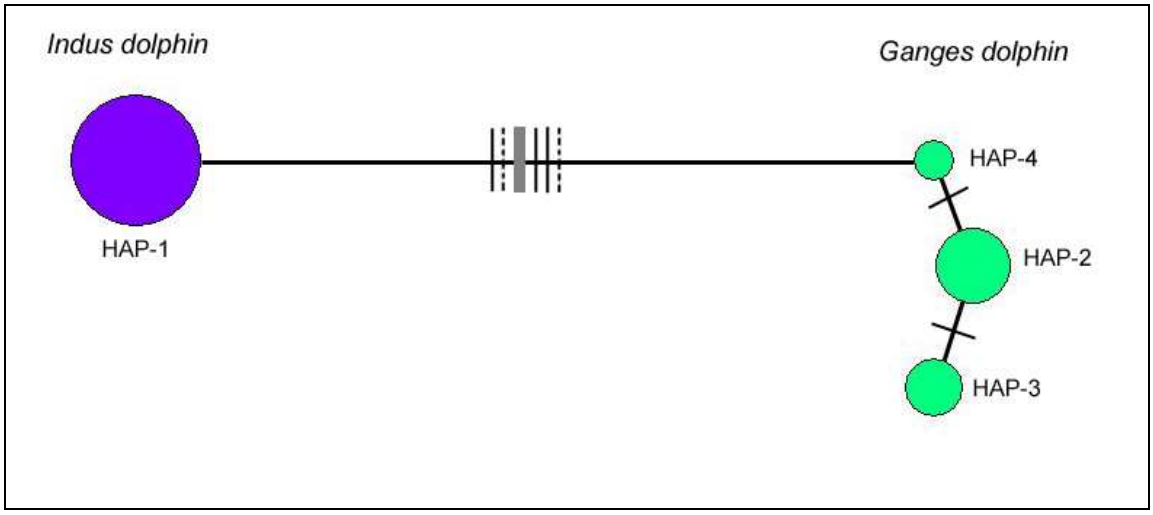

Figure 5.2. Median-joining network based on complete MtDNA control region haplotypes for the cetacean Family *Platanista*. Circle size is proportional to the number of individuals representing that haplotype and branch lengths are approximately proportional to the number of mutations. Transitions are represented by thin bars perpendicular to each branch, transversions by thick grey bars, and insertion-deletions by dotted bars.

5.3.1.3 Cytochrome b Sequences

The cytochrome b sequences were of a variety of lengths, and only a 151bp portion (position 274 to 425) was present in all 23 sequences, however this portion was of limited interest as it showed no variability. I therefore trimmed the sequences to a 541bp portion that was present in 20 of the 23 samples, by removing the 3 short Indus River dolphin sequences Gachal-2, Gachal-5 and Gachal-6 (Table 5.3).

Gene diversity and nucleotide diversity for both populations combined was 0.6474 ± 0.0720, and 0.0018 ± 0.0014, respectively, and for the Ganges River dolphin samples was 0.601 ± 0.08, and 0.0017 ± 0.0014. These diversity indices are low, and very similar to values recorded in the control region. As there were only two Indus River dolphin samples, diversity indices were not calculated, however two haplotypes were present and the genetic uniformity found in the control region was not seen in the cytochrome b gene. Although sample size was limited, similar to the control region, the Ganges River dolphin cytochrome b sequences were more diverse than the Indus River dolphin sequences.

There were four haplotypes in all the cytochrome b sequences, one unique to the Indus, two unique to the Ganges and one shared by both (Fig. 5.3). The shared haplotype was found in four individuals from Patna, Bihar, two from rivers in Bangladesh, and from one of the unpublished Indus River dolphin sequences (see Table 5.6). No differences were fixed and all haplotypes were separated by a single transition. Haplotype-3 was the most common, represented in 61% (11 out of 18) of the Ganges River dolphin specimens.

Table 5.6 – Haplotypes and variable sites in a 541bp portion of the cytochrome b gene from20 *Platanista* sequences available on GenBank or as unpublished data.

Position	***580***	***640***	***699***	***Provenance***	***No. of specimens***	***Samples***
HAP-1	G	A	C	Indus, Pakistan	1	(Arnason et al. 2004)
HAP-2	G	G	C	Indus, Pakistan	1	(Gachal 2001)
				Ganges, India	4	(Verma et al. 2004) (21-26)
				Bangladesh	2	(Verma et al. 2004)
HAP-3	A	G	C	Ganges, India	9	(Verma et al. 2004) (12-20)
					1	(Cassens et al. 2000)
					1	(Hamilton et al. 2001)
HAP-4	G	A	T	Unknown location in Bangladesh	1	(Yang et al. 2002)

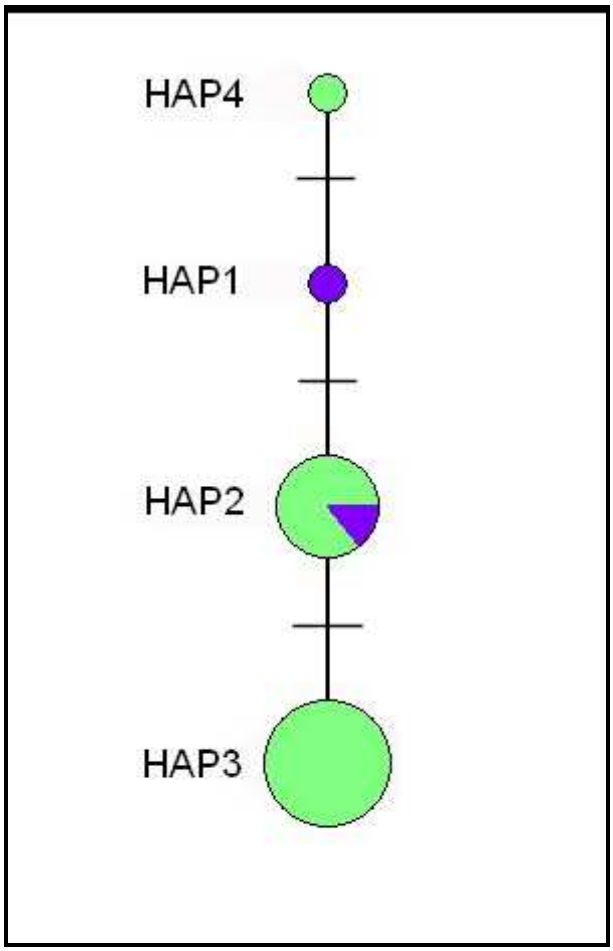

Figure 5.3. Median-joining network based on partial cytochrome b sequence haplotypes for the cetacean Family *Platanista*. Circle size is proportional to the number of individuals representing that haplotype (circles for HAP-1, and HAP-4 represent single individuals) and branch lengths are approximately proportional to the number of mutations. Transitions are represented by thin bars perpendicular to each branch. Green = Ganges River dolphin, Purple = Indus River dolphin.

5.3.2 Population Differentiation

The F_{ST} scores between Indus and Ganges River dolphins were very high, 0.921 ($p<0.0001$) when the partial sequences were considered, and 0.853 ($p=0.06$) when the full control region was tested. High F_{ST} scores reflect strong genetic differentiation between the Indus and Ganges River dolphins and indicate long-term low to non-existent gene flow between them. This indicates that following their subdivision, subsequent genetic drift to fixation has caused a loss of 85-92% of their heterozygosity. The average number of pairwise differences between populations (partial control region: 2.25 or 0.5% of base pairs, complete control region: 6.78 or 0.8% of base

pairs), was highly significantly different (p<0.0001), because the distance between populations was more than 5 times greater than that observed within each population (Ganges = 0.4 (0.00089% of base pairs); Indus = 0 (0% of base pairs). The exact test of differentiation between the two samples was highly significant (p<0.0001).

5.3.2.1 Phylogenetic Analysis

The NJ, MP and ML methods, all resulted in phylogenetic trees in structural agreement that illustrated a clear, well supported, reciprocally monophyletic separation between the Indus and Ganges River dolphins. The separation was supported in 100% of the bootstrap replicates in the ML and MP trees and in 99% of NJ trees (Fig. 5.4).

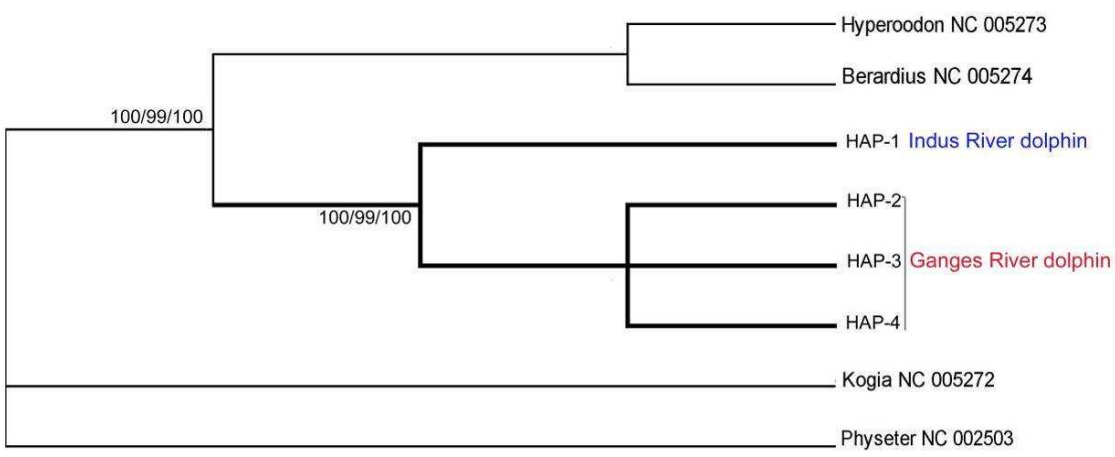

Figure 5.4. Maximum likelihood bootstrap consensus tree representing the evolutionary history of *Platanista*. The HKY evolution model, and 2000 bootstrap replicates were applied. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. Trees constructed using the neighbour-joining, maximum parsimony and maximum likelihood methods resulted in the same topology. The percentage of trees in which the associated taxa clustered together using each method, are listed next to the branches in the order: MP / NJ / ML.

5.3.3 Divergence Time

The lack of shared control region haplotypes and large number of fixed differences between the Indus and Ganges River dolphins suggests that they have been isolated

for a considerable period. This is supported by the Bayesian modelling of the divergence time between the two which estimated that they have been separated for 0.658 million years (0.174-1.200 million years 95%HPD) when using the Yule speciation prior, and for 0.660 million years (0.180-1.207 95%HPD) when the coalescent prior was used.

5.4 DISCUSSION

5.4.1 Lack of Variability

A striking result of this study is the very low amount of genetic variation in the Platanistidae Family. Only three haplotypes were identified in a 458 bp portion of mtDNA control region (n=36), and four haplotypes in a 541 bp portion of cytochrome b (n=20). The Indus River dolphin control region sequences were all the same haplotype and the Ganges River dolphin sequences were also highly conserved. The mitochondrial control region is generally considered to be the most variable part of the mammalian genome, and the total absence of variability found in the control region of Indus River dolphins is unusual. Amongst the cetaceans, control region homogeneity has only previous been recorded in the vaquita (*Phocoena sinus*) (Rosel and Rojas-Bracho 1999) and Maui's dolphin (*Cephalorhynchus hectori maui*) (Pichler and Baker 2000), both critically endangered species/subspecies with restricted ranges and small populations declining due to human activities. The low diversity in Maui's dolphin was attributed to recent population depletion caused by human activities (Pichler and Baker 2000), and genetic homogeneity in the vaquita was demonstrated to be due to historically low population size and a founder effect in its origin (Taylor and Rojas-Bracho 1999). In general, rare species contain less genetic variability than more common species (Nei et al. 1975). In addition, species with restricted geographic distributions will be exposed to a limited number of environments and are frequently less genetically diverse than those with wider distributions (Allendorf and Luikart 2007). The low genetic variability within the *Platanista* genus may be because they have a restricted habitat and are naturally not abundant. Another possibility is that historical climatic changes, such as a weakening monsoon, changes in global temperature, or fluctuating weather patterns impacted both rivers simultaneously, reducing the river dolphin population size and causing a concomitant loss of genetic variability from which the populations have yet to recover.

The mtDNA control region homogeneity in the Indus River dolphin indicates a population bottleneck that could be either recent or ancient in origin. The Indus River dolphin population is believed to have declined to perhaps just a few hundred individuals in the 1960s and early 1970s due to hunting pressure (Pilleri and Pilleri 1979; Pilleri and Zbinden 1973-74). All of the samples analysed in this study originated from a single dolphin subpopulation (4 Guddu-Sukkur) in the late 1960s and 1970s and the genetic homogeneity may therefore reflect the population bottleneck being experienced at that time. An alternate hypothesis is that the population bottleneck is due to a founder event associated with the origin of the Indus River dolphin population (Nei et al. 1975). While the Indus and Ganges River dolphins originated from a common ancestor it is not clear which habitat that ancestor occupied, it could, for example, have inhabited only one of the river systems (e.g. the Ganges) and dispersed much later to the other (e.g. the Indus) when one of the lowland tributaries was captured. Given the nature of river capture, a founder event would likely occur rapidly allowing only a few individuals to disperse, and would therefore entail a bottleneck in population size in the new population (Carson and Templeton 1984).

There is a perceived link between a lack of genetic variation and an increased risk of extinction for small populations (Gilpin and Soule 1986). Inbreeding depression is often manifested as reduced fertility and/or poor juvenile survival in other mammals (Ralls et al. 1988) and the potential importance of low genetic variability for increasing vulnerability of a species to infectious disease is also often cited (Allendorf and Luikart 2007). However, a lack of mitochondrial variability for 50,000 years in the Iberian lynx shows that low genetic variability alone does not always threaten species persistence (Rodríguez et al. 2011). At present there is no evidence of reduced reproductive success in the Indus River dolphin, since in 2006 the abundance of the largest dolphin subpopulation was reported to be increasing (Chapter 2). Although there is still some cause for concern, at present the lack of mitochondrial variability is likely not the most pressing conservation issue facing the South Asian river dolphins, as they are threatened by several other clear and immediate threats including incidental mortality in fisheries and habitat loss due to water diversion (Smith and Braulik 2008b).

5.4.2 Divergence and Speciation

The objectives of taxonomic studies are to demonstrate irreversible divergence between groups (Reeves et al. 2004). The Biological Species Concept (Mayr 1942) involves identifying whether reproductive isolation mechanisms have evolved between the putative species, however testing the ability of populations to interbreed is almost impossible in allopatric species such as South Asian river dolphins, and therefore reproductive isolation must be inferred by demonstrating divergence in multiple lines of evidence, ideally including both morphological data (e.g. external morphology, skeletal morphology and colouration) and genetic data from multiple loci (e.g. both mitochondrial and nuclear DNA) (Allendorf and Luikart 2007). These primary lines of evidence can be supported by information on geographical ranges and behaviour (Allendorf and Luikart 2007).

5.4.2.1 Reciprocal Monophyly

The most commonly applied criteria used to define phylogenetic species, or evolutionary significant units (ESU), is the necessity of reciprocal monophyly for mtDNA alleles (Moritz 1994). Reciprocal monophyly means that all DNA lineages must share a more recent common ancestor with each other than with lineages from other ESUs. The strength of this approach is that it avoids the issue of ‘how much divergence is enough?’ that plagues quantitative criteria such as allele frequency divergence and genetic distance, and it considers the pattern rather than the extent of sequence divergence (Moritz 1994). There are arguments that the neutral gene monophyly requirement is too conservative a criterion of evolutionary distinctness for the purposes of taxonomy or conservation even when applied to mtDNA (Moritz 1994, Wang et al. 2008). Wang (2008) demonstrated that recent speciation events will not be detected using the requirement of reciprocal monophyly due to the lack of fixed molecular differences between recently derived species. In the example of the recently split species of finless porpoises, *Neophocaena phocaenoides* and *Neophocaena asiaeorientalis*, speciation was relatively recent (~18,000 years ago), reciprocal monophyly was not yet present, and there were still some shared haplotypes between species due to their common ancestry. By comparison, the genetic separation of the Indus and Ganges River dolphins is much less equivocal; their separation was sufficiently long ago for many fixed differences to have evolved, for there to be no shared haplotypes in the control region, and for reciprocal monophyly to be clearly present.

When considering the entire control region, the majority of segregating sites between Indus and Ganges River dolphins were fixed (6) (including fixed insertion/deletions and transversions that are much rarer than transitions), and the number of shared polymorphisms (2) were few, clear evidence of a long-term absence of gene flow and that the length of time since separation is considerable (Wakeley 2000). However, the time to fixation and reciprocal monophyly is dependent upon population size and generation time; small populations such as the Indus and Ganges River dolphins will become reciprocally monophyletic much more rapidly than those that are large and may also speciation more rapidly (Mayr 1963; Nei et al. 1983).

The cytochrome b and control region data are in agreement, and since the control region evolves more rapidly than cytochrome b (Alter and Palumbi 2009), the greater inter-population differences observed in the control region were anticipated. The effective number of mitochondrial mtDNA genes is one quarter that of nuclear genes (only a single copy per individual, and offspring receive only the mtDNA from the mother), mtDNA sequences have a much shorter time to fixation and reciprocal monophyly than do those of nuclear genes, and the loss of mtDNA variability during population bottlenecks is relatively more pronounced. Therefore, the lack of variability discovered in this study, might not necessarily reflect low levels of genetic heterozygosity in the nuclear genome (Birky et al. 1989).

5.4.2.2 Within Versus Between Population Molecular Differences

There are difficulties creating robust phylogenetic reconstructions of the family Delphinidae because the mtDNA control region is too variable within and not divergent enough among species to produce well supported phylogenies (Kingston et al. 2009). Within the Platanistids, the reverse situation is present where there are high interspecific and low intraspecific levels of genetic variation, which generates high bootstrap support (100%) for the resulting phylogeny. 75% of the molecular variance in the current study was due to differences between Indus and Ganges populations, and only 25% was due to differences within these groups, which is similar to the differentiation within and between the newly recognised species *Sotalia fluviatilis* and *S. guianensis* (Cunha et al. 2005).

5.4.2.3 Gene Flow

Physical isolation caused by geographic distance or barriers between populations has long been identified as the principal cause of population structuring (Mayr 1942). For the effect of population subdivision to be observable the number of breeding individuals in each population per generation originating in that population must be much greater than the number migrating in from other populations (Wakeley 2000). The Indus and Ganges River dolphins are an unusual case for cetaceans, in that there is no possibility of contemporary genetic exchange between the two populations since they occur in river systems separated by hundreds of kilometres of land at their closest point. South Asian river dolphins have never been recorded in marine waters (although they may extend for a limited distance into the ocean within the river's freshwater plume) (Anderson 1879; Smith et al. 2006) and their dispersal between river systems through the ocean would involve a highly improbable journey through saline waters, of at least 4,600 km around the Indian peninsula. The exceptionally high F_{ST} statistics (0.92 and 0.82), indicate complete genetic isolation and an absence of gene flow between populations reflecting the insurmountable physical barriers between them.

5.4.2.4 Divergence

The Indus and Ganges River dolphin populations appear to have been reproductively isolated since sharing a common ancestor approximately 0.66 MY ago. Ho et al. (2008) demonstrated that divergence estimates from molecular ecological studies can be altered by the choice of calibration points, with deeper, external calibration points, such as that used in this study, sometimes leading to overestimates of times to divergence. Although internal calibration points may be more accurate, they also have a much wider degree of uncertainty, and in this case no suitable internal calibration points were available. The node used to calibrate divergence times in the current study was the split between the Platanistidae and the Ziphiidae estimated at 28.77 MY (Xiong et al. 2009). This node has also been estimated in other phylogenetic studies as 32.43 MY (27.92–37.07) (McGowen et al. 2009), 30.50 MY ±1.3 (Arnason et al. 2004), 28.9 MY ± 4.9 (Nikaido et al. 2001), and based on the fossil record as 23 MY (Hamilton et al. 2001). To test the sensitivity of our estimate of divergence to changes in the calibration point, I re-ran the Bayesian phylogenetic analysis in BEAST using the longest (32.43 MY) and shortest (23 MY) node divergence estimates. The date of the most common *Platanista* sp. ancestor was robust to changes in the calibration point; when the highest

node estimate was used *Platanista* sp. divergence was estimated at 0.74 MY (0.20-1.35 MY), and the lowest calibration node estimate generated a divergence date of 0.52 MY (95%pp: 0.14-0.96). This is similar to a divergence time of 0.51 MY (95%pp: 0.14–1.02) estimated using Indus and Ganges River dolphin cytochrome b sequences (McGowen et al. 2009).

Detailed dates of glacial events in the Himalayas, the precise timing of river capture or of variations in the strength of the monsoon that may be linked to speciation of the Platanistidae river dolphins are lacking. This is for many reasons, including that the Himalayas are so dynamic and complex, because comprehensive studies are few, and also because the scarcity of organic material in the sediments deposited in the mountains precludes the utilization of standard radio-carbon dating techniques (Owen et al. 2002). However, studies of the Indus fan show that rates of sediment accumulation with the isotopic character of the Himalaya range, far to the east of the Karakoram range drained by the Indus River, gradually increased after approximately 5 MY ago, which could only be explained by the gradual capture of many former Ganges tributaries by the Indus system (Clift and Blusztajn 2005). It is possible that these river captures allowed Ganges River dolphins to disperse to a previously uninhabited Indus River, or that dolphins were already resident in both systems and that a river capture facilitated mixing of animals sometime around 0.6-0.7 MY ago.

On a smaller-scale than the complete drainage reorganisation proposed by Clift and Blusztajn (2005), there is potential for capture of the eastern-most Indus, and western-most Ganges tributaries because of the low drainage divide separating them on the gently shelving South Asian plains (Flam 1993; Jorgensen et al. 1993). The present Sutlej (Indus tributary) and Jamuna Rivers (Ganges tributary) are separated by less than 100 km in distance, and approximately 30m in elevation, and the Jumuna is located near the top of the drainage divide (Fig. 5.5). The Jamuna is believed to have once been an Indus tributary that was captured by the Ganges around 1000 BC (Shroder Jr. 1993a), and other authors have suggested repeated capture of the Jamuna River back and forth between drainage systems (Burbank 1992; Geddes 1960). Clift (2009) also proposed that the Harappa civilisation collapsed following capture of the Ghaggar River by the Ganges around 2000 BC.

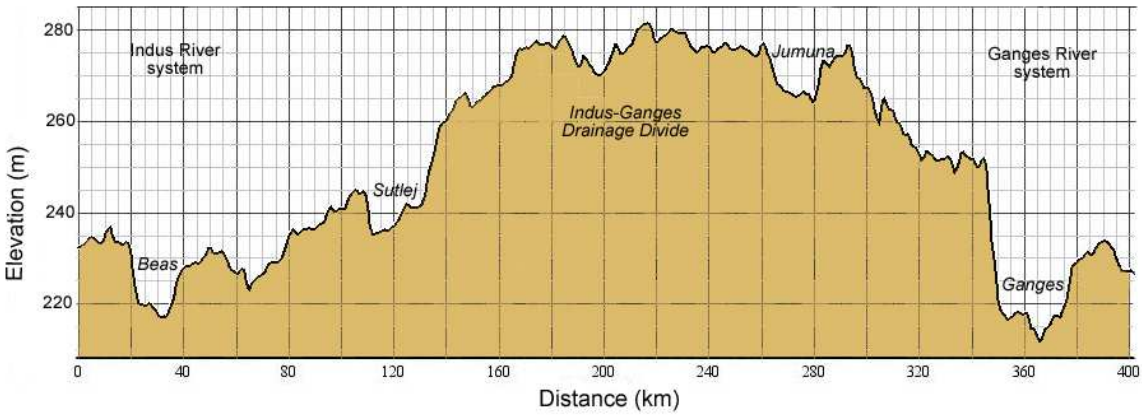

Figure 5.5 – Elevation of the Indus-Ganges River system drainage divide and of rivers near the divide (refer to Fig. 5.1 for the location of this cross-section on a plan view map of South Asia).

However, even if rivers are captured it does not necessarily mean that there were exchanges of river dolphins between systems, as capture would likely occur near the foothills of the Himalaya's where dolphins are not abundant, and are also presumably rapid events that may result in exchange of no, or only a few, individuals from one river system to another. Although there is evidence of numerous river captures on the Indo-Gangetic plain, at present no specific river capture that could explain the divergence of Indus and Ganges River dolphins around 0.6 MY ago has been discovered.

5.4.3 Conclusions

As is often the case, especially when studying endangered species, the sample size in this study was fairly small. If too few individuals are examined a haplotype may appear to be fixed because rare haplotypes may be missed (Davis and Nixon 1992). Future genetic studies of *Platanista* should maximise sample size, geographic coverage and the number of base pairs sequenced in order to present a more complete genetic picture. However, obtaining a large number of additional samples from such rare and difficult to sample animals will inevitably delay future studies by years, time that these endangered populations may not be able to afford. Although additional samples may reveal additional haplotypes or variable sites, the broad conclusions of the current study, i.e. low mitochondrial molecular diversity, well supported reciprocal monophyly in the control region and large between versus within population molecular differences, are unlikely to alter greatly with the addition of new samples.

This analysis of *Platanista* mtDNA demonstrated clear genetic distance and reciprocal monophyly between the Indus and Ganges River dolphins, however neither of these on their own are necessarily correlated with genetic isolation. There is a need to build on this work with additional genetic and morphologic studies, so that the taxonomy of the South Asian river dolphins can be satisfactorily resolved. Given the rapid decline in range of both the Indus and Ganges River dolphins in the past century and the ongoing degradation of their habitats, the conservation implications of recognition and species-level management of these distinct taxa are considerable.

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Chapter 6 – General Discussion

6.1 Thesis Synthesis

The studies conducted for this thesis were all broad-scale evaluations covering the entire current or historic range of the Indus dolphin. This opportunity is unusual in marine mammal studies, because most species have very large ranges and for practical reasons studies need to be focussed in circumscribed areas. By keeping the geographic scope of the studies as wide as possible, and using novel techniques to answer questions that are fundamental to conservation, the results are able to reveal a more complete picture of what drives the distribution and explains the decline of this endangered animal. In this chapter the scientific results, conservation implications and future research avenues are discussed.

This study was initiated to investigate the current range, distribution, abundance, habitat use, and causes of decline of the Indus River dolphin. It confirms the previously assumed range decline of approximately 80%, the extirpation of dolphins from 11 habitat fragments and continued presence of only six dolphin subpopulations. Abundance surveys indicate that probably only three of those subpopulations are large enough to be viable in the long term. The range of the Indus dolphin is fragmented and declining, however, limited survey data show no evidence that abundance is declining within the three largest dolphin subpopulations. In fact, the largest subpopulation appears to be increasing in abundance. The irrigation system and reduced dry season flows are the primary driving force behind the decline of the Indus dolphin, however an emerging threat, especially between Guddu and Sukkur barrages, is likely to be incidental mortality in fisheries (see Section 1.9.3). A lack of genetic diversity within the subspecies reflects the small population and may also be compromising their fitness or ability to adapt to a changing environment.

6.2 Abundance Estimation

One of the most striking and positive results of the research contained in this thesis, is the increase in dolphin abundance recorded between Guddu and Sukkur barrages (Chapter 2). It appears that the actions of Georgio Pilleri and the Pakistani Government in the 1970s to stop dolphin hunting were prescient and effective, and

have resulted in a gradual population recovery. Although the future of the Indus dolphin is still precarious, this is a rare conservation success story. It is instructive, that if a single, specific threat can be identified and addressed, and other threats are minimal, populations can recover quickly. The increase in abundance in this subpopulation of Indus dolphins is unparalleled among the Asian river dolphins, all of which are declining and/or threatened. Unfortunately, the threats facing many other river dolphins, are more numerous and complex than the previous situation between Guddu and Sukkur and it may be difficult to duplicate this success elsewhere.

Refining abundance estimation methods for river dolphins is a work in progress; techniques will likely become more sophisticated in time and estimates more accurate and/or precise. It is important to realise however, that while it is possible to learn from surveys of other river dolphins, because of differences in species behaviour and the types of river, a different survey approach has been selected and appears to be most appropriate in almost every circumstance. For example, surveys of Irrawaddy dolphins in the Mahakam River in Indonesia (Kreb 2002), and in the Mekong River in Cambodia have selected mark-recapture based on photo-identification as the best abundance estimation method (Beasley 2007) because these populations are schooling and individuals are identifiable. In contrast, surveys of Irrawaddy dolphins in the Ayeyarwady River of Myanmar have so far focused on direct counts because individuals there do not bear identifying marks on their bodies or dorsal fins (Smith and Tun 2007). The Yangtze and Amazon Rivers appear to be generally deeper and easier to navigate than those in South Asia and it has been possible to survey along predetermined transect lines and therefore to use line transect and/or strip transect surveys (Martin and da Silva 2004; Vidal et al. 1997; Zhao et al. 2008). South Asian river dolphins may be one of the most challenging marine mammal species to survey because of the combination of a river so shallow that it is not possible to lay out systematic transect lines and because individuals are not identifiable. Estimation of their abundance therefore requires a novel approach. The method used in this study, and also that used by Smith et al. (2006), are the first to successfully generate robust abundance estimates in these environments and therefore their success is significant. This type of double platform or tandem survey method may also be appropriate for surveying fjords, complex coastal or estuarine channels, or even narrow canyons or confined paths in terrestrial environments where line transect is not appropriate.

For Indus River dolphin abundance estimation, the area that requires the greatest focus in the future is that of calibration and correction of group size estimates. The brevity of the surface interval for South Asian river dolphins and their lack of surfacing synchronization make group size estimation difficult. The group sizes are generally small, and counts typically increase with observation period, therefore over-estimating group size is less likely than under-estimation. Two recent events have raised the definite possibility that visual river dolphin surveys dramatically underestimate group size and therefore abundance. In January 2005, a group of five dolphins were discovered trapped in a narrow irrigation canal in Sindh, Pakistan. As the canal was being drained for de-silting, numerous interested people, including some of the most experienced biologists in the country, congregated at the site to observe at close quarters the dolphin group and plan a rescue operation. Once the nets were in the water and dolphins started to be captured it became apparent that there were in fact 15 dolphins present not five as originally thought (U. Khan, pers. comm.). Similarly, in Bolivia in September 2009, a group of Amazon River dolphins, estimated to number approximately nine individuals were trapped in an isolated section of river. A programme to rescue them and move them to a safer location was enacted, but during the capture operation it was discovered that there were actually 20 individuals present (Aliaga-Rossel et al., 2011).

In Chapter 2, I suggest using a group size correction factor derived from bank-based counts, or averaging observers' independent estimates to improve group size estimates. A third option that may also be worth considered is an adaptation of cue counting. Cue counting is a method of abundance estimation that records the density of 'cues' (whale blows, dolphin surfacings etc.) in a specific area per unit time (Hiby and Hammond 1989). Effective search area is estimated by fitting a detection function to the radial distances of cues and this converted to abundance by a cue rate that is estimated independently (Buckland et al. 2001; Hammond 2010). To estimate cue rate, (in this instance surface rate), individuals must be monitored and their cue rate determined. Cue counting could be adapted for group size estimation of Indus river dolphins, so that the number of surfacings within a fixed time period in a specific location are counted, and this converted to group size based on independent studies of individual dive time (cue rate). Cue counting has been used to estimate abundance of a variety of whale species including fin, minke and humpback whales (Heide-Jørgensen and Simon 2007; Hiby 1985). Cue counting was also used by Kasuya and Nishiwaki

(1975) to estimate abundance of Indus dolphins between Guddu and Sukkur barrages in the 1970s and their method resulted in an abundance estimate approximately 30% higher than visual surveys conducted by Pilleri and Zbinden (1973-74) around that time (Table 1.2). Buckland et al (2001) state that the main weakness of this method is the ability to measure cue rate. Surfacing rate of Indus dolphins may change in different situations, for examples if calves are present that surface more frequently than adults. It will be useful to conduct experiments to compare visual group size counts, with group sizes derived from cue counting to explore this method in greater detail.

Another option that holds great promise for advancing and improving river dolphin abundance estimates is the combination of visual and acoustic methods. This has been used effectively during surveys of vaquita (Gerrodette et al. 2011) and to a more limited extent of the Yangtze finless porpoise (Zhao et al. 2008). South Asian river dolphins would be particularly suited to acoustic surveys because they appear to echolocate virtually continuously and therefore very few would be acoustically undetected (Braulik, unpublished). However, acoustic surveys would be most useful for detecting missed groups, and this was already accomplished effectively using tandem vessels and a visual survey (Chapter 2). The aspect that requires greatest refinement, group size estimation, would not be so straight forward to determine from an acoustic survey. Towing an acoustic array in such a shallow environment could be problematic, the hydrophone would likely bump into the river banks in sharp bends, it may hit the river bed in shallow areas, and there would be a constant risk of entanglement in submerged debris. Despite these challenges, exploring acoustic abundance estimation for South Asian river dolphins would be worthwhile.

Many of the difficulties of surveying river dolphins, are also found in surveying manatees in tropical freshwater systems because the water is generally tannin-stained or turbid from loose sediment, water visibility is very poor, and manatees are cryptic, and often occur in small groups and at low densities. The traditional forms of locating manatees via aerial and boat surveys, while very useful in certain habitats, yield very low numbers in tropical freshwater systems. Side-scan sonar was successfully used to detect Antillean manatees (*Trichechus manatus manatus)* and although they could not be detected beyond 20m, the use of this method was considered useful to 1) detect manatees, 2) characterize manatee habitat in ways that would not be possible otherwise, 3) identify mother-calf pairs, and 4) assist in manatee captures (Gonzalez-

Socoloske and Olivera-Gomez, 2012). It is possible, especially as technology improves, that side-scan sonar could be used for river dolphin group size calibration, or to assist in rescues of Indus dolphins from canals.

6.3 *Platanista* Speciation

One reason that the phylogeny of the Indus and Ganges River dolphins has not been assessed in detail to date, is that the two populations occur primarily in different countries. It is difficult for Indians and Pakistanis to visit each other's countries, and therefore almost all researchers work specifically with only one dolphin population, making comparative studies or even more superficial comparisons impossible. The tendency has been to assume the two dolphin populations are the same, because they look broadly similar, especially when compared to all other cetaceans. The fish fauna of the Indus and Ganges systems has also been considered to be quite similar, but is poorly studied. Several fish species thought to inhabit both the Indus and Ganges were recently split into separate species, each in a different river system. It has been suggested that as the subcontinent's fish fauna is studied in greater detail a higher amount of fish speciation will be revealed (Mirza 2003; Ng 2004). Given the genetic differentiation between Indus and Ganges dolphins described in Chapter 5, it now seems probable that other comparative studies, especially of external morphology and skeletal morphology, will also indicate more differences than originally expected. This is demonstrated by the preliminary results from a comparative study on *Platanista* skull morphology which showed different, and non-overlapping, tooth counts between individuals in each population, and clear differences in the size and shape of the nasals (Braulik, unpublished).

If it can be shown that two populations show convincing species level differentiation, their re-classification as separate species will almost certainly positively impact their conservation. The news would be particularly well received in South Asia, where the two separate populations are already believed to be very different and are already managed as such. The widespread media coverage that would result, and the increase in their ranking as distinct species of serious conservation concern would help to mobilise both financial and technical assistance for their conservation. Resolution of this issue is of high priority.

6.4 Indus Dolphin Habitat and Environmental Flows

The study of the causes of Indus dolphin range decline (Chapter 3) and of their dry season habitat use (Chapter 4) both demonstrate that reduced discharge and habitat fragmentation by barrages are the primary threats to the Indus dolphin. Realistically, irrigation barrages on the Indus River will not be removed and advocating this as an Indus dolphin conservation strategy would be unrealistic and counter-productive. If Indus dolphins are to persist, they must do so within the current configuration of habitat fragments. If only a single conservation strategy were to be suggested for the Indus dolphin, managing the Indus River so that the natural hydrological regime is at least partially restored or maintained is likely to be most successful.

Recognition of the escalating hydrological alteration of rivers on a global scale and resultant environmental degradation, has led to the establishment of the science of environmental flow assessment whereby the quantity and quality of water required for ecosystem conservation and resource protection are determined (Tharme 2003). People increasingly understand that it is important to take care of aquatic ecosystems, and the resources they provide, for long-term economic viability. Environmental flows are an integral part of modern river basin management, and mean essentially that enough water is left in a river to ensure downstream environmental, social and economic benefits (Dyson et al. 2003). It requires negotiations between stakeholders to bridge the different interests that compete for the use of water, especially in river basins, such as the Indus, where competition is already fierce. The reward is an improved management regime that guarantees the longevity of the ecosystem and finds the optimal balance between the various users and uses (Dyson et al. 2003). Environmental flows are at present almost exclusively implemented in Australia, South Africa, the UK, and USA, but there is a growing realisation that this type of management would be highly beneficial for an over-stressed system like the Indus River.

A range of methods has been developed in various countries that can be employed to define ecological flow requirements, these were classified by Dyson et al.(2003), into four categories:

1. *Look-up tables*- Water managers use hydrological indices to define water management rules and to set compensation flows below reservoirs and weirs.

Examples are maintenance of percentages of the mean flow or certain percentiles from a flow duration curve. This method is purely hydrological.

2. *Desk top analysis*– These methods can include purely hydrological data, hydraulic information, and also ecological data. Desk-top analysis methods that use ecological data tend to be based on statistical techniques that relate independent variables, such as flow, to biotic dependent variables, such as population numbers or indices of community structure calculated from species lists.
3. *Functional analysis*- Builds an understanding of the functional links between all aspects of the hydrology and ecology of the river system. This involves taking a broad view and covers many aspects of the river ecosystem, using hydrological analysis, hydraulic rating information and biological data. The basic premise is that riverine species are reliant on basic elements (building blocks) of the flow regime, including low flows and floods that maintain the sediment dynamics and the geomorphological structure of the river. An acceptable flow regime for ecosystem maintenance can thus be constructed by combining these building blocks.
4. *Habitat modelling* -This method uses data on habitat for target species to determine ecological flow requirements. The relationship between flow, habitat and species can be described by linking the physical properties of river stretches, e.g. depth and flow velocity, at different measured or modelled flows, with the physical conditions that key animal or plant species require. Once functional relationships between physical habitat and flow have been defined, they can be linked to scenarios of river flow.

It is this fourth category that would likely be most desirable for considering the flow needs of river dolphins; however, to do this properly would be a large, expensive and time consuming exercise. It would need to include specific evaluations of river discharge, hydrology and dolphin habitat use at a range of flows and seasons and ideally would include additional information on dolphin life stage, reproduction and foraging, all data that are currently lacking. Hydrology–ecology relationships frequently exhibit responses to flow that are non-linear and include important thresholds, and articulation of these thresholds can be instrumental for managing river-specific or regional environmental flow programs (Shafroth et al. 2010). Given that water

abstraction from the Indus River is one of the primary threats to Indus dolphins, and anticipating that some kind of environmental flow study will likely be conducted in the future, beginning to gather the necessary data on Indus dolphin habitat use so that it is available to feed into such a study would be prudent.

It was noted by Tharme (2003) that in developing world regions, where environmental flow research is in its infancy, water allocations for ecosystems must, for the time being at least, be based on scant data, best professional judgement and risk assessment. If, or when, an environmental flow study is conducted on the Indus, the needs of Indus River dolphins will form only a small component. Such a study would need to balance other issues of over-arching national importance such as food production through irrigated agriculture, human health linked to water borne diseases and water quality, and national security associated with honouring international agreements on water allocation between countries.

Water scarcity is felt by almost all Pakistanis. As stated by the World Bank (2005), “the survival of a modern and growing Pakistan is threatened by [lack of] water. The facts are stark.” The newspapers publish river discharge figures daily and these figures are understood by the majority of people, and are topics of conversation in the towns and cities. There is very frequent tension between the Provinces over the allocation of river water between them, with Sindh, located downstream, sensitive to its vulnerable position (Anon. 2010; Shah 2009). Despite this, there is almost no concept of water conservation; the focus is almost entirely on obtaining and capturing more water from the rivers and from ground water. The irrigation system is extremely inefficient, water delivery is unreliable and inequitable, and crop yields per cubic meter of water are much lower than international standards and compared to those in neighbouring countries (World Bank 2005). Forty percent of the water diverted from the Indus basin in Pakistan is lost in conveyance and in the late 1980s it was estimated that improvements in supply efficiency could save some 14.8 billion m^3/yr of water. Canal lining is one such improvement (World Commission on Dams 2000). Conservation and good stewardship of water resources would go a long way to improving the water resources situation in Pakistan which would have far-reaching benefits for society as well as river ecosystems and the Indus dolphin.

6.4.1 Dams

At the time the World Commission on Dams was convened in 2000, half of the world's rivers had been dammed, over 45,000 dams had been built to irrigate a third of all crops, generate a fifth of all power, control floods in wet times and store water in dry times (World Commission on Dams 2000). Yet, in the last century, large dams also disrupted the ecology of over half the world's rivers, displaced over 40 million people from their homes and left nations burdened with debt (Moore et al. 2010). The Commission concluded in its landmark report that the unprecedented expansion in large dam building over the past century, harnessing water for irrigation, domestic and industrial consumption, electricity generation and flood control has clearly benefited many people globally. Nonetheless, this positive contribution of large dams to development has been marred in many cases by significant environmental and social impacts which, when viewed from today's values, are unacceptable. One of the biggest issues surrounding dams is that they frequently entail a reallocation of benefits from local riparian users to new groups of beneficiaries, often located in urban centres, at a regional or national level. The significant social and environmental impacts are often disproportionately borne by poor people, indigenous peoples and other vulnerable groups. Lack of equity in the distribution of benefits has called into question the value of many dams in meeting water and energy development needs when compared with the alternatives (World Commission on Dams 2000). After its extensive review and consultation, the Commission noted that large dams designed to deliver irrigation services have typically fallen short of physical targets, did not recover their costs and have been less profitable in economic terms than expected. Tarbela Dam in Pakistan, which was one of the Commission's case studies, was one of the better performers (World Commission on Dams 2000).

In spite of the many negatives, there is a frenzy of dam building underway in the Himalaya to meet needs for hydropower and for irrigation. Many hundreds of dams are planned in the Himalayan region (Dharmadhikary 2008), up to 78 dams have been proposed on the Mekong River system (Ziv et al. 2012) and more than 100 on the Brahmaputra system in India (Dutta 2010). Pakistan has plans to add 10,000 MW through five projects by the year 2016, and another 14 projects totalling about 21,000 MW are under study for construction by 2025. The government and the World Bank are pushing for the immediate implementation of the massive 4,500 MW Diamer-Bhasha large dam project on the Indus River. The dependence of Pakistan on irrigated

agriculture for employment, to feed its booming population and as the major source of its international exports is immense, and large dams on the Indus River are touted as the means to satisfy demand and stimulate economic growth. Meanwhile, India have many dams in place, under construction or planned on the Indus tributaries where they flow through Indian territory. A recent study on the Mekong River found that construction of all planned tributary dams, nearly all within Lao PDR national borders, would have catastrophic impacts on fish biodiversity basin-wide and on the Cambodian and Vietnamese floodplain's fish productivity, far greater than the combined impact of six upper main-stem dams on the lower Mekong River itself (Ziv et al. 2012). Dam construction will continue on the upper Indus and the consequences for people, fisheries and dolphins located downstream are unclear.

6.5 Climate Change

One direct response of cetacean species to global climate change is that their ranges may change to remain within preferred climatic conditions (MacLeod 2009). Species and populations such as many of the river dolphins that are unable to shift their range, or that have restricted geographical distributions, with little or no opportunity for range expansion are expected to be especially vulnerable (Simmonds and Eliott 2009). Polar bears (*Ursus maritimus*) in particular, and also ringed seals (*Phoca hispida*), bearded seals (*Erignathus barbatus*), beluga whales (*Dephinapterus leucas*), narwhals (*Monodon monoceros*), and bowhead whales (*Balaena mysticetus*), all species that inhabit the polar regions, are likely to be particularly adversely affected by climate change (Ragen et al. 2008).

Palmer et al. (2008) conducted a global study to project river discharge under different IPCC (Inter-governmental Panel on Climate Change) climate and water withdrawal scenarios. The Indus basin was one of few where river discharge was predicted to increase dramatically (>90%) by 2050. This positive news is tempered by the fact that because of the great discrepancy between water availability and withdrawals for human use, it was still predicted to remain one of the most water stressed basins (Kundzewicz et al. 2008; Palmer et al. 2008). These models indicate that climate change alone is unlikely to spell doom for Indus dolphins, and, if better water conservation practices are adopted in the future, water supplies may in fact increase, signifying a positive change.

It is possible that, if global temperatures rise and a greater proportion of Indus flow is derived from rainfall as opposed to glacial melt, that river water temperatures may rise as a result of climate change. Water temperatures in the mainstem of the Indus River vary from approximately 5°C in mid-winter to at least 33°C in early summer, an annual temperature range of almost 30°C (Braulik, unpublished). Indus dolphins have evolved the capacity to cope with large temperature fluctuations because these occur predictably and naturally in their habitat, so they may be more resilient to climate change driven increases in water temperature than species with more uniform habitats.

6.6 Protected Areas

The distribution of freshwater cetaceans is not uniform within rivers, so the management of essential habitat (e.g., for foraging, calving, nursing young) within a protected area framework can be an effective tool for conservation (Kreb et al. 2010). Globally, few Protected Areas (PAs) have been created specifically for fresh waters. Instead, freshwater habitats are commonly protected only incidentally as part of their inclusion within terrestrial reserves (Saunders et al. 2002). The Sindh Dolphin Reserve is one of very few freshwater PAs designated specifically to protect river dolphins (Kreb et al. 2010). The Reserve was established in response to a specific threat, that of dolphin hunting, which at that time was determined to be the major threat to the subspecies. However, there are very few other restrictions within the dolphin reserve, including limited management of fishing activity, pollutant discharges, or vessel traffic. As human populations increase and new threats emerge, it is likely that the reserve is becoming less effective at conserving dolphins. Managers need to find ways to adapt to, and respond to, the changing situation if the Reserve is to offer dolphins some protection.

Meanwhile, there is interest to declare other stretches of river in Punjab and Khyber Phakhtunkhwa Provinces, as additional dolphin PAs. Obviously, the simple creation of Protected Areas does not guarantee the long-term survival of vital ecosystems or endangered species without carefully considered and implemented management. Among a total of 25 PAs in northern Pakistan, 16 lack basic baseline information, 22 do not have any management plan, and 19 are without any management infrastructure (Nawaz 2007). As such, they are at best ineffective at protecting the environment and at worst may actually exacerbate environmental degradation. Given that the national governance is very weak, and large parts of the river are outside of government

control, instead run by tribal landlords, government legislated protected areas will not be effective without community involvement. Many of the people who live by the river are among the very poorest communities, they have relied on the river for their livelihoods for generations, therefore community based conservation is likely to be the most effective conservation strategy whether inside or outside a formal Protected Area framework. Even in countries such as China, with historically very powerful governments, involvement of indigenous people was advantageous for the long-term maintenance of conservation goals, and it was recommended that rather than creating new Protected Areas, it would be better to support ongoing sustainable use of natural areas by the people who have lived and nurtured these environments for generations (Xu and Melick 2007). It is often assumed that economic benefits are a precondition for people's support for environmental conservation. However, as demonstrated for the Philippine crocodile (*Crocodylus mindorensis)* cultural values, such as pride, interest, and fun, can, in fact, form an important incentive to support in situ conservation, even among poor rural communities in the developing world. Environmental communication and education can foster these positive values and provide a sound foundation for community-based conservation (van der Ploeg et al. 2011). There is great potential for fostering national pride for Indus dolphin conservation in Pakistan, as many people feel pride and responsibility to protect their endemic, endangered species. These feelings increase when they discover that it is blind and therefore assume it is afflicted, requiring special help.

In a continent wide-study of the effectiveness of protected areas for preventing extinction of great apes, it was clearly demonstrated that law enforcement was the best predictor of ape survival, rather than tourism or research (Tranquilli et al. 2011). Although the habitats and species are different, this is also likely to be successful in a river dolphin protected area; the visible presence of local wildlife personnel is essential for success and of higher priority than tourism and probably also research.

It could also be argued that once a PA exists it may be easier to leverage funds both nationally and internationally to support the new PA, and once the legal and political framework is in place it may make implementing practical measures that will be beneficial to dolphins easier. Certainly, the presence of the Sindh Dolphin Reserve has created a focus for both Sindh Wildlife Department and WWF-Pakistan's dolphin conservation efforts, and this area receives far greater attention than any other location

with river dolphins in Pakistan. Although it is hard to prove that this is due to the presence of the Sindh dolphin PA, as opposed to simply a high density area with easy access, it is likely to have played a significant role.

Freshwater PAs have characteristics that are quite different to those in other ecosystems because they are affected to a very great level by activities that occur outside the PA boundary, as water arrives from upstream, or runs off from nearby terrestrial areas (Saunders et al. 2002). For example, practices such as dam building or diverting water for agriculture can occur outside park boundaries and still have negative consequences for freshwater habitats within a PA (Saunders et al. 2002). As such, PA's are likely to be most effective for river dolphin conservation when attempting to manage localised threats such as fishing, hunting, vessel traffic, or specific point sources of pollution. If the primary threats are far reaching issues of depleted river flows, dams and distant pollutant sources a PA will probably not be the most effective means of addressing these issues.

A system of community and ecosystem-based management, and zoned protected areas that include highly protected reserves in critical areas, as well as buffer zones that allow human uses such as carefully managed tourism and fishing would be ideal (Hoyt 2005). If new Protected Areas for Indus river dolphins are to be established in Pakistan, a number of activities are necessary to ensure that they are effective. The goal and objectives that the PA is expected to address should be defined, why a PA is the best approach to address the conservation issues should be described, a plan for management including community involvement should be formulated, and a strategy for funding which could include using funds from within the government and also raising matching funds from outside the country should be developed. At present, dolphin conservation activities outside of the Sindh Dolphin PA are virtually non-existent. Therefore any step forward, however small, could be seen as positive for the dolphins.

6.7 Mortality Monitoring

The study of range decline (Chapter 3), examined the persistence of entire dolphin subpopulations over a long time frame and broad geographic scale, demonstrating the link between extirpation of dolphin subpopulations and low river discharge. This is an important macro-level conclusion but there is still very little information on what are the

immediate /proximal causes of the mortality of individual Indus dolphins. In many long-lived threatened and endangered mammals, variation in mortality is a primary determinant of population growth. Therefore, describing the causes of mortality is an important component of conservation research and the initiation of conservation actions. Systematic monitoring of marine mammal strandings has the potential to provide valuable information including identification of unusual mortality events, changes in mortality rates, determination of the relative causes of mortality and also provision of life history data (Wilkinson and Worthy 1999). For example, in the USA which has a very extensive national marine mammal stranding programme, historical stranding rates were used to determine that an epizootic was affecting the USA Atlantic coastal migratory stock of bottlenose dolphins in 1987-1988 (Scott et al. 1988) and is also being used to examine possible increases in mortality due to the Deep Water Horizon oil spill in the Gulf of Mexico (Williams et al. 2011). Monitoring of strandings has also led to identification of a variety of other unusual mortalities, including epizootics amongst striped dolphins in the Mediterranean Sea (Aguilar and Raga 1993; Gomez-Campos et al. 2011; Raga et al. 2008), manatees in Florida (O'Shea et al. 1991) and bottlenose dolphins in the USA (Duignan et al. 1996), poisoning due to demoic acid in California sea lions (Goldstein et al. 2008), and mortality due to fisheries interactions (Jepson and Deaville 2009; Kuiken et al. 1994; Cox et al. 1998). Stranding response in Cambodia over the last ten years, led to the conclusion that no Irrawaddy dolphin calves had survived in the Mekong River for several years and allowed an emergency plan to be enacted to try and determine the cause/s of this mortality (Reeves et al. 2009). The realization of the potential of aquatic mammal stranding networks is dependent upon the training and education of network members, and there is a trade-off between the extent of coverage and the amount of scientific information that can be obtained (Wilkinson and Worthy 1999).

During the last decade, there were very few recorded mortalities of Indus dolphins (1-2/year), but in the last two years the number of reported strandings has risen dramatically (~30/year) (Babbar 2011; WWF-Pakistan unpublished). An important next step is to establish a stranding network so that mortalities, associated biological information, and determination of causes of death can be documented. This would involve reporting of mortalities by local communities to a designated authority, systematic collection of data that is stored in a central location, collection and analysis of tissue samples using standard protocols, and training of responders in necropsy

techniques. Until there is such a system in place it is not easy to identify or respond to peaks in mortality that may be cause for concern. Without information on the incidence of Indus dolphin mortality and the causes it is not possible to design conservation measures to reduce mortality, or easily monitor the success of existing strategies. A stranding network would also allow the collection of numerous other important life history and health data that are at present almost completely lacking.

6.8 Movement of Dolphins Through Barrages

Discussion of whether or not dolphins can, and do, move through irrigation barrages permeates most Indus dolphin conservation questions. Although there is evidence from radio-tracking that one dolphin has moved through a barrage, movement of larger numbers of individuals is still a hypothesis waiting to be evaluated. Obtaining a greater understanding of this issue is of high priority because, depending on how many individuals move and in which predominant direction, the persistence of upstream subpopulations may be threatened, and there may be a continual loss of dolphins to areas of marginal habitat downstream. Options for measuring or counting the number of dolphins that move through a barrage are limited at present because it is difficult from visual observations to determine definitively whether an individual seen on one side of the barrage has moved through unless it actually surfaces within the gates. Barrages are politically sensitive structures so attaching passive acoustic devices to them may be problematic, but this is less of an issue compared to the difficulty of anchoring such devices in the rapidly flowing water and keeping them free of debris. Previous attempts to attach a T-POD (an autonomous passive acoustic monitoring device that detects and logs cetacean clicks) to a barrage required that the device be checked and cleared of debris several times per day and even then it was submerged and almost lost numerous times (Braulik, unpublished).

Dolphin movement through barrages could be investigated in two separate ways by: 1) studying the design and operation of the barrages 2) tracking the movements of dolphins:

1. *Study of barrages*: Numerous factors associated with the river and the barrage structure and operation are likely to influence whether, when and how frequently dolphins move through barrages. The most important aspect is whether and for how long the gates are open creating a physical opening large enough for a

dolphin to pass through. For many months of the year, all gates on many barrages are closed completely and dolphin movement would be physically impossible. Other factors that may also influence whether dolphins pass through the structures include the design of the barrage, the fall in elevation between the upstream and downstream bed level which influences water turbulence within the gates, river discharge which influences water depth, velocity and turbulence, and the density of dolphins above and below the barrage. The way that these inter-related parameters influence a dolphin's ability to traverse a barrage could be examined in relation to what is known about cetacean behaviour and swimming ability. Detailed barrage operation and river discharge data can be obtained for important barrages on the Indus river and the temporal and spatial operation data used to identify the periods of the year at which there is a low, medium or high likelihood of dolphin movement through each barrage.

Figure 6.1 – View of individual barrage gates viewed from the downstream side, illustrating the differences between a gate that is fully open, and one which is partially closed: a very different barrier to dolphin movement.

2. *Tracking dolphins*– Dolphins that are stranded in irrigation canals, rescued and returned to the main river are ideal candidates for tracking. The short surfacing time of Indus and Ganges River dolphins (less than one second) is insufficient for many satellite tags to warm up, obtain a position and transmit data. When this PhD study began, the technology was not yet developed for sufficiently rapid transmission of positional data from any of the satellite tags in existence at that time, however over the last five years the situation has changed, and SPOT

satellite tags developed by Wildlife Computers can now operate in freshwater and obtain and transmit their position through ARGOS within a second. Use of the GSM phone network to transmit data seems appropriate, as there is phone coverage throughout the river, but it takes at least 12 seconds to transfer the data which would never be available unless the tagged dolphin died (McConnell et al. 2004). Pop-up tags which record satellite positions into the memory and then transmit the information after they detach from the animal and float to the surface may have potential but, because of the flowing water, actually retrieving any physical device is unlikely. To avoid continuously using the battery when an animal is underwater, most tracking devices save power by relying on a salt-water switch that is triggered when the animal surfaces. VHF radio tracking from shore has been successfully used in Pakistan in the past (Toosy et al. 2009); the transmitter stayed attached for more than 3 weeks, and tracking was successful. Movements of Amazon River dolphins have also been extensively studied using radio-tracking (Martin and da Silva 1998). However, in Pakistan it can be difficult to follow dolphins when they move into lawless areas, and it is necessary to have teams and boats available to conduct the tracking which is not always feasible. Therefore use of the newly developed satellite tags is probably the best option for long-term monitoring of the movement of individual Indus dolphins.

If tags were routinely attached to dolphins released from canals, this would provide information on dolphin movement patterns and habitat use and possibly whether they are able to move through barrages. However, due to the cost and man-power required it is unlikely that many individuals could be tagged and even if several individuals were recorded moving through barrages, it would be difficult to extrapolate this to the entire population and quantify an overall movement rate. In addition, most dolphins are rescued from canals close to Sukkur barrage when the canals are drained for maintenance, which is also the only few weeks of the year that barrage gates are fully open (Figure 6.1). Dolphins released into the river during canal closure have a far greater opportunity to move through the barrage than they would at any other time of year and tracking conducted only on rescued dolphins may therefore over estimate their ability to move through barrages.

6.9 Dolphin Translocation

Dolphin subpopulations in the Indus River system are being slowly extirpated primarily from the upstream portions of their range. The study on the temporal and spatial

dynamics of Indus dolphin decline (Chapter 3) demonstrates a 'domino effect' with subpopulations upstream disappearing prior to those immediately downstream. At present the smallest extant subpopulations are at the upstream end of the dolphin's current distribution on the Indus River, between Jinnah and Chashma and Chashma and Taunsa barrages and on the Beas River in India. The Jinnah-Chashma subpopulation has only a handful of animals and is teetering on the brink of extirpation, while the Chashma-Taunsa subpopulation is estimated at only 101 individuals (Chapter 2) and the Beas River population as approximately 10 animals (Behera et al. 2008). The possibility of translocating Indus dolphins from the high density, largest subpopulation (Guddu to Sukkur), to a subpopulation with low abundance deemed to be threatened with extirpation, has been discussed in Pakistan for more than ten years. This would be a somewhat controversial plan that would be subject to intense scrutiny, but it has some merit. Translocation programmes typically have varied goals that include bolstering genetic heterogeneity of small populations, establishing satellite populations to reduce the risk of species loss due to catastrophes, and speeding recovery of species after their habitats have been restored or recovered from the negative effects of environmental toxicants or other limiting factors (Carpenter et al. 1989). Translocations are being considered, in a 'bold management action', to improve poor juvenile survival of the Hawaiian monk seal (*Monachus schauinslandi*) within the Papahānaumokuākea Marine National Monument in the Hawaiian Islands (Gerber et al. 2011; Littnan et al. 2011).

Some key points relating to an Indus dolphin translocation programme are discussed below:

If upstream subpopulations are declining due to 'downstream migratory attrition' (Reeves 1991), and individuals are concentrating between Guddu and Sukkur barrages, then translocating dolphins out of this high density subpopulation, to supplement the low numbers in upstream areas may be sufficient to prevent the extirpation of several very small subpopulations. However, downstream migratory attrition has not been proven and it is possible that the decline of upstream subpopulations is due to other factors that would compromise the survival of translocated dolphins. Even if other factors are involved, supplementing the small subpopulations may be sufficient to ensure their persistence.

Capturing dolphins in the wide and fast-flowing river itself would be fraught with difficulty and danger to both the dolphins and the capture team and would be ill-advised (Braulik et al. 2005). However, up to twenty dolphins each year become trapped in irrigation canals and are rescued and returned to the river. These animals would die if they were not captured, and once captured, are ideal candidates for translocation. Although most dolphins rescued from canals have been released near to Sukkur barrage, several have already been released almost 200km away, upstream of Guddu barrage and there appears to be political agreement in principal to the translocation concept.

The size of the largest dolphin subpopulation between Guddu and Sukkur barrage is relatively large (Chapter 2 – 1289), occurs at high density, and appears to have been increasing in size over the last thirty years. This subpopulation could likely sustain the removal of a modest number of individuals per year. In general, translocation success is highest when animals are wild caught, originate from a high density population and from a population increasing in size (Carpenter et al. 1989).

The habitat study (Chapter 4) indicated that channel geometry and river morphology is more suitable for dolphins between Guddu and Sukkur barrages than in subpopulations upstream. However, river discharge in upstream areas is greater than in all other locations, and the range decline study suggested that these subpopulations should be able to persist for several centuries (Chapter 3). Preliminary evaluations of human threats on the Indus mainstem indicate that dolphins are under no greater threat in upstream subpopulations than in those downstream (Braulik, unpublished). Without high habitat quality, translocations have low chances of success regardless of how many organisms are released or how well they are prepared for the release (Carpenter et al. 1989). If a translocation programme were being considered seriously, detailed studies on habitat and threats in the receiving environment would be required. Even with very carefully designed scientific studies it would be difficult to demonstrate definitively that the receiving area provided sufficient suitable habitat to support the translocated animals. The question would always remain as to whether the receiving subpopulation was at low density simply because the habitat could not support more dolphins.

The genetic study (Chapter 5) demonstrated genetic uniformity in the mitochondrial control region among Indus dolphin samples collected from between Guddu and Sukkur barrages. Although it is not known whether dolphins in other subpopulations show any additional variability, it is possible that they do, in which case translocation would facilitate genetic exchange and may increase genetic variability of upstream subpopulations.

The welfare of the dolphins during capture, transport and release would need to be carefully monitored and evaluated throughout the operation. To reach various overseas dolphinariums in the 1970s dolphins were transported several thousand kilometres from their capture locations in India, Bangladesh and Pakistan by train, truck, plane, boat and in some instances rickshaws. In general this species is fairly robust during transport, the greatest mortality occurred during their capture (Haque et al. 1997; Herald et al. 1969; Pilleri 1970). This has also been seen during the dolphin rescue programme, if a dolphin survives capture, it generally survives transportation to release. A translocation programme would probably need to use a helicopter to move the dolphins over such large distances, in which case, the possible effects of the noise and vibrations on the animal would need to be carefully evaluated.

It is not possible to identify Indus dolphin individuals therefore the monitoring of the translocation programme would need to focus on 1) tracking of individuals after release to monitor their movements and possibly survival, and 2) regular population monitoring to attempt to identify changes in sub-population abundance. A translocation programme would be determined to be successful if the founder population was not severely impacted by the removals, and small subpopulations upstream either increased in size, or were maintained at current levels.

In summary, a translocation programme would need to be well-conceived and well-researched prior to implementation and would need to consider animal welfare, impacts to the founder subpopulation, habitat and threats in the receiving subpopulation, and long-term monitoring of released individuals. A formal cost-benefit risk analysis of the entire operation would be a good preliminary exercise. If conducted cautiously, I believe this to be a course of action to which it is worth giving serious consideration. Rescued dolphins provide an ideal opportunity because captured

animals are regularly available at comparatively little risk and cost. The Indus dolphin is not yet so threatened that the loss of an individual outweighs the advantages of successfully maintaining additional subpopulations. Carpenter et al. (1989) conducted a review of 93 species translocation projects and concluded that "*the greatest potential for establishing satellite populations may occur when a candidate population is expanding and numbers are moderate to high. These conditions are the ones that tend to make endangered species biologists relax; our analysis suggests that these conditions may point out the time for action*". I am inclined to agree, and believe the long-term conservation benefits of establishing and maintaining via translocation additional Indus dolphin subpopulations to be worth the possible risks associated with the conduct of such an operation.

6.10 Concluding Remarks

The research conducted in this thesis is a small step forward in increasing our knowledge of Indus River dolphins. It could be easy to become overwhelmed by how little is still understood about them. Decades could be spent collecting data to try and find the answers. However, it is important that the search for more information not become the sole focus and that concrete conservation actions also be implemented. With reference to the extinction of the baiji, the Scientific Committee of the International Whaling Commission (IWC 2008) stated that "despite extensive scientific discourse for more than two decades, little effort was made to implement any real conservation measures for this species. In hindsight, the extinction of this species is not surprising; species cannot be expected to save themselves". The conservation status of the Indus River dolphin is not yet as dire as that faced by the baiji, the vaquita, or the Mekong Irrawaddy dolphins. There is still considerable uncertainty about several of the key threats and more conservation focussed research is essential. Do dolphins move between subpopulations and through barrages? Are upstream subpopulations declining in abundance? How much water is enough to sustain a dolphin population? What is causing the recent spate of mortalities between Guddu and Sukkur? These are questions that need to be answered to improve conservation of Indus dolphins. It is possible to focus on the things that are known: that declining river flows threaten dolphins especially at the upstream end of their range and that fisheries interactions are an increasing problem, and build management actions on this strong foundation.

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Appendix I – Interview Questionnaire

River: Location: N E

Town Name:

Nearest Barrage: Date:

Name of Fishermen: Tribe:

1. How old are you? 20-30 31-40 41-50 51-60 61-70 71-80 80+
2. How many children do you have?
3. How many grand children?
4. For how many years have you been fishing?
5. Where do you fish?
6. How long have you fished or lived in this present location?
7. Do you fish: Commercially / Subsistence / Full-Time / Part-Time
8. What kind of fishing gear do you use?
9. What species of fish do you try to catch?
10. Which kinds of fish do you throw away or do you use everything?
11. Do you fish mostly during a certain season of year?
12. How good is the fishing these days?
13. What kinds of changes have you noticed over time?
14. Do you know what an Indus River dolphin is?
15. Have you ever seen an Indus River dolphin?
16. If you have seen or heard about an Indus River dolphin, please give details:
    - Who saw the dolphin? personal observation / fathers generation/ grandfathers generation / story or distant relative / other
    - Details of sighting: Date. Location. Habitat. Behaviour. Season.
17. Why do you think there are no dolphins left in this river?
18. When do you think dolphins died out here?
19. What do you think about the future of fisheries and fishermen on this river what can be done to improve the situation?

Appendix II – Local Events Calendar used to refine dates of historical dolphin sightings

Year	Years ago	Event
1886	121	Sidhnai Barrage, Ravi River completed
1892	115	Khanki Barrage, Chenab River completed
1901	106	Rasul Barrage, Jhelum River completed
1926	81	Suleimanki Barrage, Sutlej River completed
1927	80	Islam Barrage, Sutlej River completed
1932	75	Panjnad Barrage completed
1939	68	Trimmu Barrage, Chenab River completed
1947	60	Partition
1955	52	Major flood on Ravi & Sutlej
1956	51	War with India
1958	49	Ayub Khan in power
1959	48	Major flood Jhelum & Chenab
1962	45	Islamabad becomes capital
1965	42	Sidhnai Barrage 2, Ravi River completed
1965	42	War with India
1967	40	Rasul Barrage, Jhelum River completed
1967	40	Qadirabad Barrage, Chenab River completed
1968	39	Marala Barrage, Chenab River completed
1969	38	General Yaya in power
1971	36	Bangladesh independence
1971	36	Zulfikar Ali Bhutto in power
1978	29	Gen Zia president
1988	19	Zia killed
1988	19	Major flood on Ravi & Sutlej
1992	15	Major flood on Jhelum & Chenab
1998	9	Nuclear test
1999	8	Musharaf in Power
2005	2	2005 earthquake

Appendix III – Details of current and former fragments of Indus dolphin habitat

Table A1 – Current and former fragments of Indus dolphin habitat listed in chronological order of their creation. River sections highlighted grey are still present.

#	Fragment Description	Creation Date	End Date	Duration	Length	Dolphin extant
0	Former un-fragmented range	N/A	1886	N/A	3208	1
1	Sidhnai to Madhopur	1886	1917	31	380	1
2	Former range #0, minus #1	1886	1892	6	2828	1
3	Khanki to Marala	1892	2011	119	35	0
4	Former range #0, minus #1 & 3	1892	1901	9	2793	1
5	Upstream Rasul	1901	2011	110	50	0
6	Former range #0, minus #1, 3 & 5	1901	1926	25	2743	1
7	Sidhnai to Balloki	1917	2011	94	175	0
8	Upstream Suleimanki	1926	1927	1	360	1
9	Former range, minus #3, 5, 7 & 8	1926	1927	1	2383	1
10	Suleimanki to Hussainiwala	1927	2011	84	110	0
11	Upstream Hussainiwala	1927	1955	28	250	1
12	Islam to Suleimanki	1927	2011	84	145	0
13	Former range, minus everything upstream of Islam, Sidhnai, Rasul & Khanki barrages	1927	1932	5	2238	1
14	Downstream Sukkur to sea	1932	1955	23	540	1
15	Former range, minus everything upstream Islam, Sidhnai, Rasul & Khanki barrages, and downstream Sukkur	1932	1933	1	1698	1
16	Panjnad to Islam/Sidhnai/Rasul/Khani	1933	1939	6	970	1
17	All Indus River to Sukkur & Panjnad	1933	1946	13	728	1
18	Panjnad to Trimmu/Sidhnai/Islam	1939	2011	72	435	0
19	Trimmu to Rasul/Khanki	1939	1967	28	535	1
20	Upstream Jinnah	1946	2011	65	35	0
21	Jinnah to Sukkur & Panjnad	1946	1959	13	693	1
22	Sukkur to Kotri	1955	2011	56	318	1
23	Downstream Kotri to sea	1955	2011	56	222	0
24	Hussainiwala to Harike	1955	2011	56	30	0
25	Upstream Harike	1955	2011	56	220	1
26	Taunsa to Sukkur & Panjnad	1959	1962	3	403	1
27	Jinnah to Taunsa	1959	1971	12	290	1

#	Fragment Description	Creation Date	End Date	Duration	Length	Dolphin extant
28	Guddu to Sukkur	1962	2011	49	126	1
29	Taunsa to Guddu & Panjnad	1962	2011	49	277	1
30	Trimmu to Rasul & Qadirabad	1967	2011	44	490	0
31	Qadirabad to Khanki	1967	2011	44	45	0
32	Taunsa to Chashma	1971	2011	40	230	1
33	Jinnah to Chashma	1971	2011	40	60	1

Note: Lengths listed here were measured using ArcView 3.2 and satellite images, and are shorter than those recorded during vessel-based surveys in the same sections of river. N/A = Not applicable.

Appendix IV – CITES import and export permits

No. F.9-2/98-NCW
GOVERNMENT OF PAKISTAN
MINISTRY OF ENVIRONMENT
NATIONAL COUNCIL FOR CONSERVATION OF WILDLIFE

CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA (CITES)

EXPORT PERMIT

(May only be used for export within a period of six months from the date it is granted) A separate permit shall be required for each consignment of specimens.

IN PURSUANT TO ARTICLE VI OF THE CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA

Note: This export permit is issued only for the animals and its products included in Appendix-I, II, III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

EXPORTING COUNTRY PAKISTAN
EXPORT PERMIT NUMBER P.05/2008 DATED 09-04-2008

This permit is issued by National Council for Conservation of Wildlife in Pakistan, Ministry of Environment, Government of Pakistan, Islamabad, Competent Authority by virtue of functioning as Management Authority in Pakistan to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

to Pakistan Wetlands Programme (Name)

of House No. 3, Street No. 4, Sector F-7/3, Islamabad (Address)

as gives him authority to export (10) ten (Number)

specimen/product(s) of specimen(s) Tissue samples (bone, teeth and dried skin) (Type of Product)

of *Platanista minor* SPECIES (Scientific Name)

Indus River Dolphin (Common Name)

as species listed in Appendix-I/ ~~Appendix-II~~/~~Appendix-III~~ of the Convention on the export, and transit of certain species of wild animals and plants.
to be exported to United Kingdom (Country)

University of Durham, South Road, Durham, DHI 3LE,United Kingdom (Address)

r the following purpose: Scientific research (DNA analysis)

THIS PERMIT IS VALID SUBJECT TO THE PRODUCTION OF PRESCRIBED MANDATORY PROVINCIAL FEE/COMPENSATION RECEIPT/CHALLAN BEFORE THE CUSTOMS.

DESCRIPTION OF ANY MARK (S) AFFIXED ON EXPORTATION
THE APPLICANT (being aware of the Provisions of the Convention)

CITES STAMP NO. 0261416

Dated: 09-04-2008

The Authority issuing the Permit

I.G. Forests/Member Secretary
National Council for Conservation of Wildlife, Islamabad, Pakistan

IGF/MEMBER SECRETARY
National Council for Conservation of Wildlife
Ministry of Environment
Government of Pakistan
ISLAMABAD

ORIGINAL

1

1. Exporter/Re-exporter
PAKISTAN WETLANDS PROGRAMME
HOUSE NO:3 STREET NO: 4
SECTOR F-7/3
ISLAMABAD
PAKISTAN

Permit/Certificate
☒ IMPORT
☐ EXPORT
☐ RE-EXPORT
☐ OTHER

No. 307866/02

2. Last day of validity (See Condition 4)
09/10/2008

Convention on International Trade in Endangered Species of Wild Fauna and Flora

3. Importer
DR A.R. HOELZEL
DEPT. OF BIOMEDICAL SCIENCES
UNIVERSITY OF DURHAM
SOUTH ROAD
DURHAM
DH1 3LE

4. Country of (re)-export
PAKISTAN

5. Country of import
UNITED KINGDOM

6. Authorised location for live wild-taken specimens of Annex A species

7. Issuing Management Authority
Department for the Environment, Food and Rural Affairs
Wildlife Licensing and Registration Service
Floor 1, Zone 17, Temple Quay House
2 The Square, Temple Quay
Bristol, BS1 6EB
Tel 0044 (0)117 372 8749 Website www.ukcites.gov.uk

8. Description of specimen(s) (incl. marks, sex/date of birth for live animals)
Name Applied : Platanista gangetica minor
Four skin samples in formalin from Indus River Dophins in Pakistan. (10gram total for all samples)

9. Net Mass (kg): 10 g

10. Quantity: 4

11. CITES Appendix: I

12. EC Annex: A

13. Source: II

14. Purpose: M

15. Country of Origin: Pakistan

16. Permit No: P.OS/200

17. Date of issue: 09/04/2008

18. Country of last re-export:

19. Certificate No

20. Date of issue

21. Scientific name of species: Platanista gangetica

22. Common name of species: Ganges Susu

23. Special Conditions
(Permit numbers 307866/01 and 307866/02 must be used together as they refer to the same items. Please do not separate these permits.)
These samples may only be used for scientific purposes.

This permit/certificate is only valid if live animals are transported in compliance with the CITES guidelines for the transport and preparation for shipment of live wild animals or, in the case of air transport, the live animals regulations published by the International Air Transport Association (IATA)

24. The (re-)export documentation from the country of (re-)exportation
☐ has been surrendered to the issuing authority
☐ has to be surrendered to the border customs officer of introduction

NATIONAL COUNCIL FOR
CONSERVATION OF WILDLIFE
MINISTRY OF ENVIRONMENT
GOVERNMENT OF PAKISTAN
BUILDING NO. 14-D,SECOND FLOOR
F-8 MARKAZ, ISLAMABAD

25. The ☒ importation ☐ exportation ☐ re-exportation of the goods described above is hereby permitted

Signature and official stamp

Name of issuing Officer: Michael Anglin

Place and date of issue: Bristol 03 June 2008

26. Bill of Lading/Air Waybill No.:

27. For customs purposes only

Qty/Net Mass (Kg) actually imported	Number of animals dead on arrival

Customs Document
Type:
Number:
Date:

Signature and official stamp

European Communities Act 1972
The Products of Animal Origin (Third Country Imports) (England) (No.4) Regulations 2004 (as amended)

Import Authorisation

defra
Department for Environment, Food and Rural Affairs

Authorisation No.

POAO/2008/ 360

The Secretary of State for Environment, Food and Rural Affairs, in accordance with regulation 3(2) of the Products of Animal Origin (Third Country Imports) (England) (No.4) Regulations 2004 (as amended) authorises:

name and full postal address: Molecular Ecology Laboratory
University of Durham
South Road
Durham
Postcode DH1 3LE

to land in England in accordance with the conditions set out below

Product: Skin and Bone Samples of the Indus River Dolphin

from (country of origin): Pakistan

at (port of entry): London Heathrow

until (date of expiry): 29 July 2008

Unless amended, suspended or revoked by the Secretary of State by notice to the person to whom it is issued

Dated: 30 April 2008

Signed: [illegible]
Officer of the Department for Environment, Food and Rural Affairs

Conditions attached to this Authorisation

1. This authorisation is valid for **multiple consignments** and the net weight per consignment must not exceed 60 kg.
2. The products must remain in their original wrapping at all times until their arrival at Molecular Ecology laboratory, University of Durham, South Road, Durham DH1 3LE.
3. The consignment shall be taken directly from the port of entry to the above address.
4. The Divisional Veterinary Manager at the Newcastle Animal Health Office (Tel: 0191 2295400 Fax: 0191 2295413) must be advised of the arrival of the consignment in England.
5. The consignment, or its packaging, must not be allowed to come into contact with any ruminating animals, swine, poultry or horses.
6. Immediately on arrival, all outer packaging shall be destroyed by incineration at University of Durham, molecular Ecology Lab, South Road, Durham DH1 3LE.
7. **None of the material to which this authorisation relates shall be used for human consumption under any circumstances.**
8. On completion of the testing any residues of the material and the remainder of the packaging shall be incinerated at the address stated in paragraph 6.
9. The importer must confirm in writing to the address below within 7 days of the incineration taking place that the above conditions have been adhered to.
10. The products must be made available, if so required for inspection by an officer of the Department or one of its agencies at any place nominated by him for such an inspection. The importer shall afford

POAO 2 (5/06)

Appendix V

Platanista Ancient DNA extraction Laboratory Protocols

The laboratory work was conducted at the Durham University ancient DNA laboratory which is one of the only specialist laboratories for working with ancient DNA in the UK. The work was conducted by Dr. Ross Barrett under the supervision of Professor Rus Hoelzel.

DNA Extraction

All aDNA extractions were performed in a dedicated lab where no modern molecular biology or post-PCR work is undertaken. Furthermore, this is the first time *Platanista* has been studied in this laboratory and no modern material (e.g. fresh tissue or blood) was analysed that could contribute to contamination. All materials and work surfaces were bleached before use with a 10% dilution of Sodium Hypochlorite and the workspace was UV irradiated overnight. Samples of *Platanista* bone and preserved tissue were excised using a scalpel blade and then manually reduced to bone powder or macerated tissue. The powder or tissue was collected and incubated overnight on a rotator at 55°c in 500µl of extraction buffer (1M EDTA, 15mM Tris, pH8.0, 1%w/v SDS) with 8µl of Proteinase K (0.3mg.ml-1). Digested samples were then extracted using the QIAquick PCR purification method of Yang et al. (1998) as described in Nichols et al. (2007). Final eluates of aDNA were collected in 50µl of TE buffer (1mM EDTA, 10mM Tris, pH8.0) and stored at -20°C. Negative extraction controls (lacking bone powder or macerated tissue) were performed in parallel at a ratio of approximately 1:7.

DNA Amplification

The mtDNA control region was amplified in two overlapping fragments (Table 4.2). Primers were designed to be specific for *Platanista* (Arnason et al. 2004) and exclude cross-amplification of either *Homo sapiens* or *Mus musculus*, two common reagent contaminants. Each PCR used 2µl of aDNA extract in a 25µl volume with Hi-Fidelity Platinum Taq (Invitrogen, UK). PCR conditions were as follows: 25mM dNTPs, 25 mM $MgCl^2$, 1 U/µL of Taq, 10 mM of primer, plus PCR buffer made to volume with double distilled H_2O. The PCR cycling conditions were hot-start, with an initial cycle of 95°c for 5 mins used to remove an antibody bound to the Taq polymerase that prevents non-

specific amplification prior to PCR. The cycling steps for the 3F/3R primers were as follows: step 1: 95ºc for 5 mins, step 2: 95ºc for 45 secs, step 3: Ta for 45 secs; step 4: 68ºc for 45 secs; step 5. go to step 2 for 45 cycles; step 6. 68ºc for 5 mins and step 7. store at 8ºc. PCR products were purified using the QIAquick Purification kit and sequenced in both directions using ABI BigDye Terminator chemistry at Durham.

Data Authenticity

The fragmented and damaged nature of aDNA requires additional checks of authenticity. In addition to the negative extraction controls and negative PCR controls, sterile reagents and equipment, and physical isolation of the work, it was possible to compare the sequences to those previously published on GenBank (Arnason et al. 2004; Benson et al. 2004). All sequences showed complete identity with one of three haplotypes arguing strongly for their authenticity.

Table 4.2 Primers used to amplify *Platanista* mitochondrial control region.

Name Forward	5'-> 3'	Name Reverse	5'->3'	Ta	Amplicon
CRPL3F	GGTTGCGGGCCTATTCCGTCCGTGA	CRPL3R	GGGGATTAGTGGAGTACTATGTCCTGT	58	178bp
CRPL2F	TATATATGCTATGTATAATCGTGCA	CRPL2R	GAGAAATACCAACTGTACTGAGTCC	52	302bp